Ihor Gussak · Charles Antzelevitch *Editors*
Arthur A.M. Wilde · Brian D. Powell · Michael J. Ackerman
Win-Kuang Shen *Co-Editors*

Electrical Diseases of the Heart

Volume 1: Basic Foundations and Primary Electrical Diseases

Second Edition

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We dedicate this book to the thousands of investigators whose collective works have brought us to this exciting juncture in the history of science and medicine and on whose shoulders we stand. We are proud to dedicate this compendium to these pioneers of cardiac electrophysiology as well as to our mentors, collaborators, and fellows who have assisted us in advancing the field, and last but certainly not least to our families, whose understanding and support permitted us to dedicate the time and effort needed to formulate this text.

Ihor Gussak
Charles Antzelevitch

Foreword

I presented the following "case report" in the preface of the first edition of this book to illustrate the progress in cardiac electrophysiology over a period of 20-odd years. The evolution is still relevant:

She was about 35 years old when she first became my patient in 1975. She had suffered from bouts of a supraventricular tachycardia (SVT) as far back as she could remember. "In the early days," she recalled, "when I was a kid, they would give me something in the emergency room that elevated my blood pressure and damn near tore my head off. What a headache I would get! But a lot of times it didn't work. Then they stuck my head in a bucket of cold water and told me to 'bear down.' Finally, they would give me more digitalis in my vein until I started vomiting. That usually stopped the SVT."

But nothing seemed to prevent recurrences. She was on a full dose of digitoxin and was one of the first to try a β(beta) blocker (propranolol) in the late 1960s. Her episodes were fast, around 220/min, and frightened her terribly, so much so that she would ride the tractor alongside her farmer-husband all day long just to be near him in case she had a recurrence.

Then came one of the first breakthroughs. Gordon Moe had published a "case report" of a dog with probable atrioventricular node reentry (AVNRT), showing that such a tachycardia could be started and stopped by external stimuli. Clinical studies followed (though somewhat belatedly) and replicated such responses in humans. Medtronic developed an implantable pacemaker (5998 RF unit) that was triggered by an external battery-driven stimulator held over the passive receiver to deliver a burst of rapid stimuli to the epicardial electrodes implanted on her right atrium. Magic! She terminated her own SVT with unerring reliability and never precipitated atrial fibrillation. Now a free woman, she no longer needed tractor rides. But she never left her house without the RF generator and always carried a spare battery in her pocket.

Over time she discontinued her medications and gradually stopped coming for return visits because she had complete control of her SVT. About 15 years later she showed up unannounced after one of the wires in her handheld unit fractured and she no longer could stop the SVT. "Could I get her a replacement or send the broken unit for repairs?" she asked. The next day she was in the EP laboratory, had a slow pathway ablation, cure of the AVNRT, and eventual removal of the implanted unit.

This patient benefited from knowledge derived from animal and clinical research, as well as technological discoveries, over a period of some 15–20 years. And hundreds of thousands of patients like her have similarly profited from such advances. The work of basic and clinical scientists continues to uncover complex mechanisms and anatomic sites responsible for these and other arrhythmias, providing understanding ranging from the molecular to clinical level. Such advances, along with new mapping, imaging and recording modalities, and catheter and ablation innovations, help us toward our goal of translating science into improved patient care. We are also beginning to understand the pervasive role of genetics, not just for the classic inherited syndromes, but also for polygenic diseases such as sudden death in coronary disease and heart failure, and to manipulate genes for therapy.

Once again this book captures this new information, with sections on basic electrophysiology and heritable channelopathies, primary and secondary electrical diseases and sudden cardiac death, diagnostic methods and tools, risk stratification, and treatment. It is a tour de force, and one that is certain to fulfill the reading tastes and intellectual demands of both researchers and clinicians.

Congratulations to the editors and authors for creating the second edition of this popular work.

Indianapolis, IN *Douglas P. Zipes, MD*

Preface

In this second edition of *Electrical Disease of the Heart*, our goal was to embrace and highlight the explosion of knowledge that our field has witnessed since the publication of the first edition of this book. Building on the success of our first edition, our approach continues to be one of bridging basic and clinical science in an attempt to meaningfully advance our understanding of heart disease and identify the knowledge gaps that exist.

The book is organized into 77 chapters in 2 volumes. Each chapter includes up-to-date results of studies aimed at providing an understanding of the electrical function of the heart in health and disease, established and evidence-based knowledge of clinical outcomes, areas of controversy, and future trends. Our goal is to provide a contemporary and succinct distillation of the state of the art. Although many of the chapters are highly sub-specialized, this book is designed for a broad audience, ranging from medical and graduate students to clinicians and scientists.

The book is the result of a collaboration that has brought together the skills and perspectives of researchers, scientists, and clinicians. We are deeply indebted to our associate editors and to all of the authors for their valuable contributions.

Ihor Gussak
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Part I
Basic Fundamentals of Normal and Abnormal Cardiac Electrical Activity

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1
Introduction to Part I: Basic Cardiac Electrophysiology – Promises Kept and Promises to Keep

Ralph Lazzara

Abstract

Important methodological developments contributing to the great advances in basic cardiac electrophysiology in the past 100 years include the microelectrode for recording intracellular potentials, the disassociation of myocytes in the cardiac syncytium, and fluorescence imaging for monitoring intracellular calcium and the transmembrane potential. Significant advances in recent years include the description of the J wave syndromes and focal ventricular tachycardias originated from the Purkinje-myocardial junction of the papillary muscles, elucidation of the role of the autonomic nervous system in the generation of arrhythmias, investigation of the complex movements and actions of Ca^{2+} in the myocyte affecting electrophysiology and arrhythmia generation, discovery of new genetic mutations affecting the cytoskeleton and the transport, assembly and function of molecular aggregates within the myocyte and in the sarcolemma, and identification of disease- related alterations of micro RNA that result in abnormal functions of sarcolemma and intracellular protein complexes.

Keywords

J wave syndromes • micro RNA • Autonomic nervous system • Fluorescence imaging • Myocyte Ca^{2+} • Macromolecular complexes

In the introduction to the basic science section of the first edition of the Electrical Diseases of the Heart, Harry Fozzard provided a splendid historical review and thoughtful selection of landmark developments and discoveries over the past 100 years that have formed the vibrant discipline of cardiac electrophysiology, basic and clinical [1]. The germinal achievement designated by Dr. Fozzard was the invention of the electrocardiogram by Wilhelm Einthoven. The compiled list of subsequent signal contributions to the field was derived from multiple disciplines including physical chemistry, biochemistry, neurophysiology, skeletal muscle physiology, and clinical science.

To this list of grand advances I add methodological developments that proved vital to the advancement of cardiac electrophysiology apace with neurophysiology. Access to the cell interior to control transmembrane voltage and dissect and identify the separate ionic currents generating the action potential was first accomplished in the relatively gargantuan squid giant axon.

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This momentous transition from measuring voltage to measuring current, symbolically a move to the right side of the Ohm equation E = IR, was the launch that propelled electrophysiology through the latter half of the twentieth century into this century and the molecular era. Detailed analysis of individual ionic currents was an essential step towards identification and functional characterization of ion channels, the molecular entities that transmit the currents, culminating in definition of the molecular structure of ion channels.

The contracting, connected multicellular cardiac syncytium was a formidable barrier to the application of the voltage clamp technology necessary for the measurement, manipulation and analysis of individual ionic currents. Moreover the prolonged action potential of the cardiac myocyte, with its electromechanical coupling involving intricate transmembrane and intracellular movements of Ca^{2+} necessary to sustain a prolonged and modulated contraction, represented a dauntingly complex array of transmembrane ionic movements. "The little engine that could" blaze the trails to explore this system was the methodology for disassociation of cardiac myocytes with preservation of their electrophysiological and contractile integrity, allowing voltage control and recording of currents in the whole cell and in single channels in dissociated patches. The more recently developed technologies for fluorescence imaging to track transmembrane potential as well as intracellular molecular movements, notably Ca^{2+}, also have been powerful tools.

Contemporary cardiac electrophysiology, the product of these diverse but coalescent developments is expanding at an awesome pace, albeit already intimidating in its scope and depth. Testimony to the dynamic growth of the field is the call for this second edition of Electrical Diseases of the Heart just 3 years after publication of its first edition; testimony to the scope and depth of the field is the necessity for expansion in this edition of the seven sections and 63 chapters of the first edition.

In the brief period since publication of the first edition, there have been notable discoveries and insights that offer pristine visions of new knowledge and new directions for exploration as well as significant expansion in detail and depth of knowledge in established areas, sufficient for gratifying enrichment of the second edition. I offer a few subjectively selected introductory examples.

The J wave syndromes have been recently defined as a set of electrocardiographically based disorders putatively associated with lethal ventricular arrhythmias. They are unified by a common hypothetical mechanism, phase 2 reentry, which is based on an imbalance of inward and outward currents early in the action potential, roughly coincident with the action potential notch (phase I), that can generate heterogeneous early repolarization, reexcitation, and reentry. Brugada syndrome, included in the set, is the well recognized prototype in which the arrhythmia mechanism is localized in the right ventricular epicardial layers, while in the other less malignant forms the electrocardiogaphic manifestations and arrhythmia mechanisms are presumably localized in inferior and lateral walls of the left ventricle. In the forms manifesting in inferior and lateral leads, i.e., the left ventricular locations, overlap exists between the malignant phenotype and the benign normal variant long labeled as early repolarization. The definition of J wave syndromes is generating controversy as is the arrhythmia mechanism for the noncontroversial Brugada syndrome. An adversary of the original hypothesis of phase 2 reentry has appeared in the form of conventional reentry based on slowed conduction and fibrosis in the right ventricle.

Focal sites generating ventricular tachycardias and PVCs have been discovered in the papillary muscles in both the left and right ventricles. The papillary muscle foci may or may not be associated with scarring. The recording of Purkinje potentials implicates the Purkinje-myocardial junction in arrhythmia generation.

Neurocardiology, the discipline centered on the interactions between the heart and the nervous system, is undergoing an upsurge, especially in relation to the intrinsic and extrinsic autonomic systems governing the heart. Enlightenment of the autonomic cellular signaling paths continues to expand rapidly. The application of methodology to record from key neural elements in instrumented awake animals with arrhythmias has generated new insights about the relationship

between autonomic activity and arrhythmia generation. The last decade has brought new information about the workings of the intrinsic autonomic nervous system and its role in atrial fibrillation and other arrhythmias. Clinical application has occurred in the ablation laboratory where neural ablation has been added to the procedure for atrial fibrillation in some centers.

Micro RNA, regulators of mRNA production, are altered in disease states such as ischemia, hypertrophy, and heart failure. There have been recent investigations of their possible role in disease related remodeling of the functions of the sarcolemmal and intracellular protein complexes involved in ion transport, for example, ion channels, which may result in abnormal electrophysiology and generation of arrhythmias.

The complex movements and actions of Ca^{2+} integral to electromechanical coupling in the cardiac myocyte continue to be investigated with intensified vigor. Ca^{2+} has multiple diverse direct and indirect actions on electrophysiological processes which under abnormal conditions can be multifarious culprits in arrhythmia generation. Ca^{2+} is an activating binding component of Ca^{2+} – calmodulin kinase II (CaMKII), a multifunctional kinase with diverse regulatory actions at sundry sites, including sarcolemmal and sarcoplasmic reticulum membranes, the sarcomere, transcription factors, and signaling molecules. CaMKII is revved up by neurohumoral stimulation and O_2 stress in disease. Recent research revelations concerning Ca^{2+} and CaMKII related to electrophysiology and arrhythmia generation include: a role for Ca^{2+} activated small conductance K channels in atrial and ventricular fibrillation, enhancement of Ca^{2+} leak from ryanodine receptors to induce triggering by DAD and EAD, activation of Cl^- current to contribute to DAD, activation of Na^+-Ca^{2+} exchange current by an enhanced Ca^{2+} transient in the setting of abbreviated repolarization to generate EAD, activation of late Na^+ current to prolong repolarization and generate EAD, delayed reopening of the Ca^{2+} channels to promote phase 2 EAD.

Probing of the cell interior recently has uncovered many new aspects of the cytoskeleton and intracellular structural components and their roles in transcription, translation, post translational processing, trafficking, targeting, assembly and dismantling of complex molecular structures involved in ion transport. The disordered function of this matrix in acquired and genetic conditions may have severe consequences for electrophysiology and arrhythmia generation.

The roster of genetic mutations that affect the components of ion channel and ion transfer molecular assemblies, both sarcolemmal and intracellular, is multiplying almost as fast as the new molecular players and their roles in the life of the myocyte and its interaction with its neighbors. For sarcolemmal ion channels there is an expanding list of alpha units, beta and other subunits, anchoring, trafficking, and targeting partners, and dismantling units malformed and malfunctioning from defective genetic programming.

When I embarked on my personal journey into basic cardiac electrophysiology, I was guided by a master in the emerging field of cellular cardiac electrocardiography to probe what was to me the dark mysterious interior of the cardiac Purkinje fiber with the still new glass microelectode. I remember vividly the captivating images on the oscilloscope of the elegant forms of the action potentials that my microelectrode transmitted from the cell interior. Now nearly 50 years later, probing beacons are illuminating the intricate workings of a teeming multitude of molecular agents some fixed in structural units others moving, interacting, and communicating to the molecular assemblies residing in the membrane to guide the complex ionic movements that generate the action potential. I can transmit a sense of wonder at the remarkable progress in cardiac electrophysiology, eloquently expressed by Douglas Zipes [2] in the foreword to the first edition in the form of a case report illustrating the rewards to patients provided by clinical electrophysiology but enabled by knowledge acquired in basic electrophysiology.

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2
Basic Physiology of Ion Channel Function

Isabelle Baró, Denis Escande†, and Sophie Demolombe

Abstract

The present chapter aims to provide the clinical cardiologist specialized in arrhythmias with the bare essentials regarding ion channel function and mechanisms of arrhythmias either acquired or inherited and the fundamentals of antiarrhythmic drug therapy. In cardiac electrophysiology, there is a continuum of concepts between the function of ion channel molecules and the clinical phenotype. The basic principles of cardiac electrophysiology: from ion channels to ion currents, action potentials and EKG are discussed. Tightly controlled cardiac electrical activity depends on specialized properties of nodal, atrial, ventricular and Purkinje tissues and cells. This chapter reviews the identified molecular actors of the cardiac electrical activity, focusing on regional contributions and differences.

Keywords

Heart • Ion channel • Electrophysiology • High-throughput screenings • Cellular model • Computer model

Introduction

In cardiac electrophysiology, there is a continuum of concepts between the function of ion channel molecules and clinical phenotypes (Fig. 2.1). As an example, it would be almost impossible to understand heritable or iatrogenic cardiac channelopathies if one does not know what an action potential is and how it is formed. The beauty of cardiac electrophysiology is that it is the same elementary electrical signal arising from billion of single channel proteins that is summed up at the level of a single cell to generate action potentials and also summed up in time and space to generate a surface EKG. Thus cardiac electrophysiology offers the unique opportunity of

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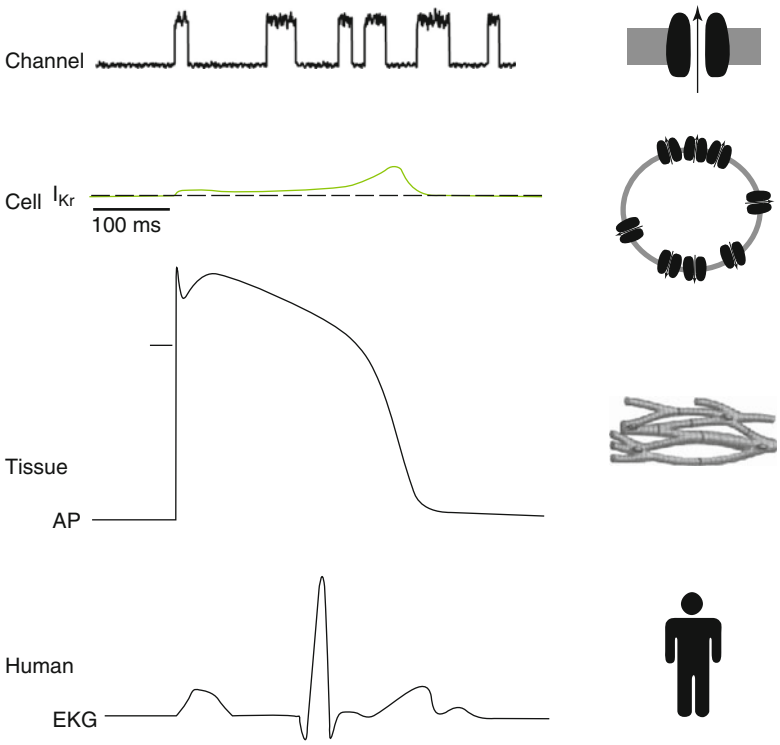

Figure 2–1. Schematic cardiac electrical activity, from molecule to patient channel: single channel current *versus* time recorded using the patch-clamp technique, upward inflection of the signal indicates outward current through a single channel; cell: outward K^+ current through the open K^+ channels present on the cell membrane; tissue: action potential resulting of the activity of all the different channels of a myocyte, recorded using the voltage-clamp technique; human: EKG resuming the electrical activity of the different regions of the heart

different levels of view to the same phenomenon either nanoscopically (at the level of a channel pore) or macroscopically (at the level of the whole organ). The present chapter aims to provide the clinical cardiologist specialized in arrhythmias the bare essentials of ion channel function needed to comprehend the mechanisms generating underlying arrhythmias either acquired or inherited and the fundamentals of antiarrhythmic drug therapy.

Basic Principles of Cardiac Electrophysiology: From Ion Channels to Ion Currents, Action Potentials and the EKG

Ion Channels

The cell membrane is made of lipids and as such is a perfectly hydrophobic milieu, across which hydrophilic ions cannot directly cross. To penetrate the cell membrane, ions need to find hydrophilic pathways, which are formed by specialized proteins (the ion channels). It happens that the ion channel pathway is not permanently available but transiently flips between open and closed states. Once a hydrophilic pathway is available (the channel is open), ions move passively across the cell membrane depending their respective electrochemical gradient (Fig. 2.2). If the gradient for a given ion species is directed inward, ions enter the cell. If the gradient is outward, ions leave the cell. "Electrochemical" means that two independent forces can move ions across the membrane: the electrical gradient and the chemical gradient. The chemical gradient causes ions to move from a compartment of higher concentration to a compartment of lower concentration (down their chemical gradient; K^+ ions move from the intracellular to the extracellular compartment; Na^+ ions move from the extracellular to the intracellular compartment). The electrical gradient causes ions to move in a direction opposite to their charge. A negatively charged compartment will attract positively-charged cations but reject negatively-charged

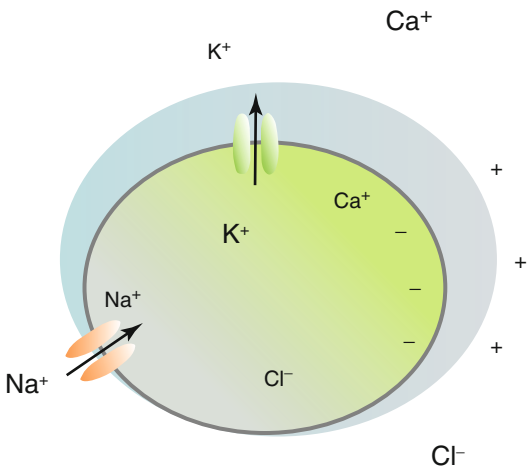

Figure 2–2. Schematic representation of the chemical and electrical transmembrane gradients of the cardiomyocyte. *Font size* is proportional to ion concentration

anions. In some cases, the electrical gradient and the chemical gradient can oppose each other and eventually be equal; in this situation the force promoting the movement of an ion in one direction equals that promoting its move in the opposite direction. Equilibrium is thus reached. Because the transmembrane potential determines the electrical gradient, the equilibrium potential is the transmembrane potential at which the electrical gradient perfectly opposes the chemical gradient and permits the equilibrium of an ion species. In a cardiac cell, the equilibrium potential is around −98 mV for K^+ ions; *i.e.* at −98 mV (inside negative compared to the outside), the force related to the chemical gradient (directed outward) equals the force related to the electrical gradient (directed inward). The equilibrium (or reverse) potential for each ion species is given by the Nernst equation:

$$E_i = R\ T / zF \ln\left(\text{Extra}\left[i\right] / \text{Intra}\left[i\right]\right)$$

where E_i is the equilibrium potential for the ion i; R, the thermodynamic gas constant; T, the absolute temperature; z, the charge/valence of the ion i; F, the Faraday (96,485.309 C/mol); Extra [i], the extracellular concentration of the ion i and Intra [i], the intracellular concentration of the ion i.

Membrane Resting Potential

A membrane that exclusively conducts a single ion species (*i.e.* K^+ ions), will polarize the transmembrane potential to the equilibrium potential for that ion species (*i.e.*–98 mV for K^+ ions). In the cardiac cell, the sodium-potassium pump enriches the cell with potassium and depletes it for sodium. It also happens that a cardiac cell at rest is predominantly permeable to K^+ and thus the resting membrane potential of −80 mV is close to the K^+ equilibrium potential. In general, a membrane potential depends on its relative permeability to different ion species. During the action potential (*see below*), the cardiac membrane potential is no longer exclusively permeable to K^+ ions, but becomes permeable to other ion species, causing it to diverge from −80 mV.

Ohm's Law

One cannot escape Ohm's law: V = RI where V is the voltage, I the current, and R the resistance of the cell membrane. It means that currents generated by ion movements through ion channels surrounded by an electrical insulator (the cell membrane) affect the membrane potential depending on the membrane resistance. Most ion channels expressed in the heart are voltage-dependent; *i.e.* they open or close in response to changes in membrane voltage. In most cases, channels open when the cell depolarizes (inside less negative in relation to the outside). Channel opening is often not instantaneous but usually that takes time (as much as hundred of milliseconds in some cases). Thus, most cardiac ion channels are both voltage and time-dependent.

When an ion channel opens, it generates an ion current, which affects the voltage (Ohm's law). This in turn affects channel opening (voltage-dependence) but not instantaneously (time-dependence). Thus, the electrical activity of the cell should be considered in a tridimensional space: voltage, current, and time. If positively charged ions (*i.e.* cations) enter the cell, this movement creates an inward current that depolarizes the cell membrane. If positively

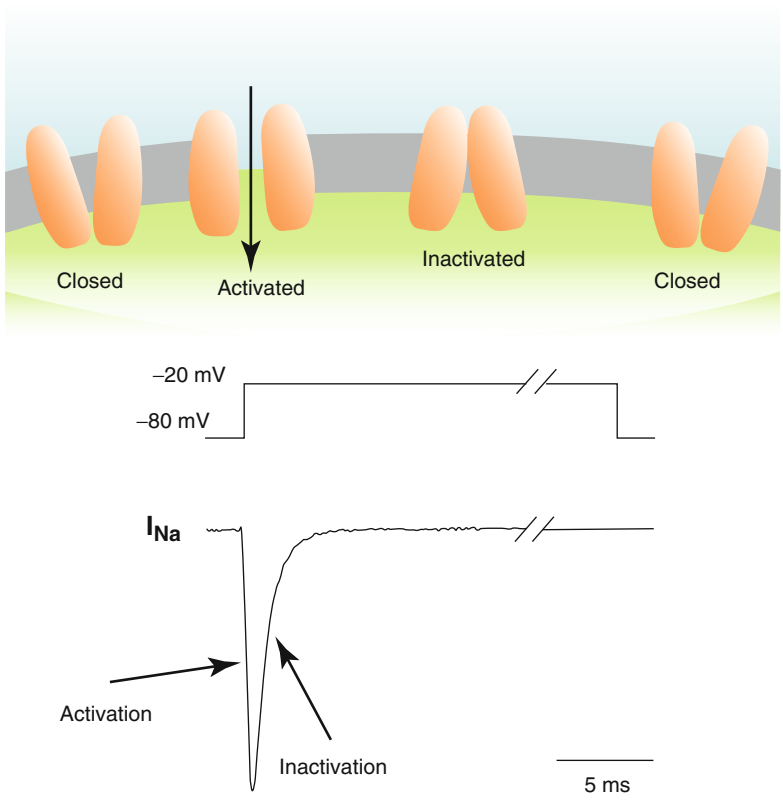

Figure 2–3. Activation and inactivation of the Na^+ current linked to the channel conformation. Schematic Na^+ current (*bottom*) recorded during a voltage pulse (*middle*). Activation of the current corresponds to an increasing number of Na^+ channels in the open conformation (*top*) due to the depolarization, inactivation corresponds to an increasing number of channels passed from the open to the inactivated conformation. Repolarization allows the inactivated channels to restore the closed conformation

charged ions leave the cell, this movement creates an outward current, which hyperpolarizes or repolarizes the cell membrane if previously depolarized.

Activation/Inactivation

Most cardiac ion channels are governed by two independent processes: activation and inactivation. The cardiac sodium channel is illustrated schematically in Fig. 2.3. At the level of the resting potential (−80 mV), the driving force for sodium ions is clearly in the inward direction (both electrical and chemical gradients are inwardly directed; the equilibrium potential for sodium ions is around +70 mV) but because sodium channel are closed, no current is generated. If the transmembrane potential is brought to values more positive than approximately −60 mV, the threshold potential, sodium channels open (voltage-dependence) and are said to *activate, thus generating sodium channel* current (I_{Na}). Activation is not instantaneous but takes a few tenths of millisecond (time-dependence). Sodium channel activation creates an inward current that further depolarizes the cell. This in turn further recruits sodium channel activation (positive feedback). The positive feedback loop is interrupted when Na^+ channels *inactivate.* Indeed, if the membrane is maintained depolarized, sodium channels do not remain open but close spontaneously. I*nactivation is* a process that is independent of activation. Thus, in response to membrane depolarization, sodium channels undergo rapid *activation* and then (less) rapid *inactivation*. Because activation is faster than inactivation, sodium channels transiently generate an inward (*i.e.* depolarizing) current. If the membrane is subsequently repolarized to the resting potential, the activation gate closes (this process is called *deactivation*) whereas the inactivation gate reopens (this process is called *reactivation* or *removal of inactivation*): the channel is ready to open in response to a new depolarization stimulus.

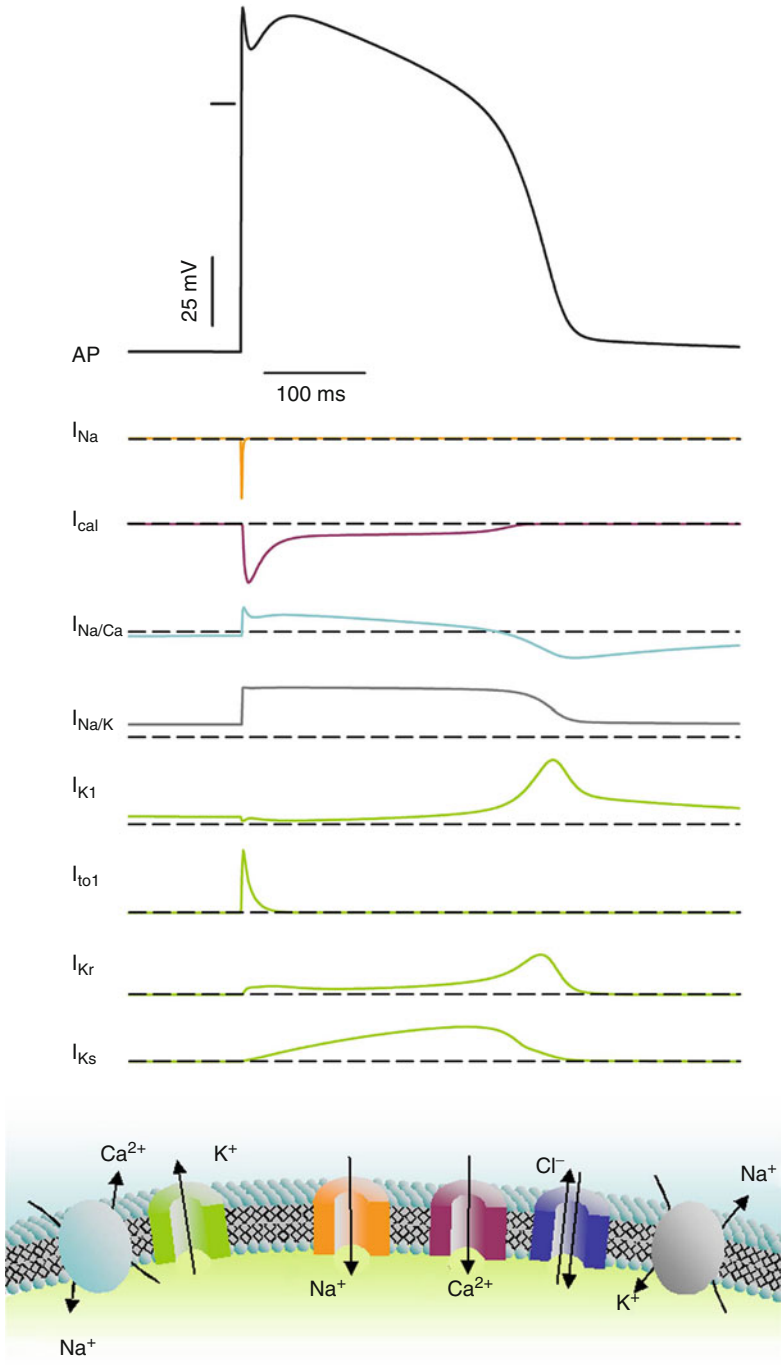

Figure 2–4. Schematic of a cardiac action potential and underlying ionic currents (*top*). This is a dynamic process: activation of the Na^+ channels generates the voltage upstroke that activates the other ionic currents. The action potential in turn modulates the timecourse of the different currents. *Bottom*: the different ion channels and transporter species. *Arrows* denote the direction of the different currents in voltage range of the action potential

Action Potential

Let's suppose that for a very few milliseconds cell membrane is much more permeable to sodium than to potassium ions (a large number of Na^+ channels activates) (Fig. 2.4). In this situation, the membrane potential is immediately attracted toward the equilibrium potential for Na^+ ions (about +70 mV). This occurs during the initial phase of the action potential (phase 0) where the membrane potential is

abruptly driven to positive values (inside positive relative to outside). The membrane potential crosses the zero line (this is called an *overshoot*). Depolarization caused by activation of Na^+ channel activates in turn other ion currents such as Ca^{2+} and K^+ currents (see Fig. 2.4), which have slower activation kinetics than Na^+ currents. Because the driving force for Ca^{2+} is inward (between 0 mV and the highly positive equilibrium potential for Ca^{2+} ions, chemical gradient attracts more Ca^{2+} into the cell than the electrical gradient limits its entry), activation of Ca^{2+} channels in response to depolarization generates an inward current, which maintains depolarization and (with other currents) creates the plateau phase of the action potential. Meanwhile, K^+ currents are also activated. In this depolarized state, the driving force for K^+ ions is outward (above 0 mV, both chemical and electrical gradients attract K^+ outside the cell). Activation of K^+ channels by membrane depolarization creates an outward current which tentatively repolarizes the cell membrane. During the plateau phase (phase 2), inward currents (mainly Ca^{2+}) equal outward currents (mainly K^+); the membrane potential is stable for a few ten milliseconds. The Ca^{2+} current then progressively inactivates whereas K^+ currents progressively activate inducing the cell membrane to repolarize (between 0 and −98 mV, the chemical gradient attracts more K^+ outside the cell than the electrical gradient retains it) toward the equilibrium potential of K^+ which is about −98 mV. Thus, when Na^+ channels are brought to threshold and open, an action potential is generated. The membrane potential is brought positive to −60 mV because an adjacent cell electrically connected through gap junctions undergoes its own action potential process. The electrical signal is thus conducted from cell to cell, from the sinus node down to the ventricular myocardium. The membrane potential can also be brought positive to the threshold potential by an external stimulus (e.g. a pacemaker).

Specialized Tissues

Action potentials of cardiac cells, specialized in automaticity (e.g. nodal cells) or in conduction (e.g. Purkinje fibers), have different shapes and characteristics (see Fig. 2.6). Automatic cells from the sinus node or from the atrio-ventricular node have a less polarized membrane potential because they express less background K^+ channels. Because of a less negative membrane potential, their Na^+ current is permanently inactivated and the depolarizing phase of their action potential relies on the activation of slow Ca^{2+} currents. As a consequence, the kinetics of the rising phase (a parameter that strongly influences conduction velocity) is much slower than in the myocardium. Another consequence of decreased K^+ current is slow depolarization of the cell membrane during diastole, termed phase 4 depolarization, bringing the membrane potential to reach spontaneously the activation voltage for Ca^{2+} current and thereafter to generate an automatic action potential. Inversely, cardiac cells specialized in conduction have a faster rate of rise of phase 0 (dV/dt max) and faster conduction velocity.

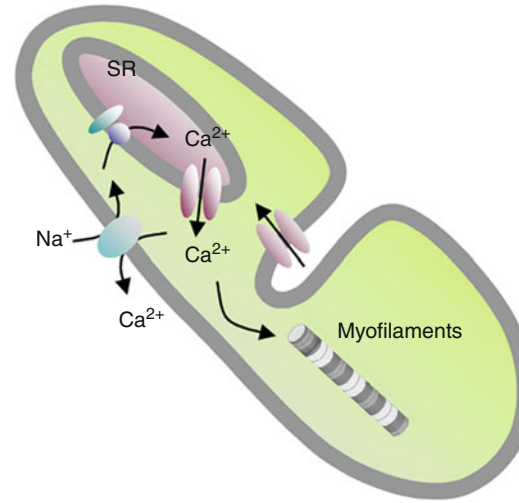

Figure 2–5. Schematic representation of the cellular mechanisms relating the action potential to contraction. Ca^{2+} enters the cell when L-type Ca^{2+}channels are activated. The local intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase activates Ca^{2+}-dependent channels localized on the sarcoplasmic reticulum (*SR*) membrane (ryanodine receptors), that liberate further Ca^{2+} into the cytoplasm. A chain reaction induces a general $[Ca^{2+}]_i$ increase that induces sarcomeric contraction. At the same time, it induces (i) the Ca^{2+}-dependent inactivation of the sarcolemmal L-type Ca^{2+} channels (ii) the re-pumping of the intracellular Ca^{2+} toward the sarcoplasmic reticulum (Ca^{2+}-ATPase) and out of the myocyte (Na-Ca exchanger), resulting in a reduction of $[Ca^{2+}]_i$ to baseline diastolic levels

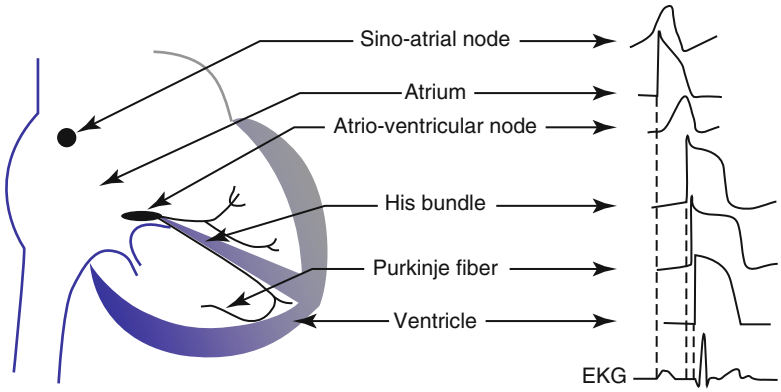

Figure 2–6. Schematic showing the diversity of action potentials morphologies in different regions of the heart. *Dotted lines* show correspondence of the activation of the different action potentials to the surface EKG

Surface EKG

As already stated in the introduction, the action potential results from the a summation in time of individual channel openings and closings. Multiple action potentials arising from the different segments of the heart are integrated in time and space to generate the extracellular signal known as surface EKG (Fig. 2.6). Because of this continuum, one might expect that an alteration in channel function can cause an anomaly of the action potential shape and therefore a change in the surface EKG. The long QT syndrome is such a situation where a loss-of-function mutation in a K^+ channel gene involved in cardiac repolarization prolongs the action potential duration (less net outward current is available to repolarize the cell membrane) and prolongs the QT interval.

Excitation-Contraction Coupling

Coupling between the electrical stimulus and contraction is ensured through movements of Ca^{2+} in and out of intracellular stores [1, 2]. The main store for Ca^{2+} in a cardiac cell is the sarcoplasmic reticulum where Ca^{2+} is buffered with specialized proteins such as calsequestrin. Diastolic free Ca^{2+} in the cytosol is maintained very low in the order of 10^{-7} M. During the action potential, a small amount of Ca^{2+} entering the cell through L-type Ca^{2+} channels, triggers the release of Ca^{2+} from the sarcoplasmic reticulum through Ca^{2+}-release channels (these are intracellular ion channels labeled by their high affinity for the alkaloid ryanodine) located in the sarcoplasmic reticulum membrane (Fig. 2.5). This mechanism called Ca^{2+}-induced Ca^{2+}-release, produces a relatively large release of Ca^{2+} from the sarcoplasmic reticulum and invasion of the cytoplasm with free Ca^{2+}. Increased cytoplasmic Ca^{2+} binds to troponin, opening the myosin binding sites on filamentous actin, and force is produced. Decreased cytoplasmic Ca^{2+} resulting from its recapture in the sarcoplasmic reticulum by Ca^{2+}-ATPase located in the sarcoplasmic reticulum membrane produces relaxation. Because the system is in perfect equilibrium, the exact amount of Ca^{2+}, which has entered the cell *via* L-type channels, is now extruded out of the cell through the Na-Ca exchanger.

Gene Correlates for Cardiac Ion Channels

Completion of the sequencing of various genomes including human and mouse has resulted in the elucidation of the complete repertoire of ion channel genes. The human genome contains approximately 25–30,000 genes. Alternative splicing should produce at least 3-times as many mRNA transcript species. However, a specialized cell, e.g. a cardiomyocyte, expresses only 1/3 of the whole genome although this proportion may not be a fixed amount but may vary with development. The human and mouse genomes contain about 230–250 genes encoding ion channel α-subunits and β-subunits. However, as stated above the heart does not express the entire collection of ion channel genes but only a subset. This

Table 2–1. Principal ionic currents, channels and auxiliary subunits, and genes expressed in the human heart

Current	Name	α-subunit or transporter	Gene	Auxilary sub-unit	Gene	Remarks
I_{Na}	Na^+ current	Nav1.5	SCN5A	β1 – β3 – β4	SCN1B – SCN3B – SCN4B	TTX insensitive channel
		Nav1.7	SCN9A			Channel in Purkinje fibers only
		Nav1.3	SCN3A			in atrial cells only
$I_{to\ fast}$	Fast transient outward K^+ current	Kv4.3	KCND3	KChiP2 – KChaP – DPP6	KCNIP2 – PIAS3 – DPP6	KChiP2 gradient in ventricle
$I_{to\ slow}$	Slow transient outward K^+ current	Kv1.4	KCNA4	Kvβ1.2 – Kvβ2	KCNAB1 – KCNAB2	
I_{CaL}	L – type Ca^{2+} current	Cav1.2	CACNA1C	β2 – α2δ1 and 2	CACNB2 – CACNA2D1 and D2	Channel absent in ventricle
		Cav1.3	CACNA1D			
I_{CaT}	T-type Ca^{2+} current	Cav3.1	CACNA1G			Absent in ventricle
I_{Kr}	Fast delayed rectifier K^+ current	HERG	KCNH2	MiRP1?	KCNE2?	
I_{Ks}	Slow delayed rectifier K^+ current	KvLQT1	KCNQ1	minK (MiRP3)	KCNE1 (KCNE4)	Gradient in ventricle
I_{Kur}	Ultra-rapid outward K^+ current	Kv1.5	KCNA5	Kvβ1.2 – Kvβ1.3	KCNAB1	Channel absent in ventricle
I_{K1}	Inward rectifier K^+ current	Kir2.1	KCNJ2			Mostly in ventricle, absent in node cells
		Kir2.2 – Kir2.3	KCNJ12 – KCNJ4			Absent in node cells
$I_{K,\ Ach}$	Acetylcholine-dependent K^+ current	Kir3.1 – Kir3.4	KCNJ3 – KCNJ5			Absent in ventricle
$I_{Na/K}$	Na-K pump current	Na-K pump	ATP1A			Responsible for K^+ and Na^+ gradient
$I_{Na/Ca}$	Na-Ca exchanger current	Na-Ca exchanger	SLC8A1			Contributes to Ca^{2+} gradient
I_f	Pacemaker current		HCN2 – HCN4			In nodal tissue

dramatic progress in our knowledge of mammalian genomes stimulated high-throughput methods (microarrays, sequencing, real-time PCR), which provide information on ion channel expression at a genome scale in various physiological and pathophysiological situations. As example, we addressed regionally ion channel expression in the non-diseased human heart with a genomic approach and highlighted significant differences (Table 2.1), with potentially important implications for understanding regional electrophysiology, arrhythmia mechanisms, and responses to ion channel blocking drugs. Concordance with previous functional studies suggests that regional regulation of cardiac ion-current expression may be primarily transcriptional. However, the processes that govern ion-channel function are complex (Fig. 2.7). Gene transcription controls the production of mRNA, which is then transported out of the nucleus to be translated into proteins. After extensive processing to optimize folding and promote trafficking by specialized chaperone proteins, the mature ion-channel protein is inserted into the cell membrane. Misfolded proteins are targeted for degradation by cellular-control machinery like proteasomes. In the cell membrane, proteins are subjected to functional regulation by enzymes like kinases and proteases, which govern the phosphorylation of key amino acids that control ion-channel activity. The channel protein can also be internalized and targeted for degradation in lysosomes or recycled back to the cell membrane. An additional layer of regulation is provided by micro-RNAs (miRNAs or miRs), which can suppress channel protein-expression by promoting mRNA degradation (which will be reflected in gene-expression profiling as reduced mRNA levels)

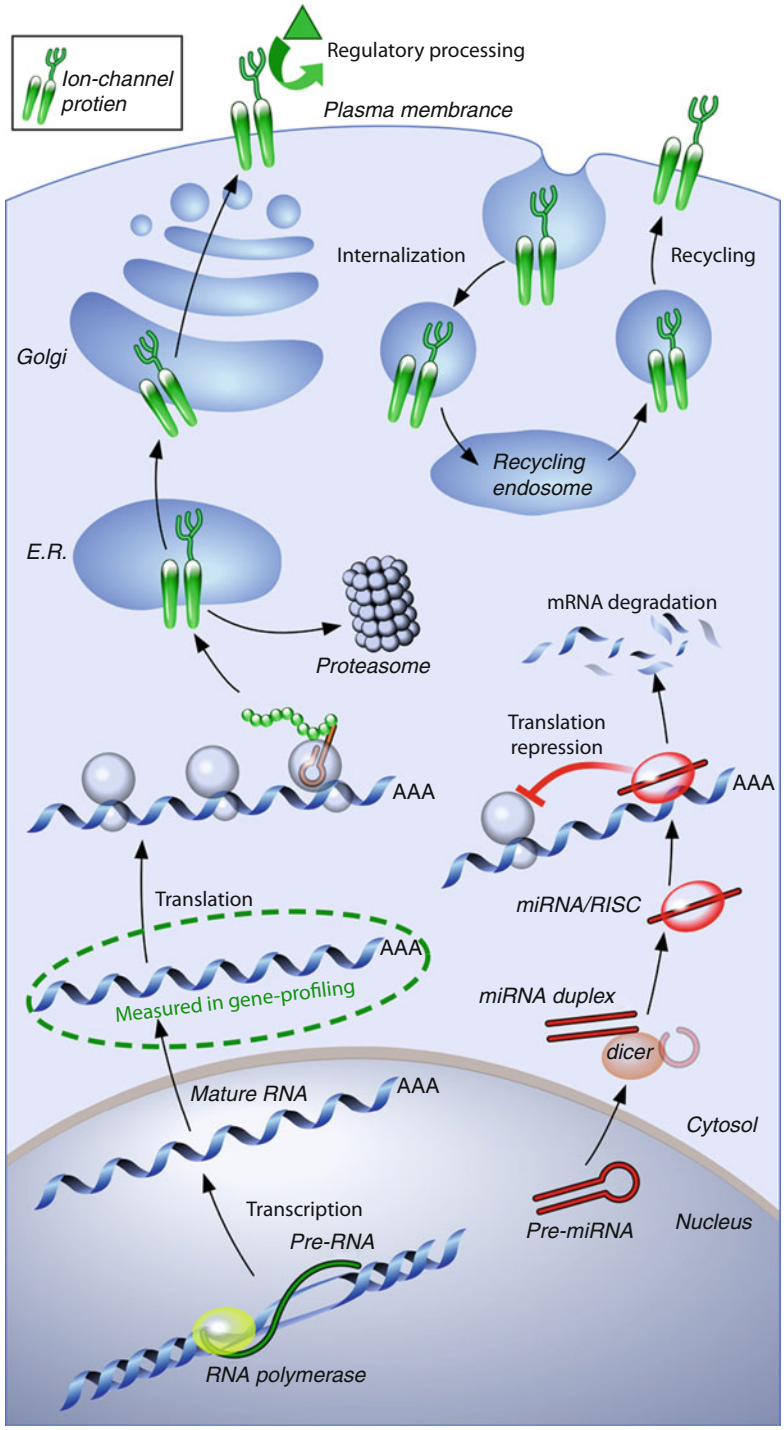

Figure 2–7. Molecular factors determining ion-channel function. Transcription of ion-channel subunit messenger-RNA (mRNA) is followed by export from the nucleus and translation into protein. The protein then undergoes a series of maturation steps in the endoplasmic reticulum (*E.R.*) and Golgi apparatus before final trafficking to the cell membrane. At the membrane, regulatory changes (e.g. control of phosphorylation) can alter channel function, and the protein can be internalized and either destroyed or recycled to the surface. Subunit-expression can also be altered by micro-RNAs (*miRNAs*, or *miRs*), which can promote mRNA degradation or impede translation by directing RNA-silencing complexes (*RISCs*) to target mRNA (From Nattel et al. [3]. Reprinted with kind permission from Elsevier)

or by repressing protein translation (which will not). Over the last few years, there has been an extensive miR research showing that miRs could be pivotal regulators in heart normal development and cardiac physiology, as well as in disease development.

Technologies to Explore Cardiac Electrophysiology

Although the first recording of a cardiac action potential was obtained about 50 years ago [4], it is the discovery and development of the patch-clamp technique (which brought the Nobel prize of medicine to E. Neher and B. Sakman in 1991), which has been key to our understanding of cardiac cellular electrophysiology [5, 6]. The technique is applied to isolated cells either freshly dissociated from cardiac tissues or maintained in culture. Depending the configuration used, the patch-clamp technique can record the electrical activity of a single channel molecule (amplitudes of a single channel current are in the order of a picoA = 10^{-12} A) or the electrical activity of the ensemble of channel protein expressed in a whole cell (current amplitudes may be in the order of one nanoA = 10^{-9} A). At the level of a single protein recording, the channel appears in a binary situation either close or open. Voltage-dependent activation is visible because the channel spends more time in the open configuration and thus less in the close configuration in response to a voltage step (usually depolarizing). Inactivation is also visible as a progressive decrease in channel firing with time at a stable voltage (see Fig. 2.3). In the current-clamp mode, variations in the voltage (action potentials) are measured whereas in the voltage-clamp mode, ion currents are measured.

Another major technical step in cardiac electrophysiology has been inherited from molecular biology techniques and relates to our capacity to make host cells (e.g. COS-7 or HEK cells) maintained in culture to express foreign genes. Cells are transfected with ion channel cDNA using routine non-viral methods and then patch-clamped after 24–72 h in culture. Recombinant ion channel proteins (in particular of human origin) are available for physiological and pharmacological investigations [7]. Site directed mutagenesis permits investigations of mutated constructs shading light into genotype-phenotype relations [8].

Genetic manipulation of ion channel genes and regulators have been successfully achieved in the mouse with either transient or permanent over-expression or invalidation of target channels [9, 10] and provided appropriate models to study complex channelopathies mechanisms [11], with important limits, though [12]. Development of *in vivo* electrophysiological methods adapted to the very small size of this animal (a mouse heart is about 100 mg) has provided similar investigations capacities as in human [13–15].

Expression of recombinant foreign channel proteins in host cell systems, as well as genetic invalidation of ion channel genes in the mouse, have been instrumental to correlate cardiac ion channels (and ion currents) with their encoding genes. In addition, these investigations have also shown that ion channels do not express in isolation in the cell membrane but rather in concert with other regulatory proteins within a channel complex. Identification of missing members of ion channel complex is the subject of active research in various laboratories with the objective to target novel candidate genes for cardiac channelopathies.

Cardiac Cellular Electrophysiology of the Human Heart

The patch-clamp technique applied to single cells dissociated from cardiac biopsies sampled during open-heart surgery in association with molecular biology techniques has provided an impressive body of information on the cellular electrophysiology of the human heart.

At the atrial level, the human action potential is initiated by a fast-activating fast-inactivating Na^+ current carried by Nav1.5 channels (encoded by the *SCN5A* gene) in association with its β1 subunits (*SCN1B*) [16, 17]. Other Na^+ channel α-subunits including Nav1.3 (*SCN3A*) [18], Nav2.1 (*SCN6A*) [19] and β3 and 4-subunits (*SCN3B* and *SCN4B*, respectively) [20] are also expressed in the human atrium although at a much lower level than Nav1.5 which is by far the predominant cardiac Na^+ channel. The Nav1.5 carried Na^+ current is responsible for the upstroke of the action potential (phase 0) and carries energy for fast conduction. Fast depolarization triggers the activation of transient outward and inward currents. The transient

outward current produces initial repolarization of the action potential and a clearly visible notch inscribed prior to the AP plateau. Transient outward current is made predominantly by Kv4.3 channel (*KCND3*) [21] in association with its regulatory β-subunits KChiP2 [22] and, putatively, DPP6 [23, 24]. Because inactivation of the transient outward current is fast, this current determines the level of the plateau phase and therefore influences the activation of other currents but does not directly influence phase 3 repolarization kinetics. Transient Ca^{2+} currents provide inward current to maintain the cell depolarized during the plateau. Two types of Ca^{2+} currents are operative: L-type (long lasting), which are target for calcium channel blockers, and T-type (fast inactivated). L-type Ca^{2+} currents are predominantly carried by Cav1.2 (*CACNA1C*) [25] and to a much lower extent by Cav1.3 (*CACNA1D*) [26] channels in conjunction with their auxiliary subunits: Cavβ2 [25], Cavα2δ2 [27], Cavα2δ1 [28]. T-type Ca^{2+} currents are brought by Cav3.1 channels (*CACNA1G*) [29]. Repolarization of the action potential (phase 3) is initiated by the delayed rectifier K^+ current, which has two components: a fast activating component termed I_{Kr} which is carried by HERG (*KCNH2*) channels and MiRP1 (*KCNE2*), its β-subunits [30] and a slow component I_{Ks} which is carried by KvLQT1 (*KCNQ1*) channels in association with the regulatory β-subunits minK (*KCNE1*), MiRP1 and MiRP2 (*KCNE3*) [31] or the A-kinase anchor protein 9 (*AKAP-9*) [32]. An ultra-rapid K^+ current (activation is 2 fold faster than I_{Kr}) is specific of the atrium in human and is carried by Kv1.5 channels (*KCNA5*) [33, 34]. Final repolarization is achieved by background time-independent currents (also called inward rectifiers), which are also responsible to maintain a negative membrane polarization during diastole. Kir2.1 channels (*KCNJ2*) are less abundant than in the ventricle accounting for the less negative resting potential in the atrium [35]. Atrial myocytes also express Kir2.2 and Kir2.3 channels (*KCNJ12* and *KCNJ4*). Specific of the atrium are Kir3.1 and Kir3.4 channels (*KCNJ3* and *KCNJ5*), which open in response to cholinergic stimulation and shorten the action potential duration [30]. Other background K^+ currents include TWIK1 (*KCNK1*) and TASK1 (*KCNK3*) currents [36]. The cardiac function of another class of ion channel is only now beginning to emerge and concerns the transient receptor potential (TRP) channels, especially the TRPC subclass. TRP channels permeate many different cations, and are most likely critical regulators of microdomain Ca^{2+} signaling in the heart in disease states such as hypertension, cardiac conduction block and cardiac hypertrophy [37]. TRPC1 and TRPC6 channels are key players in muscle mechanotransduction [38]. Other currents are related to the Na-K pump (*ATP1A1*) [39], which generates an outward current and the Na-Ca exchanger (*SLC8A1*), which generates an inward current and participates to maintain the plateau. The role of chloride channels in the human heart is still ascertained. Electrical connection between cells is ensured by the expression of connexin channel proteins (connexin 40 (*GJA5*) is the main isoform in atria while Cx43 (*GJA1*) characterizes the ventricles) [40].

The ventricular action potential shape differs from that of the atrium. In particular, the initial repolarization phase is less pronounced, the plateau phase is more positive and phase-3 repolarization is more rapid. As in the atrium, there is no spontaneous depolarization in the contractile myocardium. The ventricle exhibits almost no ultra-rapid K^+ current (Kv1.5 channels) [41, 42] or T-type Ca^{2+} current [43]. Similarly, Kir3.1 or Kir3.4 channels, which are activated under acetylcholine are not expressed whereas the background K^+ current (Kir2.1 channels) is more prominent in comparison with the atrium [35]. Consistent differences are seen between the endocardium and the epicardium with a more pronounced initial repolarization attributed to transient outward current in the epicardium [44]. These differences, which are crucial for the inscription of the normal EKG waves, have been linked to a greater KChiP2 expression in the epicardium with no Kv4.3 transmural gradient [45].

Much less information is available on the cellular electrophysiology of automatic tissues of the human heart and most of our knowledge has been obtained from animal models. However, inheritable arrhythmias have resulted in the finding of key ion channels for the activity of these specialized regions. Indeed, variations in the gene encoding for Nav1.5 and for pacemaker

channels (*HCN* genes) have been shown to cause sinus node (SAN) dysfunction (see below). Action potentials from the SAN have relatively less negative maximum diastolic potential (about −55 mV), a slow rate of rise and a spontaneous diastolic depolarization as the origin of cardiac automatism. Cholinergic and β-adrenergic stimulations slow and accelerate spontaneous sinus node, respectively. Many different ion currents are responsible for pacemaking activity with this redundancy being considered as a security system. Cells from the SAN express an inward current that activates when the cell repolarizes. This inward current called the pacemaker current is related to the specific expression of HCN channels (*HCN1, HCN2* and *HCN4* genes) [46, 47]. Other currents contribute to depolarize the cell during diastole including L-type Ca^{2+} currents (*CACNA1D* and *CACNA1C),* T-type Ca^{2+} current (*CACNA1G*) [48] and a delayed rectifier K^+ current that progressively deactivates during diastole (likely made of HERG, KvLQT1 and minK expression) [49–51]. Partial depolarization of automatic cells is explained on the basis of low expression of background Kir2.1 channels [51, 52]. Kir3.1 and Kir3.4 channels provide an additional outward current when activated by acetylcholine living less net inward current for diastolic depolarization and thereafter producing bradycardia [53, 54]. Cholinergic stimulation also decreases the availability of L-type Ca^{2+} channels [55]. β-adrenergic stimulation increases the L-type Ca^{2+} current amplitude [56] and facilitates the activation of HCN channels during repolarization [57].

The main function of the atrio-ventricular (AV) node is to slow conduction at the AV junction so as to create a delay between atrium and ventricular contraction. AV node cells have also post-repolarization refractoriness, which limits the number of impulse that can activate the ventricle. Finally, AV node cells take over the function of pacemaking when the sinus node fails to generate automaticity. AV node cells have a faster rate of rise of the action potential than SAN cells although this value remains much lower in the AV node than in the regular myocardium. As in the SAN, automaticity in the AV node is achieved through the expression of specialized channels such as HCN channels [46]. Among delayed rectifiers, I_{Kr} predominates over I_{Ks} [58]. As in the SAN, there is little background K^+ current in the AV node [59]. Also as in the SAN, the AV node expresses more Cav1.3 channels and less KChiP2 and Kv4.x channels than the regular myocardium [47].

Cardiac cells specialized in conduction belongs to the His-Purkinje system. They have a more negative resting potential than contractile fibers and a greater maximum rate of rise of depolarization. The plateau potential is more negative and the action potential is longer than in the ventricular myocardium [60]. In addition, Purkinje fibers show spontaneous diastolic depolarization responsible for the idioventricular rhythm during atrio-ventricular dissociation. Since the isolation of single Purkinje cells is difficult, research using Purkinje cells has been restricted. A review by W. Dun and P.A. Boyden concentrates on comparison of Purkinje and ventricular cells in the morphology of the action potential, ion channel function and molecular determinants by summarizing our present day knowledge of Purkinje cells [61]. Recently, gain-of-function mutations in *TRPM4* gene, encoding a calcium-activated nonselective cation channel of the transient receptor potential melastatin, and mutations in *SCN1B* gene, which encodes the function-modifying sodium channel β1 subunit, have been implicated in familial cardiac conduction disease, highlighting their participation in the Purkinje cell electrophysiology [62–64].

Cardiac Cellular Electrophysiology in Other Mammalians

Because in the obvious difficulties in obtaining un-diseased human myocardial cells, most electrophysiological and electropharmacological studies have been conducted in animal models. However, the human cardiac cell electrophysiology is unique and clearly differs from that of other mammals, especially that of the mouse. The mouse model, however, has become very

popular because of the possibilities to relatively easily manipulate its genome. A mouse heart beats 600 times per minutes; *i.e.* ten times faster than a human heart. Accordingly, the mouse has a much abbreviated action potential with virtually no plateau phase [65]. A mouse does not express sizeable delayed rectifier (I_{Ks} and I_{Kr}) [66] but relies mainly on transient outward currents (Kv4.2 in association with KChiP2 but also Kv1.5 and Kv2.1 channels) to ensure repolarization [67–69]. Thus, the mouse is not an adequate model for human pathology when repolarization is concerned. On the other hand, depolarization and conduction have comparable characteristics in the mouse and human hearts [70]. I_{Ks} and I_{Kr} are easily recorded in the guinea-pig heart with $I_{Ks} > I_{Kr}$ (the reverse of what is found in the human heart) [71, 72]. Guinea-pigs and pigs express almost no transient outward current [73, 74]. The dog has a large endocardium to epicardium transient outward current gradient similarly to human, although the biophysical characteristics of this current differ significantly between the two species [75].

As a consequence, there is no single species that can be used as a convincing model of the human heart. Depending on the problem under study, different model could be chosen. Despite the pronounced differences between the human and mouse heart, a tremendous amount of valuable information has been obtained in from species, thanks to genetic manipulations. Finally, it should be kept in mind that drosophila has been key to identify the multiple families of K^+ channel genes which later appeared as largely conserved along evolution from drosophila to human [76].

Emerging New Biological Models

Recently, the zebrafish has been used as a biomedical model as there are a large number of orthologues to human disease-causing genes found in the zebrafish genome. Despite the differences in anatomy between the fish and mammalian heart, the electrophysiology of the zebrafish is very similar to the human and larger mammals, including heart rate (between 110 and 130 beats/min), a specialized conduction system with the SA node located at the sinus venosus [77], and ventricle contraction (from the apex to the base). There is a distinct P-wave, QRS-complex and T-wave in the zebrafish EKG. The similarity between zebrafish and human electrophysiology supports the use of zebrafish in modeling human cardiac diseases such as the long QT syndrome [78].

Recently, the development of efficient non-viral transfection technology has allowed heterologous expression of foreign cDNA in neonatal (if not adult) cardiomyocytes with much less delay than methods using viruses as transfecting agents [79]. This has provided a versatile new model for the studies of WT or mutated ion channel-related protein function in a cardiomyocyte subcellular environment.

In parallel, synthetic short interfering RNAs (siRNAs or antagomirs) designed to target endogenous mRNAs, as miRs, have been developed. When transfected in cardiomyocytes (in the RNA or DNA form), this provides alternative cell models to isolated cardiomyocytes of genetically-engineered knock-down animal models much more easily achieved [80].

A very exciting new model is the human cardiomyocytes derived from induced pluripotent stem cells (iPS cells). iPS cells are obtained by ectopic expression of stemness factors in fibroblasts from affected patient's dermal biopsies [81]. After differentiation in cardiomyocytes, there represent a unique cell model with exactly the same genotype as the donors. Furthermore, human iPS cells do not carry the ethical concerns associated with human embryonic stem cells. With continuous technological improvements in genetic reprogramming, we might expect that this model will soon be readily available and affordable for experimental research of human disease. Two recent studies by Moretti et al. [82] and Itzhaki et al. [83] provide powerful proof of principle demonstrations that modeling disease in iPS cells can lead to insights on the mechanisms of long QT syndrome pathogenesis. In the near future, this model will be highly valuable, in association with genome-wide association studies, to investigate oligogenic familial arrhythmias.

Computer Models of Cardiac Cellular Electrophysiology

Each ion current is characterized by its amplitude and relation to voltage and time (activation, deactivation, inactivation, reactivation) and therefore can be fully described by a series of equations and parameters. If the equations and parameters for every ion current expressed in the heart are entered into a computer, a normal action potential can be reconstituted with relation to rate or interpolated stimulus. Such complex computerization has been achieved for several animal models [84–89] as well as human atrial [90–93], Purkinje [94, 95] and ventricular cells [96–99]. These models have proved to be of great value in helping us understand the role of single ion channel function on global electrical activity of a cell as well as to the consequences of subtle changes in ion current characteristics (as produced for example by genetically inherited mutations or drugs) [100]. The impact of some alterations in the characteristics of ion channel currents on the action potential is so complex that they cannot be easily or accurately deduced without the help of the computer. The computerized action potentials from different cells can also be integrated in two-or three-dimensional thin layers approaching the geometry of the normal heart to reconstitute its global activity on a simulated electrocardiogram [97, 101].

Conclusion

After decades of electrophysiological studies on animal and human cardiac tissues, many aspects of the molecular basis for cardiac electrogenesis have been unveiled. Although much knowledge on human ionic currents has been gathered, data from human cardiac nodes and conducting tissues are still lacking and direct extrapolations from animal data may be hazardous. Furthermore, despite the human genome sequencing achievement, function assignment for every predicted protein is still far from being achieved. As a consequence, the list of yet identified channel auxiliary proteins is largely incomplete. The quest for the Holy Grail assumes that the knowledge of every members of the orchestra would give access to the global cardiac symphony.

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3
Developmental Aspects of the Electrophysiology of the Heart: Function Follows Form

Alex V. Postma, Vincent M. Christoffels, and Antoon F. M. Moorman

Abstract

The cardiovascular system is the first organ system to form and function in the developing embryo. In a typical lifetime the heart performs roughly 2,000 million contraction-relaxation cycles (2.3×10^9), to supply the whole body and all of its organs with oxygen and nutrients. To this end, an intricate and complex organ developed, encompassing multiple chambers containing electrical and force producing components, with nodes to activate the chambers and valves to prevent regurgitation. The cardiomyocytes of a primitive heart can be considered as a nodal cell because they display automaticity and are poorly coupled, which, together with slow propagation, gives rise to peristaltic contraction. The introduction of dominant pacemaker activity at the intake of the heart perfected such a heart into a one-way pump. Subsequently, highly localized, fast-conducting cardiac chambers were added to this nodal tube, resulting in the four-chambered hearts. Concomitant with the formation of such chambers, an adult type of electrocardiogram (ECG) can already be monitored in the embryo. Thus, cardiac design, i.e. the positioning of the atrial and ventricular chambers within the nodal tube, principally explains the coordinated activation of the heart reflected in the ECG. A crucial question is why some areas of the embryonic heart tube do not participate in the formation of atrial or ventricular working myocardium and mature in a nodal direction. As a generalized hypothesis we propose that the chamber-specific program of gene expression is specifically repressed by T-box factors and by other transcriptional repressors. Consequently, aberrant expression of these factors might be at the basis of ectopic automaticity, malformations of the conduction system and congenital heart disease in general.

Keywords

Heart development • Conduction system • Transcription factor • Congenital heart disease • Nodal cell • Cardiomyocyte • Pacemaker • Cardiac design • Electrocardiogram • Electrophysiology

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Introduction

The cardiovascular system is the first organ system to form and function in the developing embryo. In a typical lifetime the heart performs roughly 2,000 million contraction-relaxation cycles (2.3×10^9), to supply the whole body

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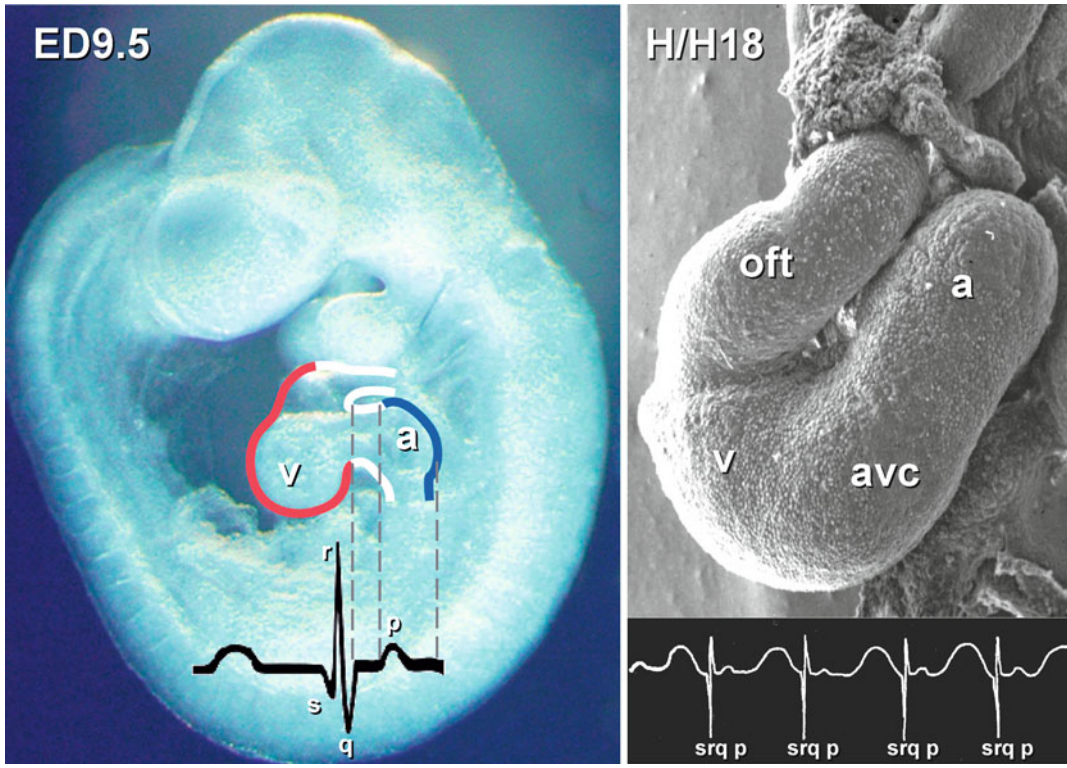

Figure 3–1. Concomitant with the formation of chambers (atria, ventricles), an adult type of electrocardiogram (ECG) can already be monitored in the embryo [2]. Scanning electron microscopic photographs of the developing chicken heart with matching electrocardiograms. At H/H 18, locally fast-conducting chamber myocardium has differentiated as reflected in the electrocardiogram. *A* atrium, *avc* atrioventricular canal, *oft* outflow tract, *V* ventricle. Note that the ECGs are displayed mirrored to match with the position of the chambers in the embryonic heart. The apparent T-wave has not been labeled since it reflects the depolarization of the muscularized outflow tract at this stage rather than the repolarization of the ventricle

and all of its organs with oxygen and nutrients. To this end, an intricate and complex organ developed, encompassing multiple chambers containing electrical and force producing components, with nodes to activate the chambers and valves to prevent regurgitation. In contrast, in early vertebrate embryos and primitive chordates, the heart merely constitutes a myocardial mantle enfolding a ventral aorta, in which the blood is propelled by peristaltic contractions. Like the nodal cells in the formed heart, the cardiomyocytes of such a primitive heart display high automaticity and are poorly coupled. This results in slow propagation of the depolarizing impulse and a matching peristaltic contraction. Due to the development of polarity along the heart tube, a dominant pacemaker activity develops at the intake of the heart, leading to the evolution of a one-way pump. Although dominant pacemaker activity implies development of sinus node function, only in mammals a morphologically distinct node actually develops [1]. The addition of highly localized, fast conducting cardiac chambers to the straight heart tube is an evolutionary novel event, and resulted in the four-chambered hearts of birds and mammals with a synchronous contraction and a dual circulation. Already with the onset of the formation of chambers, an adult type of electrocardiogram (ECG) can be monitored in the embryo (Fig. 3.1) [2]. Thus, cardiac design, i.e. the positioning of the atrial and ventricular chambers within the straight heart tube, rather than the invention of nodes, principally explains the coordinated activation of the heart reflected in the ECG. An important question to address is

why some areas of the embryonic heart tube do not participate in the formation of the atrial or ventricular working myocardium and mature in a nodal direction. In short, the chamber-specific program of gene expression is repressed by T-box factors and by other transcriptional repressors [3, 4]. Consequently, aberrant expression of these factors might be at the basis of ectopic automaticity and congenital malformations of the cardiac conduction system in the formed human heart.

Early Peristaltic Hearts

A typical circulatory system is made of pumps and transporting vessels. Nature uses two different schemes to make muscle-pumping devices. In one version, also utilized by the intestine, peristalsis is the driving force. In contrast, in adult vertebrates an alternative version involving chambers and valves is used (see next section). In the peristaltic version, a wave of contractions runs along the muscle mantel enfolding the main blood vessel, and this action pushes the encompassed fluid ahead in either direction. Such a system is not particularly efficient, but it allows the steady movement of fluids and slurries. During evolution, polarity evolved in the primitive peristaltic chordate hearts and this resulted in dominant pacemaker activity at one end of the cardiac tube, transforming such a heart into a one-way pump. All regions of peristaltic hearts possess poorly coupled cells and intrinsic automaticity, by which depolarizing impulses propagate slowly along the tube, resulting in matching peristaltic waves of contraction [5–9]. The advantage is that hearts using these slow contractions do not require well-developed contractile structures like those present in the chamber myocardium of higher vertebrates.

Development of Chambered Hearts

It is important to appreciate that the basic characteristics of muscle cells comprising a peristaltic heart are similar to those comprising the nodes of a chambered heart [4], as this facilitates the understanding that the design of chambered hearts is derived from the peristaltic heart. Though they share design characteristics, the chambered heart and its associated functional requirements are obviously far more complex than a peristaltic heart. Chambered hearts are the more powerful hearts that can cope with the increasing demands imposed by a growing microcirculatory resistance due to the evolutionary development of liver and kidneys. To achieve this, the atria became the drainage pool of the body to allow efficient filling of the ventricles, while the ventricles themselves became the power pumps. Like peristaltic hearts, chambered hearts are directional because dominant pace making activity remains localized at the intake of the heart. A logical further addition to these chambered hearts was the development of one-way valves at both the inflow and the outflow of a chamber. This permitted that, with relaxation, a chamber could be prevented from refilling from the downstream compartment, and prevented, with contraction, backflow into the preceding compartment. Remarkably, the areas in which these valves evolve display many nodal characteristics, and are the same areas that in the vertebrate embryonic heart will not, or will only later develop into chamber myocardium [10]. Thus, cardiac valves are always found in regions of nodal-like myocardium. This holds true for the sinuatrial region, atrioventricular junctional region and also for the myocardial outflow region of the embryonic heart. Interestingly, the outflow tract myocardium in human can extend as far downstream as the semi-lunar valves. Spontaneous activity and even tachycardias originating from this area have been reported [11] underscoring the notion that this myocardium is persisting embryonic nodal-like tissue.

Development of the Cardiac Chambers and Conduction System

The four-chambered heart of mammals develops from a single tube, which initially contains the precursors for the left ventricle only, or even less. With development, the precursors for the

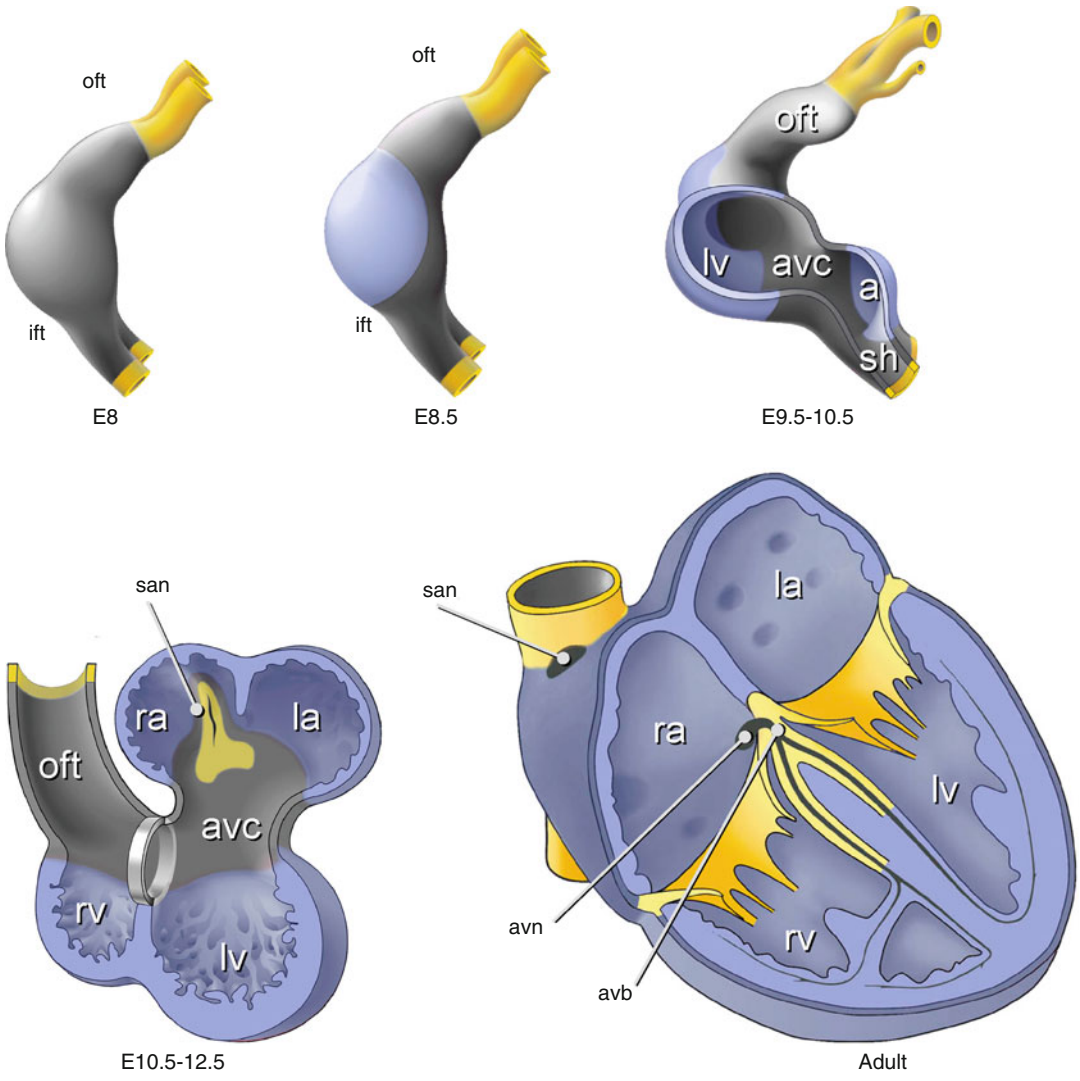

Figure 3–2. Schematic overview of heart development in higher vertebrates. Chamber myocardium (*blue*) expands from the outer curvatures of the primary heart tube, whereas non-chamber myocardium (*grey*) of the inflow tract (*ift*), sinus horns (*sh*), atrioventricular canal (*avc*), outflow tract (*oft*), and inner curvatures does not expand. Sinus horn myocardium gives rise to the sinoatrial node (*san*), atrioventricular canal myocardium to the atrioventricular node (*avn*) and atrioventricular junction. First three panels show a left-lateral view of the heart

remainder of the heart are added at the arterial and venous poles (Fig. 3.2) [12, 13]. The heart tube continues to elongate as a result of the recruitment of myocytes and bends ventrally and rightwards in a process called looping, which occurs around mouse embryonic day 8.5–9 (E8.5–9), comparable to 23 days in human development [14–16]. At this time, the heart tube has started to form a distinct ventricular chamber at its original ventral side, while the future atrial appendages differentiate slightly later at the dorsal and caudal end [17–19].

The myocardium of the original heart tube and the myocardium subsequently added to its poles, displays slow conduction and contraction, along with the property to spontaneously depolarize (automaticity), which is mainly Cx45 dependent [20], resulting in peristaltic-like, rhythmic contractions. Dominant pacemaker activity is always located in the newly formed

myocardium at the venous pole [21, 22]. This generates a unidirectional blood flow from the venous to the arterial pole. While the ventricular and atrial chamber myocardium differentiates and rapidly proliferates at specific sites within the heart tube, the remainder of the myocardium maintains the primary phenotype. Once chambers have developed, this primary myocardium can be recognized as the sinus venosus, the atrioventricular canal (AVC) and the outflow tract (OFT). The sinus node develops from a small component of the sinus venosus [22, 23] and the AVN and AV junction myocardium develop from the AVC [24, 25]. Both nodal components still display phenotypic features of the primary myocardium they develop from, such as automaticity, slow conduction and poorly developed sarcomeres and sarcoplasmatic reticuli [26]. The maintenance of the primary phenotype of the myocardium is fundamental and regulated by the transcriptional repressor genes *Tbx2* and *Tbx3* [3, 27], which are selectively expressed in the primary myocardium and developing and mature conduction system [16]. A deficiency of Tbx3 in the myocardium results in expansion of the expression of working myocardial gens *Cx40*, *Cx43*, *Nppa* and *Scn5a* into the sinus node domain. In contrast, forced expression of Tbx3 leads to development of ectopic functional pacemaker tissue [27]. Both Tbx2 and Tbx3 are required to pattern and form the AVC in a redundant fashion [28]. Thus, Tbx2 and Tbx3 inhibit differentiation into working myocardium, which allows for the development of components of the cardiac conduction system. In conclusion, the primary myocardium lies at the basis of the pacemaker tissues of the cardiac conduction system.

In contrast, formation of the chambers is marked by the differentiation of localized regions of primary myocardium of the embryonic heart tube into the fast-conducting working myocardium of the chambers. A hallmark feature of this working myocardium is the expression of a chamber-specific gene program [4]. This includes genes for rapid propagation of the action potential such as *Cx40* and *Cx43* and the secreted factor Nppa (Natriuretic precursor peptide type A, also known as Anf). Moreover, working myocardium expresses the α-subunit of the sodium channel Nav1.5, encoded by *SCN5a* and develops a functional sarcoplasmatic reticulum [29]. At the cellular level, a local increase in cell size followed by local re-initiation of proliferation marks this area [30]. At the morphological level, initiation of chamber formation is marked by the appearance of trabecules, sponge-like myocardial structures developing at the luminal side of the outer curvatures of the developing heart, particularly in the future ventricular chambers. This series of events has been dubbed the ballooning model of chamber formation [4]. Both the chamber-specific gene program and gene expression in the maintained primary myocardium are regulated amongst others by cardiac transcription factors such as Nkx2-5, Gata4 and a number of T-box transcription factors (Tbx), such as Tbx5 and Tbx20 [15]. Disruption of any of these crucial factors leads to misspecification of working myocardium or primary myocardium which, in turn, eventually can result in local heterogeneities and arrhythmias.

T-box Transcription Factors Regulate Compartmentalization of the Heart

An important question is why some areas of the (embryonic) heart do not participate in the formation of atrial and ventricular working myocardium and mature in a nodal direction, such as the sinus venosus and the atrioventricular canal. To gain insight into this process we studied the regulation of the *Nppa* gene in more detail. *Nppa* is never expressed in nodal tissues from fish to human, and in the embryonic heart it marks the developing atrial and ventricular working myocardium [31]. While investigating the mechanism behind the chamber-specific expression of *Nppa*, we established that both a single TBE site (DNA binding/recognition site for T-box transcription factors) and adjacent NKE site (Nkx2-5 binding element), are present in the *Nppa* promoter and are required for repression of Nppa in the atrioventricular canal [3] and outflow tract [17]. T-box factors are evolutionary highly conserved transcription factors, which are important regulators of (cardiac) development. Presently at least 17

different T-box genes with diverse functions in development and disease are known [32]. In a search for the T-box factors that could act as a repressor for the *Nppa* gene, we observed that *Tbx2* is expressed in inflow, atrioventricular canal, inner curvature and outflow myocardium. Moreover, expression of *Tbx2* and *Tbx3*, a transcriptional repressor with a similar role, is confined to primary (non-chamber) myocardium, remarkably mutually exclusive to *Nppa*, *Cx40*, *Cx43*, and other chamber-specific genes [3, 33, 34]. These findings point to a model in which chamber formation (e.g. atria, left and right ventricle) and differentiation is driven by broadly expressed factors, in addition to which a supplementary layer of localized repressors inhibits this process in regions where chambers do not develop [35]. *Tbx2* gain and loss of function experiments have demonstrated that *Tbx2* is indeed able and required to inhibit chamber formation and expression of chamber marker genes [3, 36]. *Tbx3* is expressed in a sub-domain of the *Tbx2* domain, and whereas it is able to block chamber formation when expressed ectopically, its deficiency does not lead to obvious defects in atrioventricular canal patterning, indicating functional redundancy with *Tbx2*.

But how do *Tbx2* and *Tbx3* exert their functions? Both factors act as repressors of transcription and share DNA binding properties and target genes [37–41]. They effectively compete with *Tbx5*, a transcriptional activator, for TBE-binding, and for *Nkx2-5* on NKE binding, thereby repressing chamber-specific genes and chamber differentiation [3, 34, 35]. Interestingly, lineage studies indicate that the sinus node develops from *Tbx18*-expressing precursors at the junction between the right sinus horn and the right atrium, whereas the atrioventricular node develops from the atrioventricular canal [23, 28]. The sinus node precursors induce expression of *Tbx3*, whereas the atrioventricular node precursors express both *Tbx2* and *Tbx3*. *Tbx2* is absent from the sinus node and sinus venosus and during development, *Tbx2* becomes down-regulated. *Tbx3* expression is maintained specifically in the nodes, thereby providing the only transcription factor found to date to be expressed specifically in the nodes (Fig. 3.3) [34, 36]. As mature nodes display many features that resemble primary myocardium in the embryo, it is attractive to hypothesize that their formation is the result of repression by *Tbx2* and *Tbx3* maintaining the primary phenotype.

Concluding, in a generalizing view one may envision that *Tbx2*, *Tbx3* and/or other transcriptional repressors suppress the chamber-specific program of gene expression, allowing the regions where these factors are expressed to further mature into the nodal direction. Aberrant expression of such factors might thus be at the basis of ectopic automaticity and congenital malformations of the cardiac conduction system in the formed human heart.

Mutations in Transcription Factors Can Cause Congenital Heart Defects and Arrhythmias

As the above mentioned transcription factors are essential for the proper development of the cardiac (conduction) system, it is hardly surprising that mutations in these key genes lead to congenital heart defects. Mutations in *Tbx5* in humans lead to the Holt-Oram syndrome (HOS), which is characterized by anterior pre-axial limb and cardiac malformations [42] (see below). Mutations in *Tbx3* cause ulnar-mammary syndrome characterized by defects in breast development, apocrine gland, limb and genital formation [43], one study reporting ventricular septal defects and pulmonary stenosis [44]. Moreover, mutations in *Tbx5* interacting partners such as *Nkx2-5* and *Gata4*, like *Tbx5* itself, are known to cause septum defects [45] and, in the case of *Nkx2-5*, atrioventricular conduction defects [46] (see below). Presently no diseases are known to be caused by *Tbx2* mutations. A more common congenital disorder called DiGeorge syndrome, is caused by a 1.5–3 megabase genomic deletions of the chromosome 22q11 region which includes the *Tbx1* gene [47]. Patients with DiGeorge syndrome are characterized by a variety of abnormalities including absence/hypoplasia of the thymus, cleft palate, facial dysmorphism and

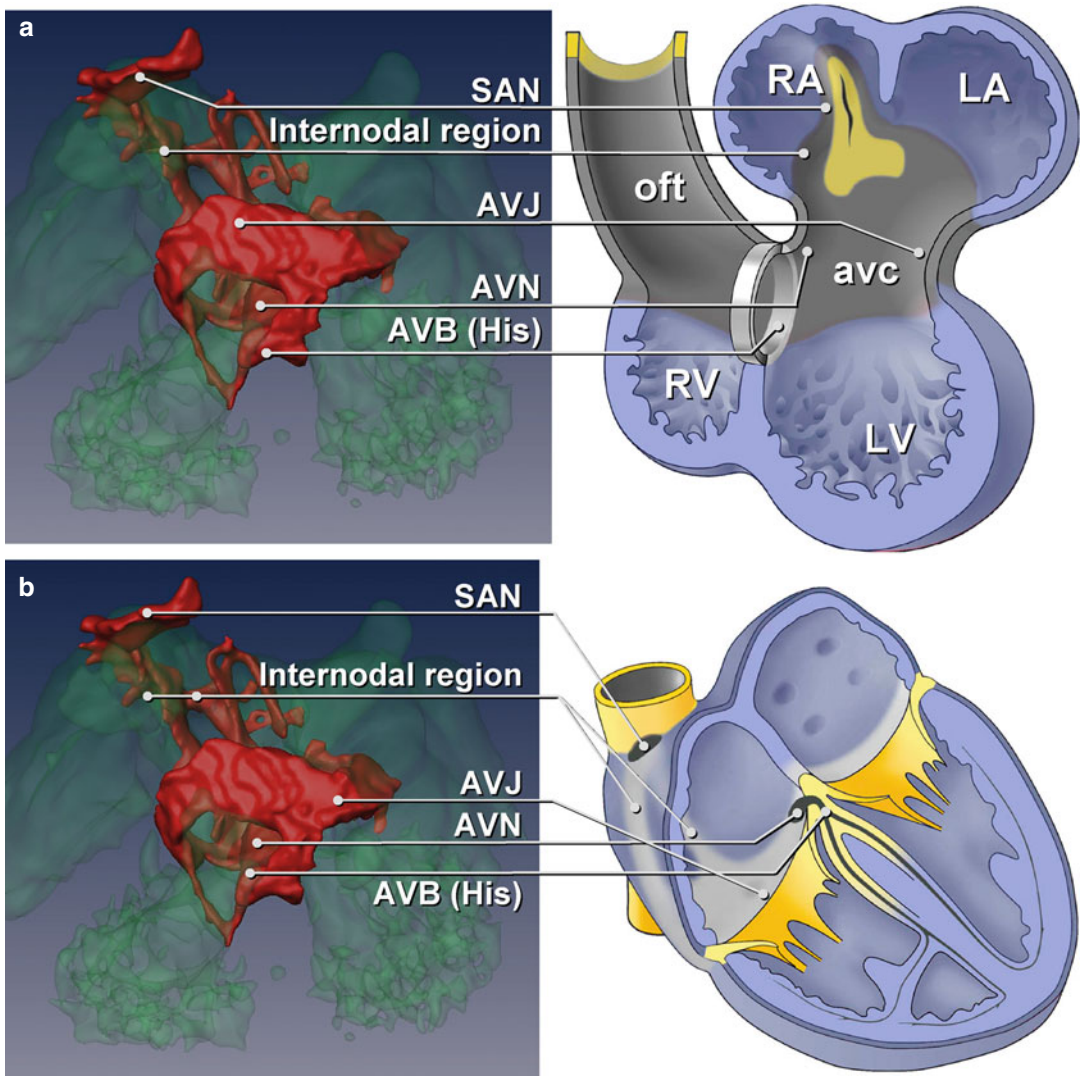

Figure 3–3. Tbx3 (*red*) expression visualized in the heart at ED10.5 (**a**). Tbx3 marks the sinoatrial node, internodal region, AV junction, AV node and the AV bundles with respect to the location of the atria, the atrioventricular canal, the ventricles and the outflow tract (**b**) and with respect to the adult heart

cardiovascular anomalies such as aortic arch malformation, outflow tract defects and VSDs. Recently it was postulated that a synergistic interaction between *Tbx1* and *Nkx2-5* might be responsible for the varying heart malformations of DiGeorge syndrome [48]. As, *Tbx1*-deficient mice phenocopy important aspects of DiGeorge including outflow tract abnormalities [49], it is believed that *Tbx1* might modulate, in part, the outflow tract defects seen in DiGeorge patients.

Nkx2-5, AV Conduction and Atrial Fibrillation

One of the most intensely studied transcription factors involved in cardiac development is Nkx2-5. This homeobox transcription factor is expressed in the heart and other tissues and is part of the core cardiac transcriptional network essential for cardiac development. Targeted disruption of *Nkx2-5* in mice causes

early embryonic lethality, with cardiac development arrested at the linear heart tube stage, prior to looping [50]. Cardiac expression of Nkx2-5 continues throughout development and into adult life. In humans dominant mutations in *NKX2-5* cause a variety of cardiac anomalies as well as atrioventricular abnormalities. Atrioventricular conduction abnormalities and atrial septal defects (ASD) are, in fact, the most common clinical presentations. However, other abnormalities such as ventricular septal defects, double-outlet right ventricle, tetralogy of Fallot and tricuspid valve abnormalities have also been noted [46, 51]. Cardiac dysfunction and sudden death have also been reported in mutation carriers. Atrioventricular conduction disease can occur in the absence of associated congenital heart defects [46, 51]. It is progressive in nature and electrophysiological studies have indicated that the conduction abnormality affects specifically the AVN. The fact that conduction defects can occur in the absence of cardiac structural malformations suggests that NKX2-5 has a function in conduction system development that is independent of its role in cardiac morphogenesis, and the fact that conduction disease is progressive with increasing age underscores the importance of normal *NKX2-5* function in maintenance of AV conduction in adult life. Recent findings that genetic variation in the region of the *NKX2.5* gene modulates the PR-interval in the general population seem to support such a role. The association signal detected at this locus however is located at some distance from the *NKX2-5* gene and further research will be required to establish an unequivocal link between the genetic variation underlying this signal, NKX2-5 expression levels and the link to the PR-interval [52]. This is further underlined by studies using transgenic mice that carry a loss of function allele (DNA-non binding mutant) for *NKX2-5*. These transgenic mice were born with structurally normal hearts but displayed progressive atrioventricular conduction defects and heart failure. PR-prolongation was observed at 2 weeks of age, rapidly progressing into complete AV block by 4 weeks of age. A dramatic decrease in expression of gap junctional channels, Cx50 and 43, probably contributed to the conduction phenotype [53]. Geno-phenotype correlations on the various Nkx2-5 mutants *in vitro* suggested that the principle determinant of the two most common phenotypes (AV block, atrial septal defect) was the total dose of Nkx2-5 capable of binding to DNA [54]. It turned out that *Nkx2-5* haploinsufficiency in the conduction system results in a significantly reduced number of normal cells, and that the specific functional defects observed in the knockout mice could be attributed to hypoplastic development of the conduction system. Thus, the postnatal conduction defects arising from *Nkx2-5* mutations may results from a defect in the development of the embryonic conduction system. This conclusion was in line with experiments on heterozygous *Nkx2-5* knockout mice with eGFP expression, knocked in the *Cx40* locus, permitting the visualization of the conduction system. Indeed hypoplasia and disorganization of the Purkinje fibers was observed in the heterozygous *Nkx2-5* mice and this was associated with abnormal ventricular electrical activation [55]. Analysis showed that maximal Nkx2-5 levels are required cell-autonomously and that a reduction in Nkx2-5 levels is associated with a delay in cell cycle withdrawal from surrounding Cx40 negative myocytes. This suggests that the formation of the peripheral conduction system is time- and dose-dependent on the transcription factor Nkx2-5, which is cell-autonomously required for the postnatal differentiation of Purkinje fibres. Experiments on Id2, a member of the Id gene family of transcriptional repressors, showing AVB-specific expression, proved that a critical transcriptional network, including Tbx5, Nkx2-5, and Id2, is required for the differentiation of ventricular myocytes into specialized cells of the conduction system [56]. Taken together, Nkx2-5 is required for development and homeostasis of atrio-ventricular conduction system (AVCS) and Purkinje fibers. In the case of the AVB some details of the molecular mechanism have been elucidated (Id2, Tbx5). It is noteworthy that Tbx3 also cooperates with Nkx2-5 [27] providing a link between this factor and Tbx3 in the AVB and AVN.

Some mutations in *Nkx2-5* are not linked to AV block, but to atrial fibrillation [57], an arrhythmia long thought to have a possible

origin in development [58]. In the majority of cases of paroxysmal atrial fibrillation, the myocardial sleeves surrounding the pulmonary veins at the orifice to the left atrium carry the triggers and possibly the substrate for the arrhythmia. Cells with presumed pacemaker activity have been found in the pulmonary veins of rat hearts [59] and in the pulmonary veins of patients with atrial fibrillation [60]. However, these observations are controversial, since lineage studies have demonstrated that the pulmonary myocardium is formed from a distinct lineage of precursor cells, different from the lineage that forms the muscle encompassing the sinus venosus (myocardium around caval veins, including the sinus node and around coronary sinus) [23, 61–63]. In contrast to the sinus muscle, pulmonary myocardium expresses from the outset transcription factor Nkx2-5 and its target gap-junction gene *Cx40*, suggesting it has an atrial working phenotype from the outset. Intriguingly, when Nkx2-5 protein levels are lowered experimentally, the pulmonary myocardium switches to a Cx40-negative, Hcn4-positive phenotype, similar to sinus nodal–like cells, resembling the sinus muscle. Thus, a reduction in the expression level of a single transcription factor, Nkx2-5, activates a gene program potentially sufficient to provide automaticity in the pulmonary myocardium. This observation suggests that inter-individual variation in Nkx2-5 dosage could be an important contributing trigger to the development of atrial fibrillation. Also, intriguingly, as mentioned above, genetic variation in the region of *NKX2.5* has been associated with the PR-interval in the general population, which when prolonged, is in turn associated with risk of AF [64]. Taken together, variations in the (regulatory) sequence of the *Nkx2-5* gene (and/or its interacting partners) are prime candidates for further research into the AV conduction and atrial fibrillation.

Tbx5, Atrial Fibrillation and Left-Right Chamber Differences

Another crucial factor in cardiac development is TBX5, which belongs to the evolutionarily conserved T-box family of transcription factors that bind DNA through a highly conserved DNA binding domain called the T-box. The consensus DNA sequences of the target genes where T-box factors bind are called T-box binding elements (TBEs) [65]. Tbx5 expression displays a posterior-anterior gradient along the heart tube with the most intense expression at the inflow tract of the heart. After chamber development has been initiated, Tbx5 expression is found in the sinus venosus, atria, AVC, left ventricle, including the left aspect of the ventricular septum, and to a lesser extent in the right ventricular trabecules [31, 66]. In humans, *TBX5* haploinsufficiency has been shown to cause Holt-Oram syndrome (HOS), which includes congenital heart defects, conduction system abnormalities and upper limb deformities [42, 67]. Heterozygous *Tbx5* knockout mice recapitulate many of the phenotypic abnormalities observed in Holt-Oram syndrome patients [68]. Expression of both *Nppa* and *Cx40*, targets of Tbx5, was also reduced in these mice during development. Complete deletion of *Tbx5* in mice leads to embryonic death (around E9.5) with failure of heart tube looping and an underdeveloped caudal part [68]. Furthermore, Tbx5 overexpression in an embryonic cell line results in significant upregulation of *Nppa* and *Cx40*, and, counter intuitively, in cells displaying a spontaneous beating phenotype [69, 70]. Moreover, as mentioned earlier, Tbx5 is required for the differentiation of the AVB in the crest of the ventricular septum [71].

Recent work further links TBX5 to arrhythmogenic mechanisms and atrial fibrillation in particular. Atrial fibrillation has occasionally been described in sporadic patients with Holt-Oram [72], though principally in the setting of congenital heart disease and the resultant hemodynamic effects (atrial enlargement). We recently reported on a family in which affected patients have mild skeletal deformations (a hallmark feature of HOS) and very few have congenital heart disease. However, the great majority presented only with paroxysmal atrial fibrillation at an unusually young age [73]. Sequencing of *TBX5* revealed a novel mutation, p.G125R, co-segregating with the disease. The mutant protein displayed significantly enhanced DNA-binding properties, which resulted in

augmented expression of *Nppa*, *Cx40*, *Kcnj2* and *Tbx3* genes in comparison to wild-type TBX5, i.e. functioned as a gain-of-function factor. This is in contrast to all known Holt-Oram mutations to date, which result in a loss-of-function phenotype. This TBX5 gain-of-function mechanism probably underlies the paroxysmal atrial fibrillation and mild HOS phenotype. The mechanism underlying atrial fibrillation in TBX5 gain-of-function mutants may involve direct stimulation of TBX5 target genes involved in familial atrial fibrillation (*NPPA*, *KCNJ2*, *CX40*). Alternatively, it may involve TBX5-stimulated *TBX3*, as it was recently shown that *TBX3* is highly sensitive to *TBX5* dosage [74], and TBX3 controls the sinus node gene program and induces pacemaker activity in atrial muscle [27]. There is thus evidence in support of a role of *TBX5* in the development of (paroxysmal) atrial fibrillation, a proposition that was recently further substantiated by the fact that genetic variation within the *TBX5* gene was found to be associated with AF [75].

The left and right ventricle have a different morphology, tissue architecture, geometry, and function, and myocytes of the right and left ventricle differentiate to similar, but not identical phenotypes [76]. Differences in the regulatory and gene programs of progenitors of the left and right ventricle probably underlie the differences in these phenotypes. Tbx5 likely contributes to this, as it is expressed in an antero-posterior gradient in the heart tube regulated by retinoic acid [77]. The left and right ventricles are specified along the antero-posterior axis, and, as a consequence, the developing left ventricle robustly expresses *Tbx5*, in contrast to the right ventricle [66]. This suggests that *Tbx5* is necessary for left ventricular identity, and provides, in part, the boundary between the left and right ventricle [78]. Consequently, target genes of *Tbx5* are likely differentially regulated between the left and right ventricles. In fact, target gene *Cx40*, mimics the pattern of Tbx5 [68] and is differentially expressed between the left and right ventricle in the developing and adult heart. Seeing that the right ventricle especially is prone to the development of various arrhythmias, such as those seen in Brugada Syndrome [79] and Arrhythmogenic Right Ventricular Dysplasia (ARVD) [80], and given the fact that *Tbx5* is actively involved in segregating between the left and right ventricle [78], it is tempting to speculate that target genes of *Tbx5* might contribute to or oppose arrhythmias. Recent work has shown that numerous genes are influenced by differences in *Tbx5* dosage, including genes expressed during heart development such as transcription factors (*Tbx3*, *Irx2*), cell-cell signalling molecules, and ion channels (*Cx40*, *KCNA5*) [74]. This, together with the link between *TBX5* and atrial fibrillation, warrants further investigation into *TBX5* and its downstream genes in relation to arrhythmias and ion channel genes.

Tbx2, Accessory Conduction Pathways and Defective Patterning and Gene Regulation Within the Atrioventricular Canal Myocardium

Wolff–Parkinson–White (WPW) syndrome is an arrhythmogenic defect characterized by a normal conduction system and one or more accessory AV connections, bypassing the AV node and His bundle, which cause ventricular preexcitation and predispose to re-entrant supraventricular tachycardia [81]. These abnormal accessory pathways may conduct faster than the AV node and this leads to ventricular preexcitation, evidenced by a short PR interval and a slurred upstroke of the QRS complex on the ECG. The majority of WPW occurs isolated and sporadic, affecting 1–3 persons per 1,000 [81], most patients with WPW syndrome have structurally normal hearts, though in a small portion of WPW patients, accessory pathways occur in association with congenital heart defects, such as Ebstein anomaly [82]. Nonetheless, autosomal dominant WPW families have been described [83, 84]. *PRKAG2* and *LAMP2* gene mutations were found in familial left ventricular hypertrophy and preexcitation, while a *PRKAG2* mutation was detected in a single family with isolated WPW [85, 86]. Patients with mutations in the *PRKAG2* gene have a variable combination of glycogen storage cardiomyopathy, progressive conduction system disease including sinus

bradycardia and atrioventricular block, ventricular preexcitation, arrhythmias, and sudden death [85]. Ventricular preexcitation is presumably caused by a disruption of the annulus fibrosus, which electrically insulates the atrial and ventricular muscle masses, distinct from the muscular-appearing bypass tracts observed in typical WPW syndrome [87]. However, in a cardiomyocyte-specific overexpression model of mutated PRKAG, accessory pathways only developed when the mutated PRKAG was overexpressed during development. When overexpression started in adulthood, glycogen storage disease and conduction system degeneration occurred but accessory pathways did not develop, suggesting that a developmental defect may underlie pre-excitation in PRKAG mutants [88]. In addition, various *NKX2-5* gene mutations in patients with CHDs are also associated with WPW, and point to a role of this critical cardiac transcription factor gene in WPW pathogenesis [51, 56].

A milder form of arrhythmias involving accessory pathways is the atrioventricular reentrant tachycardia (AVRT). This is the most common type of supraventricular tachycardia in both the fetus and the newborn [89]. Although AVRT can be potentially life-threatening and are sometimes difficult to control with antiarrhythmic drug therapy, in general these tachycardias resolve spontaneously within the first months of life, and >60 % of patients require no antiarrhythmic drug therapy and remain free of symptoms after the age of 1 year [90]. This self-resolving character of most perinatal AVRTs suggests that the majority of the accessory pathways eventually disappear after birth. Studies in animal models have shown that accessory AV myocardial connections are present until the late stages of cardiac development [91]. Moreover, a recent study on human hearts from neonates without episodes of supraventricular tachycardia found that isolation of the AV junction is a gradual and ongoing process [92]. The right lateral accessory myocardial AV connections in particular are commonly found at later stages of normal human cardiac development. These transitory accessory connections may therefore act as a substrate for AV reentrant tachycardias in fetuses or neonates.

The molecular mechanisms by which these accessory bypasses are formed are, however, poorly understood. The atrioventricular (AV) node and AV bundle are the only muscular connections that cross the annulus fibrosus. However, during heart development, in the absence of an annulus fibrosus, the AV canal myocardium still maintains adequate AV delay, allowing for the synchronized alternating contraction of the atria and ventricles [93, 94]. Remnant strands of this AV myocardium can however be observed in normal hearts around and after birth (see above). They disappear when the annulus fibrosus is fully formed [92, 95]. These strands are likely to maintain the slow conduction properties of the AV myocardium. Moreover, slow-conducting AV canal–type myocardium remains present around the orifices of the mitral and tricuspid valve in the adult heart [25, 96]. Together, this suggests that AV canal myocardium plays a central role in the AV delay and that defects in AV canal myocardium could underlie formation of functional accessory pathways. The AV canal myocardium is specified early in cardiac development by the expression of bone morphogenetic protein 2 (Bmp2) in the AV canal myocardium progenitors in the early heart tube, where it stimulates the expression of Tbx2 [97], a transcription factor required for the development of the AV canal [25]. Indeed in a recent study by Aanhaanen et al. [98], it was shown that myocardium-specific inactivation of *Tbx2* leads to the formation of fast-conducting accessory pathways, malformation of the annulus fibrosus, and ventricular preexcitation in mice. The AV accessory pathways ectopically express proteins required for fast conduction (Cx40, Cx43, and sodium channel, SCN5A). Several other genes and pathways have been implicated in AV canal development such as the Bmp and Notch pathways. Interestingly, microdeletions of BMP2 and JAGGED1, a Notch ligand, have been associated with ventricular preexcitation in human [99, 100]. Moreover, deletion of the *Alk3* receptor (activated by Bmp2) in the AV canal myocardium also results in accessory pathways and AV nodal defects in mice [101]. The fact that Notch signalling is involved in maturation of the AV canal-derived myocardium was underscored by another recent paper in which it was shown that activation of Notch

signalling in the developing myocardium of mice produces fully penetrant accessory pathways and ventricular preexcitation [102]. Conversely, inhibition of Notch signalling in the developing myocardium resulted in a hypoplastic AV node, with specific loss of slow-conducting cells expressing Cx30.2 and a loss of physiologic AV conduction delay. In conclusion, these results indicate that defective patterning and gene regulation within the AV canal myocardium, via disruption of the AV canal regulatory network, leads to malformation of the annulus fibrosus, formation of accessory AV connections, and ventricular preexcitation.

Sarcoplasmic Reticulum and Heart Development

Having discussed the building plan of vertebrate hearts and their conduction system, it is relevant to look at the developmental aspects of their most prominent features, namely contraction and electrical activity. Regular beating is already observed in the very early period of heart development from embryonic day E9 onwards [103], as mentioned above. This implies that an intracellular system of contraction and cardiac automaticity is already established. Interestingly, the atrioventricular canal and the outflow tract are characterized by slow conduction velocity [10], a low level of gap junction and connexin expression [104] and low SR activity [29]. This supports the idea that these flanking segments can function as one-way valves, because they fulfill all the requirements needed for a long contraction duration resulting in a peristaltic contraction. However, the radical change from a peristaltic slowly-contracting heart tube to one with fast-contracting chambers necessitates proper control on free intracellular calcium ions. This, in turn, requires the regulated movement of calcium ions across both the sarcolemmal and sarcoplasmic membranes. Clearance of intracellular calcium can be obtained by two ways, either by extrusion of the calcium into the extra cellular space by the Na^{+}-Ca^{2+} exchanger (NCX) or by the sarcoplasmic-endoplasmatic Ca^{2+}-ATPase (SERCA2) into the sarcoplasmic reticulum (SR). SERCA2 itself is regulated by the non phosphorylated form of another protein, phospholamban [105].

The calcium handling system was the first ionic system to be studied extensively during heart development, however mostly in the late fetal heart stage (embryonic day 16.5 and later) [105]. At this stage most calcium required for contraction is derived from the calcium influx through the voltage dependent calcium channels (L type and T type). Although a distinct SR is not morphologically observed in fetal myocytes, calcium influxes nevertheless are capable of triggering calcium releases through the calcium release channels (so-called ryanodine receptors, RYRs) [29]. It is thought that, in contrast to the mature situation, the calcium stores for the RYRs in the fetal myocytes are very small organelles, which are located far from the L and T type channels on the surface membrane. In this way, calcium flowing through the calcium membrane channels diffuses into the intracellular space, where it stimulates the immature SR. The whole chain of calcium induced calcium release is thus slowed down, and produces the slow kinetics of calcium signals in fetal myocytes.

In contrast, the expression pattern of the various genes involved in the calcium handling has been studied more thoroughly throughout embryonic heart development. SERCA2 and PLB can already be observed as early as the cardiac crescent stage (embryonic day 7.5 in the mouse), even before myocardial contraction has begun. In the forming heart tube, however, there is already polarity in expression, as SERCA2 is more abundant in the posterior region, or venous pole, of the heart and decreases towards the anterior regions, or arterial pole, whereas PLB, in contrast, shows a complementary distribution [106]. At the stage of the primitive cardiac tube (E8), several additional calcium-related genes start to be expressed. Whereas PLB and SERCA2 are expressed in opposite gradients, RYR, NCX and NaK-ATPase are distributed homogeneously over the cardiac tube. They remain homogeneously distributed throughout further embryonic development of the heart [106]. It thus seems that the control of calcium homeostasis is determined solely by the SERCA/PLB system. At the stage of cardiac looping (E8.5), when the first

signs of left/right asymmetry and identity are manifested in the embryo, the calcium related genes maintain their previous patterns of expression. In the final stage (E16.5), termed fetal heart, SERCA2 is more expressed in atrial myocardium than in the ventricular myocardium, whereas PLB is once again expressed in the opposite pattern. Additionally, SERCA2 has low expression in the AVC and OFT. Interestingly, SERCA2 and PLB display a differential expression between the trabeculated and compact layers of the ventricular myocardium. Contrarily, the expression of both genes is very weak in different components of the cardiac conduction system, atrioventricular node, and the bundle of His, which is in line with the nodal-like morphological origin of these components, as discussed earlier. The expression of the other components of the calcium metabolism, RYR, NCX and NaK-ATPase is homogenously in the different regions of the fetal heart.

Connexins and Heart Development

The propagation of cardiac impulses is mainly determined by the capacity to rapidly carry changes in the membrane potential of cardiomyocytes. This propagation is mediated by gap junctions, which are aggregates of hydrophobic cell-cell channels that allow the intercellular exchange of ions, metabolites and second messengers of up to 1 kDa in size [107]. The aqueous pores are formed by serially linked hemi-channels (connexons) provided by apposing cell membranes. One connexon hemi-channel is composed of six transmembrane proteins called connexins (Cx), which are encoded by 21 connexin genes [108]. Although the main purpose of gap junctions in the heart is the conduction of the depolarizing impulse across the myocardium, evidence exists that connexins also play a role in cardiac morphogenesis, as homozygotic Cx43-knockout mice die shortly after birth from a pulmonary outflow tract stenosis due to cardiac malformation [109]. Analogous, in humans, visceral heterotaxia and hypoplastic left heart syndrome have been found to be associated with mutations of the Cx43 gene [110, 111].

Differences in the expression of the connexins are consistent with a functional myocardium (atria and ventricles) model in which the working myocardium has the capacity to transmit the cardiac impulse more quickly than the adjacent myocardium of the inflow tract, atrioventricular canal and outflow tract. This guarantees synchronized contraction without the need for a specialized system (as discussed earlier). The expression of the main connexin, Cx43, is detected for the first time in the embryonic heart stage (E10.5). Cx40 has a similar pattern of expression although more reduced [106]. Cx40 (from ED9.5) and Cx43 mRNA (from 10.5) are detectable in atria and ventricles, but not in their flanking myocardium (inflow tract, atrioventricular canal and outflow tract) [112]. Even though Cx40 and Cx43 mRNA eventually become expressed in the inflow tract, they remain undetectable in the sinus node, the atrioventricular canal (including atrioventricular node) and outflow tract [107]. Expression of Cx40 is maximal in the fetal period and declines towards birth. At the stage of the fetal heart (E16.5), Cx43 is restricted to the ventricular myocardium and it is barely detectable in the atrial myocardium in rat, while the mouse atrium expresses a lot of Cx43 [106, 107]. Analogous to SERCA2 and PLB, Cx43 expression is low in the trabeculated layer and higher in the compact layer [106]. In contrast, Cx40 expression complements Cx43 expression, as Cx40 is expressed mainly in the atrial chambers and shows a transitory differential expression between the right and left ventricles under the influence of Tbx5, as discussed earlier.

It is interesting to note that based on the expression of Cx40 and Cx43 during development, two populations of myocytes can be distinguished, viz. cardiomyocytes that do not express Cx40 and Cx43, and cardiomyocytes that do express both. The first group includes the myocardium of the sinus node, the atrioventricular canal (including the atrioventricular node), bundle of His and the outflow tract. The second population includes the working myocardium of the atria and the ventricles, and, later in development, the myocardium of the inflow tract (excluding the sinus node). These patterns further that the structures that do not express Cx40/43 are derived from the embryonic myocardium of the sinus venosus and atrioventricular canal, respectively.

Ion Channels and Heart Development

The regulation of the action potential (AP) is determined by a large variety of ion currents. During the depolarization of the myocytes massive amounts of sodium ions are pumped into the cell, whereas the subsequent repolarizations are characterized by a balance of different potassium currents flowing into and out the myocyte. Many genes are involved in maintaining this dynamic balance, the most prominent ones being SCN5A, carrying the initial sodium current, and KCNQ1 and KCNH2, carrying the subsequent potassium currents. Several modulator genes, such as KCNE1,2 and SCN1b also play a role. As much as is known about their function and expression in the adult heart, virtually nothing is known about the different components of the action potential during the various embryonic stages. Attempts to characterize the various individual currents electrophysiologically during development have been hampered by technical difficulties though some studies were published on this topic [113–115].

In general, the action potentials and resting potentials in cardiomyocytes are altered greatly during development, e.g. both the rate of rise and the overshoot increases along with the duration of the AP. These electrophysiological changes are mainly produced by developmental changes in ion channels, e.g. changes in amount, the type and the kinetic properties. The fast sodium current (mainly encoded by SCN5a and SCN1b), which is responsible for the upstroke of the AP, is markedly increased during development. There are few functional sodium channels present at the earliest stage but the density increases progressively during development. Though the current has a significant sustained component in the earlier stages, this decreases during development, thereby contributing in part to the abbreviation of the AP [115]. The main potassium current in fetal ventricular myocytes is I_{Kr} (mainly encoded by KCNH2 and KCNE2), whereas I_{Ks} (encoded by KCNQ1 and KCNE1) is lacking or very small, though in the early neonate I_{Ks} becomes the dominant repolarizing current. I_f, or the funny current (encoded by the HCN gene family), is the pacemaker current and as such contributes prominently to cardiac rhythm [116]. In the early embryonic human heart, wide expression of the pacemaker channel HCN4 is seen at the venous pole, including the atrial chambers. The HCN4 expression becomes confined during later developmental stages to the components of the conduction system [16]. From mouse studies it is known that mice lacking HCN4 globally, as well as selectively from cardiomyocytes, die between ED9.5 and 11.5, displaying a strong reduction in I_f and bradycardia [117]. The few studies that investigated I_f electrophysiologically during development did so only in ventricular cells. They show that I_f is prominent at E9.5 in ventricular myocytes and decreases together with loss of regular spontaneous activity of ventricular cells towards the neonatal stage, which is accompanied by a subtype switch from HCN4 to HCN2 [118, 119]. So while the I_f current of the sinus node type is present in early embryonic mouse ventricular cells, the ventricle tends to lose pacemaker potency during the second half of embryonic development. Moreover, kinetics were found to be changed, as the threshold voltage to evoke I_f significantly lowers from neonatal myocytes to adult ones [120]. The developmental expression pattern of the HCN4 gene shows that it can already be detected in the cardiac crescent at ED7.5, while at ED8 it is symmetrically located in the caudal portion of the heart tube, the sinus venosus, where pacemaker activity has previously been reported [21]. Further in development, HCN4 becomes asymmetrically expressed, occupying the dorsal wall of the right atrium and eventually becomes restricted to the junction of the right atrial appendage and the superior vena cava in concordance with the site at which the sinus node is located in the postnatal and adult heart [121]. In the adult heart, the sinus node, atrioventricular node and ventricular conduction system mainly expresses HCN4, whereas expression of HCN2 is homogenously low in the heart [119]. The molecular pathways underlying the developmental regulation of cardiac HCN channels are not yet known. Recently the myocyte enhancer factor 2 (MEF2) was shown to interact with an enhancer of HCN4 and regulate its transcription [122]. As MEF2 expression is increased in the atrium, it could possibly account in part for the spatial distribution of HCN4 [123].

Likewise, NKX2-5 is a strong candidate for the developmental regulation of HCN4, as it has been implicated in HCN4 repression and its confinement to the sinus venosus in the developing heart [22].

Ion Channels Have a Possible Non-electrogenic Role in Heart Development

Recent work has demonstrated that heart development does not only rely on interactions of transcription factors and target genes, but, surprisingly, that ion channels themselves play a novel and possibly non-electrogenic role in heart development. The first evidence for this emerged from a study in which a strain of zebrafish called island beat was identified. These fish exhibited ventricular growth failure and non-contraction, while the atrium exhibited rapid, isolated, and uncoordinated contractions. Positional cloning led to the identification of mutations in the pore-forming α(alpha)1C subunit of the L-type calcium current [124]. Thus, abolishment of this current caused the ventricle to fail to grow and remain electrically silent, which demonstrated that the L-type calcium current is essential for normal cardiac form and function during embryonic development. This was followed by the discovery that knockouts for *Scn5a*, the cardiac sodium channel, led to early embryonic lethality. Hearts of knockout mice showed uncoordinated contractions, and developmental defects were seen, such as a common ventricular chamber, with reduced chamber size, reduced trabeculation of the ventricular wall, and a reduced number of thin, spindle-like cardiomyocytes [125]. This ventricular defect in *Scn5a* knockout embryos is unlikely to reflect a generalized failure of cardiac development, as the endocardial cushions of the atrioventricular canal, the common atrial chamber, and the truncus arteriosus appeared normal. This suggested the possibility that ion channels have an important influence on ventricular development. Indeed, antisense knockdown of zebrafish homologue *Scn5a* results in marked cardiac chamber dysmorphogenesis and perturbed looping [126]. Moreover, these abnormalities were associated with decreased expression of the myocardial precursor genes *Nkx2.5*, *Gata4*, and *Hand2* and significant deficits in the production of cardiomyocyte progenitors. Interestingly, these early defects did not appear to result from altered membrane electrophysiology, as prolonged pharmacological blockade of sodium current failed to phenocopy the ion channel knockdown. Moreover, embryos grown in the calcium channel blocker-containing medium (similar to the island beat experiments described earlier) had hearts that did not beat but developed normally. This demonstrated that voltage-gated ion channels have important roles in vertebrate heart development and supports the hypothesis that such roles might be mediated by nonelectrogenic functions of the channel complex. Additional evidence for the role of ion channels in heart development comes from studies on mice lacking Hcn4 (carrier of the I_f pacemaker current), either globally or specifically in the heart. Such mice die during embryonic development between E9.5 and E11.5, as mentioned earlier [117]. In summary, recent results argue that ion channels are required for heart development in addition to their canonical role as regulators of heart rhythm. Moreover, the data suggest that this developmental role is mediated by a nonelectrogenic function of the ion channel.

Conclusion

The heart evolved from a myocardial tube in primitive chordates to a four-chambered heart with synchronous contraction and dual circulation in higher vertebrates. Each cardiomyocyte of a primitive heart can be considered as a nodal cell because it displays automaticity and is poorly coupled, which, together with slow propagation, gives rise to peristaltic contraction. The introduction of dominant pacemaker activity at the intake of the heart perfected such a heart into a one-way pump. Subsequently, highly localized, fast-conducting cardiac chambers were added to this nodal tube, resulting in the four-chambered hearts. Interestingly, concomitant with the formation of such chambers, an adult type of electrocardiogram (ECG) can already be

monitored in the embryo. Thus, cardiac design, e.g. the positioning of the atrial and ventricular chambers within the nodal tube, principally explains the coordinated activation of the heart reflected in the ECG. A crucial question is why some areas of the embryonic heart tube do not participate in the formation of atrial or ventricular working myocardium and mature in a nodal direction. As a generalized hypothesis we propose that the chamber-specific program of gene expression is specifically repressed by T-box factors and by the other transcriptional repressors. Consequently, aberrant expression of these factors might be at the basis of ectopic automaticity, malformations of the conduction system and congenital heart disease in general.

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4
Anatomic and Histopathologic Characteristics of the Conductive Tissues of the Heart

Cristina Basso, Siew Yen Ho, Stefania Rizzo, and Gaetano Thiene

Abstract

The chapter deals with the anatomic and histopathologic characteristics of the Conductive Tissues of the Heart. In the first part, the authors treat the normal anatomy, histology and ultrastructural aspects of the conduction system, including the sinus node, the atrioventricular node and the internodal and interatrial myocardium with some historical notes on the discovery of these structures by early studies at the light microscope. In the second part, the pathological substrates of sino-atrial block, atrioventricular block, and ventricular preexcitation syndrome are extensively reviewed. Both congenital and acquired (inflammatory, degenerative, neoplastic, iatrogenic) diseases underlying conduction system disturbances are herein discussed in terms of gross and histological features.

Keywords

Anatomy • Anomalous pathways • Atrioventricular node • Bundle branches • Conduction system • Congenital heart diseases • His bundle • Histology • Sinus node • Internodal pathways

The cardiac conduction tissues in the human heart comprise specialised myocytes that can be differentiated from working myocardium with routine histological staining. They are located in specific regions of the heart to form the sinus node and the atrioventricular (AV) node that then extends into the AV bundle and bundle branches. Both cardiac nodes are sited in the atria with working atrial myocardium in between (so called internodal preferential pathways) (Fig. 4.1). Atrial myocardium is separated from ventricular myocardium by fibro-fatty tissues at the AV junction and rings. In the normal heart, the continuation of specialised myocytes from the AV node that forms the bundle of His, penetrating the central fibrous body, is the only muscular continuity between atrial and ventricular myocardium, allowing atrial impulses to slow down in the AV node and to be conveyed to the ventricles in orderly fashion. In this chapter, we will deal with normal and pathologic features of the conductive tissue of the heart.

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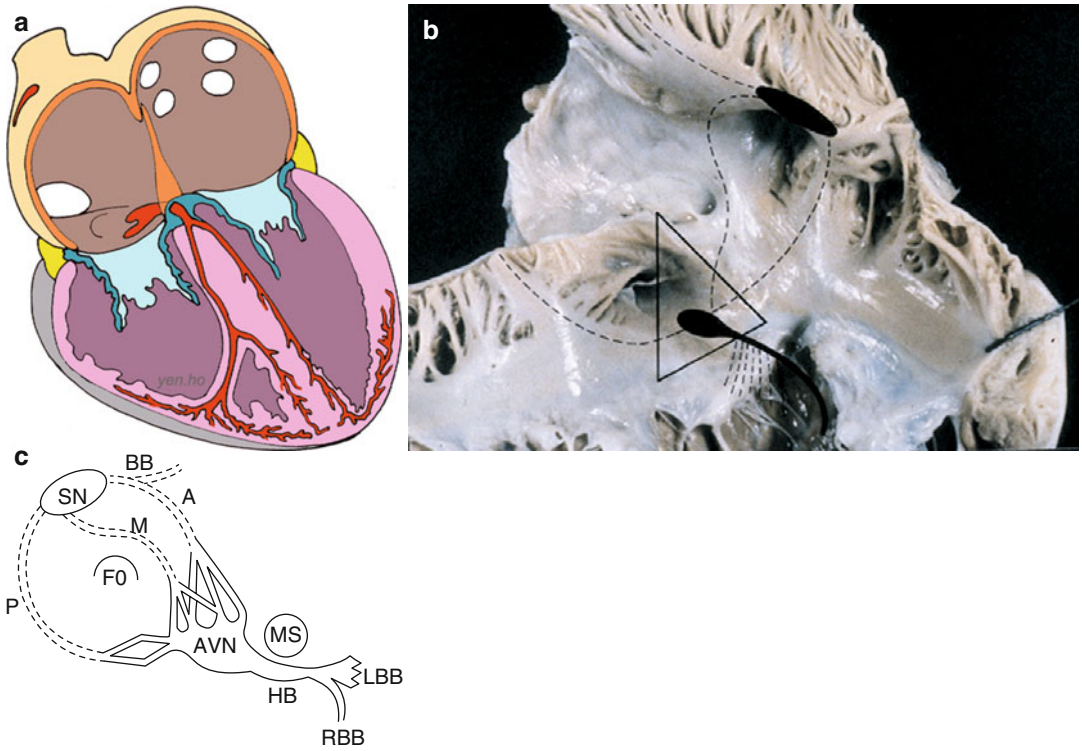


Figure 4–1. (**a**) Diagram illustrating the topography of the specialised conduction system (*red*) of the heart; (**b**) view of the right atrium: the sinus node is located at the root of the superior vena cava, lying over the crista terminalis, and the AV node within the triangle of Koch. *Dotted lines* depict internodal pathways and left bundle branch. (**c**) Diagram illustrating the internodal pathways (*A* anterior, *M* middle, *P* posterior) between the sinus node (*SN*) and the AV node (*AVN*) and the interatrial connection through the so-called Backman's bundle (*BB*). *FO* fossa ovalis, *MS* membranous septum, *HB* His bundle, *LBB* left bundle branch, *RBB* right bundle branch

Normal Anatomy

Sinus Node: Location, Anatomy and Histology

Discovered by Keith and Flack a century ago [1], the sinus node (the cardiac pace-maker) was illustrated as lying in the terminal groove (sulcus terminalis), in the lateral part of the junction between the superior vena cava and the right atrium (see Fig. 4.1). The original description reports that "*there is a remarkable remnant of primitive fibres persisting at the sino-auricular junction in all the mammalian hearts examined. These fibers are in close connection with the vagus and the sympathetic nerves, and have a special arterial supply; in them the dominating rhythm of the heart is believed to normally arise*" [1].

This lateral position was endorsed by Koch [2] and by most subsequent investigators [3–5]. A horse-shoe arrangement with the node situated anteriorly and draped over the crest of the atrial appendage as described by Hudson [6] is found in approximately 10 % of hearts [7].

The shape of the node, more commonly, is like a tadpole with a head section situated antero-superiorly and a tapering tail that extends for a variable distance inferiorly toward the entrance of the inferior vena cava (see Fig. 4.1) [2, 8]. In the subepicardium, the long axis of the node is parallel to the terminal groove, but the body and tail then penetrate intramyocardially towards the subendocardium. Thus, the fatty tissues of the terminal groove serves as the epicardial landmark whereas the terminal crest (crista terminalis) in the antero-lateral quadrant of the entrance of the superior vena cava is the endocardial landmark for the nodal head. The nodal body and tail lie in the terminal crest and are at varying depths from the endocardium [8]. In the adult the length

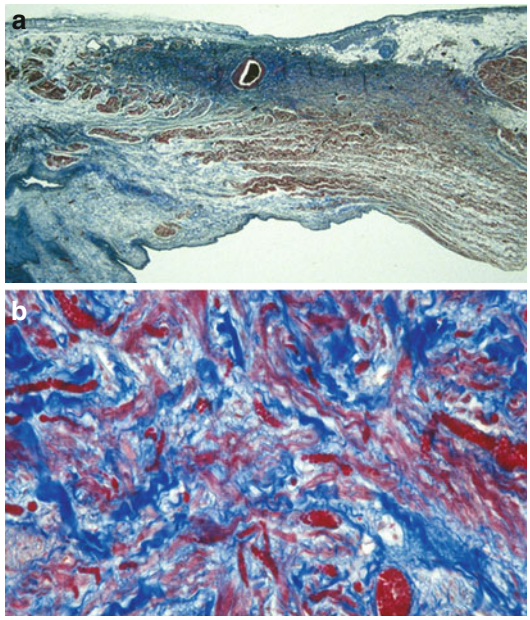


Figure 4–2. (**a**) Longitudinal section of the sulcus and crista terminalis: the sinus node is located sub-epicardially and centered by the sinus node artery. Note the abundant extracellular matrix (Heidenhain trichrome ×5); (**b**) at higher magnification, the small, interlacing pale myocytes with P and T cells are visible (Heidenhain trichrome ×40)

of the nodal body is approximately 1–2 cm but the tail portion can extend considerably longer.

The artery supplying the node is a branch from the proximal right coronary artery in 55 % of hearts and from the left circumflex coronary artery in the remainder [7]. The nodal artery approaches the node from anteriorly in majority of hearts but can also approach from posteriorly or form an arterial circle around the cavo-atrial junction [9]. Typically, the nodal artery passes centrally through the length of the nodal body.

The node is a specialised muscular structure composed mainly of small, interlacing myocytes of no definite orientation within a background of extracellular matrix, surrounded by parasymphatetic ganglionated plexus accounting for nerve supply [10]. On histology, the node appears as a dense aggregation and the specialised myocytes (P cells) appear less darkly stained than the neighboring working atrial myocardium (Fig. 4.2). These P cells have pacemaker activity due to spontaneous depolarization. At electron microscopy, P cells typically occur in small clusters of 3 or 4, surrounded by a basement membrane and contain very simple intercellular junctions (Fig. 4.3). The intercellular junctions of the P cells are composed mainly of undifferentiated regions, with only a few desmosomes and even more rarely, a very small gap (nexus).

However, the nodal margins may be discrete with fibrous separation from atrial myocardium or interdigitate through a transitional zone. In the latter, prongs of nodal (P) and transitional (T) cells extend into the atrial myocardium but actual cell-to-cell contact is uncertain. Prongs radiating from the nodal body are common [8]. Occasional prongs can be found extending toward the wall of the superior vena cava. In some hearts, the distal part of the nodal tail appears as clusters of specialised myocytes amongst fibrofatty tissues and atrial myocytes in the subendocardium [8].

Internodal and Interatrial Myocardium

The transmission of the cardiac impulse from the sinus node to the AV node is often depicted as through three internodal tracts in Cardiology texts. These are portrayed as cable-like structures that pass anteriorly, medially and posteriorly in the right atrium (Fig. 4.1b, c). However, light microscopy and electron microscopy examinations have not revealed discrete specialised bundles in the atrial walls that could satisfy the criteria of conduction tissue tracts. Apart from the occasional caudal extension of the sinus node into the crista terminalis, there are no histologically recognizable specialised pathways. This is also true for the interatrial conduction through the so-called Backman's bundle [11]. Instead, the walls of the atria are made up of broad bands of working myocardium that are separated by orifices of the veins, foramen ovale, and the AV valves. Parts of the wall, for example the crista terminalis and the anterior rim around the foramen ovale, show a better alignment of the myocytes than other parts, allowing preferential propagation of the cardiac impulse. Thus, the spread of excitation from pacemaker to AV node as well as to the left atrium is along broad wavefronts in these muscular bands. Through its transitional cell zone, the AV node acts as the 'receiver' that then

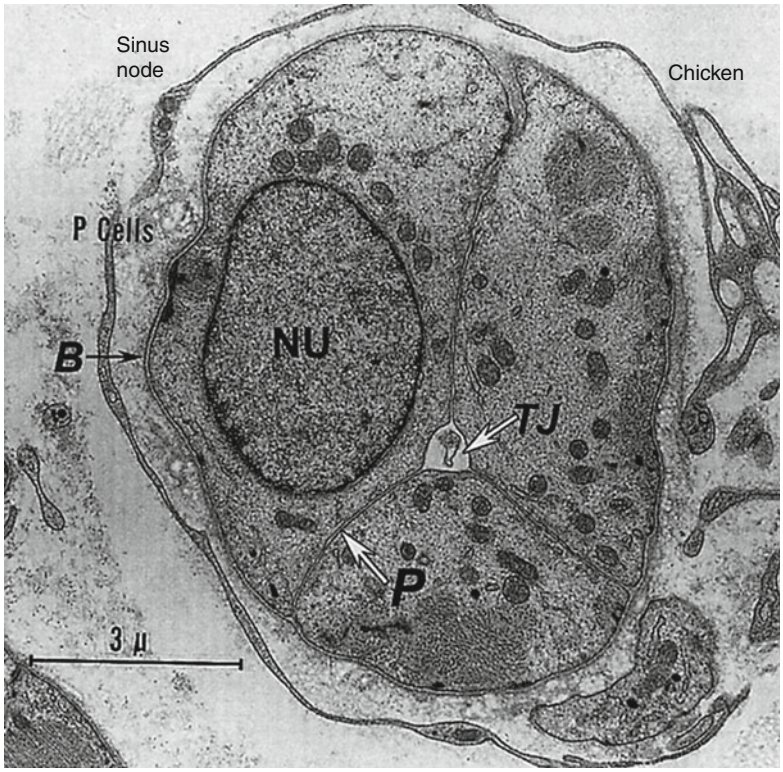


Figure 4–3. Clusters of P-cells in the chicken sinus node. Note a basement membrane (*B*) surrounding the entire P-cell cluster. Junctions between the three P cells here are composed entirely of "undifferentiated regions," with simple apposition of plasma membranes (*P*) at a constant distance from each other (*TJ* triadic junction, *NU* nucleus) (From James [10]. Reprinted with permission from Elsevier Limited)

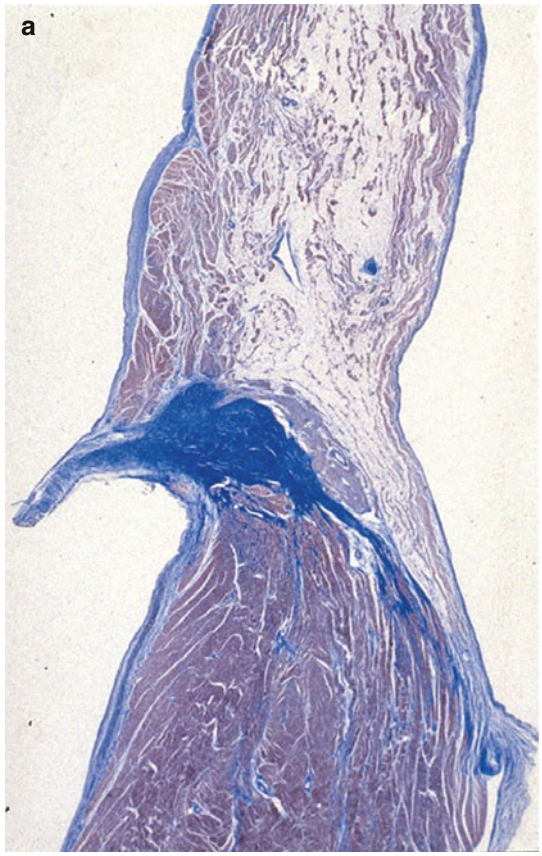
channels the impulse to the ventricles via the specialised conduction bundle and bundle branches.

AV Conduction System: Location, Anatomy and Histology

The pioneering work of Tawara [12] a century ago likened the AV system to a tree, with its roots in the atrial septum, and its branches ramifying within the ventricles. He recognized a collection of histologically distinct cells at the base of the atrial septum that he termed the "knoten", and that has subsequently become known as the AV node.

Being the atrial component of the AV conduction system, the AV node receives, slows down and conveys atrial impulses to the ventricles. It is an interatrial structure located on the right side of the central fibrous body and when considered from the right atrial aspect it is situated within the triangle of Koch (see Fig. 4.1). The triangle described by Koch [2] is bordered anteriorly by the 'annulus' of the septal leaflet of the tricuspid valve, posteriorly by the tendon of Todaro that runs within the sinus septum (Eustachian ridge or crista dividens), and inferiorly by the orifice of the coronary sinus and the atrial vestibule (see Fig. 4.1). The vestibule is recognised by arrhythmologists as the so-called 'septal isthmus'. This is the target for ablating the slow pathway in patients with AV nodal reentrant tachycardia [13]. The central fibrous body itself is comprised of a thickened area of fibrous continuity between the leaflets of the mitral and aortic valves, termed the right fibrous trigone (Fig. 4.4), together with the membranous component of the cardiac septum. The tendon of Todaro inserts into the central fibrous body that lies at the apex of the triangle (Fig. 4.5) [14]. The 'annulus' of the septal leaflet of the tricuspid valve crosses the membranous septum (Fig. 4.6a).

In the original description by Tawara, it is reported that "*the system is a closed muscle bundle that resembles a tree, having a beginning, or root, and branches… The system connects with the ordinary ventricular musculature for the first time at the terminal ramifications*" [12]. Moreover, in 1893 His was the first to observe the bundle as



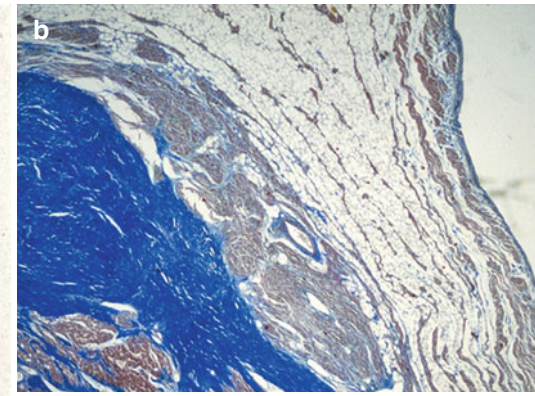


Figure 4–4. (**a**) The AV node is located on the right side of the central fibrous body, which extends to the fibrous mitro-aortic continuity (Heidenhain trichrome ×3); (**b**) close-up of the AV node, with compact and transitional zones, centered by the AV nodal artery (Heidenhain trichrome ×12)

follow: " *I have succeeded in finding a muscle bundle which unites the auricular and ventricular septal walls…The bundle arises from the posterior wall of the right auricle near the auricular septum, in the atrioventricular groove; attaches itself along the upper margin of the ventricular septal muscle…proceeds on top of this toward the frontal until near the aorta it forks itself into a right and left limb*" [15].

The compact node, approximately 5 mm long, 5 mm wide and 0.8 mm thick in adults [16], is adjacent to the central fibrous body on the right side but is uninsulated by fibrous tissue on its other sides, allowing contiguity with atrial myocardium (see Fig. 4.4). Owing to the lower level of attachment of the tricuspid valve relative to the mitral valve, the AV node 'leans' toward the right atrial side and is a few millimetres far from the endocardium (see Fig. 4.4).

From the node extends the AV bundle of His that passes through the fibrous core of the central fibrous body (see Fig. 4.5). The bundle veers leftward as it penetrates the central fibrous body, taking it away from the right atrial endocardium and toward the ventricular septum. In majority of hearts it emerges to the left of the ventricular septal crest but is insulated from ventricular myocardium by fibrous tissue and from atrial myocardium by the membranous septum itself (Fig. 4.6b). Viewed from the left ventricle, the landmark for the AV bundle is the area of fibrous continuity between aortic and mitral valves that is adjacent to the membranous septum. Viewed from the aorta, the interleaflet fibrous triangle between the right and the non-coronary sinuses adjoins the membranous septum and the AV bundle passes beneath that part of the septum (Fig. 4.7).

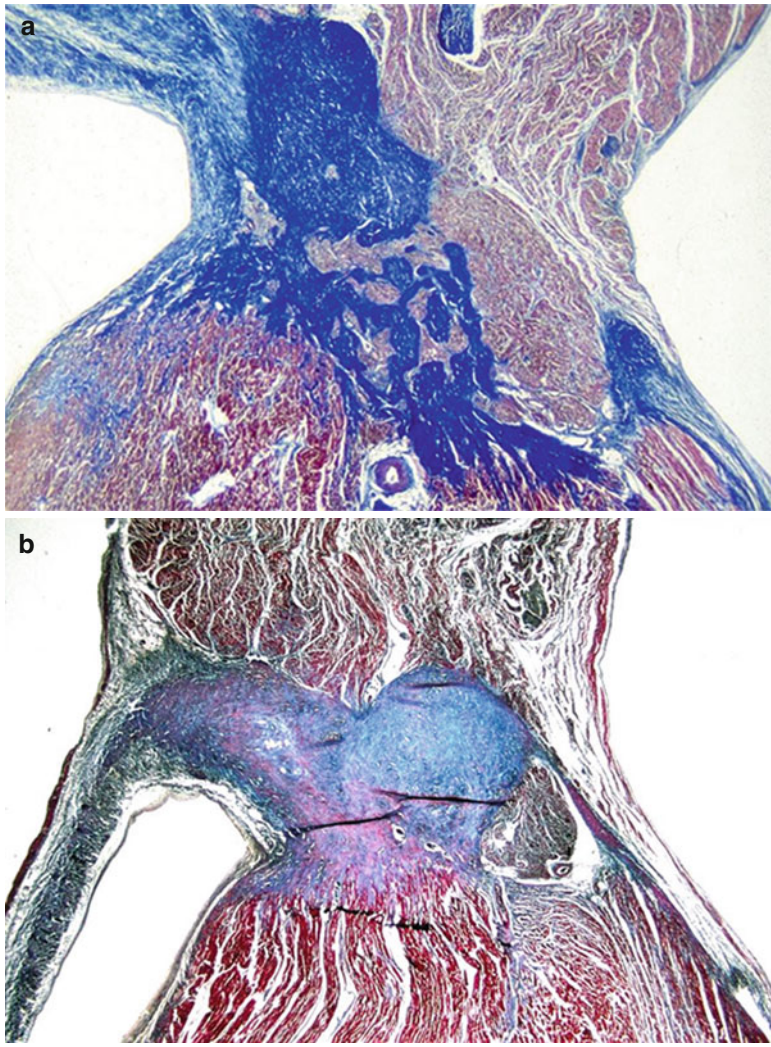


Figure 4–5. (**a**) Penetrating AV bundle: note on the top the tendon of Todaro, approximating the central fibrous body (Heidenhain trichrome ×12); (**b**) common AV bundle running within the fibrous body on the right side and surrounded by a fibrous sheat (Heidenhain trichrome ×12)

Progressing forward, the AV bundle divides into left and right bundle branches, still ensheathed by fibrous tissue until the bundle branches have descended approximately halfway down the septum. Descending in the subendocardium, the left bundle branch fans out into interconnecting fascicles as depicted in the original drawings by Tawara [12]. The fascicles then ramify into thinner and thinner strands toward the apex. Sometimes its proximal subendocardial course is visible due to the glistening sheen of its fibrous sheath. Owing to its fan-shape, its proximal portion is considerably more extensive than that of the right bundle branch. The right bundle branch, a cord-like structure, is a direct continuation of the AV bundle. In majority of hearts, since the AV bundle is usually to the left of the ventricular septal crest instead of being astride the crest, the right bundle branch passes through the septal myocardium before reaching the subendocardium of the right side of the ventricular septum (Fig. 4.6). The anatomical landmark for its emergence is the base of the medial papillary muscle (Lancisi muscle). From there its proximal portion can often be seen as a white line in the subendocardium of the septomarginal trabeculation where it is still within a fibrous sheath. Distally, ramifications of the right bundle branch extend to the apex of the heart, and are also carried across the ventricular cavity through the moderator band and other muscular bundles (see Fig. 4.1). In some hearts, an additional bundle arises from the branching bundle, in between the bundle

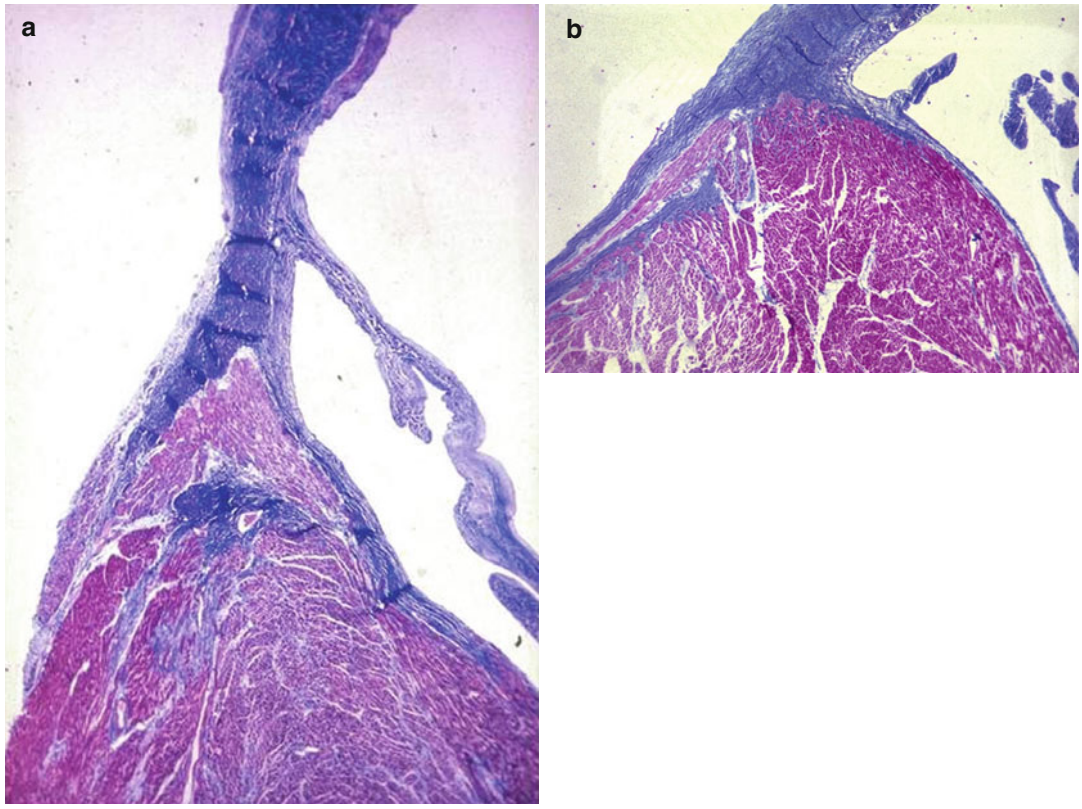


Figure 4–6. (**a**) Bifurcating bundle astride the ventricular septal crest, underneath the membranous septum: note the insertion of the septal leaflet of the tricuspid valve dividing the membranous septum in interventricular and AV components (Heidenhain trichrome ×4); (**b**) Course of the bifurcating bundle on the left side of the ventricular septal crest: note the insulation of the bundle by fibrous tissue and the intramyocardial course of the proximal right bundle branch (Heidenhain trichrome ×8)

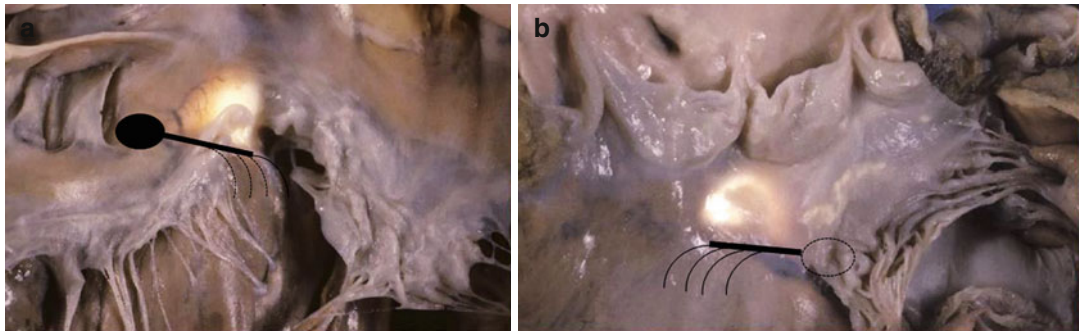


Figure 4–7. The "core" of the heart in correspondence of the membranous septum, where the specialised AV junction is located (**a**, right side view; **b**, left side view). The landmark of the AV bundle from the left side is the continuity between the aortic and mitral valve, adjacent to the membranous septum, which is located underneath the interleaflet triangle between the right and posterior non-coronary cusps

branches, and extends forward. This is described as the 'dead end tract' and is more often seen in fetal and infantile hearts than in adult hearts [17]. It continues from the main bundle anterosuperiorly toward the root of the aorta.

Under the microscope, the specialised AV conduction bundle and its main branches are readily identifiable by their encasing fibrous sheaths using basic histological stains. In keeping with Tawara's work [12], it is the continuity from

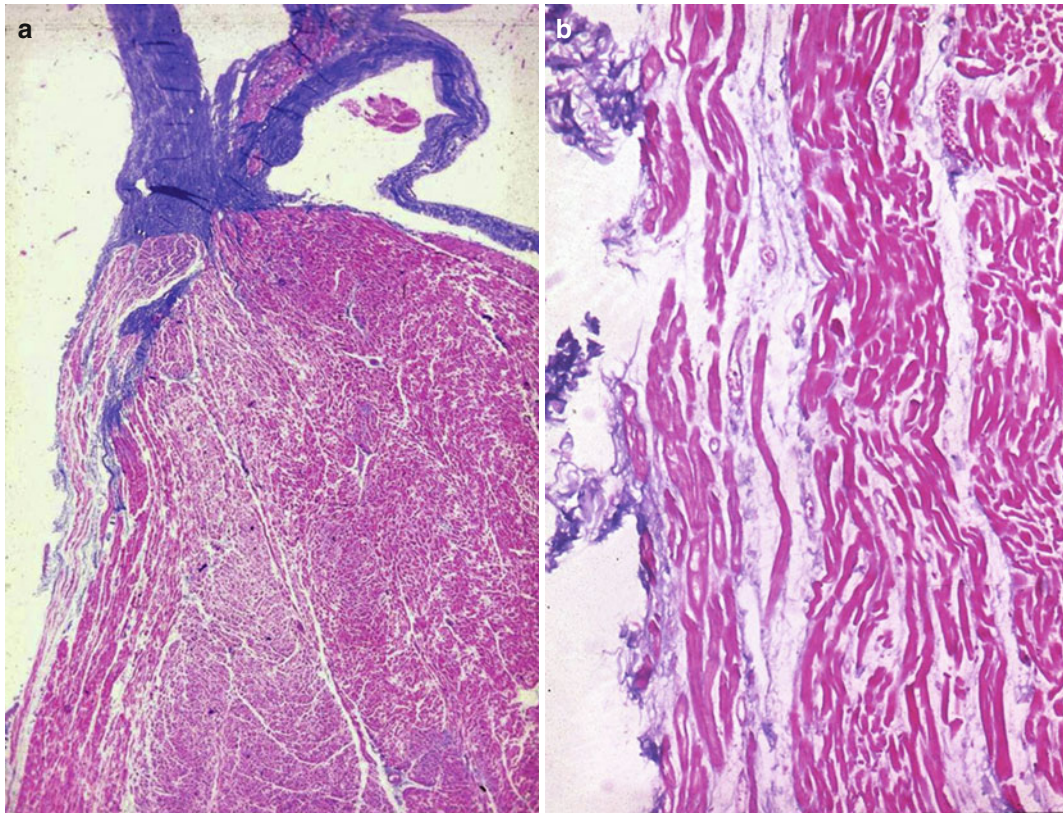


Figure 4–8. (**a**) The course of the left bundle branch under the subendocardium of the left side of the ventricular septum (Heidenhain trichrome ×5); (**b**) Close-up of the Purkinje-like cells of the left bundle branch (Heidenhain trichrome ×25)

section to section that serves as the most reliable method for histological localisation of the AV conduction system. Beginning with the AV node, which has the inherent function of delaying the cardiac impulse, the human node has a compact portion, and zones of transitional cells (see Fig. 4.4). The compact node is recognizable, when seen in cross-sections, as a half-moon shaped structure hugging the central fibrous body. The nodal cells are smaller than atrial myocytes. Like the cells of the sinus node, the compact nodal cells are closely grouped, and frequently arranged in interweaving fashion. In many hearts, the compact node has a stratified appearance with a deep layer overlain by a superficial layer. When traced inferiorly, toward the base of Koch's triangle, the compact area separates into two prongs, usually with the artery supplying the node running in between. The prongs bifurcate toward the tricuspid and mitral annuli respectively. Their lengths vary from heart to heart and in recent years the rightward prongs have been implicated in so-called slow pathway conduction in AV nodal re-entrant tachycardia [18]. Interposing between the compact node and the working atrial myocardium is a zone of transitional cells (see Fig. 4.4b). These cells are histologically distinct from both the cells of the compact node and the working cells of the atrial myocardium, and are not insulated from the surrounding myocardium. The cells are long, attenuated, and have a wavy appearance. They tend to be separated from one another by thin fibrous strands. According to established definitions, transitional cells do not represent conducting tracts but they provide the crucial bridge between the working and the specialised myocardium. Transitional cells interpose between the left and right margins of the compact node and the myocardium from the left and right sides of the atrial septum. Wider extensions of transitional cells are present inferiorly and posteriorly between the compact node and the mouth of the coronary sinus and into the Eustachian ridge. The right

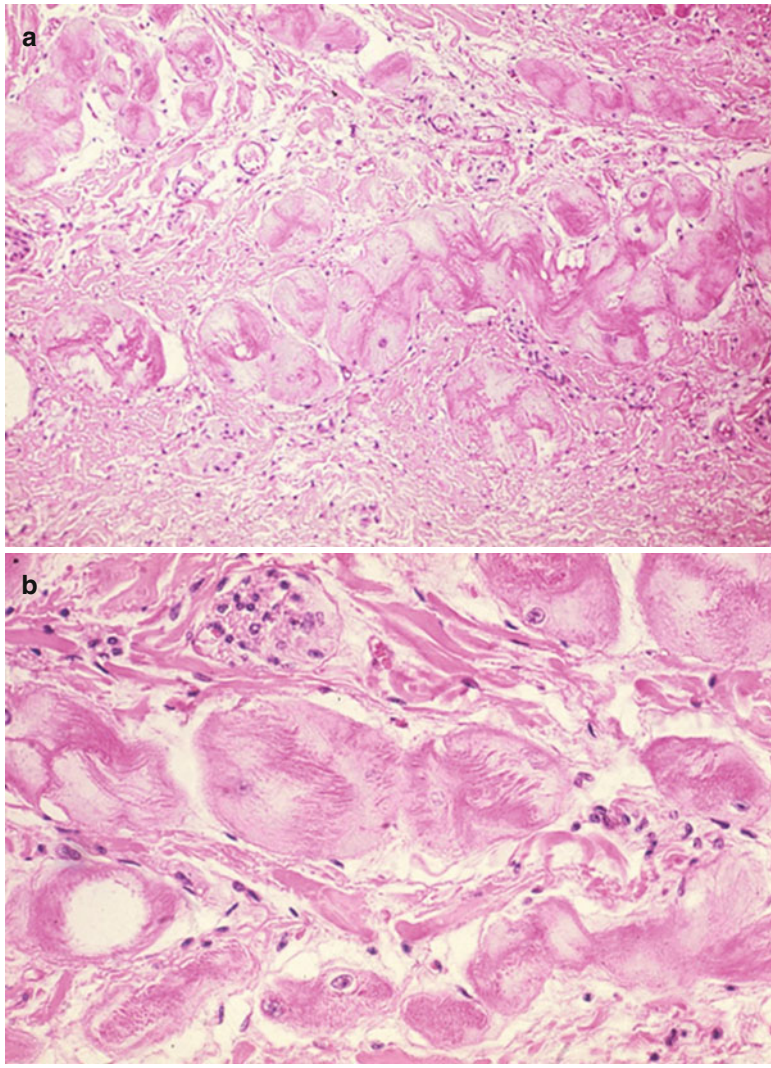


Figure 4–9. Purkinje cells in ungulates' heart. (**a**) Large, swollen and pale specialised cardiac myocytes as compared to the surrounding working myocardium (haematoxylin-eosin ×40); (**b**) Purkinje cells at higher magnification (haematoxylin-eosin ×120)

margin of the node faces the vestibule of the right atrium. Here, an overlay of working myocytes in the subendocardium from the atrial wall in front of the fossa ovalis streams over the layer of transitional cells.

When the conduction system is followed distally from the compact node into the penetrating bundle of His, there can be little difference in the cellular composition in the two areas. The specialised cells themselves, however, become aligned in a more parallel fashion distally. Even so, Tawara [12] proposed that the distinction be made on purely anatomical grounds. The key change from node to bundle is that the bundle is insulated by fibrous tissue from the adjacent myocardium (see Fig. 4.6b), preventing atrial activity from bypassing the node. Thus, all atrial activity must be channeled via the AV node.

Being surrounded by fibrous tissue, the penetrating bundle is the first part of the axis which qualifies as a conducting tract (see Fig. 4.5b). The cells are marginally larger than compact nodal cells and they increase in size as the penetrating bundle continues into the AV bundle and branching bundle (Fig. 4.8). Here, the cells are very similar in size to ventricular myocytes. Swollen cells or Purkinje cells are not characteristic of specialised myocytes in the human heart and are seldom seen. However, they are typically seen in ungulates (Fig. 4.9) [12, 19, 20]. The AV bundle, branching bundle and proximal parts of the bundle branches are recognizable by the fibrous

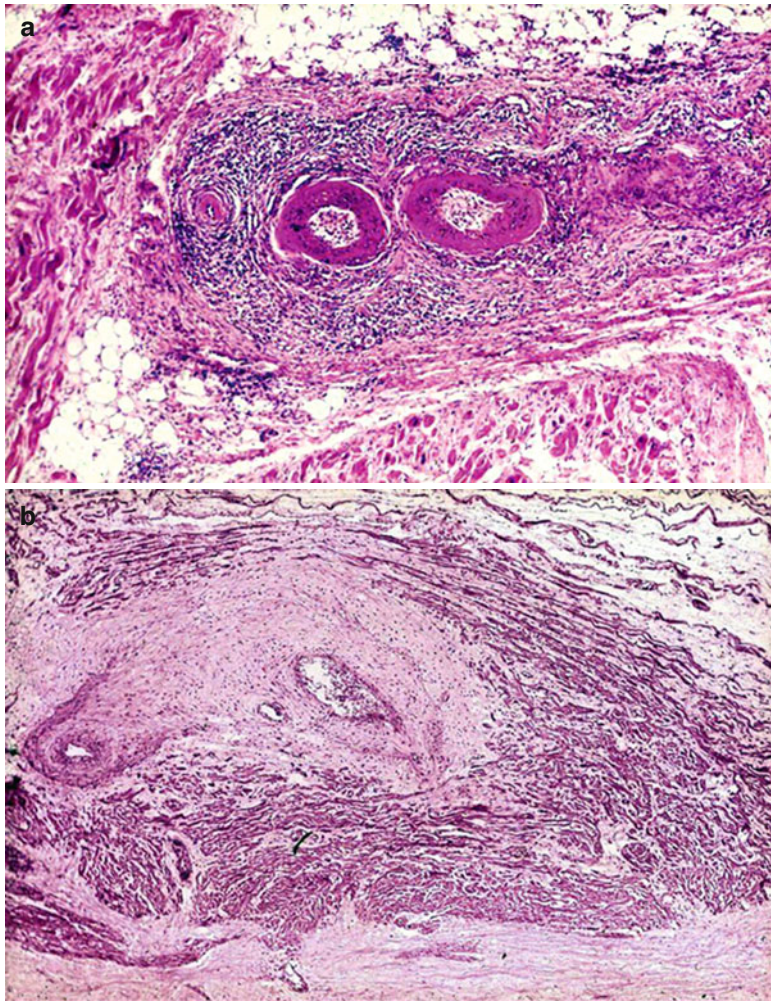


Figure 4–10. (**a**) Arteritis of the sinus node artery in polyarteritis nodosa: note the inflammation extended to the nodal specialised myocardium (haematoxylin-eosin ×15); (**b**) AV node in polyarteritis nodosa: the AV nodal artery shows aneurysm and recanalized thrombus with extensive fibrotic replacement of conductive cells (haematoxylin-eosin ×15)

sheaths that encase them, insulating them from the adjacent working ventricular myocardium. When the bundles loose their fibrous sheaths distally, it is no longer possible to distinguish conduction tissues from working myocardium.

Pathology

Sino-Atrial Block and Sinus Arrest

The atrial activation may be impaired (atrial standstill) for two main reasons: impulses are not generated from the sinus node (sinus arrest) or their propagation to the atria is impeded (sinus block). In the etiology of sino-atrial block, several lesions of the sinus node and its innervation have been described, besides neurovegetative changes (vagal stimulation), drug sensitivity – intoxication and hyperkalemia.

From the pathological viewpoint, abnormalities of the sinus node artery, of the specialised myocardium of the sinus node and/or of its connections with the atrial myocardium (nodal approaches), and of the nodal ganglionated plexus have been reported. Myocardial infarction due to occlusion of the right coronary artery, proximal to the origin of the sinus node artery, remains the main cause of sinus node dysfunction, causing severe damage of the node and its atrial approaches in terms of necrosis, leukocytic

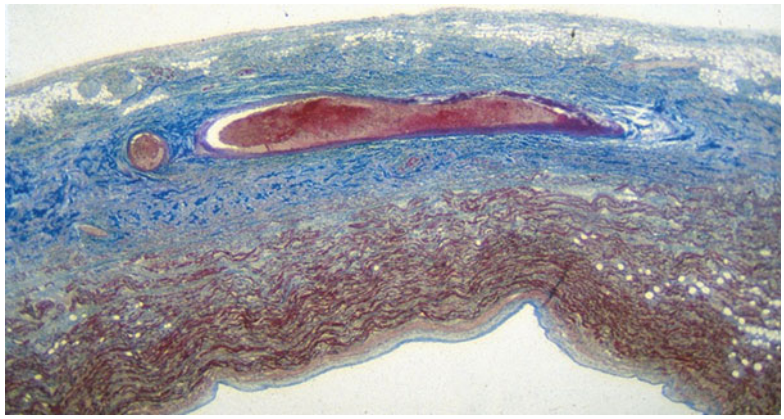


Figure 4–11. Massive infarction of the sinus node and crista terminalis by occlusive thromboembolism of the nodal artery (Heidenhain trichrome ×6)

infiltrates and haemorrhage. Sinus node artery perfusion can be also altered as a consequence of arteritis (Fig. 4.10) and embolism (Fig. 4.11), amyloid deposition and connective tissue disorders [21–25]. Recipient sinus node in cardiac transplantation undergoes to massive infarction due to nodal artery transection during surgical procedure and the donor sinus node artery may show obstructive intimal proliferations due to allograft vasculopathy (chronic rejection) (Fig. 4.12) [26].

AV Block

Any disease, either acute or chronic, that affects the myocardium may produce AV block, which may occur at the level of AV node approaches, AV node itself, penetrating or branching part of the bundle, and bundle branches [21–23, 27].

Morgagni, Adams and Stokes share the merit of having individuated the clinical entity of AV block and, after the recognition of the anatomic basis of the AV conduction axis by His and Tawara, Mahaim did the first clinico-pathologic assessment of this entity [28].

Pathologically, AV block may be classified as being caused by congenital or acquired diseases [27]. As far as the *congenital* AV block in an otherwise normally developed heart, this is usually a benign condition, mainly due to a lack of connection between the atria and the peripheral conduction system, with fatty replacement of the AV node and nodal approaches [29]. Moreover, the AV bundle may present marked fragmentation and septation. Maternal lupus with an immune mechanism plays a major etiopathogenetic role [30, 31].

Acquired AV block may be caused by *acute myocardial ischemia or infarction*. Inferior myocardial infarction may be complicated by third-degree AV block due to ischemic injury of the AV node itself. In particular, in postero-septal myocardial infarction, due to right coronary artery thrombotic occlusion in right dominant pattern, ischemic damage may involve atrial approaches to the AV node, AV node and His bundle. However, since the conducting tissues are resistant to ischemia, pathologic changes may be reversible and the AV block transient. Anterior myocardial infarction usually is associated with third-degree block due to ischemia or infarction of bundle branches; the branching bundle and bundle branches are often involved by the necrotic process, and by inflammatory infiltrates of the surrounding working ventricular myocardium [32]. Chronic ischemic heart disease, with or without infarction, may also be characterized by AV block, due to fibrotic changes of the bifurcating bundle and bundle branches, as well as of the crest of the ventricular septum [27].

The heart may be the target of *angioitides and collagen diseases*. Polyarteritis nodosa is a medium-large vessel vasculitis, with cardiac involvement in up to 80 % of cases. It typically manifests as pericarditis, coronary arteritis, myocardial infarction, arrhythmias and conduction disturbances [24]. Nodal arteries may be of

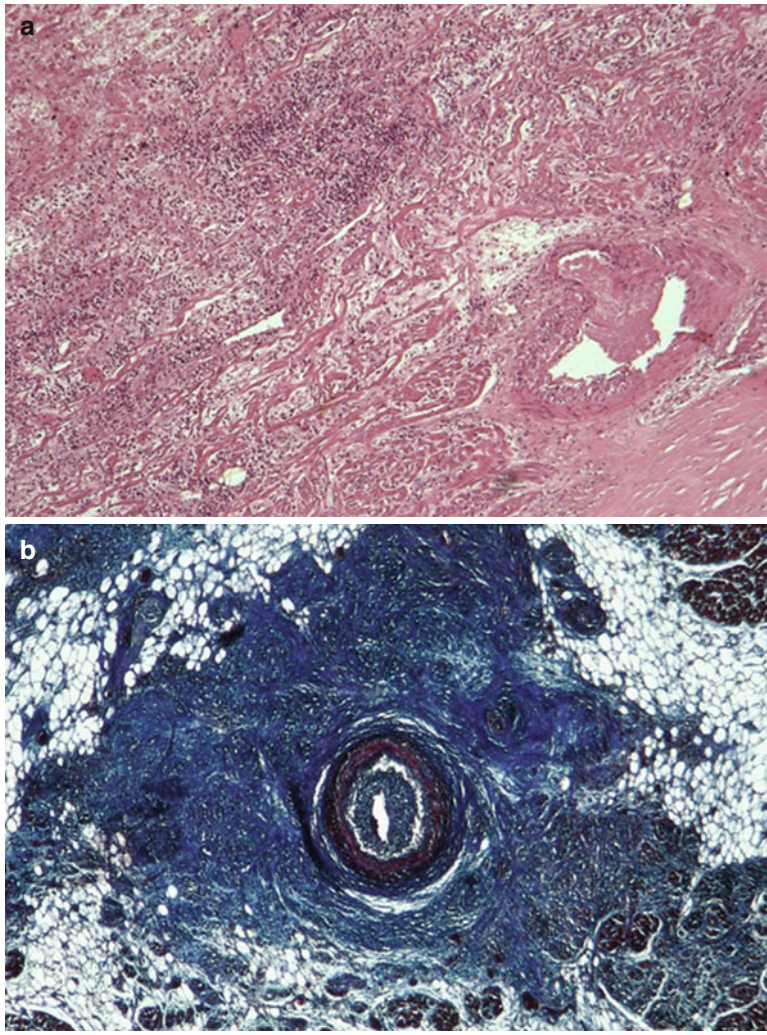


Figure 4–12. Conduction system pathology in the transplanted heart. (**a**) AV node shows severe acute rejection and thrombosis of the AV node artery (haematoxylin-eosin stain, original magnification **a** ×60); (**b**) Histologic section of the donor sinus node artery in a patient with allograft vasculopathy (chronic rejection) who died 1,996 days after cardiac transplantation: note the obstructive, concentric intimal proliferation (Trichrome Heidenhain stain ×30)

the proper size to be affected and the surrounding conductive tissue involved as well (Fig. 4.10b).

Cardiac involvement has been reported in 8–44 % of cases of Wegener's granulomatosis. It typically manifests as pericarditis, coronary arteritis and myocarditis with granulomas involving the conduction system.

Similarly, cardiac involvement is seen in up to 70 % of cases of systemic lupus erythematosus. The most common findings are pericarditis, myocarditis, and Libman-Sacks endocarditis. As previously mentioned, congenital heart block is a peculiar feature of neonatal lupus syndrome, which is associated with transplacental transfer of anti-Ro and anti-La antibodies [30, 31]. In rheumatoid arthritis, rheumatoid granulomas may affect the myocardium, endocardium, and valves as well as the conductive tissues; conduction disturbances and heart block have been reported [33].

Myotonic dystrophy, particularly type 1 myotonic dystrophy (Steinert's disease), which is the commonest muscular dystrophy in adults, is characterized by myotonia, muscle weakness and a variety of other symptoms. Cardiac involvement is a frequent manifestation, the most prominent feature being conduction disturbances and arrhythmias, at risk of sudden death [34].

Myocarditis, especially in the acute phase, may present with AV block. It is usually transient and relates to inflammatory infiltrates and

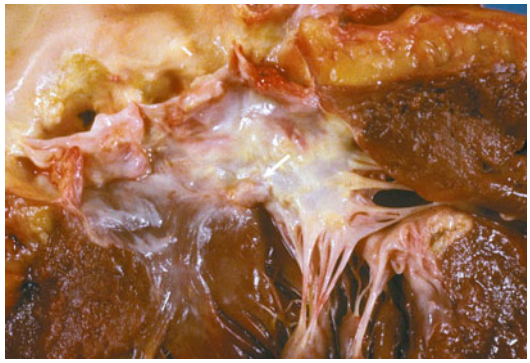


Figure 4–13. Calcific aortic valve stenosis with dystrophic calcification extended to the mitro-aortic continuity, where the His bundle is coming out

interstitial edema of the working myocardium surrounding the specialised conducting pathways, although involvement of the sinus node and AV node themselves have been reported. In *acute rheumatic carditis*, all the structures of the heart may be involved by the inflammatory process, conduction system included. In terms of rhythm disturbance, besides tachyarrhythmias, varying degrees of heart block, mostly first degree, are frequently recorded. Similar features with transient AV block may complicate *acute rejection* of the specialised conducting tissues themselves after cardiac transplantation (see Fig. 4.12) [26].

In *infective endocarditis* of the aortic and/or mitral valve, the inflammatory process may extend to the central fibrous body, thereby disrupting the AV node and His bundle as to produce AV block.

Complete AV block occurs also in the setting of *hypertensive heart disease*, and it can results from a combination of direct mechanical injury to the origin of the main left bundle branch at the crest of the ventricular septum and ischemic heart disease.

Calcific aortic stenosis has long been recognized as a cause of AV block, and many of the patients originally reported by Stokes correspond to this entity. The His bundle penetrates the central fibrous body in close proximity to both the aortic and mitral valve fibrous continuity, which is an usual site of dystrophic calcification, and extension of calcification can directly involve the His bundle and/or the origin of the left bundle branch (Fig. 4.13).

Degenerative changes in the AV node or bundle branches are the most common cause of nonischemic AV block. The term Lenègre-Lev syndrome has been used to indicate an acquired complete heart block due to idiopathic fibrosis and calcification of the AV conduction system of the heart. However, we should keep distinct the two entities.

Lev disease is most commonly seen in the elderly, and is often described as senile degeneration of the conduction system [35]. It may imply, among others, degenerative changes at the summit of the ventricular septum, mitro-aortic fibrous continuity and membranous septum, consisting of fibrosis, hyalinisation, loss of conducting fibers, with or without calcification. The pathologic degenerative process, which has been traditionally considered the result of stress and strain, may affect mainly the branching bundle and the proximal right and left bundle branches. In the classical Lev disease, the origin of the left bundle branch and adjacent bifurcating bundle are destroyed with preservation of peripheral conduction system.

In contrast, AV block due to *Lenègre disease* occurs in younger people and the histopathologic features are in keeping with a primary myocardial disease that selectively destroys the right and left bundle branch conduction fibers, extending well down into the periphery (Fig. 4.14) [36]. It is a form of inherited cardiomyopathy confined in the specialised myocardium and should be classified among cardiomyopathies [37]. In 1999, Lenègre disease with AV block was linked to mutations of the *SCN5A* sodium channel gene, the same gene which may account also for congenital long QT syndrome type 3 and to Brugada syndrome [38].

Among *infiltrative myocardial diseases*, sarcoidosis is frequently associated with AV block due to sarcoid granulomas involving the specialised axis [39].

AV block may complicate aortic dissection when the dissecting haematoma infiltrates the atrial septum along with the retrograde extension, thus creating atrionodal discontinuity (Fig. 4.15) [40].

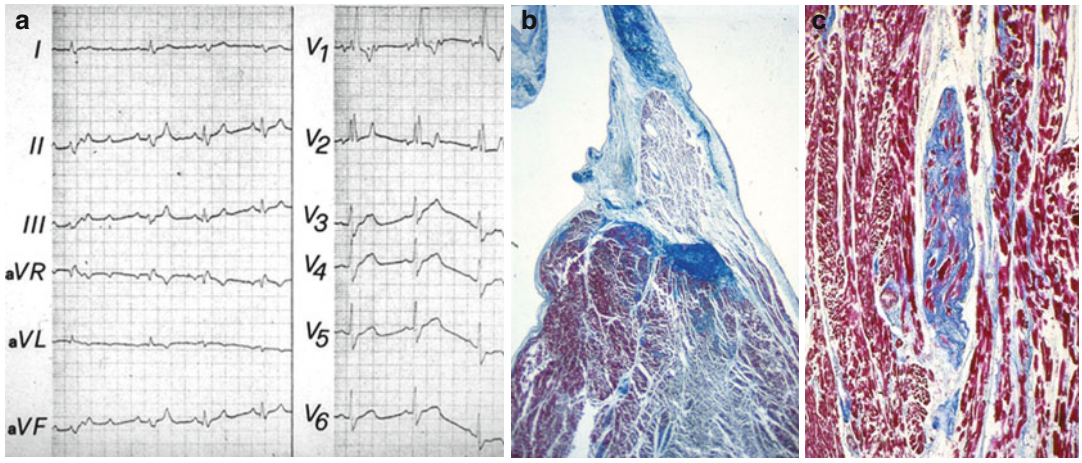


Figure 4–14. Lenègre disease with AV block. (**a**) 12 lead ECG tracing with intermittent AV block; (**b**) Sclero-atrophy of the origin of the left bundle branch from the His bundle (Heidenhain trichrome ×6); (**c**) Fibrotic interruption of the intramyocardial tract of the right bundle branch (Heidenhain trichrome ×60)

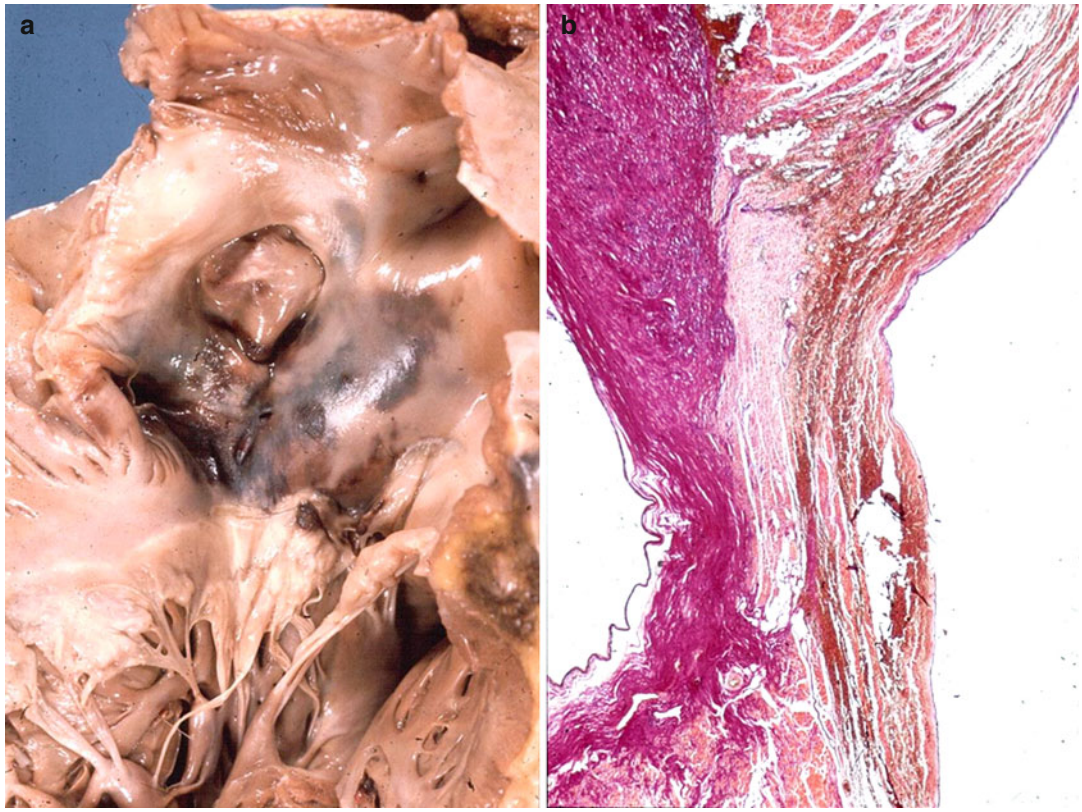


Figure 4–15. Type A aortic dissection with AV block. (**a**) Gross view of the interatrial septum with haemorrhage due to retrograde extension of dissecting haematoma seen from the right side; (**b**) At histology, atrio-nodal discontinuity due to haemorrhagic infiltration of the interatrial septum and AV node atrial approaches (Heidenhain trichrome ×15)

The term *celothelioma of the AV node*, known also as Tawarioma or cystic tumor of the AV node, refers to a tumor with heterotopic epithelial replacement of the AV node with multicystic appearance (Fig. 4.16). It is a rare entity and, thus, an unusual cause of supra-His AV block, at risk of sudden death. Definitive histologic diagnosis is made at autopsy or in explanted hearts from cardiac transplantation [41, 42].

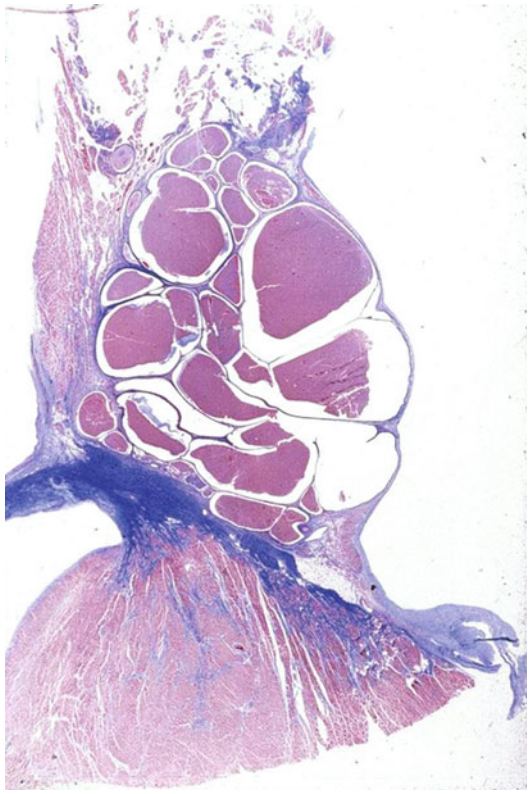


Figure 4–16. Cystic tumor of the AV node: note the multicystic neoplasm at the level of the AV node (Heidenhain trichrome ×5)

Complete AV block can appear in the setting of other *cardiac tumors*, including metastatic carcinoma of the heart, primary or secondary sarcoma and lymphoma, by direct infiltration or compression of the conducting tissue [43].

By studying a series of 177 cases with permanent AV block, Davies [27] showed that idiopathic bilateral bundle branch fibrosis is the commonest single cause (33 %), followed by ischaemic damage (17 %), cardiomyopathies (14 %) and calcific AV block (10 %). The remaining causes of chronic AV block were individually very rare ranging through tumour involvement, congenital defects, collagen diseases and surgical or traumatic damage.

As far as the site is concerned, according to Rossi [21] the frequency of AV block-producing lesions seems to increase in a downwards direction along the AV conducting pathway. Among 400 cases of AV block, which he studied by the serial section histological technique, the AV conduction discontinuity accounting for block could be ascribed to distal lesions of both bundle branches and/or bifurcating bundle in 60–70 % of cases, of the common His bundle in 15 %, of the AV node in 10–14 % and of the atrio-AV nodal approaches in 5–11 % (Fig. 4.17).

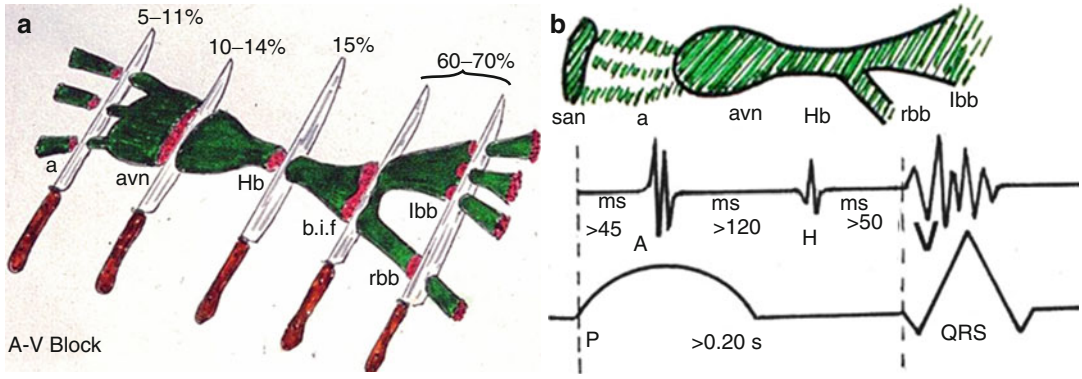


Figure 4–17. Site of AV blocking lesions: 60–70 % of the interruption of the AV conduction axis is located in the bifurcating proximal bundle branches, accounting for prolongation of HV interval

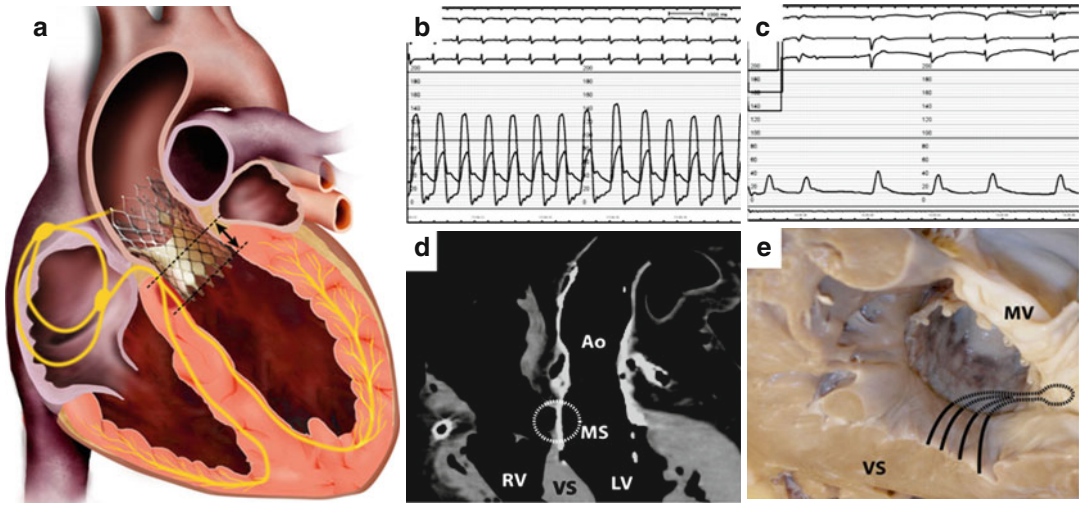


Figure 4–18. Iatrogenic AV block as a complication of transcatheter aortic valve implantation (TAVI) with CoreValve prosthesis. (**a**) Diagram illustrating anatomic relation between a deep prosthesis implantation into left ventricular outflow tract *a*ffecting electrical conduction system; (**b**) electrocardiogram showing right bundle branch block and 60 mmHg transaortic gradient at baseline. (**c**) electrocardiogram showing complete AV block immediately after TAVI. (**d**) computed tomography scan of heart explanted at autopsy showing the deep positioning of CoreValve within left ventricular outflow tract, overlapping membranous septum (*dotted circle*) and crest of interventricular septum. (**e**) gross anatomic view of left ventricular outflow seen from below: the expansion of prosthesis frames in subaortic region compresses the ventricular septum and overlaps proximal branching of left bundle branch (*dotted lines*). *Ao* aorta, *LV* left ventricle, *MS* membranous septum, *MV* mitral valve, *RV* right ventricle, *VS* ventricular septum

Iatrogenic

AV block may occur following surgical or interventional manipulation of the conducting system. Surgical (aortic valve replacement, congenital septal defects repair, septal myectomy) or other therapeutic procedures (alcohol septal ablation in patients with obstructive hypertrophic cardiomyopathy, AV ablation in patients with supraventricular arrhythmias, and transcatheter aortic valve implantation) may be complicated by AV block [44–46]. In particular, in the setting of TAVI with prostheses protruding into the left ventricular outflow tract, independent predictors of pacemaker implantation are the depth of prosthesis implantation and pre-existing right bundle branch block (Fig. 4.18) [44]. Moreover, iatrogenic AV block with pacemaker implantation, may be a therapy of atrial fibrillation with high rate ventricular response. Electric (direct current-DC) or radiofrequency ablation of the AV node or His bundle may be selectively accomplished through the right atrium via the inferior vena cava, by delivering energy in the triangle of Koch at the level of the AV node or His bundle (Fig. 4.19).

The Conduction System in Congenital Heart Diseases

The site and course of the conduction system in congenital heart disease is related to the type of visceral symmetry, ventricular looping and septal defect.

In situs inversus with AV concordance the conduction system is located in a mirror image of the normal heart with sinus node on the left-sided morphologically right atrium.

In case of visceral symmetry, two sinus nodes are present in right atrial isomerism because of bilateral morphologically right atrium with two cristae terminalis, whereas no sinus node is present in left atrial isomerism since two morphologically left atria with no crista terminalis did develop. In the latter case the pacemaker activity is taken over by the AV node (Fig. 4.20) [47, 48].

In congenitally corrected transposition, with situs solitus and AV discordance (l-loop), the AV conduction system is located anteriorly, with an antero-lateral AV node giving rise to a His bundle penetrating the mitro-pulmonary fibrous

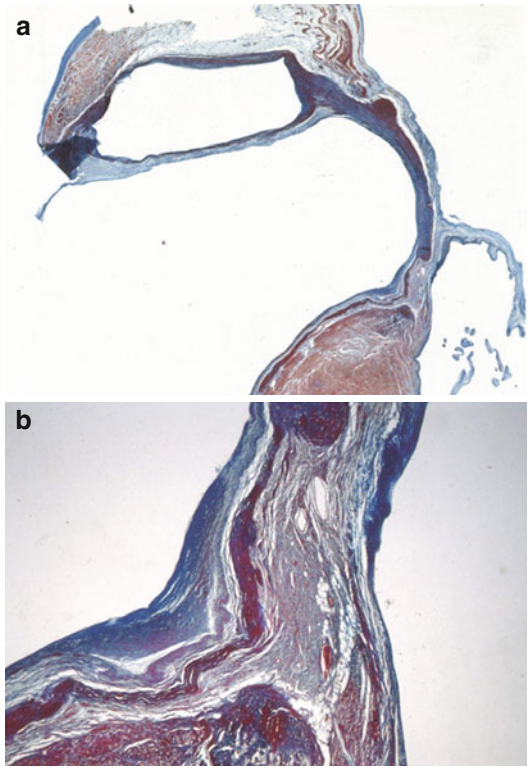


Figure 4–19. Ablation of the AV junction (iatrogenic AV block) to threat atrial fibrillation with high ventricular response. (**a**) At histology, the bifurcating bundle underneath the membranous septum appears interrupted by granulation tissue (Heidenhain trichrome ×5). (**b**) Close up of the same (Heidenhain trichrome ×20)

continuity and running anteriorly on the right-side of the ventricular septum, underneath the pulmonary valve (Fig. 4.21) [49]. In situs inversus with AV discordance (congenitally corrected transposition with situs inversus) the AV axis has been observed posteriorly, probably because of the d-loop in this setting [50].

In perimembranous ventricular septal defects, the His bundle and bifurcation are located in the postero-inferior rim, whatsoever the malformation (isolated VSD, tetralogy of Fallot, complete transposition of the great vessels, truncus arteriosus) (Figs. 4.22 and 4.23) [51]. They are at risk at the time of surgical closure if stitches are inserted in the infero-posterior rim (Fig. 4.24). A favorable condition is when the postero-inferior rim of the VSD is reinforced by the posterior limb of the trabecula septo-marginalis.

In AV canal, whether partial (ostium primum plus mitral cleft) or complete (common AV valve and orifice), the AV conduction axis is displaced well posteriorly and the penetration of the His bundle occurs at the postero-inferior rim of the huge AV septal defect; stitches in this site are at risk of AV block (Fig. 4.25) [52].

In valve malformation, the AV node may be located on the left side of the atrial septum, thus at risk of injury during mitral valve replacement [53].

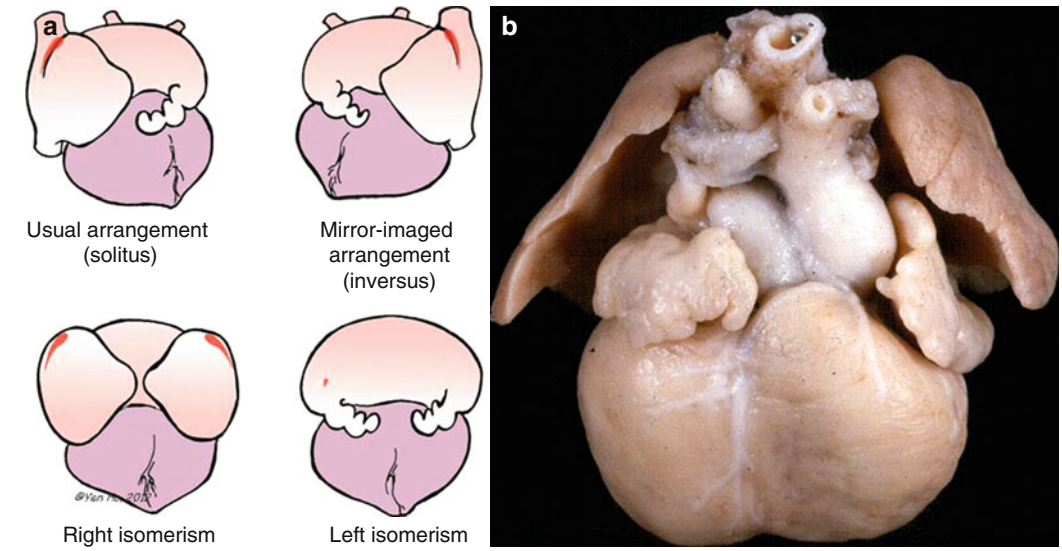


Figure 4–20. Site of the sinus node according to atrial symmetry. (**a**) Diagram illustrating the usual atrial arrangement (situs solitus), mirror-imaged arrangement (situs inversus), right isomerism and left isomerism. (**b**) Gross view of a heart specimen with left atrial isomerism (note the two left auricular appendices) and absent sinus node

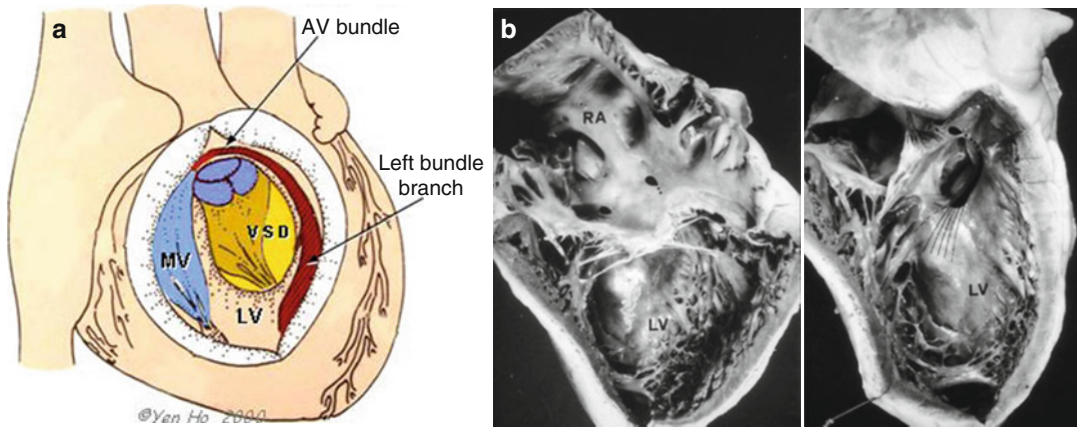


Figure 4–21. Conduction system in congenitally corrected transposition of the great arteries with situs solitus. (**a**) Diagram illustrating the AV conducting system displaced anteriorly. *MV* mitral valve, *VSD* ventricular septal defect; (**b**) gross view of the anatomical specimen with atrioventricular discordance (right atrium –RA to left ventricle-LV) and ventriculo-arterial discordance (left ventricle-LV to pulmonary artery): note the anterior location of the AV conducting axis

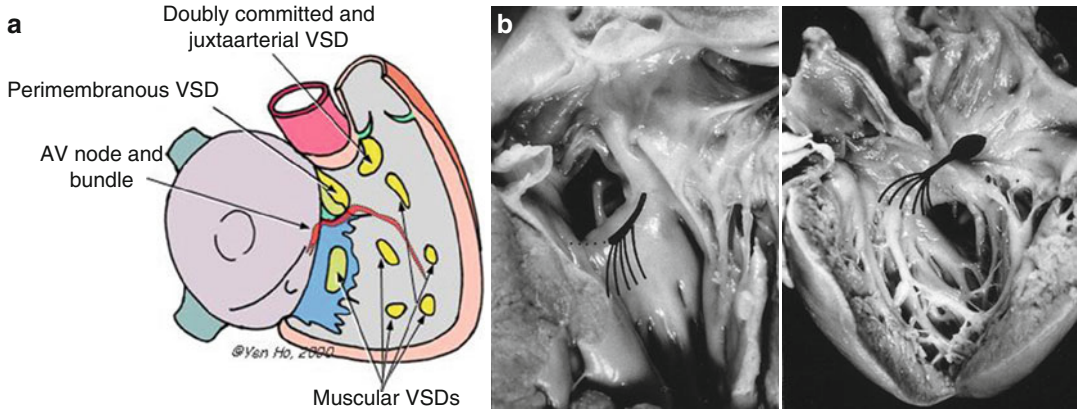


Figure 4–22. AV conduction system in ventricular septal defects (*VSD*). (**a**) Diagram illustrating the location of the AV conducting axis related to various types of VSD: doubly committed and juxtaarterial, perimembranous, muscular; (**b**) Gross view of heart specimens with perimembranous and muscular VSD: note the location of the AV node, bundle and left branch seen from the *left sides*

Ventricular Preexcitation and Enhanced AV Conduction

Accessory AV connections accounting for ventricular preexcitation may be "direct" (working to working myocardium) when located outside the specialised AV junction and connecting directly the atrial and ventricular myocardium (so-called "Kent fascicle") or "mediated" (working to specialised myocardium or vice versa) when they involve the specialised AV junction and connect either the septal atrial myocardium with the His bundle (James or Brechenmacher fibers) or the AV conduction axis with the ventricular myocardium (Mahaim fibers) [54].

A rare condition that promotes early ventricular excitation is the enhanced AV conduction (so-called Lown-Ganong-Levine syndrome [55]). The impulse runs very quickly through the AV node and His bundle, with a short PR interval and a normal QRS complex. Two histological backgrounds have been reported to

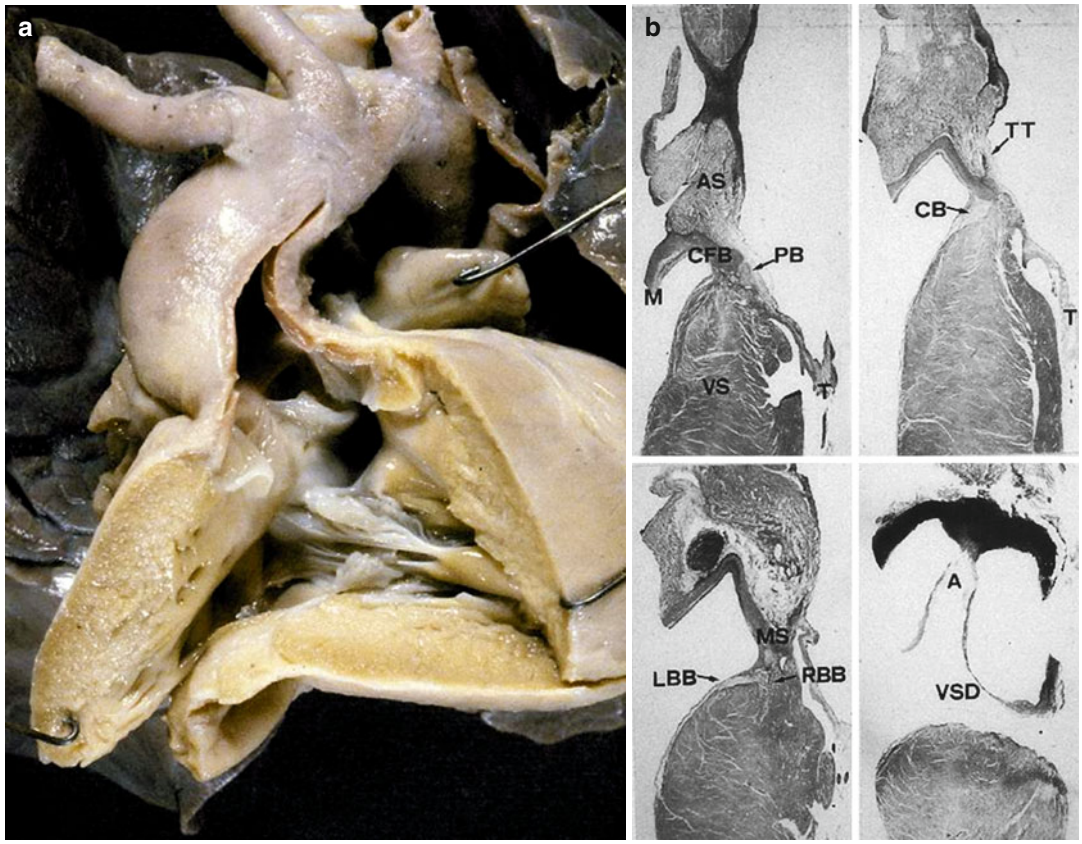


Figure 4–23. AV conduction system in tetralogy of Fallot. (**a**) Gross view of right ventricular outflows in a specimen with pulmonary atresia and perimembranous VSD; (**b**) At histology, note the course of common bundle (*CB*) and bifurcating bundle dividing into left and right branches at the postero-inferior rim of the defect (*A* aorta, *AS* atrial septum, *CFB* central fibrous body, *LBB* left bundle branch, *M* mitral valve, *MS* membranous septum, *PB* penetrating bundle, *RBB* right bundle branch, *T* tricuspid valve, *TT* tendon of Todaro, *VS* ventricular septum)

explain the missed delay at the specialised AV junction: (a) a congenitally hypoplastic AV node, with a lessened bulk of specialised tissue to slow down impulse transmission from atria to ventricles (Fig. 4.26) [56]; (b) the presence of an atrio-His bundle of working myocardium that bypasses the AV node and transmits the activation signal directly to the His bundle without any delay at the nodal level. In both substrates, the onset of atrial fibrillation, with one to one AV conduction, may trigger ventricular fibrillation, as it occurs in the Wolff-Parkinson-White syndrome.

In Wolff-Parkinson-White syndrome, an aberrant working myocardium ("Kent fascicle") joins directly the atria to the ventricles out of the specialised AV junction (Fig. 4.27) [57, 58].

Such myocardial bridges between atrial and ventricular myocardium, accessory to the normal AV conducting tissue, have been reported either in structurally normal hearts or in hearts with congenital heart diseases, like Ebstein's anomaly and congenitally corrected transposition [59]. This aberrant fascicle of working myocardium can be located all around the left and right AV rings, with the exception of the mitro-aortic fibrous continuity area. Accessory pathways in the septal area are less common and are located primarily on the right side. The "Kent fascicle" usually consists of a thin

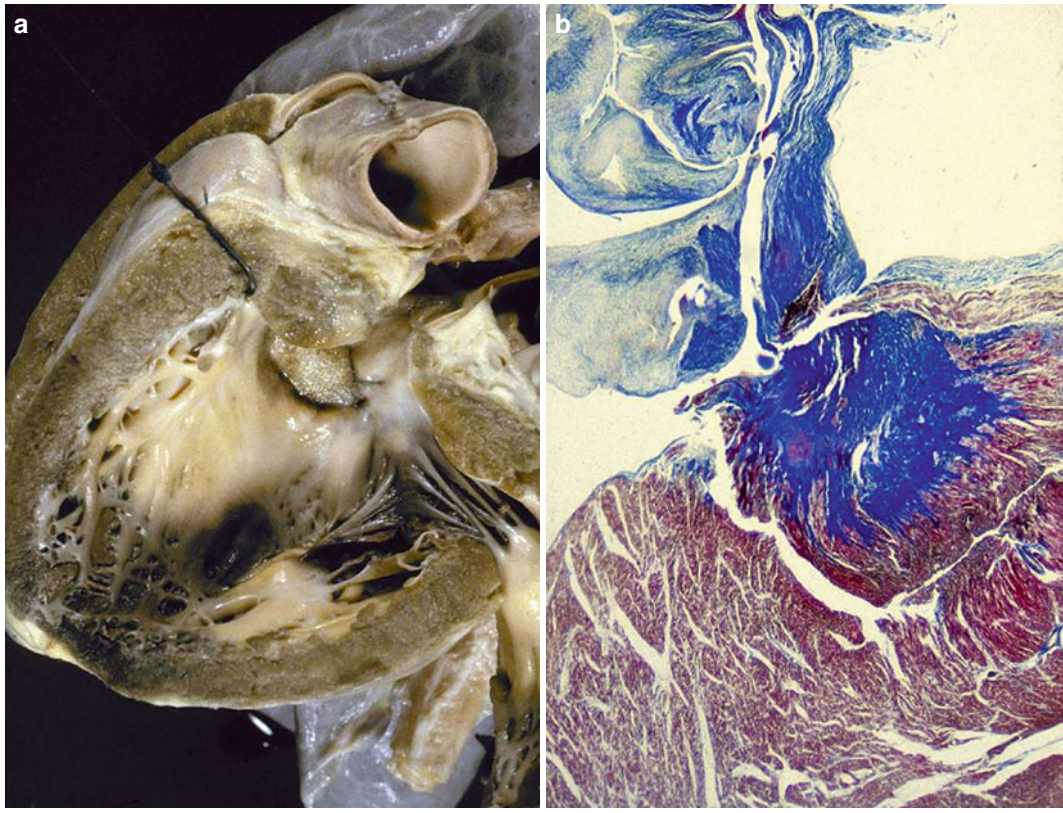


Figure 4–24. Iatrogenic AV block complicating surgical closure of a perimembranous septal defect. (**a**) Gross view of the cardiac specimen with the septal patch: note the stitches in the postero-inferior rim. (**b**) At histology, the surgical stitches cuts the AV bundle accounting for AV block (Heidenhain trichrome)

(mean 300 μm in thickness) bundle of working myocardium and, as such, does not possess decremental conduction properties. It may serve not only as bypass tract for ventricular preexcitation (thus explaining the short PQ interval and the delta wave of the QRS) but also as a limb for an AV reentry circuit, which accounts for a reciprocating supraventricular tachycardia, typical of Wolff-Parkinson-White syndrome. Impedance mismatch between the tiny anomalous fibers and the ventricular muscle bulk, in addition to fibrosis of the accessory fascicle, may explain impaired antegrade conduction and intermittent preexcitation. Preexcitation syndromes are a not so minor cause of sudden death [58–60]. The mechanism is believed to be paroxysmal atrial fibrillation, with one to one conduction, which may degenerate into ventricular fibrillation and cardiac arrest. In these conditions, atrial myocarditis may trigger the onset of life-threatening lone atrial fibrillation [58]. The accessory fascicle along the AV sulcus is always located closer to the endocardium than to the epicardium; size and site are such that "Kent's fascicle" is easily amenable to endocardial transcatheter ablation, which is the current procedure to interrupt the preexcitation and to reestablish the sole regular electrical connection through the His bundle.

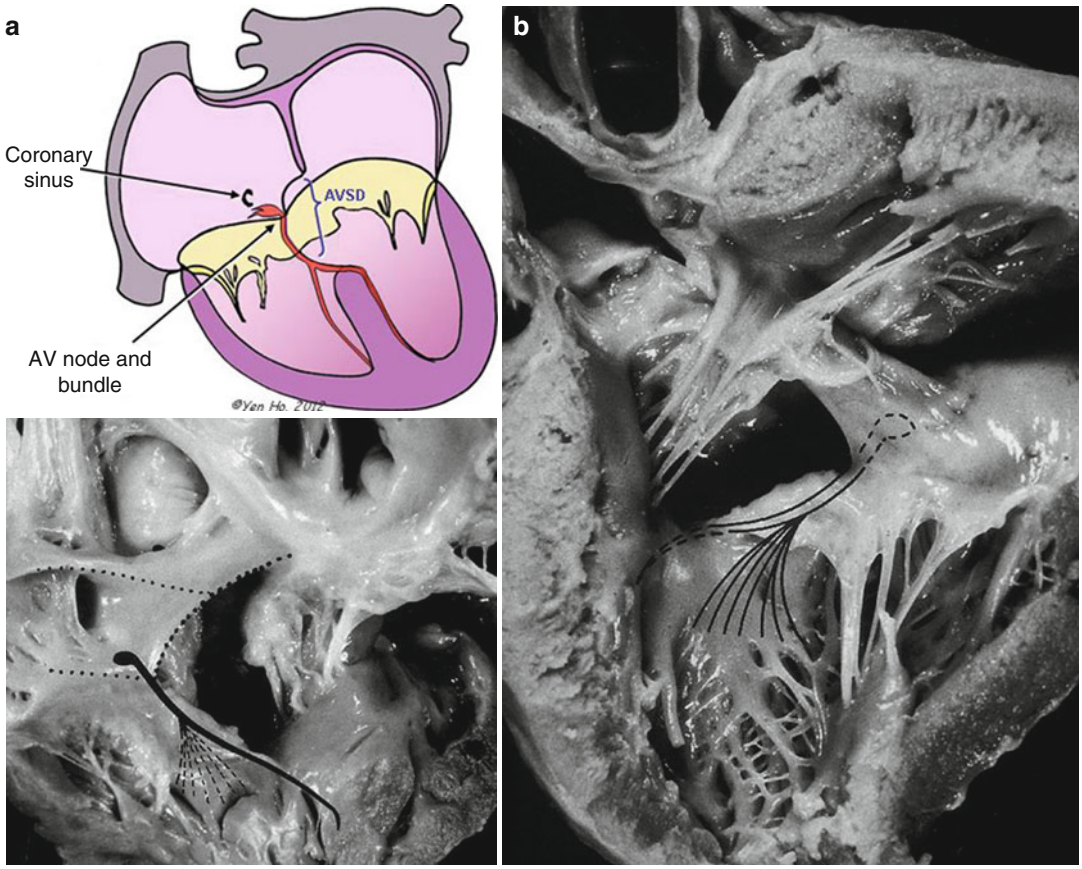


Figure 4–25. Conduction system in complete AV canal. (**a**) Diagram showing the course of the AV axis (*AVSD* AV septal defect). (**b**) Gross views of the anatomical specimen showing the location of the AV node and bundle in relationship to the AV septal defect, seen from the left side

Purkinje Cell Tumour (Also Called Histiocytoid Cardiomyopathy, Idiopathic Infantile Cardiomyopathy, Purkinjoma)

This tumour is a rare cause of severe and intractable tachyarrhythmias early in infancy as to be mostly diagnosed at post-mortem [61–63]. It is currently considered a hamartoma arising from the cardiomyocyte or the Purkinje cell, while others consider it as a peculiar type of mitochondrial cardiomyopathy. Macroscopically, these masses present as focal yellowish nodules or areas of discoloration composed of vacuolated histiocyte-like cells within the myocardium. Their size varies from 1 mm to 1.5 cm in diameter. The most common locations are conduction system and the left ventricle, but they may be also found in the right atrium and ventricle, mostly in the subendocardial layer. Microscopically, these masses contain large oval cardiac myocytes with a coarse granular pale cytoplasm (Fig. 4.28). The cytoplasm is filled with bizarre looking mitochondria. The term oncocytic cardiomyopathy describes the process of the granules (mitochondria) replacing the working myofibrils.

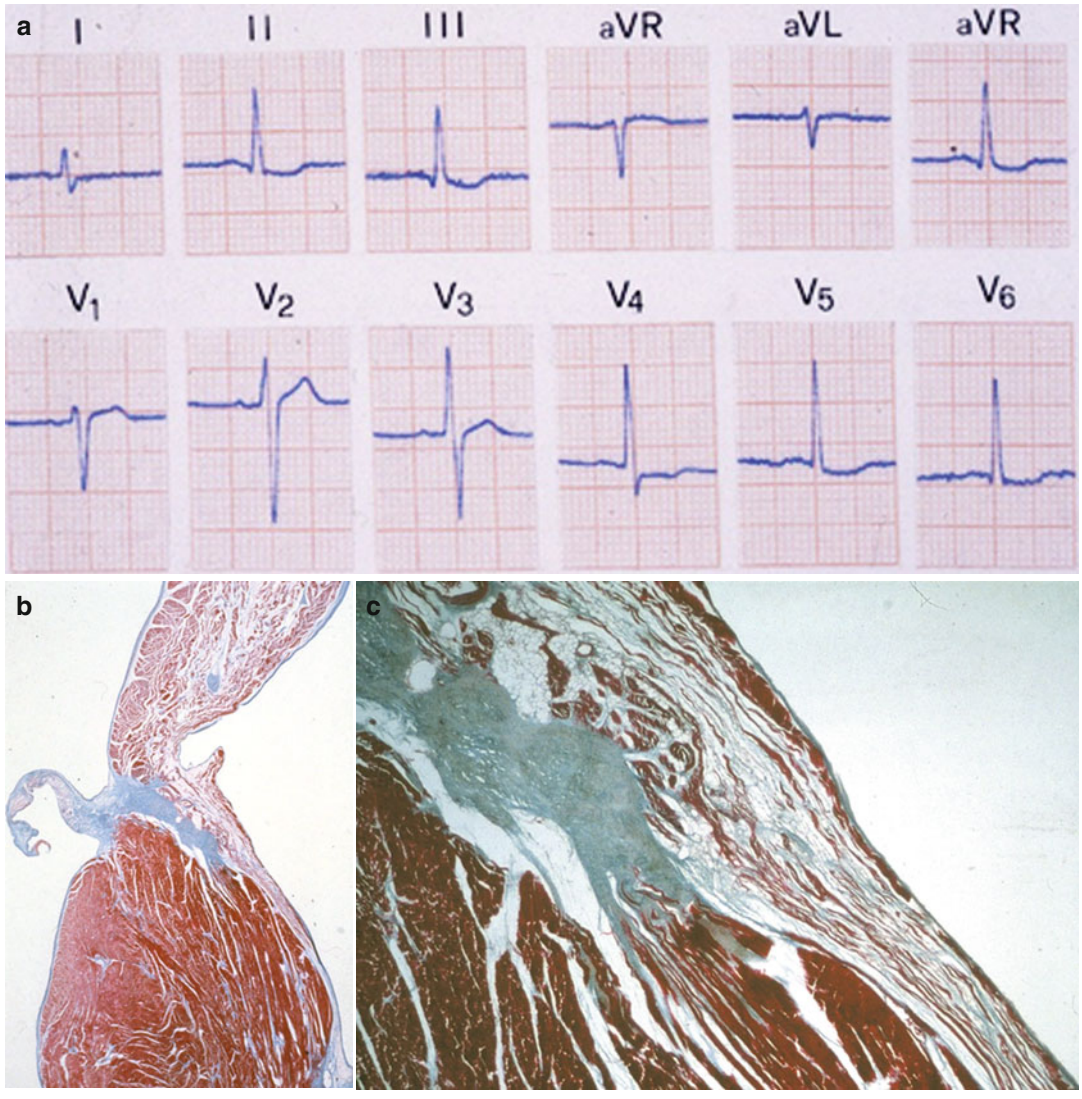


Figure 4–26. Lown-Ganong-Levine syndrome. (**a**) 12 lead ECG tracing with short PR interval and normal QRS complex; (**b**) extremely hypoplastic AV node (Heidenhain trichrome ×6); (**c**) close-up of B (Heidenhain trichrome ×15)

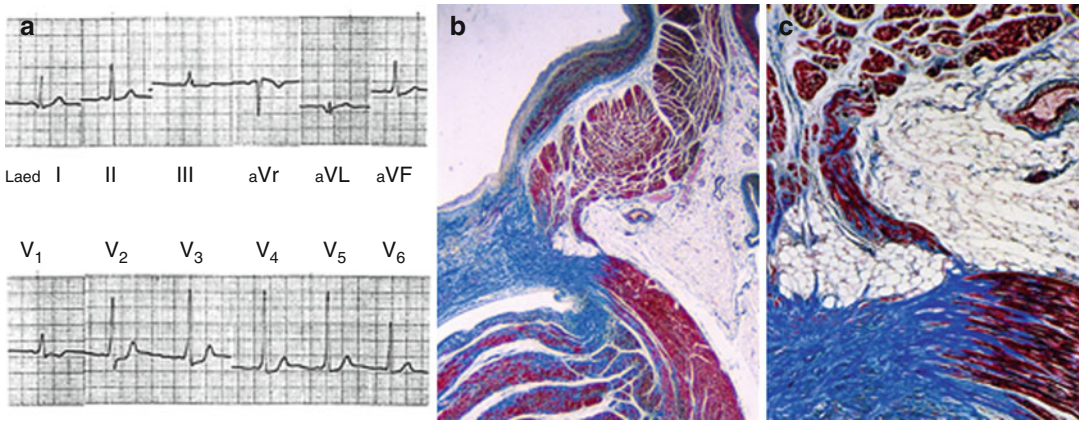


Figure 4–27. Wolff- Parkinson-White syndrome. (**a**) 12 lead ECG tracing with intermittent short PR interval and delta wave; (**b**) the Kent fascicle, located close to the endocardium, joins the working atrial and ventricular myocardium (Heidenhain trichrome ×6); (**c**) close-up of B, with mild fibrosis of the accessory bundle (Heidenhain trichrome ×18)

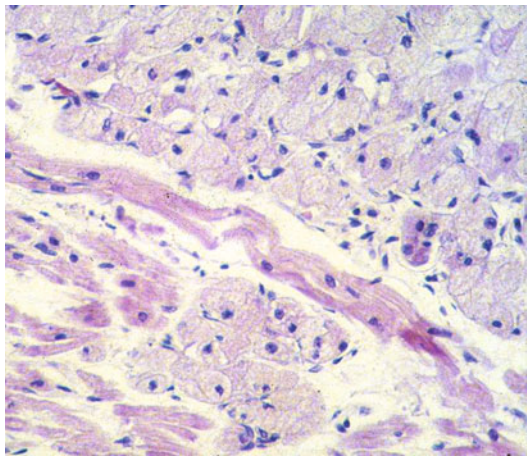


Figure 4–28. Purkinje cell tumor in a child who died suddenly. Histology shows focal islands of Purkinje-like cells, clearly distinct from the normal surrounding cardiomyocytes (Hematoxylin-Eosin stain)

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5
Neural Regulation of the Heart in Health and Disease

Richard L. Verrier and Alex Tan

Abstract

Understanding of the role of autonomic nervous system activity in health and disease has continued to evolve in a fascinating and productive manner. Significant strides have been made in recent years to elucidate the intricacies of neural control of heart rhythm and the mechanisms whereby excess sympathetic nerve activity can provoke life-threatening arrhythmias. Autonomic factors impact the genesis of both atrial and ventricular arrhythmias. Of particular significance is our increased recognition of the importance of the process of neural remodeling following myocardial infarction, which has provided important clues regarding the factors that impact on recovery of risk for sudden death. The role of behavioral influences including intense emotion and sleep states are also reviewed.

New clinical tools have been developed for evaluating neurocardiac interactions including heart rate turbulence, deceleration capacity, and T-wave alternans. These noninvasive ECG-based parameters have proved to be clinically useful in identifying individuals who are at risk for life-threatening arrhythmias.

Promising nerve stimulation strategies including vagus nerve activation and spinal cord stimulation have progressed from animal testing to clinical trials. Progress in both the experimental and clinical domains is reviewed.

Keywords

Autonomic nervous system • Sudden cardiac death • Myocardial and neural remodeling • T-wave alternans • Behavioral stress

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Introduction

Neural influences on heart rhythm are not only potent but also diverse. The complexity derives from integration at multiple levels in the brain, a network of intrinsic cardiac nerves, and autonomic reflexes, all of which interact with a cardiac substrate altered by advancing age and disease. In patients with ischemic heart disease, which is the major factor underlying risk for sudden cardiac death (SCD) [1], neural

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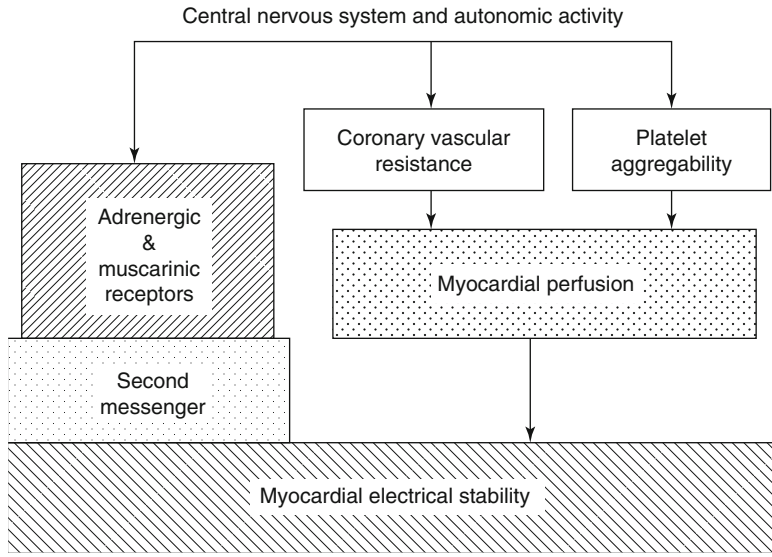

Figure 5–1. The interaction between neural triggers and cardiovascular substrate during autonomic activation [2]. Stimulation of beta$_1$-adrenergic receptors can decrease electrical stability directly as a result of changes in second messenger formation and alterations in ion fluxes. This deleterious influence is opposed by muscarinic receptor stimulation, which inhibits presynaptically the release of norepinephrine and opposes its action at the receptor level. Catecholamines may also alter myocardial perfusion by complex means, including alpha-receptor stimulation of coronary vessels and platelets and by impairing diastolic perfusion time due to adrenergically mediated sinus tachycardia (From Verrier [2]. Reprinted with permission from Springer Dordrecht)

influences can predispose to arrhythmias both directly through effects on excitable properties of the heart and its specialized conducting system and more indirectly by impairing myocardial perfusion through effects on coronary vascular function and platelet aggregability (Fig. 5.1) [2]. Neural influences may also be arrhythmogenic in patients with channelopathies including the long QT and Brugada syndromes.

Useful noninvasive tools have been developed to explore the influence of autonomic factors on arrhythmogenesis, including heart rate variability [3], baroreceptor sensitivity [3], heart rate turbulence [4], and deceleration capacity [5]. The direct effects of neural stimuli on the electrical instability of the myocardial substrate can be assessed by monitoring repolarization indices, notably T-wave alternans, measured by either the Spectral or Modified Moving Average (MMA) Methods [6].

Integration of Neural Control of Cardiac Electrical Activity

Regulation of cardiac neural activity is highly integrated and is achieved by circuitry at multiple levels (Fig. 5.2) [7]. Higher brain centers operate through elaborate pathways within the hypothalamus and medullary cardiovascular regulatory sites. Baroreceptor mechanisms have long been recognized as integral to autonomic control of the cardiovascular system, as evidenced by heart rate variability and baroreceptor sensitivity testing of both cardiac patients and normal subjects. The intrinsic cardiac nerves and fat pads provide local neural coordination independent of extrinsic cardiac nerves and higher brain centers. Newly recognized is the phenomenon of electrical remodeling attributable to nerve growth and degeneration. At the level of the myocardial cell, autonomic receptors

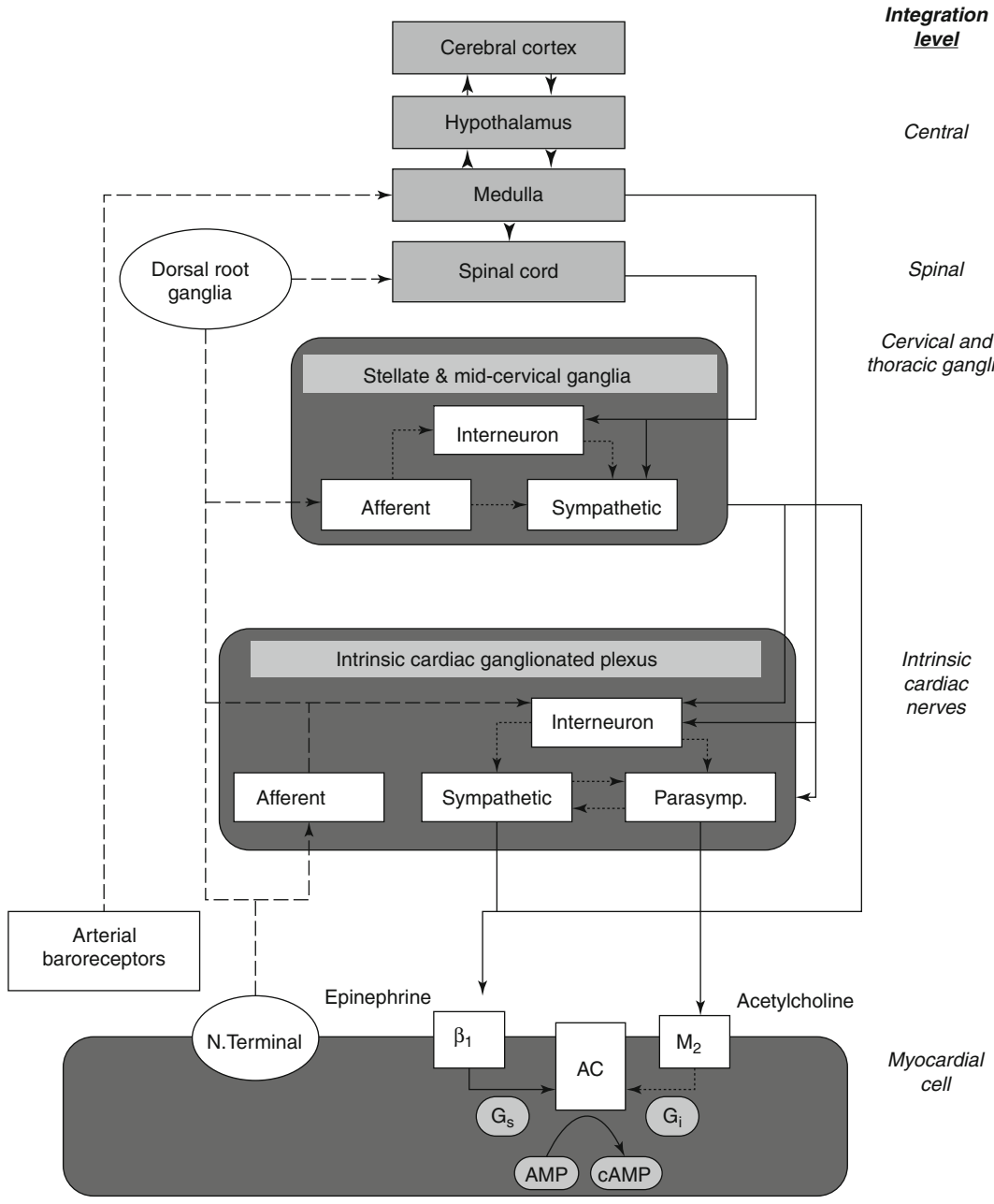

Figure 5–2. Synthesis of new and present views on levels of integration important in neural control of cardiac electrical activity [7]. More traditional concepts focused on afferent tracts (*dashed lines*) arising from myocardial nerve terminals and reflex receptors (e.g., baroreceptors) that are integrated centrally within hypothalamic and medullary cardiostimulatory and cardioinhibitory brain centers and on central modulation of sympathetic and parasympathetic outflow (*solid lines*) with little intermediary processing at the level of the spinal cord and within cervical and thoracic ganglia. More recent views incorporate additional levels of intricate processing within the extraspinal cervical and thoracic ganglia and within the cardiac ganglionic plexus, where recently described interneurons are envisioned to provide new levels of noncentral integration. Release of neurotransmitters from postganglionic sympathetic neurons is believed to enhance excitation in the sinoatrial node and myocardial cells through norepinephrine binding to beta$_1$-receptors, which enhances adenyl cyclase (*AC*) activity through intermediary stimulatory G-proteins (G_s). Increased parasympathectomy outflow enhances postganglionic release and binding of acetylcholine to muscarinic (M_2) receptors, and through coupled inhibitory G-proteins (G_i), inhibits cyclic AMP production (*cAMP*). The latter alters electrogenesis and pacemaking activity by affecting the activity of specific membrane Na, K, and Ca channels. New levels of integration are shown superimposed on previous views and are emphasized here to highlight new possibilities for intervention (From Lathrop and Spooner [7]. Reprinted with permission from John Wiley and Sons)

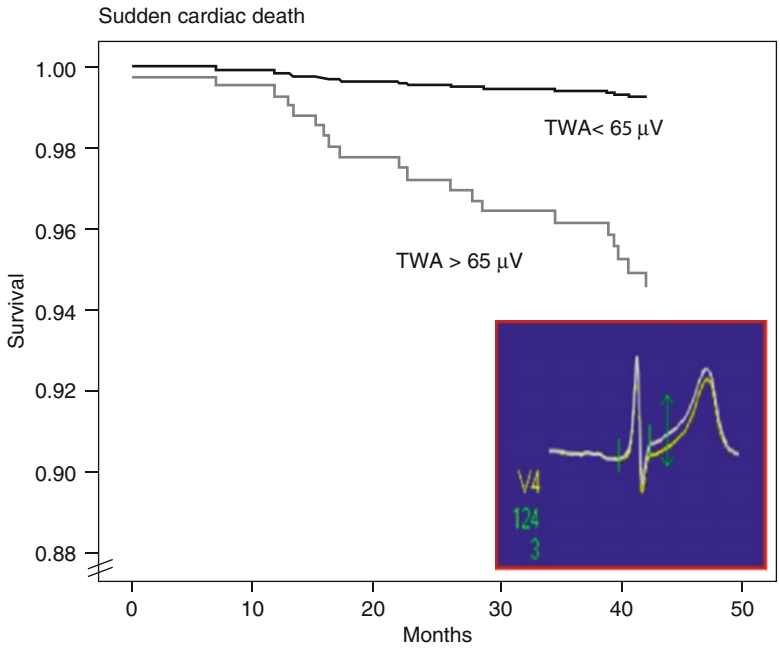

Figure 5–3. Survival curves from the Finnish Cardiovascular Study (FINCAVAS), which enrolled >1,000 consecutive patients referred for routine exercise testing [12]. *Inset* is a high-resolution QRS-aligned template from a FINCAVAS patient illustrating T-wave alternans (*TWA*) as the separation between successive beats as measured by the Modified Moving Average method [13] (From Nieminen et al. [12]. Reprinted with permission from Oxford University Press. Inset from Minkkinen et al. [13]. Reprinted with permission from John Wiley and Sons)

influence G proteins to control ionic channels, pumps, and exchangers. Finally, studies of behavioral state provide evidence that markers of arrhythmia vulnerability can be monitored noninvasively in combination with autonomic parameters during emotional and physical stressors and sleep states to identify individuals at heightened risk of lethal cardiac arrhythmias.

T-Wave Alternans as a Tool for Noninvasive Assessment of Neurocardiac Interactions

TWA, defined as a repeating ABAB pattern in the morphology and amplitude of the ST-segment or T wave, has been widely used in experimental and clinical studies to assess neural influences on susceptibility to cardiac arrhythmias. Furthermore, this parameter has proved to be useful in stratification of arrhythmia risk in prospective clinical studies enrolling >12,000 patients, as recently reviewed in a clinical consensus guidelines statement [6]. The mechanistic basis for TWA's predictivity has been extensively discussed [8–10]. TWA appears to reflect spatiotemporal heterogeneity of repolarization, is sensitive to perturbations in intracellular calcium handling, and serves as a mechanism of arrhythmogenesis by amplifying repolarization heterogeneity. Diverse physiologic factors influence TWA, including heart rate, neurotransmitters, myocardial ischemia, and heart failure. The changes in TWA magnitude correlate with the pro- and antiarrhythmic effects of diverse interventions including pharmacologic agents [11]. The clinical presentation of TWA and its relationship to sudden death risk is illustrated in Fig. 5.3 [12, 13].

Adrenergic Influences on Cardiac Vulnerability

It is well established that adrenergic inputs constitute the primary neural trigger for ventricular arrhythmias. Activation of the sympathetic nerve structures, including the posterior hypothalamus or stellate ganglia, increases susceptibility to ventricular fibrillation. Infusion of epinephrine or norepinephrine is also profibrillatory. Proof of the triggering role of sympathetic nerve discharge in ventricular arrhythmias was provided by Zhou et al., who performed direct recording of left stellate ganglion in ambulatory dogs with chronic myocardial infarction to demonstrate that ventricular

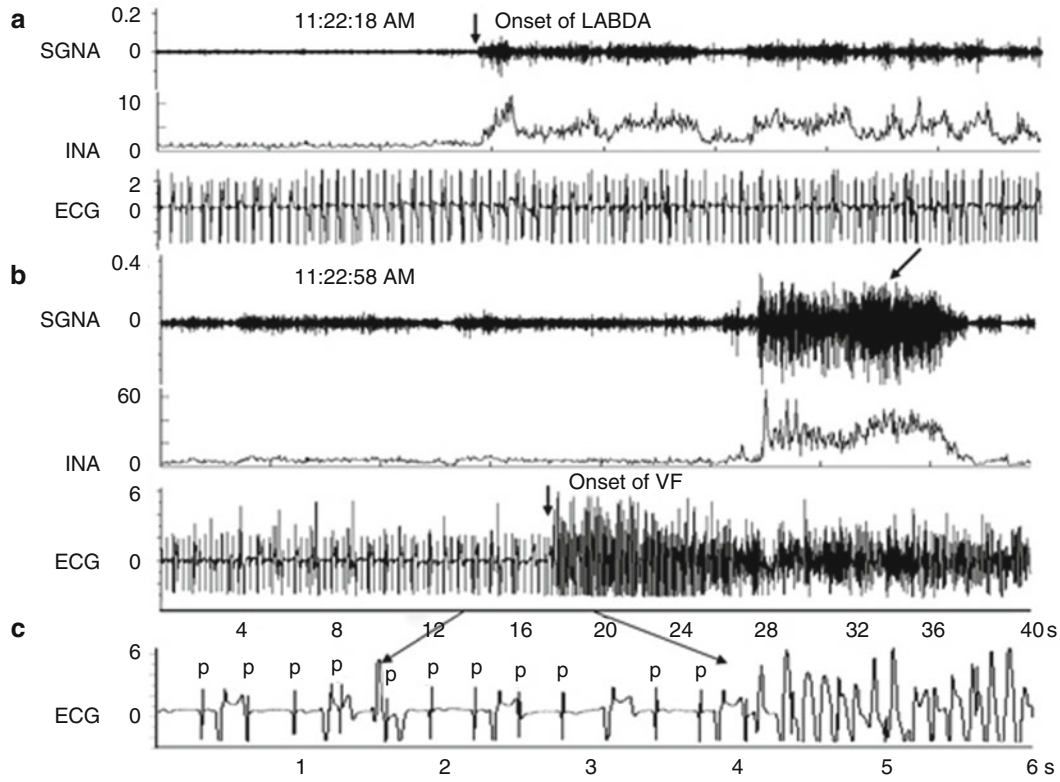

Figure 5–4. Example of increased left stellate ganglion nerve activity (*SGNA*) preceding ventricular fibrillation (*VF*) and sudden cardiac death [14]. (**a**) Increased low-amplitude burst discharge activity (*LABDA*) resulted in accelerated idioventricular rhythm. (**b**) VF occurred approximately 40 s later. *Panels* **a** and **b** are continuous. (**c**) A 6-s recording from *panel* **b**. *INA* integrated nerve activity, *P* P wave, which is dissociated from ventricular activation due to complete AV block. Units for integrated nerve activity are millivolts (From Zhou et al. [14]. Reprinted with permission from Elsevier)

tachycardia and sudden death are immediately preceded by spontaneous sympathetic nerve discharge (Fig. 5.4) [14]. Such striking surges in sympathetic nerve activity also occur within a few minutes of experimental left anterior descending (LAD) coronary artery occlusion and are associated with a marked increase in susceptibility to ventricular fibrillation, as evidenced by a fall in ventricular fibrillation threshold (Fig. 5.5) [15], as well as by spontaneous occurrence of the arrhythmia and correlated increase in TWA magnitude [16, 17]. Upon reperfusion, a second peak in ventricular arrhythmia vulnerability and TWA occurs, probably due to washout products of cellular ischemia [15–18]. Stellectomy significantly blunts the surge in vulnerability to ventricular fibrillation during occlusion but enhances its magnitude during reperfusion [16]. These findings are consistent with the facts that adrenergic factors play a key role during ischemia [19] and that stellectomy increases the reactive hyperemic response to release-reperfusion, which in turn probably leads to greater liberation of ischemic byproducts.

In the atrium, simultaneous sympathovagal activation facilitates the onset of paroxysmal atrial fibrillation [20, 21] by a mechanism termed "calcium transient triggering." Sympathetic nerve activation prolongs intracellular calcium transients, and vagus nerve activation shortens cardiac action potentials. The discrepancy between the normally tightly coupled action potential duration and calcium transients leads to increased forward Na/Ca exchanger current, which contributes to the generation of early afterdepolarizations towards the end of phase 3 of the cardiac action potential [22]. These triggers are particularly common in highly innervated pulmonary veins [23, 24].

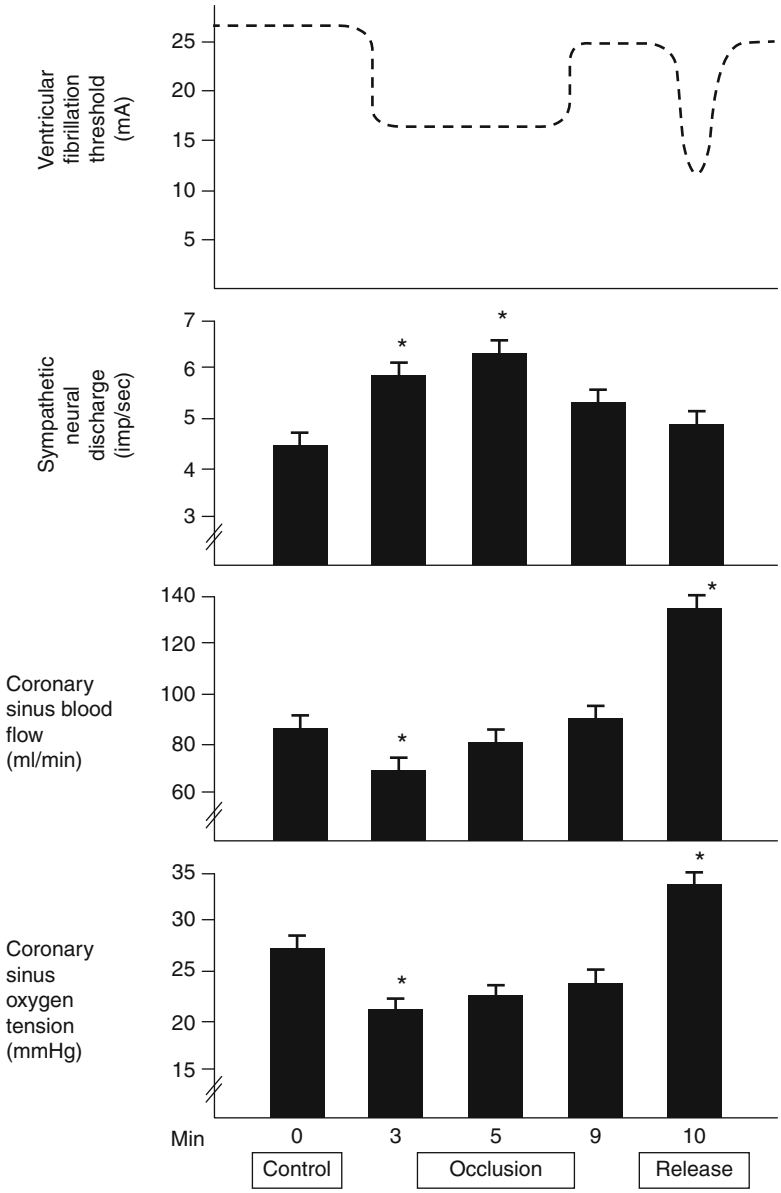

Figure 5–5. Effects of a 10-min period of left anterior descending (LAD) coronary artery occlusion and release on neural sympathetic activity, coronary sinus blood flow, and oxygen tension [15]. A schematic representation of the time course of changes in ventricular fibrillation threshold is also displayed. LAD coronary artery occlusion results in a consistent activation of sympathetic preganglionic fibers, which corresponds with the period of maximal increase in vulnerability to ventricular fibrillation (* = $p < 0.05$ compared to control period). The concomitant changes in coronary sinus blood flow and reperfusion are also displayed (From Lombardi et al. [15]. Reprinted with permission from Elsevier)

Mechanisms Responsible for Arrhythmogenesis During Beta-Adrenergic Receptor Activation

The mechanisms whereby enhanced sympathetic nerve activity increases cardiac vulnerability in the normal and ischemic heart are complex. The major indirect effects include impairment of oxygen supply–demand ratio due to increased cardiac metabolic activity, alpha-adrenergically mediated coronary vasoconstriction, especially in vessels with damaged endothelium, and changes in preload and afterload. The direct arrhythmogenic effects on cardiac electrophysiologic function, which are primarily mediated through $beta_1$-adrenergic receptors, are multifold. They include derangements in impulse formation, conduction, repolarization alternans, and heterogeneity of repolarization, with the potential for culmination in ventricular tachycardia and fibrillation (Fig. 5.6) [25, 26]. Increased levels of catecholamines stimulate

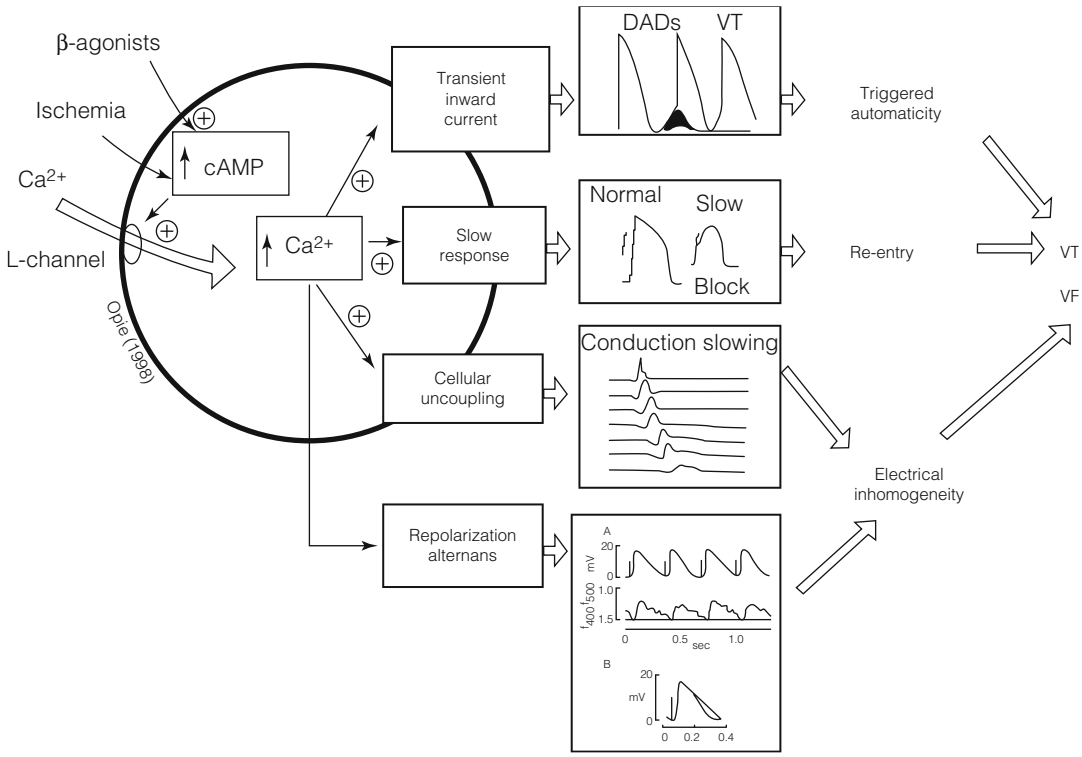

Figure 5–6. The cardiac beta-adrenergic signaling system mediating ventricular arrhythmogenesis. The central pathways include links between cyclic adenosine 3′,5′-monophosphate (*cAMP*), cytosolic calcium, and specific calcium-mediated electrophysiologic abnormalities that predispose to ventricular tachycardia (*VT*) and ventricular fibrillation (*VF*). The *lowest panel* is based on a study from Lee et al. [26], indicating that simulated ischemia results in alternation in calcium transients, which appears to underlie action potential alternans (Adapted from Opie [25] and used with his permission; Lee [26] figure reprinted with permission from Wolters Kluwer Health)

beta-adrenergic receptors, which in turn alter adenylate cyclase activity and intracellular calcium flux. These effects are probably mediated by the cyclic nucleotide and protein kinase regulatory cascade, which can alter spatial heterogeneity of calcium transients and consequently provoke TWA and dispersion of repolarization. The effect of increased intracellular calcium, with the potential for overload and impaired intracellular calcium cycling by the sarcoplasmic reticulum may be compounded and become especially arrhythmogenic during concurrent myocardial ischemia, which further predisposes to intracellular calcium excess [16, 26–28]. The net effect is an increase in vulnerability to ventricular fibrillation. The converse is also true: reduction of cardiac sympathetic neural drive by stellectomy provides an antifibrillatory influence in animals and humans.

Cardiac $beta_1$-adrenergic receptor blockade is capable of negating the profibrillatory effect of direct sympathetic nerve stimulation [29] by an action at the neurocardiac effector junction. But, cardiac $beta_2$-adrenergic receptors do not appear to play a significant role in modulating ventricular excitable properties. The role of cardiac $beta_3$-adrenergic receptors has been enigmatic. $Beta_3$ receptors are present in human cardiac myocytes in smaller quantities and are activated at higher concentrations of catecholamines than are $beta_1$ and $beta_2$ receptors. Zhou and coworkers [30] have provided evidence that during conditions of sympathetic hyperinnervation, there is a significant and dynamic response in $beta_3$-adrenoreceptor expression. They also found that $beta_3$ receptor stimulation in canines following myocardial infarction reduces the incidence of ventricular arrhythmias [31], suggesting that $beta_3$ receptors serve a rescue function in

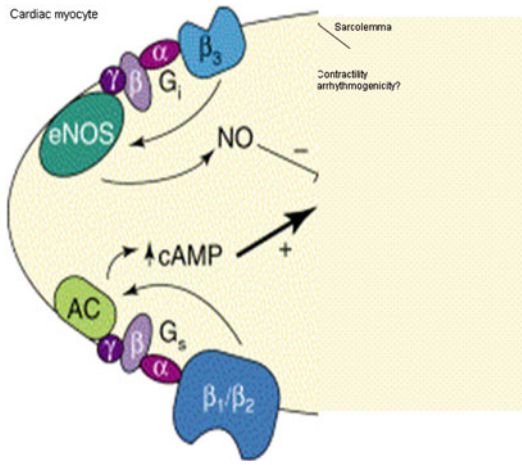

Figure 5–7. Countervailing mechanisms proposed by Gauthier and coworkers [32] to account for the interaction between beta$_1$- and beta$_2$-adrenoceptors and the beta$_3$-adrenoceptor subtype. In normal heart, beta$_1$- and beta$_2$-adrenoceptors mediate the classic positive inotropic effect of catecholamines via cAMP. In an opposing manner, stimulation of the beta$_3$-adrenoceptor exerts a negative inotropic response that occurs through activation of a constitutively expressed endothelial nitric oxide synthase (*eNOS*). They propose that the beta$_3$-adrenoceptor can provide a "rescue" function, which occurs particularly in disease conditions associated with hyperadrenergic activity, especially in heart failure. Little is known about the influence of these receptors on susceptibility to arrhythmias (Adapted from Gauthier et al. [32] by Verrier [33] and reprinted with permission from Elsevier)

response to catecholamine excess not only with respect to mechanical contractility (Fig. 5.7) [32, 33] but also to arrhythmia vulnerability. The mechanisms by which beta$_3$ receptor agonism are antiarrhythmic is the subject of further investigation.

Alpha-Adrenergic Receptors

Elucidation of the role of alpha-adrenergic receptors has been challenging because these agents exert direct actions not only on myocardial excitable properties but also on platelet aggregability and coronary hemodynamic function [34, 35]. In the normal heart, alpha-adrenergic receptor stimulation or blockade does not appear to affect ventricular electrical stability, as evidenced by the fact that administration of alpha-adrenergic agonists such as phenylephrine or methoxamine does not influence excitable properties when the pressor response is controlled to prevent reflex changes in autonomic tone [36, 37]. In the setting of myocardial ischemia, alpha-adrenergic blockade may alleviate coronary vasoconstriction and reduce platelet aggregability.

Sympathetic-Parasympathetic Interactions

Vagal influences are contingent on the prevailing level of adrenergic tone [38–42]. When sympathetic tone to the heart is augmented by thoracotomy [39], sympathetic nerve stimulation [39], myocardial ischemia, or catecholamine infusion [41], vagal activation exerts a protective effect on ventricular vulnerability. Vagus nerve stimulation alone is without effect on ventricular vulnerability when adrenergic input to the heart is ablated by beta-adrenergic blockade [39]. Levy and coworkers termed this phenomenon "accentuated antagonism." The basis for this antagonism of adrenergic effects is presynaptic inhibition of norepinephrine release from nerve endings [43] and a muscarinically mediated action at the second messenger level, attenuating the response to catecholamines at receptor sites. Also, importantly, vagal influences provide indirect protection against ventricular fibrillation by reducing excess heart rates [39], which can otherwise critically compromise diastolic perfusion time during acute myocardial ischemia to increase ischemic insult. However, the beneficial effects of vagus nerve activity may be annulled if profound bradycardia and hypotension ensue. Vagus nerve stimulation has been shown in experimental studies to protect against ventricular arrhythmias during myocardial ischemia but its protection during reperfusion is attributable to decreased heart rate [44]. Finally, myocardial infarction may damage nerve pathways, thereby limiting the potential of the vagus nerve to be activated. Vanoli and colleagues demonstrated the antifibrillatory effect of vagus nerve stimulation during exercise-induced ischemia in canines with a healed myocardial infarction [45]. Direct stimulation of the right cervical vagus through a chronically implanted electrode at 15 s after onset of

exercise-induced acute myocardial ischemia reduced the incidence of ventricular fibrillation by 92 %. This effect was only partly due to the attendant heart rate reduction, as in half of the animals, the efficacy of vagal stimulation persisted despite maintenance of constant heart rate by atrial pacing.

In the atrium, simultaneous activation of the sympathetic and parasympathetic nervous systems is thought to be highly conducive to atrial arrhythmias, due to synergistic effects on action potential characteristics, refractoriness, and propensity for triggered activity [20, 21]. This critical interaction between sympathovagal activation and cellular electrophysiology is most pronounced at sites of high concentrations of autonomic nerves at pulmonary vein-atrial junctions [24, 46]. In clinical studies using heart rate variability parameters, it was estimated that 10–25 % of atrial arrhythmias are facilitated by sympathovagal nerve influences, as atrial fibrillation was immediately preceded by a sharp increase in vagus nerve tone occurring on a background of increased sympathetic nerve tone [47]. Experimentally, *in vitro* infusion of acetylcholine in the sinus node artery invariably induced atrial fibrillation in dogs; acetylcholine-mediated atrial fibrillation was facilitated by the β(beta)-adrenoceptor agonist isoproterenol, which decreased the threshold concentration of acetylcholine required for arrhythmia induction and also prolonged episodes of atrial fibrillation [48].

Baroreflexes and Arrhythmias

The classic studies by Billman et al. [49] drew attention to the importance of baroreceptor function on susceptibility to life-threatening arrhythmias associated with myocardial ischemia and infarction. In their initial investigations in canines, they demonstrated that the more powerful was the baroreflex response, the less vulnerable animals were to ventricular fibrillation during myocardial ischemia superimposed on prior myocardial infarction. The protective effect of the baroreceptor mechanism has been linked primarily to the antifibrillatory influence of vagus nerve activity. The latter effect improves diastolic coronary perfusion, minimizing the ischemic insult from coronary artery occlusion. The importance of baroreceptor sensitivity (BRS) was subsequently documented in human subjects in whom baroreceptor function was evaluated with the pressor agent phenylephrine. LaRovere and colleagues [3] demonstrated that post-myocardial infarction patients were less likely to experience sudden cardiac death if their baroreceptor function was not depressed.

Experimental evidence indicates that exercise training improves depressed BRS in high-risk post-MI dogs and prevents VF during acute myocardial ischemia [50] and that it also provides antifibrillatory protection in high-risk dogs with a normal heart [51]. These findings paved the way for clinical studies to assess whether increasing vagal activity by exercise training is capable of significantly improving long-term prognosis. Ninety-five post-myocardial infarction patients, matched for all major variables, were randomized to a 4-week endurance-training period or to no training. During a 10-year follow-up, cardiac mortality among the trained patients who had an exercise-induced increase in baroreflex sensitivity >3 ms/mmHg was strikingly lower compared to that of the trained patients without such a baroreflex response and to that of the non-trained patients (Fig. 5.8) [52].

In the last few years, BRS testing has been pursued by noninvasive monitoring of heart rate turbulence (HRT) [4]. This phenomenon refers to fluctuations of sinus-rhythm cycle length after a single ventricular premature beat (VPB) and appears to be mechanistically linked with BRS [53]. The basic principle, introduced by Schmidt and coworkers, is that the reaction of the cardiovascular system to a VPB and the subsequent decrease in arterial blood pressure is a direct function of baroreceptor responsiveness, since reflex activation of the vagus nerve controls the pattern of sinus rhythm (Fig. 5.9) [54]. Several studies confirm that in low-risk patients, after a VPB, sinus rhythm exhibits a characteristic pattern of early acceleration and subsequent deceleration. By contrast, patients at high risk exhibit essentially a flat, nonvarying response to the VPB, indicating inability to activate vagal

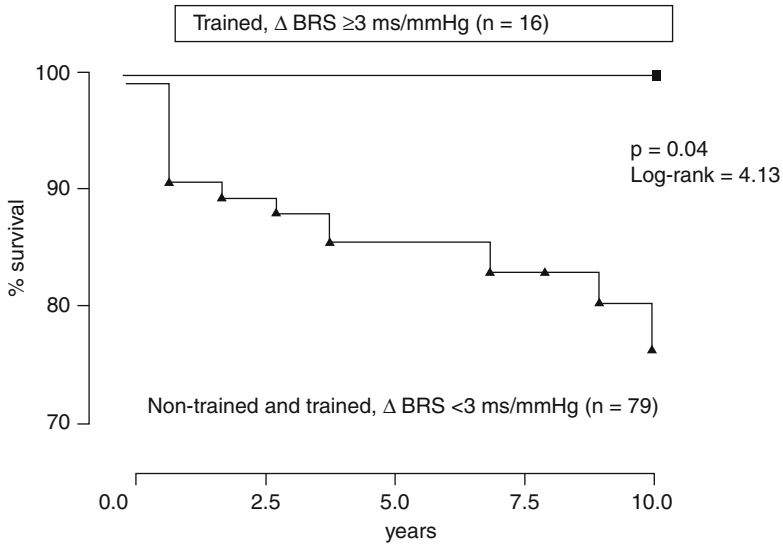

Figure 5–8. Cardiac mortality estimated by the Kaplan-Meier method among the patients with a training-induced increase in BRS ≥3 ms/mmHg and the group including patients who trained without the same BRS increase and non-trained patients [52] (From La Rovere et al. [52]. Reprinted with permission from Wolters Kluwer Health)

nerves and their cardioprotective effect. The method appears to be a promising independent predictor of total mortality in patients with ischemic heart disease, myocardial infarction, and/or heart failure [54–56]. Heart rate deceleration capacity, a related and even more comprehensive marker of autonomic control than HRT, may be of considerable clinical value in assessing overall autonomic regulation of the heart in patients with diverse types of cardiovascular disease [5].

Intrinsic Cardiac Innervation

In the late 1970s, Armour [57] and his colleagues introduced and investigated the elaborate intrinsic neural network within the heart, which provides local, independent heart rhythm control. Randall, Zipes, Chen and their respective coworkers [58–61] subsequently verified this important advance, drawing attention to the fact that components of this innervation system reside within discrete fat pads. Myocardial ischemia can compromise the functional capacity of cardiac intrinsic neurons residing in the fat pad and thus has the potential to increase electrical inhomogeneity and susceptibility to arrhythmias [62]. Intrinsic innervation is also vulnerable to diabetic neuropathy, which accordingly could exacerbate vulnerability to arrhythmias [63]. Surgical incisions through the atrial walls and radiofrequency ablation may isolate SA node pacemaker cells and damage the fat pads and result in proarrhythmia due to iatrogenically induced autonomic imbalance [64]. Heterogeneity of fibers within and without the fat pads contributes to dispersion of electrical activity, which in turn can predispose to arrhythmogenesis in adjacent atrial tissue [65]. Finally, another region that is richly innervated by the autonomic nervous system is the pulmonary vein-atrial junction, which contains a high density of co-localized sympathetic and parasympathetic ganglionated plexi from which atrial fibrillation may frequently arise [46].

Nerve Growth and Degeneration

Whereas the concept of remodeling has been well established with respect to the heart, the importance of restructuring of cardiac innervation has only recently received due attention, with fundamental contributions from the laboratories of Zipes [66, 67] and Chen [68–71]. In particular, Jayachandran et al. [67] demonstrated in a canine model of atrial fibrillation induced by rapid, prolonged pacing, that atrial electrical remodeling was associated with spatially heterogeneous uptake of the postganglionic sympathetic indicator hydroxyephedrine

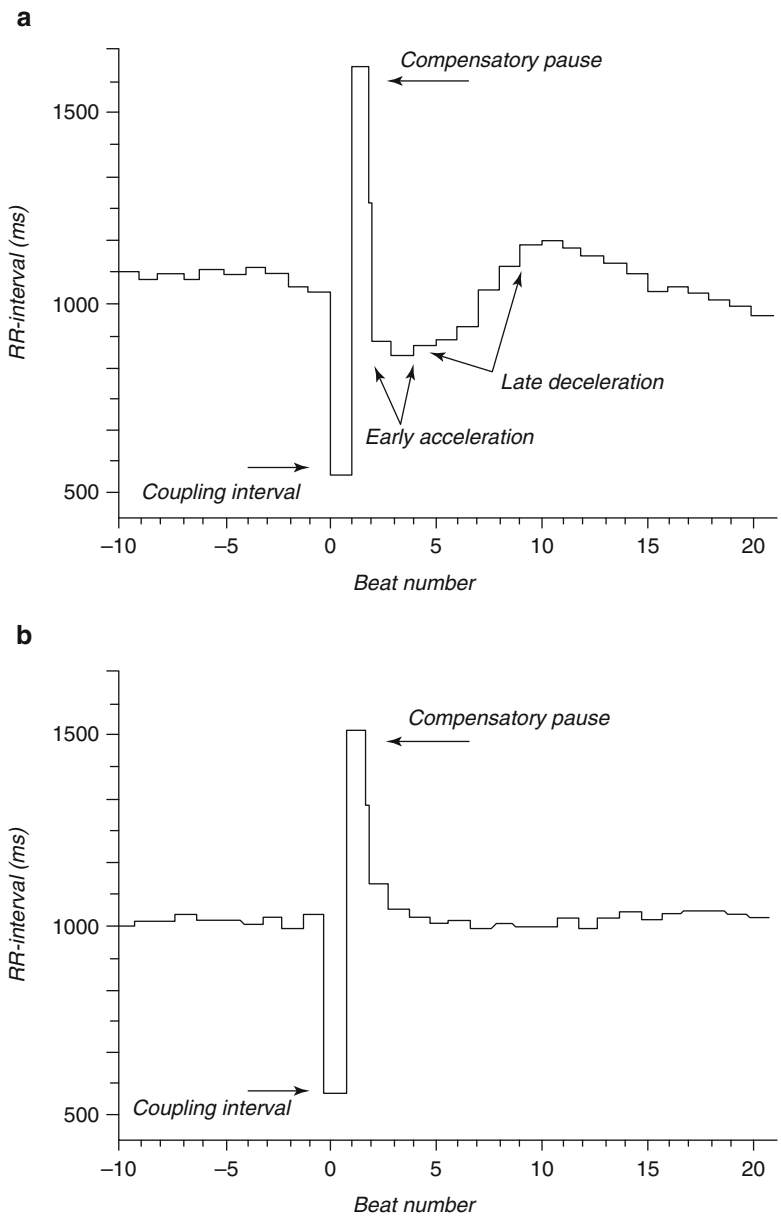

Figure 5–9. (**a**) Heart rate turbulence in a low-risk post-myocardial infarction (post-MI) patient. (**b**) Blunted heart-rate turbulence in a high-risk post-MI patient [54] (From Guzik and Schmidt [109]. Reprinted with permission from Springer)

into the nerve terminals within the sinus node, crista terminalis, and myocardium. Importantly, increased uptake was accompanied by electrical heterogeneity and augmented norepinephrine tissue levels. Studies by Chang et al. [69] and Olgin et al. [66] provided further evidence in favor of the concept of injury-induced neural repair with selective sympathetic remodeling and the attendant potential for induction and perpetuation of atrial arrhythmias.

Chen and coworkers [70] documented evidence that nerve sprouting could apply to ventricular as well as to atrial arrhythmogenesis and potentially to sudden cardiac death. These investigators demonstrated a significant correlation between increased sympathetic nerve density as reflected in immunocytochemical markers and history of ischemia in native hearts of human transplant recipients. In a canine model, they determined that induction of nerve sprouting

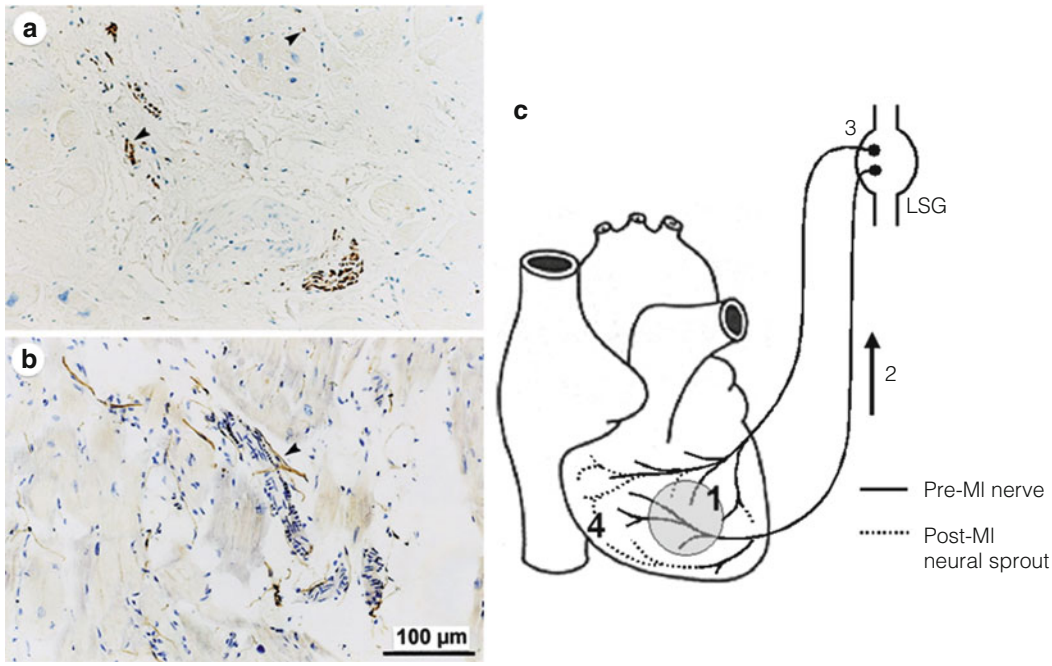

Figure 5–10. Nerve sprouting after myocardial infarction. *Panels* **a** and **b** demonstrate TH-positive nerve fibers (*arrowheads*) in injured areas or around coronary arteries and in a patient with coronary artery disease [68] (From Cao et al. [68]. Reprinted with permission from Wolters Kluwer Health). *Panel* **c** Signaling of neural remodeling after myocardial infarction [72]. Myocardial injury (*shaded area*) results in early local nerve growth factor (NGF) release, presumably from damaged cells, followed by upregulated NGF and growth-associated protein 43 (GAP43) expression, especially in the infarct area (*1*). These signal proteins are then retrogradely transported (*2*) to the nerve cell bodies in the ganglia (*3*) where they stimulate the sprouting of new cardiac nerve endings in the heart (*4*), predominantly in noninfarcted regions, leading to heterogeneous hyperinnervation (From Verrier and Kwaku [72]. Reprinted with permission from Wolters Kluwer Health)

with nerve growth factor resulted in increased incidence of ventricular tachycardias and sudden death, with concomitant TWA [71], consistent with this parameter's capacity to track arrhythmia vulnerability [6]. Significantly, the predisposition to arrhythmias was linked to immunocytochemical evidence of a heterogeneous pattern of sympathetic nerve reinnervation (Fig. 5.10) [72]. More recently, Liu and coworkers [73] demonstrated in rabbits that hypercholesterolemia can produce neural and electrophysiological remodeling that is highly arrhythmogenic and is associated with important changes in ionic currents including I_{Ca}. Collectively, this evidence points to the lability of autonomic innervation and the intricate changes that may be responsible for derangements in neural activity. This adverse effect of heterogeneous remodeling of sympathetic innervation to the heart is likely to play a role in the increased risk for life-threatening arrhythmias [72]. The term "neural remodeling" should be employed alongside "myocardial remodeling" in the conceptual framework of the pathophysiology of acute infarction.

Behavioral State

Stress and Arrhythmogenesis

Behavioral models have been developed to define the impact of behavioral state on cardiac electrical stability [28, 74–76]. These have included both aversive behavioral conditioning paradigms and models eliciting natural emotions, notably anger and fear. Aversive conditioning of dogs in a Pavlovian sling with mild chest shock on three consecutive days showed that subsequent exposure to the environment without shock elicited a reduction in the repetitive extrasystole threshold >30 % [74]. The

same paradigm elicited a threefold increase in the occurrence of spontaneous ventricular fibrillation when coronary artery occlusion was carried out in the aversive sling compared to the nonaversive cage environment. In dogs recovering from myocardial infarction, exposure to the aversive environment consistently elicited ventricular tachycardia for several days during the healing process [75]. After this time, the animals continued to exhibit signs of behavioral stress in the aversive environment but no longer experienced ventricular arrhythmias, indicating that the arousal state required a substrate of cardiac electrical instability for the induction of rhythm disturbances. The stress-induced changes in cardiac excitable properties were largely obtunded by beta-adrenergic receptor blockade with propranolol or metoprolol.

In a separate series of experiments, an experimental canine model was developed to emulate anger [76], which is the emotion most commonly associated with myocardial infarction and sudden death [77, 78]. A standardized food-access-denial paradigm provoked intense arousal, which elicited a sizeable increase in TWA in precordial V_5 ECG. A 3-min period of coronary artery occlusion potentiated arrhythmia risk, as it more than doubled the magnitude of anger-induced TWA [28]. The stress-related effects were significantly lessened by metoprolol, further implicating a major role of $beta_1$-adrenergic receptors in sympathetic nerve induction of cardiac vulnerability and TWA.

The view that behavioral factors may predispose to malignant arrhythmias has gained strong support in recent years because of batteries of psychometric tests for behavioral testing and indicators of cardiac electrical instability including defibrillator discharge frequency and TWA. In patients with implantable cardioverter-defibrillators (ICDs), Lampert and colleagues [79] systematically examined the linkage between emotional and physical stressors in provoking spontaneous ventricular arrhythmias. Subjects completed detailed diaries of mood states and physical activity during two periods preceding spontaneous, appropriate ICD shocks and during control periods 1 week later. A total of 107 documented ICD shocks were reported by 42 patients, the majority of whom had coronary artery disease. In the 15-min period preceding shocks, there was a significant incidence of high levels of anger, with odds ratios of 1.83. Other mood states, notably anxiety, worry, sadness, and happiness, did not trigger ICD discharge. Physical activity was also associated with increased incidence of shocks. Correlative findings were reported by Fries et al. [80], who found sevenfold increased risk of ICD shock with high levels of physical activity and ninefold increased risk of ICD shock with acute mental stress. These observations are consistent with a recent demonstration in ICD patients that mental stress as well as exercise is capable of significantly increasing TWA, independent of effects on heart rate (Fig. 5.11) [81]. By contrast, normal matched control subjects did not experience significant exercise- or mental stress-induced increases in TWA. Such surges in TWA presage the onset of VT/VF, as they indicate high levels of cardiac electrical instability [6, 82–85]. Given the important influence of daily activity in arrhythmia vulnerability, it is advantageous that TWA can be assessed using time-domain methods during ambulatory ECG monitoring. In fact, several studies have shown the capacity of ambulatory-ECG based TWA in predicting arrhythmia in post-MI patients [6, 86, 87], in individuals with preserved or depressed ejection fraction [6, 88, 89], as well as in patients with ischemic and nonischemic cardiomyopathy [90].

Sleep as an Autonomic Stress Test for the Heart

In healthy individuals, sleep is generally salutary and restorative. Ironically, during sleep in patients with respiratory or heart disease, the brain can precipitate breathing disorders, myocardial ischemia, arrhythmias, and even death. An estimated 250,000 nocturnal myocardial infarctions and 38,000 nocturnal sudden deaths occur annually in the U.S. population, as 20 % of myocardial infarctions and 15 % of sudden deaths occur during the period from midnight to 6:00 a.m. [91]. Thus, sleep is not an entirely protected state. The distribution of deaths and myocardial infarctions during nighttime is nonuniform, a pattern consistent with provocation by pathophysiologic triggers. Precise characterization of the precipitating factors for nocturnal

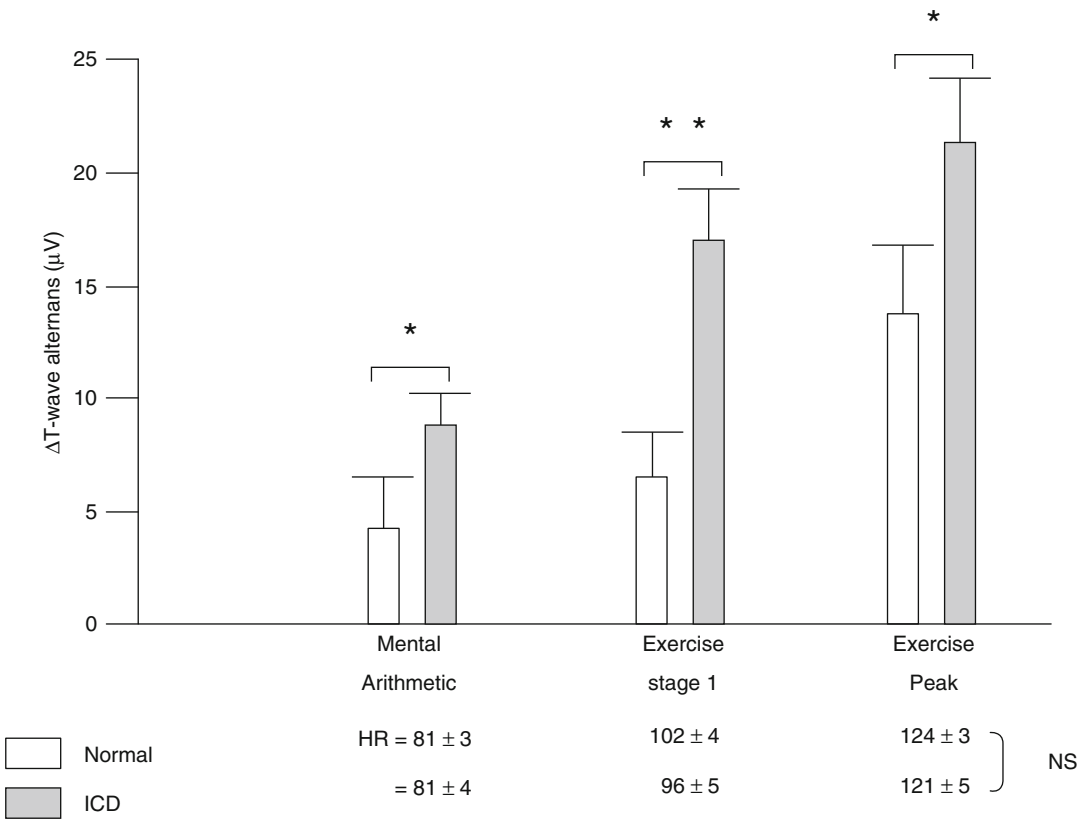

Figure 5–11. Comparison of ICD patients with controls in T-wave alternans (TWA) responses to mental stress and exercise (Δ = change from baseline) [81]. Although heart rates were not significantly different among the groups, increases in TWA were higher in ICD patients than in controls during mental arithmetic ($p = 0.043$), exercise stage 1 ($p = 0.0004$), and peak exercise ($p = 0.038$). *$p < 0.05$, **$p < 0.01$, ICD versus control (From Kop et al. [81]. Reprinted with permission from Wolters Kluwer Health)

cardiac events is incomplete. High-risk populations for nocturnal cardiorespiratory events include a number of sizeable patient groups (Table 5.1) [92].

The two main factors that have been implicated in nocturnal cardiac events are sleep-state dependent surges in autonomic nervous system activity and depression of respiratory control mechanisms, which impact on a vulnerable cardiac substrate. The brain, in subserving its needs for periodic reexcitation during rapid eye movement (REM) sleep and dreaming, imposes significant demands on the heart by inducing bursts in sympathetic nerve activity, which reaches levels higher than during wakefulness. In susceptible individuals, this degree of sympathetic nerve activity may compromise coronary artery blood flow, as metabolic demand outstrips supply, and may trigger sympathetically mediated life-threatening arrhythmias. Obstructive sleep apnea, which impairs ventilation during sleep and can generate reductions in arterial oxygen saturation, afflicts five to ten million Americans, or 2–4 % of the population [93]. This condition has been strongly implicated, when severe, in the etiology of hypertension, ischemia, arrhythmias, myocardial infarction, and sudden death in individuals with coexisting ischemic heart disease. Takasugi and colleagues [94] reported in patients with congestive heart failure that sleep apnea induces cardiac electrical instability manifested as TWA, indicating increased risk of nocturnal SCD and supporting the utility of nighttime ECG monitoring.

Autonomic or respiratory disturbances during sleep may trigger atrial fibrillation in certain patient populations. A challenge is also presented by NREM sleep, when malperfusion of the heart

Table 5–1. Patient groups at potentially increased risk for nocturnal cardiac events [92]

Condition (U.S. patients/year)	Possible mechanism
Angina, MI, arrhythmias, ischemia, or cardiac arrest at night [2 % of MIs (~250,000 cases/year) and 15 % of sudden deaths (~49,000 cases/year) occur between midnight and 6:00 a.m.]	The nocturnal pattern suggests a sleep state–dependent autonomic trigger or respiratory distress
Unstable angina	Nondemand ischemia and angina peak between midnight and 6:00 a.m.
Acute MI (1.5 million)	Disturbances in respiration and autonomic balance may be factors in nocturnal arrhythmogenesis. Nocturnal onset of MI is more frequent in older and sicker patients and carries a higher risk of congestive heart failure
Heart failure (5.3 million)	Sleep-related breathing disorders are pronounced in the setting of heart failure and may contribute to its progression and to mortality risk
Spousal or family report of highly irregular breathing, excessive snoring, or apnea in patients with coronary disease (15 million U.S. patients with apnea)	Patients with hypertension or atrial or ventricular arrhythmias should be screened for the presence of sleep apnea, which conduces to hypertension, ischemia, and arrhythmias, and is a risk factor for lethal daytime events, including myocardial infarction
Long QT3 syndrome, Brugada syndrome, Sudden Unexplained Nocturnal Death Syndrome (SUNDS)	The profound cycle-length changes associated with sleep may trigger pause-dependent torsades de pointes in these genetically related syndromes
Near-miss or siblings of victims of SIDS (2,500 total SIDS deaths annually in the U.S. or 1 death per 2,000 live births)	SIDS commonly occurs during sleep with characteristic cardiorespiratory symptoms
Atrial fibrillation (2.2 million) [29 % of episodes occur between midnight and 6:00 a.m.]	Respiratory and autonomic mechanisms are suspected

From Verrier and Josephson [92]. Reprinted with permission from Wolters Kluwer Health

and brain may result from hypotension and decreased blood flow in stenosed vessels. These conditions may be confounded by medications that cross the blood–brain barrier, alter sleep structure and/or provoke nightmares with severe cardiac autonomic discharge. Finally, an insidious component of the problem of nocturnal risk results from the fact that many individuals are unaware of their respiratory or cardiac distress at night and therefore take no corrective action. Thus, sleep presents unique autonomic, hemodynamic, and respiratory challenges to the diseased myocardium that cannot be monitored by daytime diagnostic tests. The importance of monitoring nocturnal arrhythmias extends beyond identifying sleep-state dependent triggers of cardiac events, as nighttime ischemia, arrhythmias, autonomic activity, and respiratory disturbances carry predictive value for daytime events [92].

Autonomic Modulation as an Antiarrhythmic Strategy

Autonomic denervation of intrinsic cardiac nerves around the base of the pulmonary veins has been proposed as an adjunctive procedure or even as a stand-alone treatment for patients with atrial fibrillation [95–99]. However, its effectiveness as an adjunctive therapeutic strategy to catheter ablation has been inconsistent in part because the interactions between the autonomic nervous system and atrial fibrillation are complex and still incompletely understood, with wide individual variability in the autonomic triggers [96]. Nevertheless, results of a small randomized, multicenter trial demonstrated that patients who underwent combined ganglionated plexi ablation and pulmonary vein isolation had fewer recurrences within 12 months after the procedure than did patients who underwent pulmonary vein isolation alone [99]. In selected populations, especially those with identifiable autonomic triggers [96], these adjunctive procedures might add value to standard catheter ablation techniques.

Ablation of extrinsic cardiac nerves such as the left stellate ganglion is known to reduce the incidence of ventricular arrhythmias in patients with long QT syndrome [100, 101], but less is known of its clinical effects on atrial tachyarrhythmias. In a canine model, Tan et al. [21] demonstrated that ablation of the stellate ganglion and cardiac branches of the left vagal nerve eliminated all episodes of paroxysmal atrial tachyarrhythmias.

Recently, novel methods of atrial autonomic denervation have been developed to suppress vagally induced atrial fibrillation. Oh et al. injected botulinum toxin into epicardial fat pads, resulting in short-term autonomic denervation and suppression of vagally induced atrial fibrillation [102]. Similarly, Stavrakis and colleagues reported suppression of atrial fibrillation by injection into the epicardial fat pads of vasostatin-1, a cardioregulatory peptide with strong antiadrenergic effects that are mediated via the release of nitric oxide [103]. Yu and coworkers demonstrated that magnetically guided particles containing a neurotoxin can be used to inhibit ganglionic tissue selectively without affecting atrial tissue [104]. These techniques potentially offer less invasive strategies than radiofrequency ablation for elimination of autonomic nerves.

Finally, direct stimulation of nerve structures has been reported to reduce ventricular tachyarrhythmias. Spinal cord stimulation reduces ischemia-induced arrhythmias in canines [105] and TWA in patients with ischemic cardiomyopathy [106]. Chronic vagus nerve stimulation prevented ventricular fibrillation and sudden cardiac death in conscious dogs with a healed myocardial infarction [45]. In humans, chronic vagus nerve stimulation improves left ventricular function in patients with advanced heart failure [107], raising the possibility of concurrent improvement in mechanical function and reduction in arrhythmia risk.

Neural stimulation has more recently been shown to be antiarrhythmic at the atrial level. Shen and coworkers [108] used direct nerve recordings to demonstrate that continuous low-level vagus nerve stimulation suppressed paroxysmal atrial tachyarrhythmias in ambulatory, conscious dogs through the reduction of stellate ganglion nerve activity.

Conclusion

Our understanding of the role of the autonomic nervous system has continued to evolve in a fascinating and productive manner. A number of direct benefits have been achieved in terms of understanding of the function of the autonomic nervous system in health and disease. Quantification of autonomic tone through heart rate variability and baroreceptor function testing by heart rate turbulence analysis shows considerable promise in terms of sudden death risk stratification, underscoring the crucial role of autonomic reflexes in maintaining cardiac health. Exercise-induced changes in autonomic function also appear capable of disclosing latent cardiac electrical instability, as evidenced by heightened levels of TWA, which is predictive of susceptibility to sudden cardiac death and cardiovascular mortality [6].

From the basic science perspective, there appears to be great promise in understanding the organization and function of the intrinsic nervous system and the dynamic nature of nerve sprouting and neural remodeling. The pattern of local neurocircuitry is likely to play a critical role in influencing heterogeneity of repolarization, a fundamental factor in arrhythmogenesis. A number of promising therapeutic approaches based on pharmacologic and electrical targeted neuromodulation to decrease cardiac sympathetic while augmenting vagus nerve tone are being pursued.

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6
Mechanisms of Cardiac Arrhythmia

Charles Antzelevitch and Alexander Burashnikov

Abstract

A cardiac arrhythmia simply defined is a variation from the normal heart rate and/or rhythm that is not physiologically justified. Cardiac arrhythmias are associated with increased morbidity and mortality. Treatment is often based on the underlying cause. Recent years have witnessed important advances in our understanding of the electrophysiologic mechanisms underlying the development of a wide variety of cardiac arrhythmias. Progress relative to our mechanistic understanding of these phenomena has been fueled by advances in our understanding of the genetic basis and genetic predisposition to electrical dysfunction of the heart. The mechanisms responsible for cardiac arrhythmias are generally divided into two broad categories: (1) enhanced or abnormal impulse formation (i.e., focal activity) and (2) conduction disturbances (i.e., reentry). Often these mechanisms act in concert to generate arrhythmias. An understanding of these arrhythmogenic mechanisms is important to providing patient- and mechanism-specific therapy. This chapter provides the fundamentals of our current understanding of the mechanisms underlying the development of cardiac arrhythmias.

Keywords

Sudden cardiac arrest • Electrophysiology • Tachycardia • Fibrillation • Reentry • Triggered activity • Automaticity • Conduction disturbance

Introduction

A cardiac arrhythmia simply defined is a variation from the normal heart rate and/or rhythm that is not physiologically justified. Recent years have witnessed important advances in our understanding of the electrophysiologic mechanisms underlying the development of a variety of cardiac arrhythmias. The mechanisms responsible for cardiac arrhythmias are generally divided into two major categories: (1) enhanced or abnormal impulse formation (i.e., focal activity) and (2) conduction disturbances (i.e., reentry) (Fig. 6.1).

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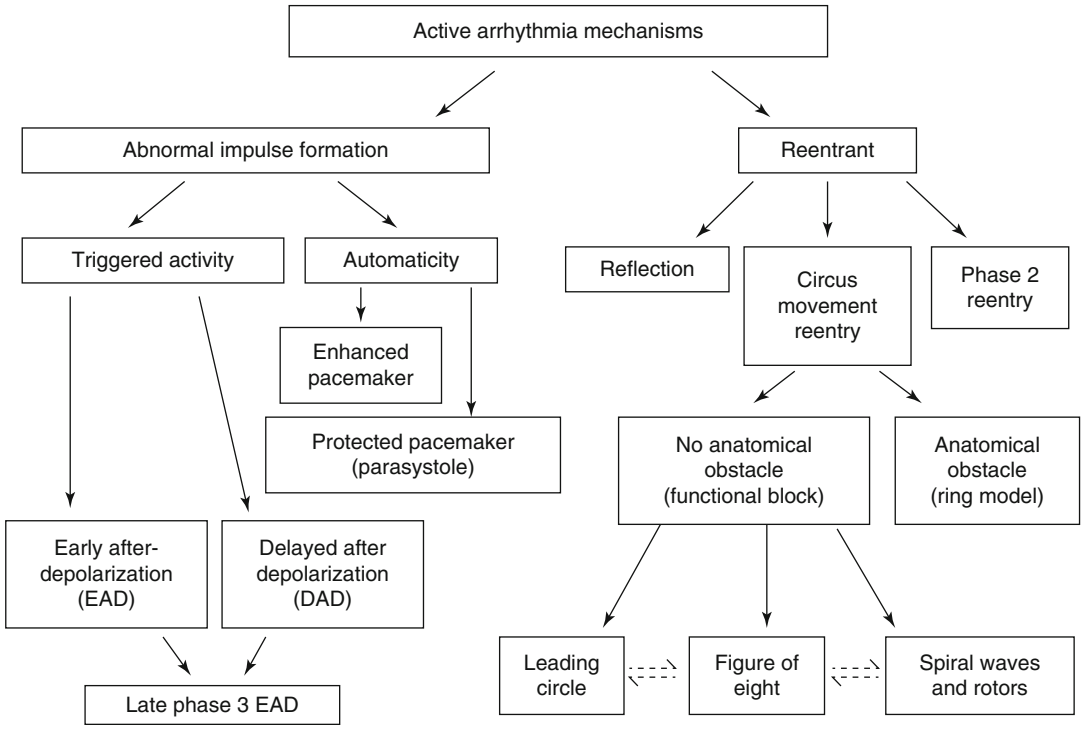

Figure 6–1. Classification of active cardiac arrhythmias

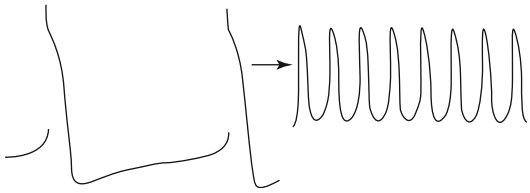

Figure 6–2. Transition of normal to abnormal automaticity (depolarization-induced low voltage activity) in a Purkinje fiber

Abnormal Impulse Formation

Normal Automaticity

Automaticity is the property of cardiac cells to generate spontaneous action potentials. Spontaneous activity is the result of diastolic depolarization caused by a net inward current during phase 4 of the action potential, which progressively brings the membrane potential to threshold (Fig. 6.2). The sino-atrial (SA) node normally displays the highest intrinsic rate. All other pacemakers are referred to as subsidiary or latent pacemakers because they take over the function of initiating excitation of the heart only when the SA node is unable to generate impulses or when these impulses fail to propagate.

The Voltage and Calcium Clocks

Lakatta, Maltsev and their collaborators [1, 2] used the terms sarcolemma voltage clocks and the subsarcolemmal Ca clocks to describe the mechanisms of SA node automaticity. The voltage clock is formed by voltage-sensitive membrane currents, such as the hyperpolarization-activated pacemaker current (I_f) [3]. This current is also referred to as a "funny" current because, unlike the majority of voltage-sensitive currents, it is activated by hyperpolarization (from −40/−50 to −100/−110 mV) rather than depolarization. At the end of the action potential, the I_f is activated and depolarizes the sarcolemmal membrane [3]. I_f is a mixed Na-K inward current modulated by the autonomic nervous system through cAMP. The depolarization activates $I_{Ca,L}$ which provides Ca to activate

the cardiac ryanodine receptor (RyR2). The activation of RyR2 initiate sarcoplasmic reticulum (SR) Ca release (Ca induced Ca release), leading to contraction of the heart, a process known as EC coupling. The intracellular Ca (Ca_i) is then pumped back into SR by the SR Ca-ATPase (SERCA2a) and complete this Ca cycle. In addition to I_f, multiple time- and voltage-dependent ionic currents have been identified in cardiac pacemaker cells, which contribute to diastolic depolarization. These currents include (but not limited to) $I_{Ca\text{-}L}$, $I_{Ca\text{-}T}$, I_{ST}, and various types of delayed rectifier K currents [2]. Many of these membrane currents are known to respond to β(beta)-adrenergic stimulation. All these membrane ionic currents contribute to the regulation of SA node automaticity by changing the membrane potential.

Another important ionic current that can depolarize the cell is the sodium-calcium exchanger current (I_{NCX}). In its forward mode, I_{NCX} exchanges three extracellular Na^+ with one intracellular Ca^{2+}, resulting in a net intracellular charge gain. This electrogenic current is active during late phase 3 and phase 4 because the Ca_i decline outlasts the SA node action potential duration. Recent studies showed that I_{NCX} may participate in normal pacemaker activity [4]. The sequence of events includes spontaneous rhythmic SR Ca release, Ca_i elevation, the activation of I_{NCX} and membrane depolarization. This process is highly regulated by the cAMP and the autonomic nervous system [2]. These studies suggest that sympathetic stimulation accelerates heart rate by phosphorylation of proteins that regulate Ca_i balance and spontaneous SR Ca cycling. These proteins include phospholamban (PLB, a SR membrane protein regulator of SERCA2a), L-type Ca channels and RyR2. Phosphorylation of these proteins controls the phase and size of subsarcolemmal SR Ca releases. The resulting I_{NCX} contributes to both basal and reserve cardiac pacemaker function.

Subsidiary Pacemakers

In addition to SA node, the atrioventricular (AV) node and Purkinje system are also capable of generating automatic activity. The contribution of I_f and I_K differs in SA node/AV nodes and Purkinje fiber because of the different potential ranges of these two pacemaker types (i.e., −70 to −35 mV and −90 to −65 mV, respectively). The contribution of other voltage-dependent currents can also differ among the different cardiac cell types. Whether or not Ca clock plays a role in pacemaking of AV node and Purkinje cells remain unclear.

Cells in the SA node possess the fastest intrinsic rates. Thus the SA node is the primary pacemaker in the normal heart. When impulse generation or conduction within or out of the SA node is impaired, latent or subsidiary pacemakers within the atria or ventricles take control of pacing the heart. The intrinsically slower rates of these latent pacemakers generally result in bradycardia. Both atrial and AV junctional subsidiary pacemakers are under autonomic control, with the sympathetic system increasing and parasympathetic system slowing the pacing rate. Although acetylcholine produces little in the way of a direct effect, it can significantly reduce Purkinje automaticity by means of the inhibition of the sympathetic influence, a phenomenon termed *accentuated antagonism* [5]. Simultaneous recording of cardiac sympathetic and parasympathetic activity in ambulatory dogs confirmed that sympathetic activation followed by vagal activation may be associated with significant bradycardia [6, 7].

Automaticity as a Mechanism of Cardiac Arrhythmias

Abnormal automaticity includes both reduced automaticity, which causes bradycardia, and increased automaticity, which causes tachycardia. Arrhythmias caused by abnormal automaticity can result from diverse mechanisms (see Figs. 6.1 and 6.2). Alterations in sinus rate can be accompanied by shifts of the origin of the dominant pacemaker within the sinus node or to subsidiary pacemaker sites elsewhere in the atria. Impulse conduction out of the SA mode can be impaired or blocked as a result of disease or increased vagal activity leading to development of bradycardia. AV junctional rhythms occur

when AV junctional pacemakers located either in the AV node or in the His bundle accelerate to exceed the rate of SA node, or when the SA nodal activation rate was too slow to suppress the AV junctional pacemaker.

Hereditary Bradycardia

Bradycardia can occur in structurally normal hearts due to genetic mutations that result in abnormalities of either membrane clock or Ca clock mechanisms of automaticity. One example is the mutation of hyperpolarization-activated nucleotide-gated channel (HCN4), which is part of the channels that carry I_f. Mutations of the HCN4 may cause familial bradycardia as well [8, 9].

Secondary SA Node Dysfunction

Common diseases, such as heart failure and atrial fibrillation, may be associated with significant SA node dysfunction. Malfunction of both membrane voltage clocks and Ca clocks might be present in both of these common diseases. Zicha et al. [10] reported downregulation of HCN4 expression contributes to heart failure-induced sinus node dysfunction. An A450V missense loss of function mutation in *HCN4* has recently been shown to underlie familial sinus bradycardia in several unrelated proband of Moroccan Jewish descent [9, 11–13].

Enhanced Automaticity

Atrial and ventricular myocardial cells do not display spontaneous diastolic depolarization or automaticity under normal conditions, but can develop these characteristics when depolarized, resulting in the development of repetitive impulse initiation, a phenomenon termed *depolarization-induced automaticity* (see Fig. 6.2) [14]. The membrane potential at which abnormal automaticity develops ranges between −70 and −30 mV. The rate of abnormal automaticity is substantially higher than that of normal automaticity and is a sensitive function of resting membrane potential (i.e., the more depolarized resting potential the faster the rate). Similar to normal automaticity, abnormal automaticity is enhanced by β(beta)-adrenergic agonists and by reduction of external potassium.

Depolarization of membrane potential associated with disease states is most commonly a result of either: (1) an increase in extracellular potassium, which reduces the reversal potential for I_{K1}, the outward current that largely determines the resting membrane or maximum diastolic potential; (2) a reduced number of I_{K1} channels; (3) a reduced ability of the I_{K1} channel to conduct potassium ions; or (4) electrotonic influence of neighboring cells in the depolarized zone. Because the conductance of I_{K1} channels is a sensitive to extracellular potassium concentration, hypokalemia can lead to major reduction in I_{K1}, leading to depolarization and the development of enhanced or abnormal automaticity, particularly in Purkinje pacemakers. A reduction in I_{K1} can also occur secondary to a mutation in *KCNJ2*, the gene that encodes for this channel, leading to increased automaticity and extrasystolic activity presumably arising from the Purkinje system [15, 16]. Loss of function KCNJ2 mutation gives rise to Andersen-Tawil syndrome, which is characterized among other things by a marked increase in extrasystolic activity [17–19].

Overdrive Suppression of Automaticity

The automaticity of most pacemakers within the heart is inhibited when they are overdrive paced [20]. This inhibition is called *overdrive suppression*. Under normal condition all subsidiary pacemakers are overdrive-suppressed by SA nodal activity. A possible mechanism of overdrive suppression is intracellular accumulation of Na leading to enhanced activity of the sodium pump (sodium-potassium adenosine triphosphatase [Na^+-K^+ ATPase]), which generates a hyperpolarizing electrogenic current that opposes phase 4 depolarization [21]. The faster the overdrive rate or the longer the duration of overdrive, the greater the enhancement of sodium pump activity, so that the period of quiescence after cessation of overdrive is directly related to the rate and duration of overdrive.

Parasystole and Modulated Parasystole

Latent pacemakers throughout the heart are generally reset by the propagating wavefront initiated by the dominant pacemaker. An exception to this rule occurs when the pacemaking tissue is protected from the impulse of sinus nodal origin. A region of entrance block arises when cells exhibiting automaticity are surrounded by ischemic, infarcted, or otherwise compromised cardiac tissues that prevent the propagating wave from invading the focus, but which permit the spontaneous beat generated within the automatic focus to exit and activate the rest of the myocardium. A pacemaker region exhibiting entrance block, and exit conduction is referred to as a parasystolic focus. The ectopic activity generated by a parasystolic focus is characterized by premature ventricular complexes with variable coupling intervals, fusion beats and inter-ectopic intervals that are multiples of a common denominator. This rhythm is relatively rare and is usually considered benign, although a premature ventricular activation of parasystolic origin can induce malignant ventricular rhythms in the ischemic myocardium or in the presence of a suitable myocardial substrate.

Modulated parasystole, a variant of classical parasystole, was described by Moe and co-workers [22, 23]. This variant of the arrhythmia results from incomplete entrance block of the parasystolic focus. Electrotonic influences arriving early in the pacemaker cycle delayed and those arriving late in the cycle accelerated the firing of the parasystolic pacemaker, so that ventricular activity could entrain the partially protected pacemaker. As a consequence, at select heart rate, extrasystolic activity generated by the entrained parasystolic pacemaker can mimic reentry, generating extrasystolic activity with fixed coupling [22–26].

Afterdepolarization and Triggered Activity

Oscillatory depolarizations that attend or follow the cardiac action potential and depend on preceding transmembrane activity for their manifestation are referred to as afterdepolarizations (Figs. 6.3, 6.4, and 6.5). Two subclasses traditionally recognized: (1) early, and (2) delayed. Early afterdepolarization (EADs) interrupt or retard repolarization during

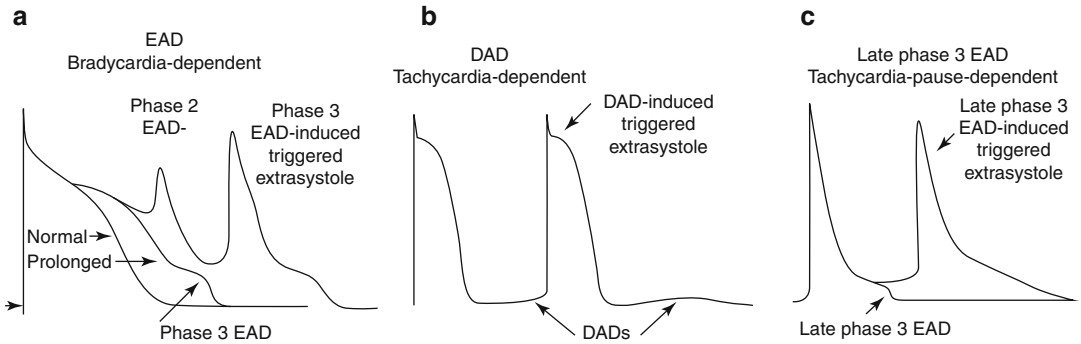

Figure 6–3. Early (EAD) and delayed (DAD) afterdepolarizations and EAD- and DAD-induced triggered action potentials (AP). (**a**) Phase 2 EAD and phase 3 EAD-induced APs in canine isolated Purkinje fiber preparation treated with d-sotalol (I_{Kr} block). The conditional phase of EAD is defined as the time interval spanning from the moment when membrane potential starts to deviate from normal coarse to the moment that immediately precedes the EAD upstroke or down-stroke. (**b**) DAD- and DAD-induced triggered activity in canine ventricular preparation induced by rapid pacing in the presence of isoproterenol (β-adrenergic agonist, augmenting intracellular calcium activity). (**c**) Late phase 3 EAD-induced triggered beat in canine right atrium in the condition of abbreviated repolarization (in the presence of acetylcholine, a parasympathetic agonist). Shown are the first sinus beat and following late phase 3 EAD-inducing triggered beat after a period of rapid activation (*Panels* **a** and **b** reproduced from [27] with permission from John Wiley and Sons, and *panel* **c** from [28] with permission from Wolters Kluwer Health)

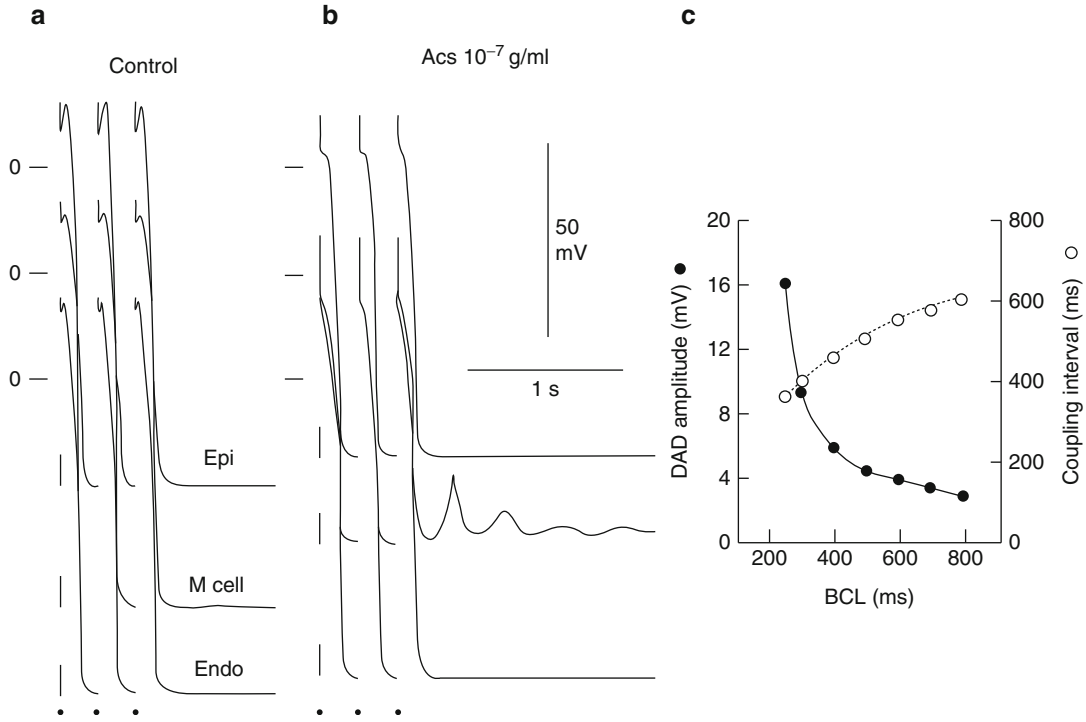

Figure 6–4. Digitalis-induced delayed afterdepolarizations in M cells but not epicardium or endocardium. Effects of acetylstrophanthidin (*AcS*) on transmembrane activity of an epicardial (*Epi*), endocardial (*Endo*) and M cell preparation. $[K^+]_o = 4$ mM. (**a**) Control. (**b**) Recorded after 90 min of exposure to 10^{-7} g/ml AcS. Each panel shows the last three beats of a train of ten basic beats elicited at a basic cycle length (*BCL*) of 250 ms. Each train is followed by a 3 s pause. AcS induced prominent delayed afterdepolarizations (*DADs*) in the M cell preparation but not in epicardium or endocardium. (**c**) Rate-dependence of coupling interval and amplitude of the AcS-induced DADs. Measured is the first DAD recorded from the M cell (From [29] with permission from John Wiley and Sons)

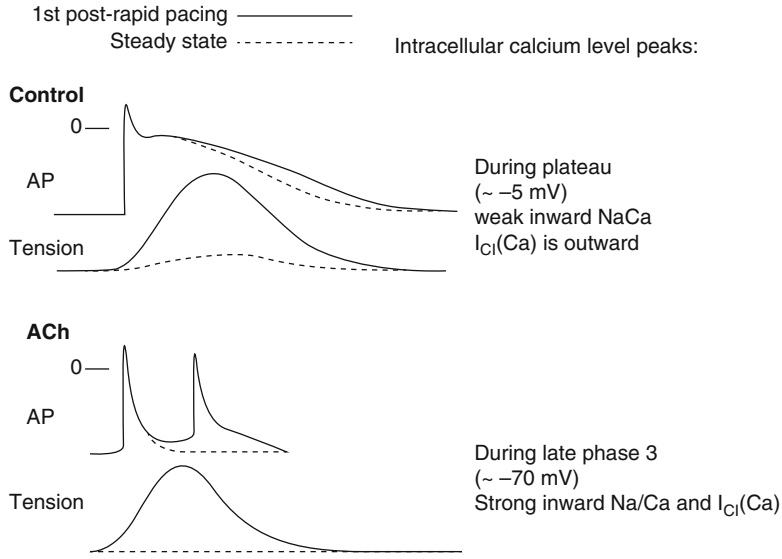

Figure 6–5. Proposed mechanism for the development of late phase 3 EADs. Shown are superimposed action potential (*AP*) and phasic tension recordings obtained under steady state conditions and during the first regular post-rapid pacing beat in control and in the presence of acetylcholine. See text for further discussion (Reproduced from [28] with permission from Wolters Kluwer Health)

phase 2 and/or phase 3 of the cardiac action potential, whereas delayed afterdepolarization (DADs) occur after full repolarization. When EAD or DAD amplitude suffices to bring the membrane to its threshold potential, a spontaneous action potential referred to as a triggered response is the result (see Figs. 6.3, 6.4, and 6.5). These triggered events giver rise to extrasystoles, which can precipitate rapid cardiac arrhythmias.

Early Afterdepolarizations and Triggered Activity

EADs are observed in isolated cardiac tissues exposed to injury, altered electrolytes, hypoxia, acidosis, catecholamines, and pharmacologic agents, including antiarrhythmic drugs. Ventricular hypertrophy and heart failure also predispose to the development of EADs [30]. EAD characteristics vary as a function of animal species, tissue or cell type, and the method by which the EAD is elicited. Although specific mechanisms of EAD induction can differ, a critical prolongation of repolarization accompanies most, but not all, EADs. Drugs that inhibit potassium currents or which augment inward currents predispose to the development of EADs [31]. Phase 2 and phase 3 EADs sometimes appear in the same preparation.

EAD-induced triggered activity is sensitive to stimulation rate. Antiarrhythmic drugs with class III action generally induce EAD activity at slow stimulation rates [14]. In contrast, β(beta)-adrenergic agonist–induced EADs are fast rate-dependent [32]. In the presence of rapidly activating delayed rectifier current (rapid outward potassium current [I_{Kr}]) blockers, β(beta)-adrenergic agonists, and/or acceleration from an initially slow rate transiently facilitate the induction of EAD activity in ventricular M cells, but not in epicardium or endocardium and rarely in Purkinje fibers [33].

Cellular Origin of Early Afterdepolarizations

EADs develop more commonly in midmyocardial M cells and Purkinje fibers than in epicardial or endocardial cells when exposed to APD-prolonging agents. This is due to the presence of a weaker I_{Ks} and stronger late I_{Na} in M cells [34, 35]. Block of I_{Ks} with chromanol 293B permits the induction of EADs in canine epicardial and endocardial tissues in response to I_{Kr} blockers such as E-4031 or sotalol [36]. The predisposition of cardiac cells to the development of EADs depends principally on the reduced availability of I_{Kr} and I_{Ks} as occurs in many forms of cardiomyopathy. Under these conditions, EADs can appear in any part of the ventricular myocardium [37].

Ionic Mechanisms Responsible for the EAD

EADs develop when the balance of current active during phase 2 or 3 of the action potential shifts in the inward direction. If the change in current–voltage relation results in a region of net inward current during the plateau range of membrane potentials, it leads to a depolarization or EAD. Most pharmacological interventions or pathophysiological conditions associated with EADs can be categorized as acting predominantly through one of four different mechanisms: (1) A reduction of repolarizing potassium currents (I_{Kr}, class IA and III antiarrhythmic agents; I_{Ks}, chromanol 293B or I_{K1}); (2) an increase in the availability of calcium current (Bay K 8644, catecholamines); (3) an increase in the sodium-calcium exchange current (I_{NCX}) caused by augmentation of Ca_i activity or upregulation of the I_{NCX}; and (4) an increase in late sodium current (late I_{Na}) (aconitine, anthopleurin-A, and ATX-II). Combinations of these interventions (i.e., calcium loading and I_{Kr} reduction) or pathophysiological states can act synergistically to facilitate the development of EADs.

Delayed Afterdepolarization-Induced Triggered Activity

DADs and DAD-induced triggered activity are observed under conditions that increase intracellular calcium, $[Ca^{2+}]_i$, such as after exposure to toxic levels of cardiac glycosides (digitalis) [38–40] or catecholamines [32, 41, 42]. This activity is also manifest in hypertrophied and failing

hearts [43, 44] as well as in Purkinje fibers surviving myocardial infarction [45]. In contrast to EADs, DADs are always induced at relatively rapid rates. Recent studies have shown that ouabain-induced activation of calcium calmodulim-dependent protein kinase II (CaMKII) gives rise to an augmentation of late I_{Na}, thus contributing to the development of calcium overload and digitalis toxicity [46]. Ranolazine, a late I_{Na} blocker, prevented the ouabain-induced Ca overload and toxic manifestations. Yao and coworkers further showed that $Na_v1.5$-dependent increase in sodium influx leads to activation of CaMKII, which in turn phosphorylates $Na_v1.5$, further promoting sodium influx and that inhibition of either CaMKII or $Na_v1.5$ can ameliorate cardiac dysfunction associated with excessive sodium influx [47].

Role of Delayed Afterdepolarization-Induced Triggered Activity in the Development of Cardiac Arrhythmias

An example of DAD-induced arrhythmia is the catecholaminergic polymorphic ventricular tachycardia (CPVT), which may be caused by the mutation of either the type 2 ryanodine receptor (RyR2) or the calsequestrin (CSQ2) [48]. The principal mechanism underlying these arrhythmias is the "leaky" ryanodine receptor, which is aggravated during catecholamine stimulation. A typical clinical phenotype of CPVT is bidirectional ventricular tachycardia, which is also seen in digitalis toxicity. Wehrens et al. [49] demonstrated that heterozygous mutation of FKBP12.6 leads to leaky RyR2 and exercise-induced VT and VF, simulating the human CPVT phenotype. RyR2 stabilization with a derivative of 1,4-benzothiazepine (JTV519) increased the affinity of calstabin2 for RyR2, which stabilized the closed state of RyR2 and prevented the Ca leak that triggers arrhythmias. Other studies indicate that delayed afterdepolarization-induced extrasystoles serve to trigger catecholamine-induced VT/VF, but that the epicardial origin of these ectopic beats increases transmural dispersion of repolarization, thus providing the substrate for the development of reentrant tachyarrhythmias, which underlie the rapid polymorphic VT/VF [50]. Heart failure is associated with structural and electrophysiological remodeling, leading to tissue heterogeneity that enhances arrhythmogenesis and the propensity of sudden cardiac death [51].

Late Phase 3 Early Afterdepolarizations and Their Role in the Initiation of Fibrillation

In 2003, Burashnikov and Antzelevitch described a novel mechanism giving rise to triggered activity, termed "late phase 3 EAD", which combines properties of both EAD and DAD, but has its own unique character (see Figs. 6.3 and 6.5) [27, 28]. Late phase 3 EAD-induced triggered extrasystoles represent a new concept of arrhythmogenesis in which abbreviated repolarization permits "normal SR calcium release" to induce an EAD-mediated closely coupled triggered response, particularly under conditions permitting intracellular calcium loading [27, 28]. These EADs are distinguished by the fact that they interrupt the final phase of repolarization of the action potential (late phase 3). In contrast to previously described DAD or Ca_i-depended EAD, it is *normal,* not spontaneous SR calcium release that is responsible for the generation of the EAD. Two principal conditions are required for the appearance of late phase 3 EAD: an APD abbreviation and a strong SR calcium release [28]. Such conditions may occur when both parasympathetic and sympathetic influences are combined. Simultaneous sympathovagal activation is also known to be the primary trigger of paroxysmal atrial tachycardia and AF episodes in dogs with intermittent rapid pacing [6].

Late phase 3 EAD-induced extrasystoles have been shown to initiate AF in canine atria, particularly following spontaneous termination of the arrhythmia (IRAF, immediate reinduction of AF) [28]. The appearance of late phase 3 EAD immediately following termination of AF or rapid pacing has been reported by in the canine atria *in vivo*[52] and pulmonary veins in vitro [53]. The pattern of initiation of some forms of paroxysmal AF in humans (i.e., tachycardia- or pause- dependent AF initiation) is consistent with behavior of late phase 3 EAD [54]. In addition to the atrial arrhythmias, late phase 3 EAD may also be

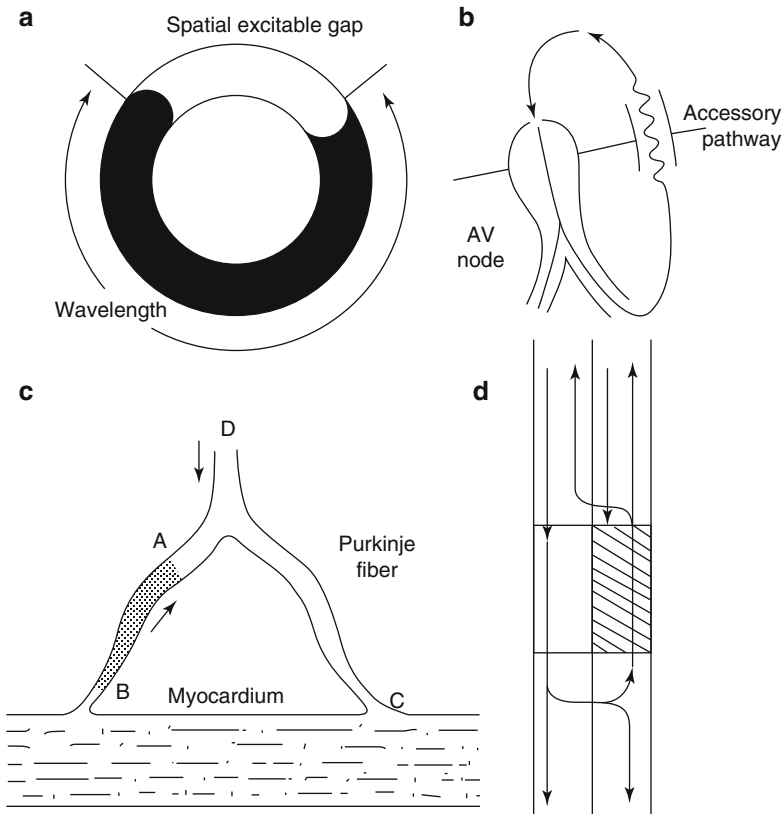

Figure 6–6. Ring models of reentry. (**a**) Schematic of a ring model of reentry. (**b**) Mechanism of reentry in the Wolf-Parkinson-White syndrome involving the AV node and an atrioventricular accessory pathway (AP). (**c**) A mechanism for reentry in a Purkinje-muscle loop proposed by Schmitt and Erlanger. The diagram shows a Purkinje bundle (*D*) that divides into two branches, both connected distally to ventricular muscle. Circus movement was considered possible if the stippled segment, *A B*, showed unidirectional block. An impulse advancing from *D* would be blocked at *A*, but would reach and stimulate the ventricular muscle at *C* by way of the other terminal branch. The wavefront would then reenter the Purkinje system at *B* traversing the depressed region slowly so as to arrive at *A* following expiration of refractoriness. (**d**) Schematic representation of circus movement reentry in a linear bundle of tissue as proposed by Schmitt and Erlanger. The upper pathway contains a depressed zone (*shaded*) serves as a site of unidirectional block and slow conduction. Anterograde conduction of the impulse is blocked in the upper pathway but succeeds along the lower pathway. Once beyond the zone of depression, the impulse crosses over through lateral connections and reenters through the upper pathway (*Panels* **c** and **d** are from Schmitt and Erlanger [56], reprinted with permission from Springer Science + Business Media)

responsible for the development recurrent VF in failing hearts [55].

Reentrant Arrhythmias

Reentry is fundamentally different from automaticity or triggered activity in the mechanism by which it initiates and sustains cardiac arrhythmias. Circus movement reentry occurs when an activation wavefront propagates around an anatomical or functional obstacle or core, and reexcites the site of origin (Fig. 6.6). In this type of reentry, all cells take turns in recovering from excitation so that they are ready to be excited again when the next wavefront arrives. In contrast, reflection and phase 2 reentry occur in a setting in which large differences of recovery from refractoriness exists between one site and another. The site with delayed recovery serves as a virtual electrode that excites its already recovered neighbor, resulting in a reentrant re-excitation. In addition, reentry can also be classified as anatomical and functional,

although there is a grey zone in which both functional and anatomical factors are important in determining the characteristics of reentrant excitation.

Circus Movement Reentry Around an Anatomical Obstacle

The ring model is the prototypical example of reentry around an anatomical obstacle (see Fig. 6.6). It first emerged as a concept shortly after the turn of the last century when Mayer reported the results of experiments involving the subumbrella tissue of a jellyfish (*Sychomedusa cassiopeia*) [57]. The muscular disk did not contract until ringlike cuts were made and pressure and a stimulus applied. This caused the disc to "spring into rapid rhythmical pulsation so regular and sustained as to recall the movement of clockwork." Mayer demonstrated similar circus movement excitation in rings cut from the ventricles of turtle hearts, but he did not consider this to be a plausible mechanism for the development of cardiac arrhythmias. His experiments proved valuable in identifying two fundamental conditions necessary for the initiation and maintenance of circus movement excitation: (1) unidirectional block—the impulse initiating the circulating wave must travel in one direction only; and (2) for the circus movement to continue, the circuit must be long enough to allow each site in the circuit to recover before the return of the circulating wave. Mines [58] was the first to develop the concept of circus movement reentry as a mechanism responsible for cardiac arrhythmias. He confirmed Mayer's observations and suggested that the recirculating wave could be responsible for clinical cases of tachycardia [59]. The following three criteria developed by Mines for identification of circus movement reentry remains in use today:

1. An area of unidirectional block must exist.
2. The excitatory wave progresses along a distinct pathway, returning to its point of origin and then following the same path again.
3. Interruption of the reentrant circuit at any point along its path should terminate the circus movement.

It was recognized that successful reentry could occur only when the impulse was sufficiently delayed in an alternate pathway to allow for expiration of the refractory period in the tissue proximal to the site of unidirectional block. Both conduction velocity and refractoriness determine the success or failure of reentry, and the general rule is that the length of the circuit (pathlength) must exceed or equal that of the wavelength, the wavelength being defined as the product of the conduction velocity and the refractory period or that part of the pathlength occupied by the impulse and refractory to reexcitation. The theoretical minimum path length required for development of reentry was therefore dependent on both the conduction velocity and the refractory period. Reduction of conduction velocity or APD can both significantly reduce the theoretical limit of the pathlength required for the development or maintenance of reentry.

Circus Movement Reentry Without an Anatomical Obstacle

In 1924, Garrey suggested that reentry could be initiated without the involvement of anatomic obstacles and that "natural rings are not essential for the maintenance of circus contractions" [60]. Nearly 50 years later, Allessie and coworkers [61] provided direct evidence in support of this hypothesis in experiments in which they induced a tachycardia in isolated preparations of rabbit left atria by applying properly timed premature extra-stimuli (Fig. 6.7). Using multiple intracellular electrodes, they showed that although the basic beats elicited by stimuli applied near the center of the tissue spread normally throughout the preparation, premature impulses propagate only in the direction of shorter refractory periods. An arc of block thus develops around which the impulse is able to circulate and reexcite its site of origin. Recordings near the center of the circus movement showed only subthreshold responses. The authors proposed the term "leading circle" to explain their observation [62]. They argued that the functionally refractory region that develops at the vortex of the circulating wavefront prevents the centripetal waves from short circuiting

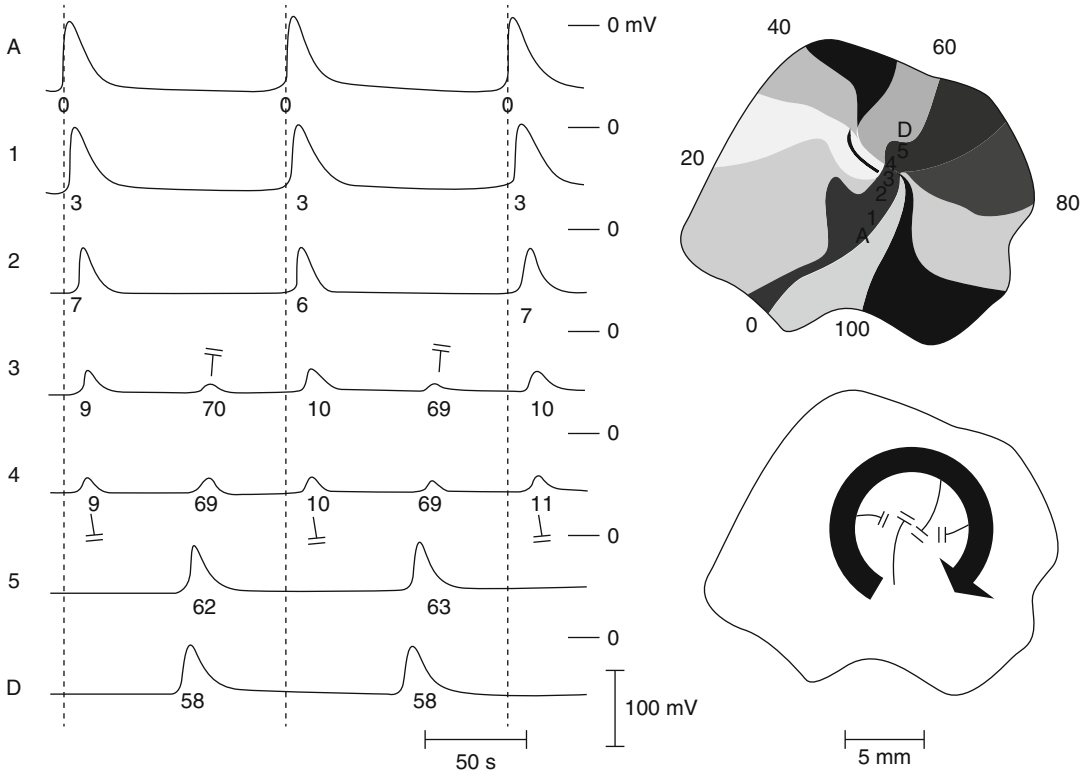

Figure 6–7. Leading circle model of reentry. Activation maps during steady-state tachycardia induced by a premature stimulus in an isolated rabbit atrium (*upper right*). On the *left* are transmembrane potentials recorded from seven fibers located on a straight line through the center of the circus movement. Note that the central area is activated by centripetal wavelets and that the fibers in the central area show double responses of subnormal amplitude. Both responses are unable to propagate beyond the center, thus preventing the impulse from short-cutting the circuit. *Lower right*: the activation pattern is schematically represented, showing the leading circuit and the converging centripetal wavelets. Block is indicated by *double bars* (From Allessie et al. [62] with permission from Wolters Kluwer Health)

the circus movement and thus serves to maintain the reentry. The authors also propose that the refractory core was maintained by centripetal wavelets that collide with each other. Because the head of the circulating wavefront usually travels on relatively refractory tissue, a fully excitable gap of tissue may not be present; unlike other forms of reentry the leading circle model may not be readily influenced by extraneous impulses initiated in areas outside the reentrant circuit and thus may not be easily entrained. Although the leading circle reentry for a while was widely accepted as a mechanism of functional reentry, there is significant conceptual limitation to this model of reentry. For example, the centripetal wavelet was difficult to demonstrate either by experimental studies with high resolution mapping or with computer simulation studies.

First introduced by Rosenblueth and Weiner in 1946 [63], the concept of spiral waves (rotors) has attracted a great deal of interest over the past decade. Originally used to describe reentry around an anatomic obstacle, the term *spiral wave reentry* was later adopted to describe circulating waves in the absence of an anatomic obstacle [64, 65]. Because spiral waves of excitation is a well described phenomenon in many excitable media [66], the application of the spiral waves of excitation to cardiac tissues is met with great enthusiasm. Spiral wave theory has advanced our understanding of the mechanisms responsible for the functional form of reentry. Although leading circle and spiral wave reentry

are considered by some to be similar, a number of distinctions have been suggested. The curvature of the spiral wave is the key to the formation of the core [67]. The curvature of the wave forms a region of high impedance mismatch (source-sink mismatch), where the current provided by the reentering wavefront (source) is insufficient to charge the capacity and thus excite larger volume of tissue ahead (sink). A prominent curvature of the spiral wave is generally encountered following a wave break, where the wavefront meets the wavetail and a large curvature (and short action potential) is present. Due to a very small source in part related to a short action potential (wavefront and wavetail meets), the broken end of the wave moves most slowly. Figure 6.8 shows the formation of the spiral wave by wave front interaction with the refractory tail of a previous activation [70]. This three dimensional computer simulation study reproduced the wavebreak observed in the optical mapping studies of VF in swine ventricle. The wavebreak occurs when the wavefront encountered refractory tail of a previous activation, inducing two spiral waves (Panel A). A three dimensional view of the scroll wave is shown in Panel B. Panel C is a blow up of the wavebreak. Note that the newly formed wavebreak has a very high curvature. As curvature decreases along the more distal parts of the spiral, propagation speed increases. The high curvature prevents the wave from propagating in the direction of wavebreak. The wavefront then circles around the wavebreak site to form circus movement. In three dimensions, there are two new scroll waves formed by these interactions. Another difference between the leading circle and spiral wave is the state of the core; in former the core is refractory because of repetitive centripetal wavelet that invades the core. In latter the core remains unexcited because the source-sink mismatch prevented the propagation of the wavefront into the core.

The term *spiral wave* is usually used to describe reentrant activity in two dimensions. The center of the spiral wave is called the *core* and the distribution of the core in three dimensions is referred to as the *filament*. The three-dimensional form of the spiral wave forms a scroll wave (see Fig. 6.8b). In its simplest form, the scroll wave has a straight filament spanning the ventricular wall (i.e., from epicardium to endocardium). Theoretical studies have described three major scroll wave configurations with curved filaments (L-, U-, and O-shaped), although numerous variations of these three-dimensional filaments in space and time are assumed to exist during cardiac arrhythmias.

Spiral wave activity has been used to explain the electrocardiographic patterns observed during monomorphic and polymorphic cardiac arrhythmias as well as during fibrillation. Monomorphic VT results when the spiral wave is anchored and not able to drift within the ventricular myocardium. In contrast, a meandering or drifting spiral wave causes polymorphic VT and VF like activity [71]. VF seems to be the most complex representation of rotating spiral waves in the heart. VF is often preceded by VT. One of the theories suggests that VF develops when a single spiral wave responsible for VT breaks up, leading to the development of multiple spirals that are continuously extinguished and recreated [72].

Figure-Eight Reentry

In the late 1980s, El-Sherif and coworkers delineated a figure-eight reentry in the surviving epicardial layer overlying an area of infarction produced by occlusion of the left anterior descending artery in canine hearts [73]. The same patterns of activation can also be induced by creating artificial anatomical obstacles in the ventricles [74], or during functional reentry induced by a single premature ventricular stimulation [75]. In the figure-eight model, the reentrant beat produces a wavefront that circulates in both directions around a line of conduction block rejoining on the distal side of the block. The wavefront then breaks through the arc of block to reexcite the tissue proximal to the block. The reentrant activation continues as two circulating wavefronts that travel in clockwise and counterclockwise directions around the two arcs in a pretzel-like configuration. Figure-eight reentry can be anatomical, functional, or spiral wave reentry. Thus, figure-eight reentry is a two-dimensional representation of all possible spatiotemporal representations of these reentrant mechanisms.

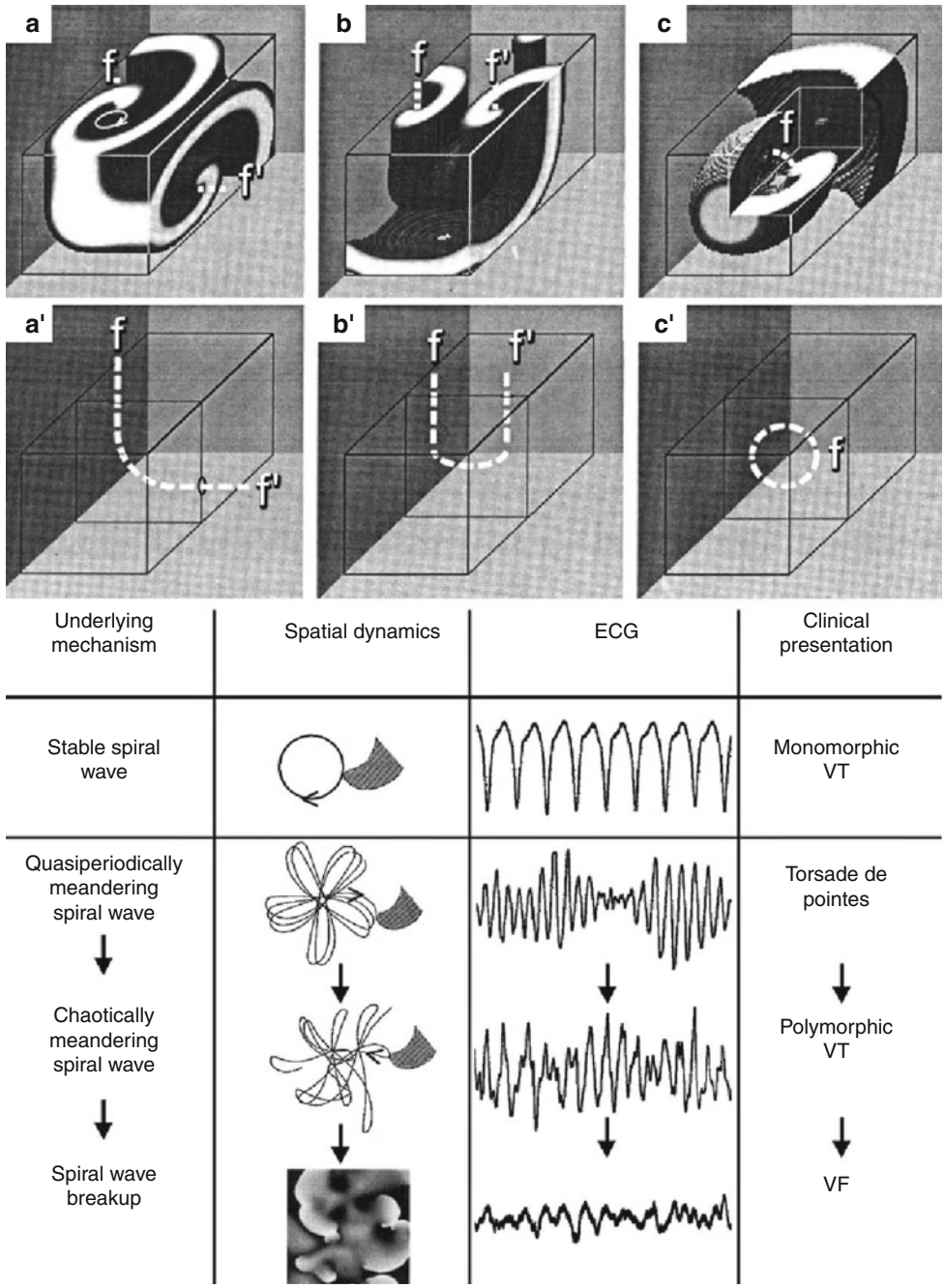

Figure 6–8. Schematic representation of basic scroll-type reentry in 3-D and spiral wave phenotypes with their possible clinical manifestations. *Upper panel*: Basic configurations of vortex-like reentry in three dimensions. (**a**, **a**′) L-shaped scroll wave and filament, respectively. The scroll rotates in a clockwise direction (on the *top*) about the L-shaped filament (*f*, *f*′) shown in (**a**′). (**b**, **b**′) U-shaped scroll wave and filament, respectively. (**c**, **c**′) O-shaped wave and filament, respectively (From Pertsov and Jalife [68] with permission). *Bottom panel*: Four types of spiral wave phenotypes and associated clinical manifestations. A stable spiral wave mechanism gives rise to monomorphic ventricular tachycardia (*VT*) on the ECG. A quasi-periodic meandering spiral wave is responsible for Torsade de Pointes, whereas a chaotically meandering spiral wave is revealed as polymorphic VT. A ventricular fibrillation (*VF*) pattern is caused by spiral wave breakup. Second column, spiral wave are shown in *gray*; the path of their tip are shown as *solid lines* (From Garfinkel and Qu [69], with permission)

Cardiac Fibrillation: Multiple Wavelets or Single Source?

There are two major theories to explain AF/VF generation. The first, originally suggested by Gordon Moe and colleagues, proposes that cardiac fibrillation is maintained by multiple unstable reentrant wavelets [76]. The multiple reentrant wavelet hypothesis of cardiac fibrillation was the dominant theory from 1960 to 1990. Starting in the 1980's, studies employing activation mapping techniques provided evidence for the presence of multiple reentrant wavelets during atrial and ventricular fibrillation [72, 77–80]. Starting in the 1990s, with a growing number of groups conducting mapping studies as well as with an improvement of mapping technologies, it became evident that multiple reentrant wavelets could not be readily observed during fibrillation. In fact, results of most mapping studies revealed that during fibrillation, a full reentrant circuit is either not recorded at all, observed rarely as a single short-lived rotor, or manifest as a single stable or meandering rapidly revolving micro-reentrant circuit, leading to disorganized activity due to fibrillatory conduction in the rest of the atria or ventricles [71, 81–90]. These data promoted the revival of another theory for the maintenance of VF/AF known as the "single source hypothesis", which was first suggested 50 years earlier by Scherf et al. [91] and Prinzmetal et al. [92]. This theory proposes that AF/VF can be maintained by a single high frequency source, giving rise to impulse propagation with variable conduction block in the remainder of the ventricle (i.e., fibrillatory conduction), which accounts for the AF/VF pattern in the ECG. Jalife and co-workers were among the strongest advocates for the single source hypothesis, with a "mother rotor" being the driving force for AF/VF [87, 88, 93].

Most of the fibrillation activation mapping data have been obtained from epicardial and/or endocardial surfaces, where a full reentrant circuit is not readily observed [71, 81–85, 90, 94, 95]. A common interpretation of these results is that reentrant circuit(s) or part(s) of the circuit(s) travels intramurally, and, thus, may not be readily observed on the cardiac surfaces. Indeed, some of the studies that used both epicardial and endocardial mapping have revealed that a focal pattern of activation on the surface can be due to a reentrant circuit involving epicardial, intramural, and endocardial pathways [96, 97]. Some studies using intramural plunge electrode mapping techniques presented evidence for intramural scroll waves during VF [78, 90]. Factors that reduce the chance of detecting driver(s) of AF/VF include limited mapping resolution and/or cardiac area available for mapping. Taking into account these limitations and given that AF/VF generation may involve a complex 3-D spatiotemporal electrical activation (even in the case of relatively thin atrial structures [96, 98]), it is often difficult to decisively prove or disprove the presence of a single or multiple sources. Moreover, while a reentrant mechanisms are believed to be primary, a rapid focal source may also maintain some forms of atrial and ventricular fibrillation [83, 99–103]. While the weight of available evidence appears to point to a single driving source as the primary mechanism for the maintenance of AF/VF [90, 93], it is well recognized that mechanisms of cardiac fibrillation are unlikely to be the same in all cases, and that fibrillations may be maintained by diverse mechanisms [93, 97, 100, 104].

Reflection

Reentry can occur without circus movement. Reflection and phase 2 reentry are two examples of non-circus movement reentry. The concept of reflection was first suggested by studies of the propagation characteristics of slow action potential responses in K^+-depolarized Purkinje fibers [105]. In strands of Purkinje fiber, Wit and coworkers demonstrated a phenomenon similar to that observed by Schmitt and Erlanger in which slow anterograde conduction of the impulse was at times followed by a retrograde wavefront that produced a "return extrasystole" [105]. They proposed that the nonstimulated impulse was caused by circuitous reentry at the

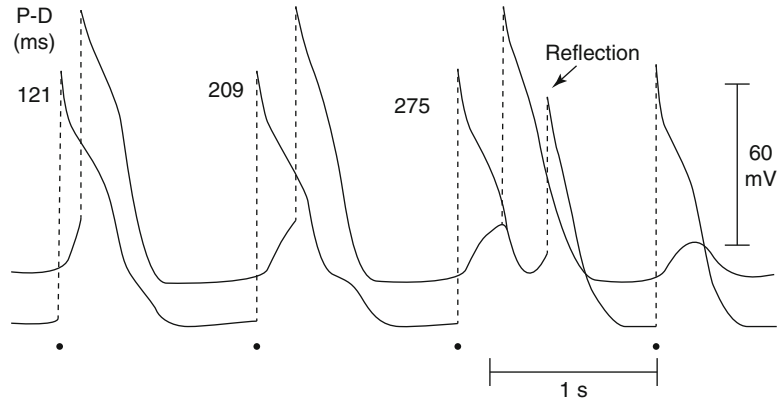

Figure 6–9. Delayed transmission and reflection across an inexcitable gap created by superfusion of the central segment of a Purkinje fiber with an *ion-free* isotonic sucrose solution. The two traces were recorded from proximal (*P*) and distal (*D*) active segments. P–D conduction time (indicated in the *upper portion* of the figure, in ms) increased progressively with a 4:3 Wenckebach periodicity. The third stimulated proximal response was followed by a reflection (From Antzelevitch et al [108], with permission from Elsevier Limited)

level of the syncytial interconnections, made possible by longitudinal dissociation of the bundle, as the most likely explanation for the phenomenon but also suggested the possibility of reflection. Direct evidence in support of reflection as a mechanism of arrhythmogenesis was provided by Antzelevitch and coworkers in the early 1980s [106, 107]. A number of models of reflection have been developed. The first of these involves use of *ion-free* isotonic sucrose solution to create a narrow (1.5–2 mm) central inexcitable zone (gap) in unbranched Purkinje fibers mounted in a three-chamber tissue bath (Fig. 6.9) [108]. In the sucrose-gap model, stimulation of the proximal (P) segment elicits an action potential that propagates to the proximal border of the sucrose gap. Active propagation across the sucrose gap is not possible because of the ion-depleted extracellular milieu, but local circuit current continues to flow through the intercellular low resistance pathways (an Ag/AgCl extracellular shunt pathway is provided). This local circuit or electrotonic current, very much reduced on emerging from the gap, gradually discharges the capacity of the distal (D) tissue thus giving rise to a depolarization that manifests as a either a sub-threshold response (last distal response) or a foot-potential that brings the distal excitable tissue to its threshold potential. Active impulse propagation stops and then resumes after a delay that can be as long as several 100 ms. When anterograde (P–D) transmission time is sufficiently delayed to permit recovery of refractoriness at the proximal end, electrotonic transmission of the impulse in the retrograde direction is able to reexcite the proximal tissue, thus generating a closely coupled reflected reentry. Reflection therefore results from the to-and-fro electrotonically-mediated transmission of the impulse across the same inexcitable segment; neither longitudinal dissociation nor circus movement need be invoked to explain the phenomenon.

A second model of reflection involved the creation of an inexcitable zone permitting delayed conduction by superfusion of a central segment of a Purkinje bundle with a solution designed to mimic the extracellular milieu at a site of ischemia [107]. The gap was shown to be largely comprised of an inexcitable cable across which conduction of impulses was electrotonically mediated. Reflected reentry has been demonstrated in isolated atrial and ventricular myocardial tissues as well [109–111]. Reflection has also been demonstrated in Purkinje fibers in which a functionally inexcitable zone is created by focal depolarization of the preparation with long duration constant current pulses [112]. Reflection

is also observed in isolated canine Purkinje fibers homogeneously depressed with high K^+ solution as well as in branched preparations of *normal* Purkinje fibers [113].

Phase 2 Reentry

Another reentrant mechanism that does not depend on circus movement and can appear to be of focal origin is Phase 2 reentry [114–116]. Phase 2 reentry occurs when the dome of the action potential, most commonly epicardial, propagates from sites at which it is maintained to sites at which it is abolished, causing local reexcitation of the epicardium and the generation of a closely coupled extrasystole. Severe spatial dispersion of repolarization is needed for phase 2 reentry to occur.

Phase 2 reentry has been proposed as the mechanism responsible for closely coupled extrasystoles that precipitate VT/VF associated with Brugada and early repolarization syndromes [117, 118].

Spatial Dispersion of Repolarization

Studies conducted over the past 20 years have established that ventricular myocardium is electrically heterogeneous and comprised of at least three electrophysiologically and functionally distinct cell types: epicardial, M, and endocardial cells [119, 120]. These three principal ventricular myocardial cell types differ with respect to phase 1 and phase 3 repolarization characteristics (Fig. 6.10). Ventricular epicardial and M, but not endocardial, cells generally display a prominent phase 1, because of a large 4-aminopyridine (4-AP)-sensitive transient outward current (I_{to}), giving the action potential a spike and dome or notched configuration. These regional differences in I_{to}, first suggested on the basis of action potential data [123], have now been directly demonstrated in a ventricular myocytes from a wide variety of species including canine [121], feline [124], guinea pig [125], swine [126], rabbit [127] and humans [128, 129]. Differences in the magnitude of the action potential notch and corresponding differences in I_{to} have also been described between right and LV epicardium [130]. Similar interventricular differences in I_{to} have also been described for canine ventricular M cells [131]. This distinction is thought to form the basis for why the Brugada syndrome is a right ventricular disease.

Myocytes isolated from the epicardial region of the left ventricular wall of the rabbit show a higher density of cAMP-activated chloride current when compared to endocardial myocytes [132]. I_{to2}, initially ascribed to a K^+ current, is now thought to be largely comprised of a calcium-activated chloride current ($I_{Cl(Ca)}$) which contributes to the action potential notch, but it is not known whether this current, differs among the three ventricular myocardial cell types [133].

Between the surface epicardial and endocardial layers are transitional cells and M cells. M cells are distinguished by the ability of their action potential to prolong disproportionately relative to the action potential of other ventricular myocardial cells in response to a slowing of rate and/or in response to action potential duration (APD)-prolonging agents [119, 134, 135]. In the dog, the ionic basis for these features of the M cell include the presence of a smaller slowly activating delayed rectifier current (I_{Ks}) [34], a larger late sodium current (late I_{Na}) [35] and a larger Na-Ca exchange current (I_{NCX}) [122]. In the canine heart, the rapidly activating delayed rectifier (I_{Kr}) and inward rectifier (I_{K1}) currents are similar in the three transmural cell types. Transmural and apical-basal differences in the density of I_{Kr} channels have been described in the ferret heart [136]. Amplification of transmural heterogeneities normally present in the early and late phases of the action potential can lead to the development of a variety of arrhythmias, including Brugada, long QT, and short QT syndromes as well as catecholaminergic VT. The genetic mutations associated with these inherited channelopathies are listed in the Table 6.1. The resulting gain or loss of function underlies the development of the arrhythmogenic substrate and triggers.

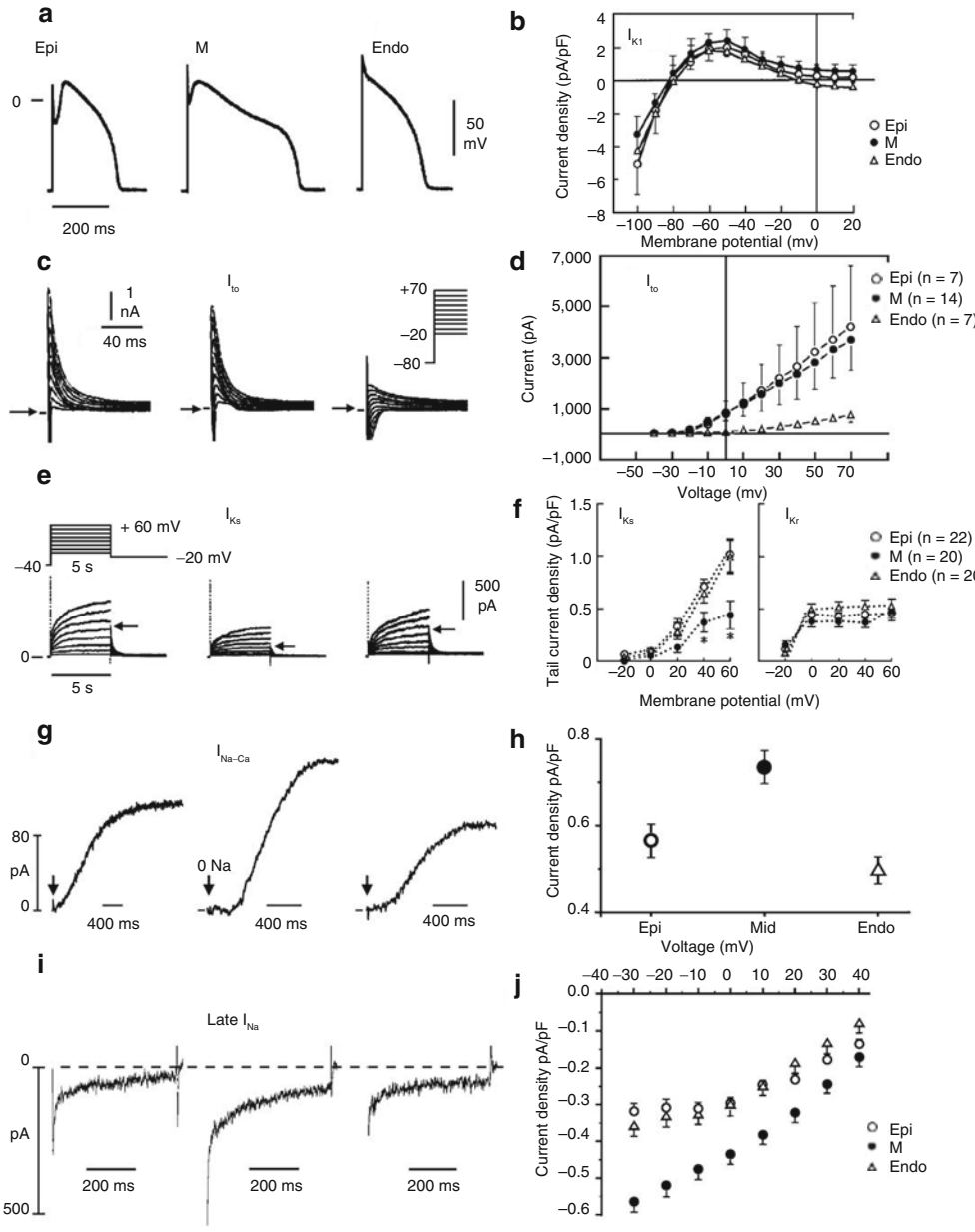

Figure 6–10. (**a**) Ionic distinctions among epicardial, M and endocardial cells. Action potentials recorded from myocytes isolated from the epicardial, endocardial and M regions of the canine left ventricle. (**b**) I–V relations for I_{K1} in epicardial, endocardial and M region myocytes. Values are mean ± S.D. (**c**) Transient outward current (I_{to}) recorded from the three cell types (current traces recorded during depolarizing steps from a holding potential of −80 mV to test potentials ranging between −20 and +70 mV). (**d**) The average peak current–voltage relationship for I_{to} for each of the three cell types. Values are mean ± S.D. (**e**) Voltage-dependent activation of the slowly activating component of the delayed rectifier K^+ current (I_{Ks}) (currents were elicited by the voltage pulse protocol shown in the inset; Na^+-, K^+- and Ca^{2+}- free solution). (**f**) Voltage dependence of I_{Ks} (current remaining after exposure to E-4031) and I_{Kr} (E-4031-sensitive current). Values are mean ± S.E. * $p < 0.05$ compared with Epi or Endo (From [34, 121, 122], with permission). (**g**) Reverse-mode sodium-calcium exchange currents recorded in potassium- and chloride-free solutions at a voltage of −80 mV. I_{Na-Ca} was maximally activated by switching to sodium-free external solution at the time indicated by the *arrow*. (**h**) Midmyocardial sodium-calcium exchanger density is 30 % greater than endocardial density, calculated as the peak outward I_{Na-Ca} normalized by cell capacitance. Endocardial and epicardial densities were not significantly different. (**i**) TTX-sensitive late sodium current. Cells were held at −80 mV and briefly pulsed to −45 mV to inactivate fast sodium current before stepping to −10 mV. (**J**) Normalized late sodium current measured 300 ms into the test pulse was plotted as a function of test pulse potential (Modified from [122] with permission from Wolters Kluwer Health)

Table 6–1. Genetic Disorders Causing Cardiac Arrhythmias In The Absence Of Structural Heart Disease (Primary Electrical Disease)

		Rhythm	Inheritance	Locus	Ion channel	Gene/protein
LQTS	(RW)	TdP	AD			
	LQT1			11p15	IKs	*KCNQ1*, KvLQT1
	LQT2			7q35	IKr	*KCNH2*, HERG
	LQT3			3p21	INa	*SCN5A*, Nav1.5
	LQT4			4q25		*ANKB, ANK2*
	LQT5			21q22	IKs	*KCNE1*, minK
	LQT6			21q22	IKr	*KCNE2*, MiRP1
	LQT7	(Andersen-Tawil Syndrome)		17q23	IK1	*KCNJ2*, Kir 2.1
	LQT8	(Timothy Syndrome)		6q8A	ICa	*CACNA1C*,Cav1.2
	LQT9			3p25	INa	*CAV3*, Caveolin-3
	LQT10			11q23.3	INa	*SCN4B*. Navb4
	LQT11			7q21-q22	IKs	*AKAP9*, Yotiao
	LQT12			20q11.2	INa	*SNTA1*, a-1 Syntrophin
	LQT13			11q24	I**K-ACh**	KCNJ5, Kir3.4
LQTS	(JLN)	TdP	AR	11p15	IKs	*KCNQ1*, KvLQT1
				21q22	IKs	*KCNE1*, minK
BrS	BrS1	PVT	AD	3p21	INa	*SCN5A*, Nav1.5
	BrS2	PVT	AD	3p24	INa	*GPD1L*
	BrS3	PVT	AD	12p13.3	ICa	*CACNA1C*,CaV1.2
	BrS4	PVT	AD	10p12.33	ICa	*CACNB2b, Cavb2b*
	BrS5	PVT	AD	19q13.1	INa	*SCN1B*, Navb1
	BrS6	PVT	AD	11q13-14	ICa	*KCNE3*. MiRP2
	BrS7	PVT	AD	11q23.3	INa	*SCN3B*, Navb3
	BRS8	PVT	AD	12p11.23	IK-ATP	*KCNJ8*, Kir6.1
	BrS9	PVT	AD	7q21.11	ICa	*CACNA2D1*, Cav**a2d1**
	BrS10	PVT	AD	1p13.3	IK-ATP	KCND3, KV4.3
	BrS11	PVT	AD	17p13.1	INa	*MOG1*
	BrS12	PVT	AD	3p21.2-p14.3	INa	*SLMAP*
	BrS12	PVT	AD	12p12.1	IK-ATP	A*BCC9*, SUR2A
ERS	ERS1	PVT	AD	12p11.23	I**K-ATP**	*KCNJ8*, Kir6.1
	ERS2	PVT	AD	12p13.3	ICa	*CACNA1C*,CaV1.2
	ERS3	PVT	AD	10p12.33	ICa	*CACNB2b*, Cavb2b
	ERS4	PVT	AD	7q21.11	ICa	*CACNA2D1*, Cav**a2d1**
	ERS5	PVT	AD	12p12.1	IK-ATP	A*BCC9*, SUR2A
	ERS6	PVT	AD	3p21	INa	*SCN5A*, Nav1.5
SQTS	SQT1	VT/VF	AD	7q35	IKr	*KCNH2*, HERG
	SQT2		AD	11p15	IKs	*KCNQ1*, KvLQT1
	SQT3		AD	17q23.1-24.2	IK1	*KCNJ2*, Kir2.1
	SQT4		AD	12p13.3	ICa	*CACNA1C*,CaV1.2
	SQT5		AD	10p12.33	ICa	*CACNB2b*, Cavb2b
	SQT6		AD	7q21.11	ICa	*CACNA2D1*, Cav**a2d1**
Catecholaminergic polymorphic VT						
	CPVT1	VT	AD	1q42-43		*RyR2*
	CPVT2	VT	AR	1p13-21		*CASQ2*

Abbreviations: *AD* autosomal dominant, *AR* autosomal recessive, *BrS* Brugada syndrome, *ERS* Early Repolarization Syndrome, *JLN* Jervell and Lange–Nielsen, *LQTS* long QT syndrome, *RW* Romano-Ward, *TdP SQTS* Short QT syndrome, Torsade de Pointes, *VF* ventricular fibrillation, *VT* ventricular tachycardia

Mechanisms Underlying Channelopathies

In the section below we will briefly discuss the how reentrant and triggered mechanisms contribute to development of VT/VF associated with the long QT, short QT and J wave syndromes.

J Wave Syndromes

Because they share a common arrhythmic platform related to amplification of I_{to}-mediated J waves, and because of similarities in ECG characteristics, clinical outcomes and risk factors, congenital and acquired forms of Brugada (BrS) and early repolarization (ERS) syndromes have

been grouped together under the heading of J wave syndromes [117].

Brugada Syndrome

In 1992, Pedro and Josep Brugada [137] reported a new syndrome associated with ST elevation in ECG leads V1-V3, right bundle branch appearance during sinus rhythm, and a high incidence of ventricular fibrillation (VF) and sudden cardiac death. Brugada syndrome has been associated with mutations in 11 different genes. Mutations in *SCN5A* ($Na_v1.5$, BrS1) have been reported in 11–28 % of BrS probands [138–140]. Mutations in *CACNA1C* ($Ca_v1.2$, BrS3), *CACNB2b* (Ca_vß(Beta)2b, BrS4) and *CACNA2D1* (Ca_vα(alpha)2δ(delta)1, BrS9) are found in approximately 12–13 % of probands [141, 142]. Mutations in glycerol-3-phosphate dehydrogenase 1-like enzyme gene (*GPD1L*, BrS2), *SCN1B* (β(beta)1-subunit of Na channel, BrS5), *KCNE3* (MiRP2; BrS6), *SCN3B* (β(beta)3-subunit of Na channel, BrS7), *KCNJ8* (BrS8) and *KCND3* (BrS10) are more rare [143–148]. Mutations in these genes lead to loss of function in I_{Na} and I_{Ca}, as well as to a gain of function in I_{to} or $I_{K\text{-}ATP}$. MOG1 was recently described as a new partner of $Na_V1.5$, playing a role in its regulation, expression and trafficking. A missense mutation in MOG1 was also associated with BrS (BrS11) [149].

The mechanisms of arrhythmogenesis in Brugada syndrome can be explained by the heterogeneous abbreviation of APD on the right ventricular epicardium (Fig. 6.11) [118]. In regions of the myocardium exhibiting a prominent I_{to}, such as the epicardium of the right ventricular outflow tract, accentuation of the action potential notch secondary to a reduction of calcium or sodium channel current or an increase in outward current, results in a transmural voltage gradient that leads to coved ST segment elevation, which is the only form of ST segment elevation diagnostic of BrS (see Fig. 6.11b). Under these conditions, there is little in the way of an arrhythmogenic substrate. However, a further outward shift of the currents active during the early phase of the action potential can lead to loss of the action potential dome, thus creating a dispersion of repolarization between epicardium and endocardium as well as within epicardium, between region at which the dome is maintained and regions where it is lost (see Fig. 6.11c). The extent to which the action potential notch is accentuated leading to loss of the dome depends on the initial level of I_{to} [151–153]. When I_{to} is prominent, as it is in the right ventricular epicardium [130, 151, 153], an outward shift of current causes phase 1 of the action potential to progress to more negative potentials at which the L-type calcium current ($I_{Ca,L}$) fails to activate, leading to an ***all-or-none*** repolarization and loss of the dome (see Fig. 6.11c). Because loss of the action potential dome is usually heterogeneous, the result is a marked abbreviation of action potential at some sites but not others. The epicardial action potential dome can then propagate from regions where it is maintained to regions where it is lost, giving rise to a very closely coupled extrasystole via phase 2 reentry (see Fig. 6.11d) [114]. The extrasystole produced via phase 2 reentry often occurs on the preceding T wave resulting in an R-on-T phenomenon. This in turn can initiate polymorphic ventricular tachycardia (VT) or ventricular fibrillation (see Fig. 6.11e, f).

Potent sodium channel blockers like ajmaline, procainamide, pilsicainide, propafenone, and flecainide can be used to induce or unmask ST segment elevation in patients with concealed J wave syndromes because they cause an outward shift of current active in the early phases of the action potential [154–156]. Sodium channel blockers like quinidine, which also inhibits I_{to}, can effectively reduce the magnitude of the J wave and ST segment elevation, thus suppressing the phase 2 reentry [151, 157]. Agents that boost calcium channel current (I_{Ca}) such as isoproterenol and dobutamine [151, 158, 159] as well as the phosphodiesterase III inhibitor, cilostazol [159], act to restore the action potential dome and are thus effective in suppressing the substrate and triggers underlying BrS. Isoproterenol, sometimes in combination with quinidine, has been shown to be effective in normalizing ST segment elevation in patients with the Brugada syndrome and in controlling electrical storms, particularly in children [160–166].

Recent studies point to a role of depolarization impairment resulting in local conduction delay in the RV [167–169]. Sodium channel mutations, in particular, have been implicated in

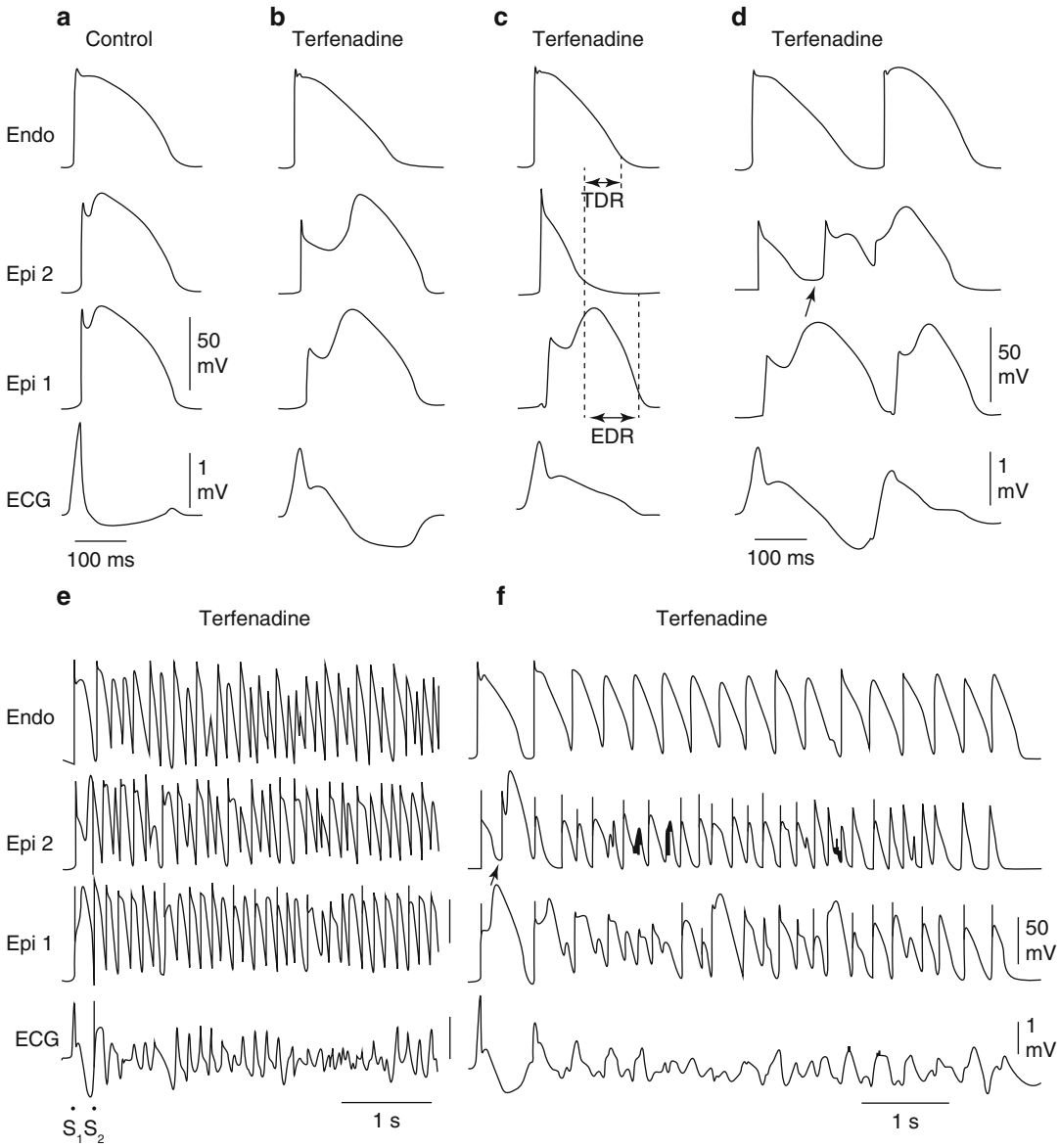

Figure 6–11. Cellular basis for electrocardiographic and arrhythmic manifestation of BrS. Each panel shows transmembrane action potentials from one endocardial (*top*) and two epicardial sites together with a transmural ECG recorded from a canine coronary-perfused right ventricular wedge preparation. (**a**) Control (Basic cycle length (BCL) 400 ms). (**b**) Combined sodium and calcium channel block with terfenadine (5 μM) accentuates the epicardial action potential notch creating a transmural voltage gradient that manifests as a ST segment elevation or exaggerated J wave in the ECG. (**c**) Continued exposure to terfenadine results in all-or-none repolarization at the end of phase 1 at some epicardial sites but not others, creating a local epicardial dispersion of repolarization (EDR) as well as a transmural dispersion of repolarization (TDR). (**d**) Phase 2 reentry occurs when the epicardial action potential dome propagates from a site where it is maintained to regions where it has been lost giving rise to a closely coupled extrasystole. (**e**) Extrastimulus (S1–S2 = 250 ms) applied to epicardium triggers a polymorphic VT. (**f**) Phase 2 reentrant extrasystole triggers a brief episode of polymorphic VT (Modified from Fish and Antzelevitch [150], with permission from Elsevier Limited)

the development of conduction impairment giving rise to a Brugada-like syndrome in infants [170]. The role of conduction delay in the RV as a primary factor in the electrocardiographic and arrhythmic manifestations of BrS however remains a matter of hot debate [171].

Early Repolarization Syndrome

An early repolarization (ER) pattern, consisting of a J point elevation, a notch or slur on the QRS (J wave), and tall/symmetric T waves, is commonly found in healthy young males and has traditionally been regarded as totally benign [172, 173]. A report in 2000 that an ER pattern in the coronary-perfused wedge preparation can easily convert to one in which phase 2 reentry gives rise to polymorphic VT/VF, prompted the suggestion that ER may in some cases predispose to malignant arrhythmias in the clinic [117, 174]. Many case reports and experimental studies have long suggested a critical role for the J wave in the pathogenesis of idiopathic ventricular fibrillation (IVF) [175–183]. A definitive association between ER and IVF was presented in the form of two studies published in the New England Journal of Medicine in 2008 [184, 185]. These were followed by another study from Viskin and co-workers [186] that same year and large population association studies [187–191].

The high prevalence of ER in the general population suggests that it is not a sensitive marker for sudden cardiac death (SCD), but that it is a marker of a genetic predisposition for the development of VT/VF via and Early repolarization syndrome (ERS). Thus, when observed in patients with syncope or malignant family history of sudden cardiac death, ER may be prognostic of risk. We recently proposed a classification scheme for ERS based on the available data pointing to an association of risk with spatial localization of the ER pattern [117]. In this scheme, Type 1 is associated with ER pattern predominantly in the lateral precordial leads; this form is very prevalent among healthy male athletes and is thought to be largely benign. Type 2, displaying an ER pattern predominantly in the inferior or infero-lateral leads, is associated with a moderate level of risk and Type 3, displaying an ER pattern globally in the inferior, lateral and right precordial leads, appears to be associated with the highest level of risk and is often associated with electrical storms [117]. Because ER is commonly observed only for a brief period of time just preceding the development of the electrical storm, Type 3 may often appear as Type 2 ERS. Of note, BrS represents a fourth variant in which ER is limited to the right precordial leads.

In ERS, as in BrS, the dynamic nature of J wave manifestation is well recognized. The amplitude of J waves, which may be barely noticeable during sinus rhythm, may become progressively accentuated with increased vagal tone and bradycardia and still further accentuated following successive extrasystoles and compensatory pauses giving rise to short long short sequences that precipitate VT/VF [117, 185, 192].

Studies examining the genetic and molecular basis for ERS are few and data regarding genetic effects are limited (Table 6.1). Haissaguerre and co-workers were the first to associate *KCNJ8* with ERS [193]. Functional expression of the S422L missense mutation in *KCNJ8* was not available at the time but was recently reported by Medeiros-Domingo and co-workers [146]. The authors genetically screened 101 probands with BrS and ERS and found one BrS and one ERS proband with a S422L-*KCNJ8* (Kir6.1) mutation; the variation was absent in 600 controls. The authors co-expressed the *KCNJ8* mutation with ATP regulatory subunit *SUR2A* in COS-1 cells and measured $I_{K\text{-}ATP}$ using whole cell patch clamp techniques. A significantly larger $I_{K\text{-}ATP}$ was recorded for the mutant vs. WT in response to a high concentration of pinacidil (100 μ(mu)M). The presumption is that the S422L-*KCNJ8* mutant channels fail to close properly at normal intracellular ATP concentrations, thus resulting in a gain of function. The prospect of a gain of function in $I_{K\text{-}ATP}$ as the basis for ERS is supported by the observation that pinacidil, an $I_{K\text{-}ATP}$ opener, has been shown to induce both the electrocardiographic and arrhythmic manifestation of ERS in LV wedge preparations [117]. Direct support for this hypothesis was recently provided by Barajas-Martinez and co-worker [194]. Using patch clamp techniques applied to inside-out patches of membrane excised from TSA201 cells co-expressed with *KCNJ8*-S422L and *SUR2A*-wild type, we observed a significantly greater IC_{50} for ATP in the mutant channels (785.5 ± 2 vs. 38.4 ± 3 μM, $n = 5$; $p < 0.01$) pointing to incomplete closing of the K_{ATP} channels under normoxic conditions. These results provided support the hypothesis that *KCNJ8* is a susceptibility gene for Brugada and early repolarization syndromes and point to S422L as a possible hotspot mutation. These findings suggest that the S422L-induced gain of function in $I_{K\text{-}ATP}$ is due to reduced sensitivity to intracellular ATP.

ERS has thus far been associated with mutations in six different genes. A mutation in *KCNJ8*, responsible for the pore-forming subunit of the $I_{K\text{-}ATP}$ channel [146, 193, 194] and loss of function mutations in I_{Ca} secondary to mutations in $Ca_v1.2$, $Ca_V\beta$(Beta) and Caα(alpha)2δ(delta)1 subunits of the cardiac L-type calcium channel (*CACNA1C, CACNB2, and CACNA2D1*) [142]. The most recent addition to the genes associated with ERS is SCN5A, the gene that encodes the α subunit of the cardiac sodium channel. Watanabe and coworkers reported loss-of-function mutations in *SCN5A* in patients with idiopathic ventricular fibrillation associated with ERS [195]. We recently presented preliminary data for the involvement of mutations in *ABCC9*, which encodes SUR2A, the ATP-regulatory subunit, of the $I_{K\text{-}ATP}$ channel, giving rise to a gain of function in $I_{K\text{-}ATP}$ [196].

The ECG and arrhythmic manifestation of ERS are thought to be due to mechanisms similar to those operative in BrS. In ERS, the outward shift of current may extend beyond the action potential notch thus leading to an elevation of the ST segment akin to early repolarization. Activation of the ATP-sensitive potassium current ($I_{K\text{-}ATP}$) or depression of inward calcium channel current (I_{Ca}) can effect such a change [142]. Transmural gradients generated in response to response to I_{Ca} loss of function or $I_{K\text{-}ATP}$ gain of function could manifest in the ECG as a diversity of ER patterns including J point elevation, slurring of the terminal part of the QRS and mild ST segment elevation. The ER pattern could facilitate loss of the dome due to other factors and thus lead to the development of ST segment elevation, phase 2 reentry and VT/VF.

It should be noted that the use of the term early repolarization as well as the mechanisms underlying ERS have been the subject of recent debate [197–199].

The Long QT Syndrome

The long QT syndromes (LQTS) are phenotypically and genotypically diverse, but have in common the appearance of long QT interval in the ECG, an atypical polymorphic ventricular tachycardia known as Torsade de Pointes, and, in many but not all cases, a relatively high risk for sudden cardiac death [200–202]. Congenital LQTS has been associated with 13 genes in at least seven different ion genes and an structural anchoring protein located on chromosomes 3, 4, 6, 7, 11, 17, 20, 21 (Table 6.1) [203–210]. Timothy syndrome, also referred to as LQT8, is a rare congenital disorder characterized by multiorgan dysfunction including prolongation of the QT interval, lethal arrhythmias, webbing of fingers and toes, congenital heart disease, immune deficiency, intermittent hypoglycemia, cognitive abnormalities, and autism. Timothy syndrome has been linked to loss of voltage-dependent inactivation due to mutations in $Ca_v1.2$, the gene that encodes for an α(alpha) subunit of the calcium channel [211]. The most recent gene associated with LQTS is *KCNJ5* which encodes Kir3.4 protein, the protein that encodes the α subunit of the $I_{K\text{-}ACh}$ channel. Mutations in this gene produce a loss of function which produces an LQT phenotype via a mechanism, which is not clearly understood [212].

Two patterns of inheritance have been identified in LQTS: (1) a rare autosomal recessive disease associated with deafness (Jervell and Lange-Nielsen), caused by two genes that encode for the slowly activating delayed rectifier potassium channel (KCNQ1 and KCNE1); and (2) a much more common autosomal dominant form known as the Romano Ward syndrome, caused by mutations in 13 different genes (see Table 6.1).

Acquired LQTS refers to a syndrome similar to the congenital form but caused by exposure to drugs that prolong the duration of the ventricular action potential [213] or QT prolongation secondary to cardiomyopathies such as dilated or hypertrophic cardiomyopathy, as well as to abnormal QT prolongation associated with bradycardia or electrolyte imbalance [214–218]. The acquired form of the disease is far more prevalent than the congenital form, and in some cases may have a genetic predisposition.

Amplification of spatial dispersion of repolarization within the ventricular myocardium has been identified as the principal arrhythmogenic substrate in both acquired and congenital LQTS. The accentuation of spatial dispersion, typically secondary to an increase of transmural, trans-septal or apico-basal dispersion of repolarization, and the development of early afterdepolarization (EAD)-induced triggered activity underlie the substrate and trigger for the development of Torsade de Pointes arrhythmias observed under LQTS

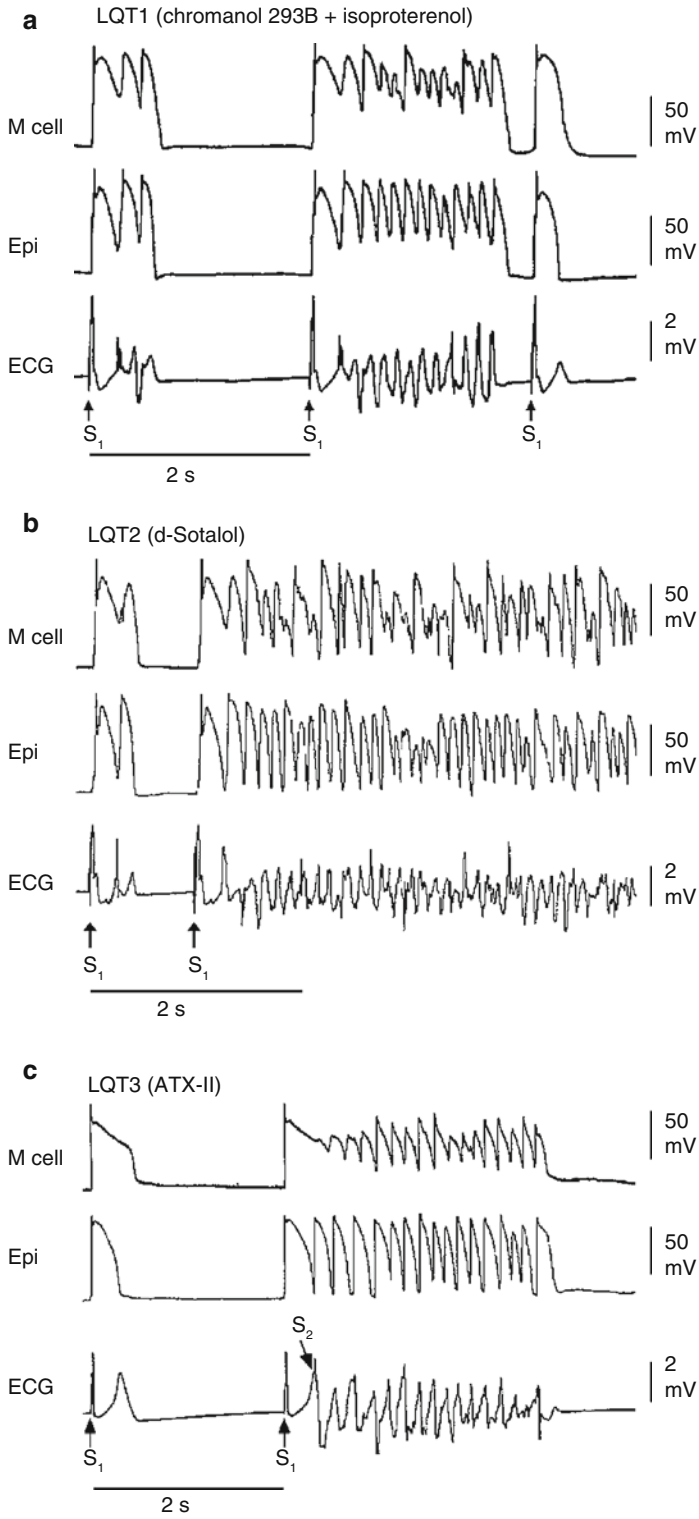

Figure 6–12. Polymorphic ventricular tachycardia displaying features of Torsade de Pointes (TdP) in the LQT1 (**a**), LQT2 (**b**), and LQT3 (**c**) models (arterially-perfused canine left ventricular wedge preparations). Isoproterenol + chromanol 293B, d-sotalol, and ATX-II are used to mimic the 3 LQTS syndromes, respectively. Each trace shows action potentials simultaneously recorded from M and epicardial (Epi) cells together with a transmural ECG. The preparation was paced from the endocardial surface at a BCL of 2,000 ms (S1). (**a**, **b**) Spontaneous TdP induced in the LQT1 and LQT2 models, respectively. In both models, the first groupings show spontaneous ventricular premature beat (or couplets) that fail to induce TdP, and a second grouping that show spontaneous premature beats that succeed. The premature response appears to originate in the deep subendocardium (M or Purkinje). (**c**) Programmed electrical stimulation-induces TdP in the LQT3 model. ATX-II produced very significant dispersion of repolarization (first grouping). A single extrastimulus (S2) applied to the epicardial surface at an S1–S2 interval of 320 ms initiates TdP (second grouping) (Modified from [221, 222] with permission)

conditions [219, 220]. Models of the LQT1, LQT2, and LQT3, and LQT7 forms of the long QT syndrome have been developed using the canine arterially perfused left ventricular wedge preparation (Fig. 6.12) [16, 223, 224]. Data from these studies suggest that in LQTS,

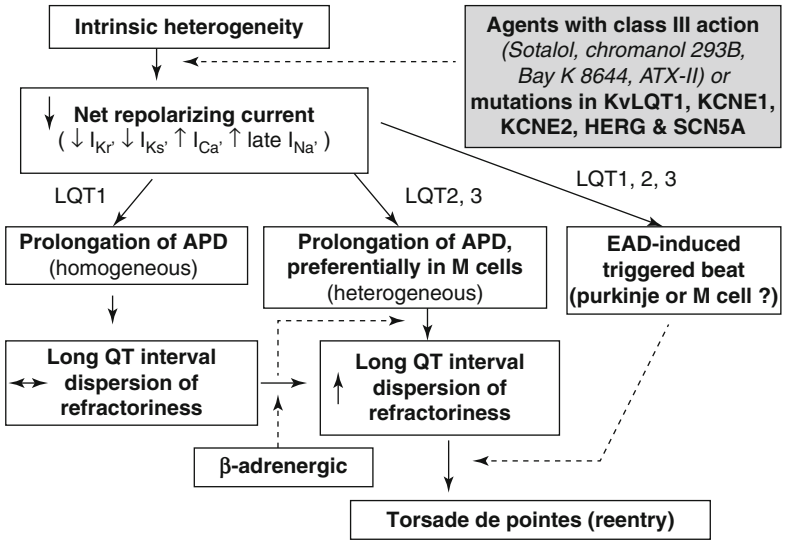

Figure 6–13. Proposed cellular and ionic mechanisms for the long QT syndrome

preferential prolongation of the M cell APD leads to an increase in the QT interval as well as an increase in transmural dispersion of repolarization (TDR), which contributes to the development of spontaneous as well as stimulation-induced Torsade de Pointes (TdP) [221, 222, 225]. The unique characteristics of the M cells, i.e., the ability of their action potential to prolong more than that of epicardium or endocardium in response to a slowing of rate [134, 226, 227], is the heart of this mechanism. Figure 6.13 presents our working hypothesis for our understanding of the mechanisms underlying LQTS-related TdP based on available data. The hypothesis presumes the presence of electrical heterogeneity in the form of transmural dispersion of repolarization under baseline conditions and the amplification of TDR by agents that reduce net repolarizing current via a reduction in I_{Kr} or I_{Ks} or augmentation of I_{Ca} or late I_{Na}. Conditions leading to a reduction in I_{Kr} or augmentation of late I_{Na} lead to a preferential prolongation of the M cell action potential. As a consequence, the QT interval prolongs and is accompanied by a dramatic increase in transmural dispersion of repolarization, thus creating a vulnerable window for the development of reentry. The reduction in net repolarizing current also predisposes to the development of EAD-induced triggered activity in M and Purkinje cells, which provide the extrasystole that triggers TdP when it falls within the vulnerable period. β(Beta) adrenergic agonists further amplify transmural heterogeneity (transiently) in the case of I_{Kr} block, but reduce it in the case of I_{Na} agonists [225, 228].

Short QT Syndrome

The short QT syndrome (SQTS), first proposed as a clinical entity by Gussak et al. in 2000 [229], is an inherited syndrome characterized by a QTc ≤ 360 ms and high incidence of VT/VF in infants, children and young adults [230, 231]. The familial nature of this sudden death syndrome was highlighted by Gaita et al. in 2003 [232]. Mutations in six genes have been associated with SQTS: *KCNH2, KCNJ2, KCNQ1, CACNA1c, CACNB2b and CACNA2D1* (Table 6.1) [141, 233–236]. Mutations in these genes cause either a gain of function in outward potassium channel currents (I_{Kr}, I_{Ks} and I_{K1}) or a loss of function in inward calcium channel current (I_{Ca}).

Experimental studies suggest that the abbreviation of the action potential in SQTS is heterogeneous with preferential abbreviation of either ventricular epicardium or endocardium, giving rise to an increase in TDR [237, 238]. In the atria, the I_{Kr} agonist PD118057 causes a

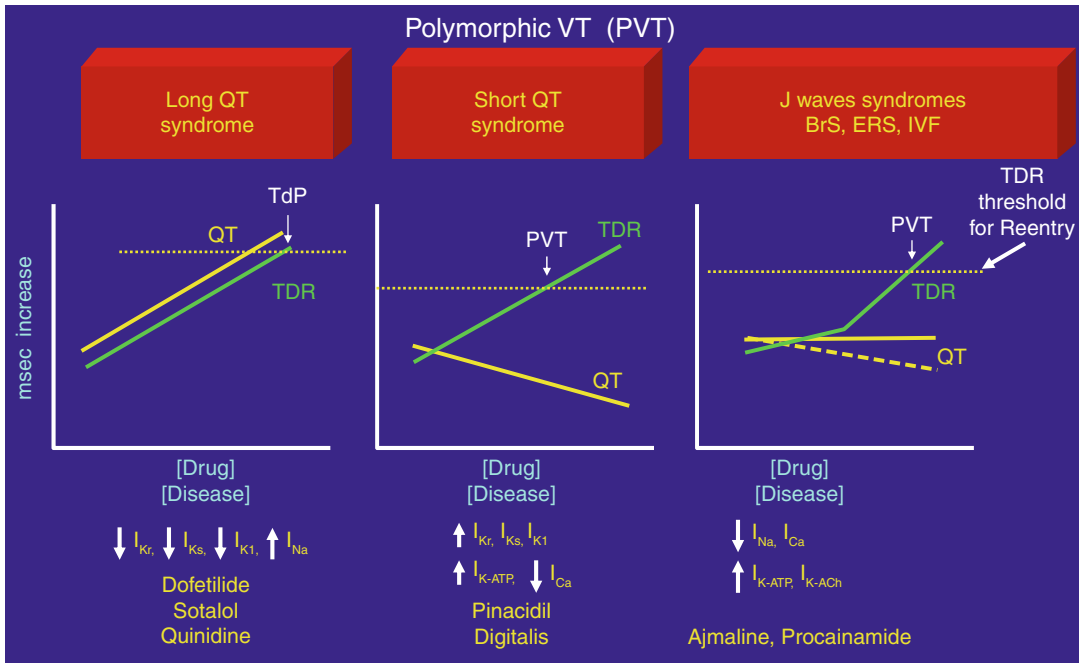

Figure 6–14. The role of transmural dispersion of repolarization (TDR) in Channelopathy-induced Sudden Death. In the long QT syndrome, QT increases as a function of disease or drug concentration. In the J wave syndromes (Brugada and early repolarization syndromes), it remains largely unchanged or is moderately abbreviated and in the short QT syndrome QT interval decreases as a function of disease of drug. The three syndromes have in common the ability to amplify TDR, which results in the development of polymorphic VT (PVT) or Torsade de Pontes (TdP) when dispersion reaches the threshold for reentry

much greater abbreviation of the action potential in epicardium when compared to cristae terminalis, thus creating a marked dispersion of repolarization in the right atrium [239]. Dispersion of repolarization and refractoriness serve as substrate for reentry by promoting unidirectional block. The marked abbreviation of wavelength (product of refractory period and conduction velocity) is an additional factor promoting the maintenance of reentry. T_{peak}-T_{end} interval and T_{peak}-T_{end}/QT ratio, an electrocardiographic index of spatial dispersion of ventricular repolarization, and perhaps TDR, have been reported to be significantly augmented in cases of SQTS [240, 241]. Interestingly, this ratio is more amplified in patients who are symptomatic [242].

Evidence supporting the role of augmented TDR in atrial and ventricular arrhythmogenesis in SQTS derives from experimental studies involving the canine left ventricular wedge and atrial preparations [237–239, 243].

The Role of Spatial Dispersion of Repolarization in Channelopathy-Mediated Sudden Death

The inherited and acquired sudden death syndromes discussed above differ with respect to the behavior of the QT interval. In the long QT syndrome, QT increases as a function of disease or drug concentration (Fig. 6.14). In the Brugada and early repolarization syndromes, it remains largely unchanged or is abbreviated and in the short QT syndrome, QT interval decreases as a function of disease or drug. What these three syndromes have in common is an amplification of TDR, which results in the development of polymorphic VT when TDR reaches the threshold for reentry. In the setting of a prolonged QT, we refer to it as Torsade de Pointes. It is noteworthy that the threshold for reentry decreases as APD and refractoriness are reduced, thus requiring a shorter pathlength for reentry, making it easier induce.

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7
Mechanisms of Action of Antiarrhythmic Drugs in Ventricular Arrhythmias

Shingo Murakami and Yoshihisa Kurachi

Abstract

Most antiarrhythmic drugs act on ion channels and alter the electrical properties of cardiac tissues, which is beneficial for preventing or treating cardiac arrhythmias. This chapter provides a basic understanding of anti-arrhythmic agents, especially on the electrophysiological properties of cardiac excitation.

Keywords

Arrhythmia • Antiarrhythmic drugs • Refractory period • Reverse frequency dependence • Reentry • Delayed afterdepolarization • Early afterdepolarization • Use dependence

Introduction

Antiarrhythmic drugs have been used as an effective measure to treat or prevent mainly tachyarrhythmias including ventricular tachycardia and fibrillation in clinics for a long time. Arrhythmias refer to changes from the normal sequence of electrical impulses and conduction, causing abnormal heart rhythms. They can be classified into two categories; bradyarrhythmias and tachyarrhythmias. Both can make the heart pump less effective and, more seriously, cause sudden death. Possible treatments include electrical defibrillation, radiofrequency ablation, implantable cardioverter defibrillators, artificial pacemakers, and medication. All these are used to prevent or terminate arrhythmias. Among them, arrhythmia medication is a nonsurgical and effective treatment and its major target has been tachyarrhythmias mainly in the ventricle, including ventricular tachycardia and fibrillation. Of course, recently treatment of atrial tachyarrhythmias such as atrial fibrillation is also one of the major interests. Since usage of antiarrhythmic drugs tended to rely on clinicians' experience and to be based on clinical practice, the effects of antiarrhythmic drugs had been understood empirically. Accumulated studies dealing with the mechanism of anti-arrhythmic agents, however, have provided much basic understanding of drug action, especially on the electrophysiological properties of cardiac excitation. This will not only help clinicians to select proper antiarrhythmic drugs, but will also help in the development of new antiarrhythmic drugs.

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Most antiarrhythmic drugs act on ion channels and alter the electrical properties of cardiac tissues, including excitation and conduction, which is beneficial for preventing or treating cardiac arrhythmias, though some of the effects are sometimes proarrhythmic. Therefore, it is necessary to understand the effects of various antiarrhythmic agents on the cardiac ion channels and thus on excitation and conduction. Ion channels are generally specific for ion species (e.g. Na^+, K^+, Ca^{2+}), and movement of ions though ion channels generates current across the membrane and forms the action potential (AP). Cardiac AP (typically the ventricular AP) consists of four phases: shape depolarization from the resting potential due to Na^+ ions entering the myocyte through the Na^+ channel, phase 0; rapid initial repolarization, phase 1; due to a K^+ channel current and Cl^- channel current, depolarization was maintained for 100 ms because of the Ca^{2+} channel current, phase 2; repolarization due to K^+ channel currents, phase 3; and the resting potential, phase 4 (Fig. 7.1a). Different types of ion channels contribute to each phase of AP and mediate the transduction from membrane depolarization to the contraction of the cell, a process called excitation-contraction (EC) coupling.

A number of classification systems of antiarrhythmic drugs based on their interaction with ion channels and receptors have been proposed including that by Vaughan-Williams [1]. Vaughan-Williams first proposed a scheme based on electrophysiological mechanisms of drug action to classify antiarrhythmic drug action [2]. Although knowledge about the electrophysiological bases of arrhythmias and drug action at that time were limited, this classification is still commonly used. Vaughan-Williams classification divides antiarrhythmic drugs into four major groups (Class I–IV) according to whether their major effect is to block Na^+ channel (Class I drug), β-adrenergic receptor (Class II drug), K^+ channel (Class III drug) or Ca^{2+} channel (Class IV drug) (Table 7.1). In general, by blocking the Na^+ channel, Class I drugs reduce maximum rate of rise in phase 0 of the AP without changing the resting potential, but have different effects on action potential duration (APD). The Class I drugs are further divided into three subgroups (Class Ia, Ib, Ic) according to their different effects on APD: Class Ia drugs prolong APD, Class Ib drugs shorten APD, and Class Ic drugs do not have significant effects on APD (Fig. 7.1b). Class II drugs block β-adrenergic receptors. Class III drugs block K^+ channels of delayed-rectifier type and prolong APD. Class IV drugs block Ca^{2+} channels. Vaughan-Williams classification has the virtue of simplicity. However, many anti-arrhythmic drugs may block more than one type of ion-channel. This classification cannot account for such complex phenomena and, moreover some antiarrhythmic agents including digitalis and adenosine cannot be covered by the four groups. One important classification of many others proposed to deal with these problems is the Sicilian Gambit [3]. The working group of the European Society of Cardiology met in Taormina, Sicily to consider the classification of anti-arrhythmic drugs [3]. They criticized the Vaughan-Williams classification because of the following reasons: (1) The classification is a hybrid. A single class effect can

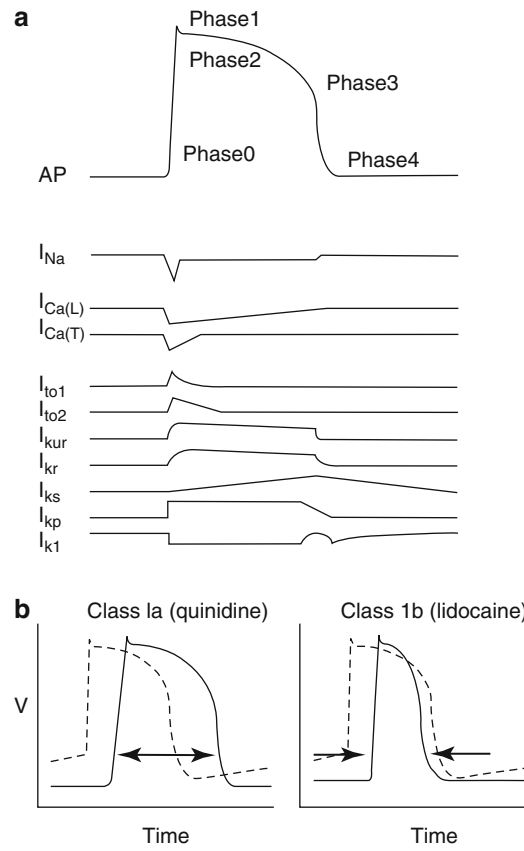

Figure 7–1. (**a**) Phases of a cardiac action potential and ion channel currents. (**b**) blocking effects of Lidocaine and Quinidine on action potential duration

I	Na^+ channel block	Ia	Prolong APD	Quinidine Procainamide Ajmaline Disopyramide Cibenzoline Pirmenol
		Ib	Shorten APD	Lidocaine Mexiletine Tocainide Aprindine(no effect on APD)
		Ic	No effect on APD	Flecainide Pilsicainide Propafenone
II	β-adrenergic receptor block			Propranolol Metoprolol Atenolol
III	K^+ channel block	APD prolongation		Amiodarone Bretylium Sotalol Dofetilide E-4031
IV	Ca^{2+} channel block			Nifedipine Verapamil Diltiazem Bepridil

Table 7–1. Vaughan Williams classification of antiarrhythmic drug actions

be produced by multiple mechanisms and some drugs have several classes of actions. (2) Activation of channels or receptors is not considered. (3) The classification is incomplete. For example, α-adrenergic blockers, cholinergic agonists, digitalis and adenosine are not included. The Sicilian Gambit group proposed that the Vaughan-Williams classification system be replaced with a new classification. In the new classification, the vulnerable parameters associated with specific arrhythmic mechanisms are identified and the effects of each drug on each parameter are listed to characterize the profile of each drug in the termination or suppression of the arrhythmia depending on its underlying mechanisms (Table 7.2). Thus, a most effective drug with possible minimal possible adverse effects would be expected. This system can account for multiple drug actions and provides more flexibility for classifying anti-arrhythmic drugs. However, this multidimensional classification system is significantly more complex than the standard Vaughan-Williams classification and may not be suitable for a basic understanding of antiarrhythmic drug actions. Because of its simplicity, the Vaughan-Williams classification will be used in this chapter to explain the mechanisms of antiarrhythmic drug-action in ventricular arrhythmias.

General Arrhythmia Suppression Mechanisms in Ventricular Arrhythmias

Antiarrhythmic drugs can block cardiac arrhythmias by suppressing underlying mechanisms, such as abnormal automaticity, delayed afterdepolarization (DAD), early afterdepolarization (EAD), and reentry (Fig. 7.2). Typically, the abnormal automaticity is caused by decrease of resting membrane conductance and/or enhancement of inward currents such as Ca^{2+} channel current. The DAD is due to the overload of intracellular calcium ions (Fig. 7.2a). EAD is caused by the excessive prolongation of APD (Fig. 7.2b). And Establishment of microreentry requires the unidirectional conduction block and slow conduction of AP (Fig. 7.2c). Abnormal automaticity can be altered by either changing the diastolic phase 4 slope, the threshold potential for rapid initial upstroke in phase 0, or APD. Generally speaking, Na^+ channel blockers and Ca^{2+} channel blockers elevate the threshold potential for AP initiation, K^+ channel blockers prolong the

Table 7–2. Sicilian Gambit

Drug	Channels						Receptors				Pumps
	Na			Ca	K	I_f	α	β	M_2	P	Na/K ATPase
	Fast	Med	Slow								
Lidocaine	○										
Mexiletine	○										
Tocainide	○										
Moricizine	Ⓘ										
Procainamide		Ⓐ			●				○		
Disopyramide		Ⓐ			●				○		
Quinidine		Ⓐ			●		○		○		
Propafenone		Ⓐ						●			
Flecainide			Ⓐ		○						
Encainide			Ⓐ								
Bepridil	○			●	●						
Verapamil	○			●			●				
Diltiazem				●							
Bretylium					●		◐	◐			
Sotalol					●			●			
Amiodarone	○			○	●		●	●			
Alinidine					●	●					
Nadolol								●			
Propranolol	○							●			
Atropine									●		
Adenosine										□	
Digoxin									□		●

○, ●, ● relative blocking potency of low, moderate and high, separately, □ agonist, ◐ agonist/antagonist, *A* activated state blocker, *I* inactivated state blocker

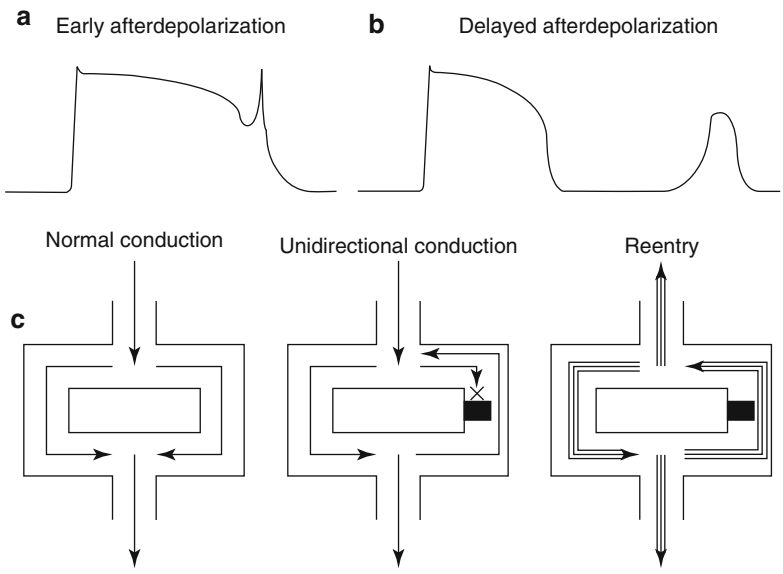

Figure 7–2. (**a**) Early afterdepolarization (**b**) Delayed after depolarization (**c**) Normal conduction, unidirectional conduction and Reentry

APD, adenosine hyperpolarizes the cell membrane potential and shorten the APD, and β-adrenergic receptor blockers decrease phase 4 slope. Similarly, Na^+ channel blockers and Ca^{2+} channel blockers may suppress DAD by interfering with its upstroke. The EAD can be inhibited by shortening APD. Reentry may be terminated by prolonging the refractory period, i.e. by blocking delayed rectifier K^+ channels or slowing recovery of Na^+ channel from inactivation.

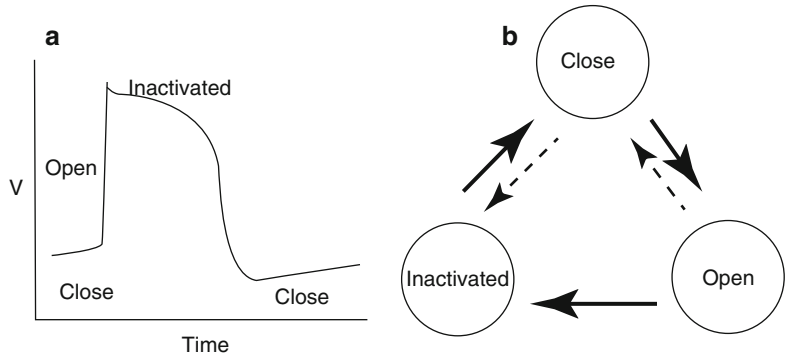

Figure 7–3. (**a**) States of Na^+ channel during a cardiac action potential. (**b**) State diagram of the Na^+ channel

The main purpose of this chapter is to describe the basic mechanisms of each class of drug affects cardiac ion channel activity.

Na^+ Channel Blocker (Class I Drugs)

Class I drugs in the Vaughan-Williams classification are primary Na^+ channel blockers and, historically, the blockers' effect on the Na^+ channel was first investigated to elucidate the mechanisms of antiarrhythmic drugs, before other antiarrhythmic drugs. Na^+ channels in many excitable cells generate a rapid regenerative upstroke of action potential and the Na^+ channel current is tetrodotoxin (TTX)-sensitive and/or μ-conotoxin sensitive. Two independent gating mechanisms of Na^+ channel (activation and inactivation) give a rise to fast onset and decay of the Na^+ channel current after depolarizing pulse (Fig. 7.3a). The Na^+ channel in atria and ventricle accounts for the initial rapid depolarization (phase 0), which is responsible for the fast conduction of excitation in cardiac tissues. Its maximum upstroke slope (dV/dtmax) is approximately proportional to the Na^+ channel current amplitude. And thus it has been used as the parameter to evaluate the effect of Class I drugs on the Na^+ channel. Therefore, the primary effect of Class I drugs is to slow the upstroke of cardiac AP. Class I drugs are further divided into three subgroups (Ia, Ib, Ic), according to their electrophysiological effects on APD. For example, Class Ia drugs such as quinidine slow the maximum upstroke slope and in addition prolong APD (see Fig. 7.1b). Because of its APD prolongation effect, quinidine is also known to cause so-called quinidine-shock, i.e., quinidine-related torsades de pointes type of polymorphic ventricular tachycardia. On the other hand, Class Ib drugs, such as lidocaine, also make maximum upstroke slope gentle but shorten the APD (see Fig. 7.1b). Class Ic drugs slow the maximum upstroke slope but do not have a significant effect on APD. These differences among the Class I subtype drugs can be accounted for either by the kinetic properties of the drug action on Na^+ channels and/or by action on other ion channels such as K^+ channels.

Drug Pathway to the Na^+ Channel Binding Site and Modulated Receptor Hypothesis

The action of Class I drugs (Na^+ channel blockers) should be understood in two different ways: one is the pathway by which the drugs reach their binding site in the Na^+ channel to be blocked, and the other is the state of the Na^+ channel which the drugs interact with. The most important theory for this is the modulated receptor hypothesis. Difference of drug action among antiarrhythmic drugs, such as use-dependent block, can be explained by the modulated receptor hypothesis. To understand the mechanism of antiarrhythmic drugs on the cardiac Na^+ channel, and that of local anesthesia on the nerve Na^+ channel, the modulated receptor hypothesis has been proposed [4, 5]. This hypothesis features the following: (1) Na^+ channel block by drugs is caused by a binding between a drug molecule and a receptor in the channel pore or nearby, and (2) the affinity between a receptor and a drug depends on the channel state, such as rested, open and inactivated (Fig. 7.4). This hypothesis was developed by comparing a Class Ib open

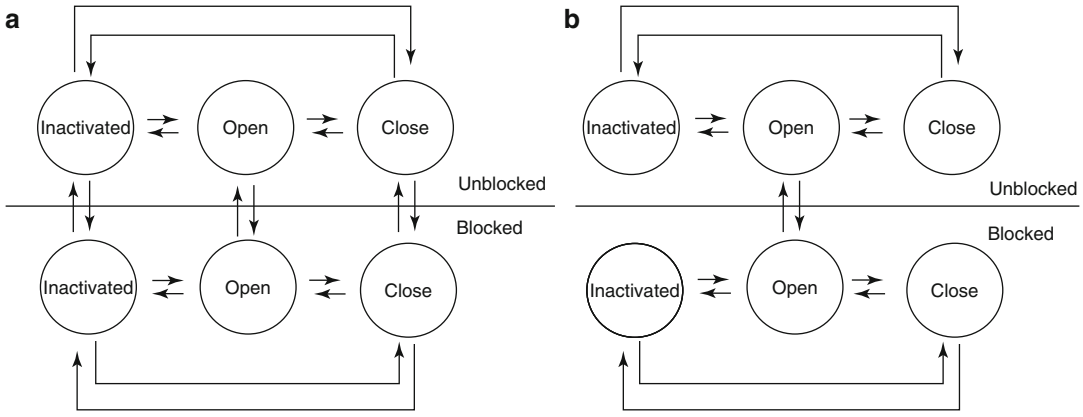

Figure 7–4. State-dependent ion channel block (**a**) Hydrophobic drug (**b**) Hydrophilic drug

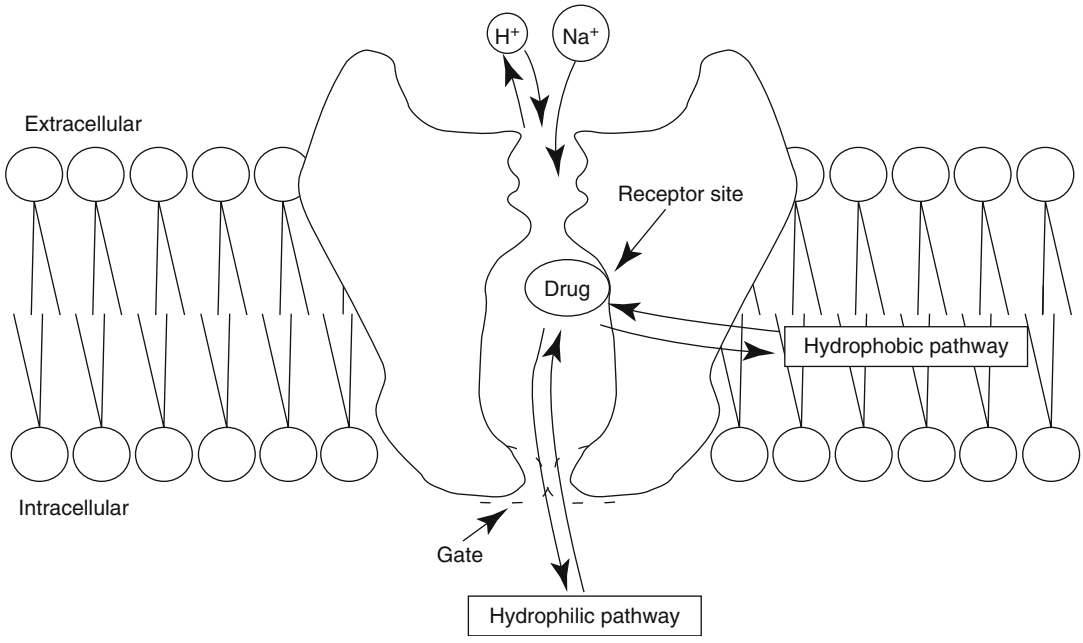

Figure 7–5. Binding site of Na^+ channel blockers and hydrophobic and hydrophilic pathways

(and inactivated) channel blocker, lidocaine, and a quaternary derivative of lidocaine, QX-314. QX-314 is a charged molecular and cannot go through the membrane. It blocks neuronal Na^+ channels only from the intracellular side and application of QX-314 from extracellular space does not block the Na^+ channel at resting potential. This is because QX-314 can go through only hydrophilic pathway (i.e. ion channel pores) (Fig. 7.5). When applied from the inside site, the drug can block the channel. However, the drug still requires the channel to open for its action (see Fig. 7.4b). Blockers with this kind of state-dependent block are generally called open channel blockers. In the application of open channel blockers, the magnitude of the Na^+ channel current at the first pulse is almost the same as that in the control, but it decreases gradually with additional pulses to a certain steady level. Independent of stimulus-frequency, the drug effect accumulates at each AP and reaches a certain steady state. The unblocking of QX-314 also requires

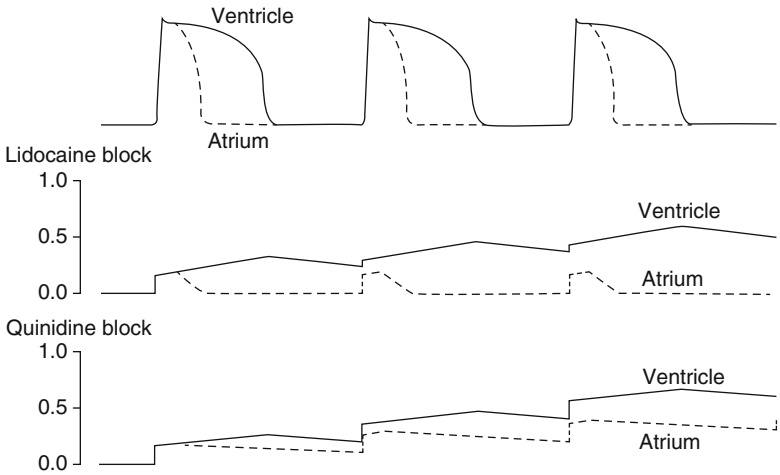

Figure 7–6. Accumulation of block effects of lidocaine and quinidine in the atria and ventricle action potentials

the opening of the Na^+ channel. As long as the Na^+ channel is closed, the drug remain in the channel pore and blocks the Na^+ channel in the channel pore. When the Na^+ channel is open, the drug may go out from the channel pore and unblock the Na^+ channel. Thus, the action of QX-314 on the Na^+ channel is use-dependent but frequency-independent. On the other hand, the block by lidocaine, a neutral molecule, can either develop or recover even when the Na^+ channel is closed or inactivated (see Fig. 7.4a). This is because lidocaine can go through not only the hydrophilic pathway (i.e. ion channel pores) but also the hydrophobic pathway (i.e. lipid bilayer) (see Fig. 7.5). Thus, the action of lidocaine is frequency-dependent due to the balance between the development and recovery of action. These properties of the drug pathway are important to characterize the state dependence of each drug.

In addition to the drug-pathway, the voltage-dependent properties of the interaction between the drug and the binding site in the channel determine the net action of drugs on the Na^+ channel. Even if the drug goes through channel pore and can bind to a receptor, the actual occurrence of the binding depends on transmembrane potential. QX-314 strongly suppresses Na^+ channel current at the depolarized potential and weakly at the hyperpolarized potential. In the same way, state-dependent unblocking can be accounted for by a similar explanation.

The analysis also suggests that the Na^+ channels blocked by Na^+ channel blockers also have three states (open state, inactivated state, and rested) and each drug has a different preference for each channel state (see Fig. 7.4). These are determined by the properties of the drug in the access pathway and the interaction with the binding site. For example, quinidine shows open state block but not significant inactivated state block. Lidocaine shows both open state block and inactivated state block. These are probably due to the difference of these drugs in the access pathway to the binding site. Recovery from block with lidocaine is much faster than with quinidine, therefore, lidocaine induces use-dependent block and exhibits stronger block at higher stimulus frequency, but quinidine does so even at a relatively low frequency (Fig. 7.6). Since the open state block is dominant in quinidine and its recovery is slow (~5–7 s), quinidine is effective in both atria where APD is short and in the ventricle where APD is long. In the case of lidocaine, open state block and inactivated state block work more or less in the same order. But the recovery of block is fast at a speed of ~100–200 ms. Therefore, lidocaine is also effective in ventricle where APD is long, but is not so effective in atria where APD is so short that accumulation of block is not likely to happen.

This different behavior between charged and neutral drug molecules also underlies the effect of extracellular pH on drug-action. Charged drug molecules, such as quinidine, have relatively stable properties as an open channel blocker at wide pH. Therefore, the effect on inactivated state channel is weak and recovery is slow. In contrast, although lidocaine at neutral pH has

the property of a neutral molecule and the effect on inactivated state channels is strong and recovery is fast, lidocaine at low pH becomes charged. Thus, at low pH it works as an open channel blocker and the effect on inactivated state channels becomes weak and recovery becomes slow. The effects of the extracellular acidification on the drugs are related to the phenomenon that the drugs inhibit excitability and conductance effectively in ischemic cardiac myocyte.

Class I drugs may have other actions than Na^+ channel block. For example, Class Ia drugs, quinidine and disopyramide can work as K^+ blockers and prolong APD. Because of this, quinidine is well known for starting drug-induced torsades de pointes. In addition, many Class I drugs, such as quinidine and disopyramide, have anticholinergic effects. Anticholinergic effects are due either to drug-induced inhibition of acetylcholine secretion from the nervous system or to inhibition of the effect of acetylcholine in the heart. The latter can be achieved by inhibition of M_2-muscarinic receptor and/or inhibition of muscarinic K^+ channel expressed in sinoatrial and atrioventricular nodes and atria. Therefore, the Class Ia drugs may speed up the conduction in the atrioventricular node and could cause serious adverse effect in atrial flutter. One of the reasons why digitalis is given to patients with atrial flutter is to slow down the conduction is in the atria before fibrillation treatment.

β-Adrenergic Blocker (Class II Drugs)

Stimulus of the β-adrenergic receptor enhances the Ca^{2+} channel current, Cl^- channel current and I_h, and may induce DAD- and EAD-related arrhythmias. Therefore, β-adrenergic receptor antagonists, such as propranolol, metoprolol and atenolol, may work as antiarrhythmic drugs by decreasing sympathetic activity on the heart, and are classified as Class II drugs. β-adrenergic blockers prolong atrioventricular nodal conduction time and refractoriness, and are useful in preventing or terminating reentrant arrhythmias involving atrioventricular node in the reentrant pathways.

K^+ Channel Blocker (Class III Drugs)

Reentry, one of the causes of arrhythmia, can be suppressed by removing the heterogeneity of the refractory period. For example, amiodarone is the best established antiarrhythmic drug for the treatment of ventricular arrhythmia in ischemic heart diseases. Amiodarone has multiple drug actions: it has β-adrenergic block action and Ca^{2+} channel block action on the sinoatrial and atrioventricular nodes, acts on the Na^+- and K^+ channel to increase the refractory period, and acts on the Na^+ channel to slow down intracardiac conduction of the cardiac AP. But which action is mainly responsible for its antiarrhythmic effect has not been yet fully determined. However, amiodarone is generally classified as a Class III drug and prolongation is thought to be one of its major effects for effective antiarrhythmic action. As a result of this, a number of Class III drugs have been developed for prolonging APD and thus the refractory period for treatment of cardiac arrhythmia.

Most of the existing class III drugs such as dofetilide (which has been utilized to treat atrial fibrillation) and E-4031, and nifekalant, target the rapid delayed rectifier current (I_{Kr}), which is one of the most important components of phase 3 repolarization. I_{Kr} is a fast component of delayed rectifier K^+ currents and is presumably due to current flowing through the channel pore whose subunit is encoded by human ether-à-go-go related gene (HERG). The I_{Kr} block effects of many class III drugs were studied as possible antiarrhythmic drugs and I_{Kr} block-related adverse effects, such as torsades de pointes, were found in many Class III drugs blocking specifically I_{Kr} (Table 7.3) [6]. These I_{Kr} blockers generally have a tendency to prolong APD as the stimulus frequency is decreased. More prominent prolongation of APD at lower stimulus frequency may cause excessive prolongation of QT at bradycardia, which results in ventricular tachycardia such as torsade de pointes. This reverse frequency dependent nature of APD propagation by I_{Kr}-blockers is known to be one of the underlying mechanisms that induce the life-threatening arrhythmias, torsade de pointes. Candidates as for clinically effective I_{Kr} blockers

Table 7–3. Cardiac and non-cardiac drugs reported to block I_{Kr}, cause torsades de pointes, induce EADs and increase dispersion of ventricular repolarization

Drug	Blocks I_{Kr}	Prolongs QT interval	TdP reported	Induces EADs	Increases dispersion of repolarization
Anti-arrhythmics					
Almokalant	+	+	+	+	+
Amiodarone	+	+	+	−	±
Azimilide	+	+	+	+	+
Dofetilide	+	+	+	+	+
Ibutilide	+	+	+	+	+
Quinidine	+	+	+	+	+
D-Sotalol	+	+	+	+	+
Antihistamines					
Astemizole	+	+	+	+	+
Terfenadine	+	+	+	+	+
Antibiotics					
Erythromycin	+	+	+	+	+
Clarithromycin	+	+	+	+	+
Ca^{2+} channel blockers					
Diltiazem	+	±	−	−	−
Verapamil	+	±	−	−	−
Mibefradil	+	+	+	+	−
Bepridil	+	+	+	+	+
Psychotherapeutics					
Sertindole	+	+	+	+	+
Droperidol	+	+	+	+	?
Fluoxetine	+	±	+	−	?
Miscellaneous					
Cisapride	+	+	+	+	+
Sodium pentobarbital	+	+	−	−	−
Ketanserin	+	+	+	+	+

Modified from Belardinelli et al. [6]

without this adverse effect include those that do not cause the reverse frequency-dependent prolongation of APD.

Two factors may be involved in the reverse-frequency-dependent prolongation of APD upon I_{Kr}-blockade. The first factor is the relative contribution of I_{Kr} to the total current for repolarization of cardiac AP (phase 3) at various stimulus frequencies. The membrane current for AP repolarization is mainly composed of I_{Kr} and I_{Ks} (a slow component of the delayed rectifier K^+ current) [7]. I_{Ks} is composed of KvLQT1and minK. Activation of I_{Kr} is relatively fast (in the order of 10s milliseconds) and can be fully activated even with low stimulus frequency. Since deactivation is also fast, I_{Kr} can recover fully before the next stimulus. On the other hand, the activation of Iks is relatively slow and I_{Ks} cannot be fully activated during AP with low stimulus frequency. However, once it is activated, due to the slow deactivation, I_{Ks} cannot be fully deactivated during resting at high stimulus frequency and some of its fraction still remains to be activated at the next stimulus. Therefore, if the stimulus frequency is increased, I_{Ks} will be accumulated. And thus the relative contribution of I_{Ks} in the total repolarization current (I_{Ks} plus I_{Kr}) will be increased. That is, when stimulus frequency is low, the dominant repolarization current is I_{Kr} and when stimulus frequency is high, it is I_{Ks}. If a Class III drug blocks only and completely I_{Kr}, APD will be more prolonged as the frequency of stimulus becomes lower.

The second factor is that the speed of the recovery of open channel blockers of I_{Kr} may affect the reverse frequent dependence. Open channel blockers with slow recovery, such as E-4031 and dofetilide, have a stimulus frequency independent effect after their blocks reach steady-states. However, I_{Kr} blockers with fast recovery, such as vesnarinone, exhibit clear use-dependent block at high stimulus frequency

while they exhibit only limited use-dependent block at low stimulus frequency. Therefore, the fast recovery of and consequent counterreverse frequency dependence work toward the cancellation of the reverse frequency dependence and this type of I_{Kr} blocks are expected to prolong APD without the reverse frequency dependence.

In addition to the reverse frequency dependence of APD prolongation by the I_{Kr}-blockers, the heterogeneity of depolarization in tissue caused by them is thought to be another underlying mechanism which causes torsade de points. In fact, it is know that amiodarone does not cause heterogeneity of excitation in tissue despite prominent prolongation of APD (Table 7.3).

Ca^{2+} Channel Blocker (Class IV Drugs)

Cardiac Ca^{2+} channels activate at more depolarized potential than Na^+ channels and their activation and inactivation are slower than Na^+ channels. Together with outward K^+ channel currents, inward currents through the activated Ca^{2+} channel shape the action potential plateau in phase 2. Because of these characteristics of Ca^{2+} channel current, its blockade leads to the reduction of amplitude and length of phase 2. The first generation of Ca^{2+} antagonists inhibits specifically L-type Ca^{2+} channels, and is categorized to class IV drugs in the Vaughan-Williams classification. These Ca^{2+} channels antagonists are divided into three subclasses: benzothiazepine class (e.g. diltiazem), papaverine derivatives (e.g. verapamil), and dihydropyridines (e.g. nifedipine). These three first generation Ca^{2+} blockers also have prominent effect on blood vessel smooth muscle and have been used as antihypertensive drugs. The second generation of Ca^{2+} blockers has extended-release mechanisms and the third generation of Ca^{2+} blockers have slow onset and long acting hemodynamic effect over days. Some of them have other effects than L-type Ca^{2+} channel blockade: amlodipine and cilnidipine inhibits N-type Ca^{2+} channel, and efonidipine inhibits T-type Ca^{2+} channel.

Binding Site of Ca^{2+} Channel Blockers

After Numa and colleagues revealed the structure of $\alpha 1$ subunit of the L-type Ca^{2+} channel from skeletal muscle, Ca^{2+} channels from various tissues including cardiac myocytes were cloned. With these results, binding positions of Ca^{2+} blockers were elucidated. The three different blockers are known to bind to three different sites of $\alpha 1$ subunit: the 1,4-dihydropyridine (DHP) site, the phenylalkylamine site and the benzothiazepine site. What makes the Ca^{2+} channel blocker different from the Na^+ channel blocker is that the combined application of these Ca^{2+} channel can enhance or the weaken block effect. The multiple binding sites for the Ca^{2+} channel blocker account for this phenomenon.

Use Dependence

Each of the three different first generation Ca^{2+} blockers shows different use dependence. Verapamil does not exhibit resting block and opening of the channel accumulates the block effect. Diltiazem has mild resting block and use-dependent block, which is strengthened with depolarizing pulse. Nifedipine shows distinct resting block and virtually no use-dependent block. The underlying mechanisms of these Ca^{2+} channel blockers can also be understood by the modulated receptor hypothesis. Verapamil may be a charged molecule, nifedipine may be a neutral molecule, and diltiazem may be between a charged and neutral molecule.

And as with the case of Na^+ channel blockers, both hydrophilic and hydrophobic pathways exist for Ca^{2+} channel blockers. For example, the block effect of verapamil is obviously use-dependent and verapamil can enter and exit from intracellular space through pores only when channels are open. Therefore, the block effect of verapamil lasts a long time. Because verapamil and diltiazem enhance their use-dependence under high frequency stimulus, they presumably have a high affinity with activated state channels. Some dihydropyridine

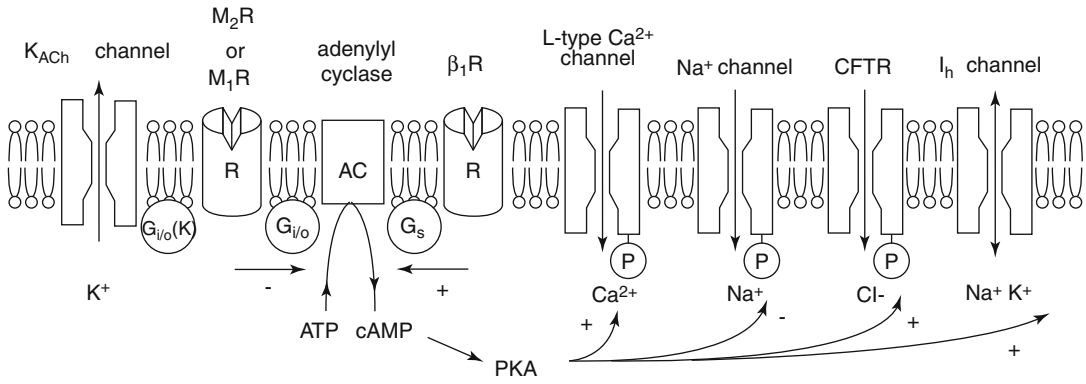

Figure 7–7. Intracellular signals transduction controlling cardiac ion channels

derivatives enhance L-type Ca^{2+} channel current, which cannot be explained by simple blockade of the channel pore, and have allosteric mechanisms with the concept of mode. Antagonist of dihydropyridines (nifedipine) move Ca^{2+} channel mode I to mode 0, and agonist of dihydropyridines (e.g. BAY K8644) move Ca^{2+} channel mode I to mode II.

Digitalis

The primary action of digitalis, such as digoxin, is to inhibit Na^+-K^+-ATPase, which results in an inotropic action on heart tissues and also an increase of vagal tone. As for the treatment of cardiac arrhythmia, digitalis is used to decrease the atrio-ventricular conduction. Thus, digitalis is used to (1) decrease the conduction of atrial excitation to the ventricle in the tachyatrial fibrillation and (2) prevent paroxysmal supraventricular tachycardia (PSVT) with a Ca^{2+} channel blocker, verapamil.

Adenosine

Adenosine is a metabolite of ATP and is not included in the list of Vaughan-Williams's classification. This substance is able to stop the paroxysmal supra-ventricular tachycardia, which includes the atriaventricular node in its circuit and some of adrenaline (or excise)-induced ventricular tachycardia. Adenosine binds to the A_1-purinergic receptor of cardiac myocytes. A_1-purinergic receptor couples to either adenylyle cyclase (AC) or inwardly rectifying muscarinic-K^+ channels via trimeric pertussis toxin sensitive $G_{i/o}$ proteins. Thus adenosine exhibits two actions; (1) an increase of K^+ conductance in sino-atrial and atrio-ventricular nodes and atria but not in the ventricle, and (2) inhibition of AC to reduce intracellular cyclic AMP in all types of cardiac myocytes including nodal, atrial and ventricular myocytes (Fig. 7.7).

Adenosine (5–10 mg) should be applied intravenously with a one-shot bolus injection, because the substance will be rapidly absorbed into cells via a cell membrane transporter. Thus, the time of action is transient and very short (less than several tenths of seconds). Dipyridamole prolongs the action of adenosine by disturbing the action of the transporter. The A_1-receptor antagonists, such as theophylline and aminophylline, abolish the action. ATP can also be used for the same purposes, because this substance will be metabolized to adenosine quickly in the blood. However, ATP may also binds to P_2-purinergic ATP-receptors prior to be metabolized to adenosine. P2-receptors either form ligand-gated non-selective cation channels (P2X) or couple to G_q proteins and Ca^{2+} mobilization signal (P2Y). Thus, ATP causes abnormal excitation of cardiac tissues, such as premature ventricular excitation, in addition to the actions of adenosine mediated A_1-receptor actions.

Cessation of PSVT: Adenosine injected intravenously transiently increases a K^+ conductance and causes bradycardia and various degrees of atrio-ventricular conduction block. Therefore, the tachyarrhythmia due to the macrocircuit reentrant involving the atrioventricular node can be transiently stopped because of the atrioventricular conduction block. A Ca^{2+}-channel blocker, verapamil, is also often used for the same purpose because this drug prolongs atrioventricular conduction. However, it is necessary to be careful to use this Ca^{2+} channel blocker for this purpose, because verapamil possesses a prominent negative inotropic action by blocking sarolenmal Ca^{2+} channel and the wash-out of action takes a very long time (3–7 h).

Cessation of exercise-induced ventricular tachycardia: Excise-induced or adrenaline-induced ventricular tachycardia is caused by an increase of intracellular cAMP due to β-adrenergic stimulation [8]. β_1-Adrenergic receptors in cardiac myocytes stimulate AC via G_s proteins. Stimulation of AC by G_s proteins is antagonizied with $G_{i/o}$ proteins (dual control of AC by G proteins). Because adenosine A_1-receptor couples to $G_{i/o}$ proteins, adenosine applied intravenously can decrease cAMP enhanced by β adrenergic stimulation and thus stop the ventricular tachycardia.

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8
Mechanisms of Action of Antiarrhythmic Drugs in Atrial Fibrillation

Alexander Burashnikov and Charles Antzelevitch

Abstract

The principal goal of antiarrhythmic therapy in the management of atrial fibrillation (AF) is to prolong the effective refractory period (ERP), which makes rapid activation of the atria impossible. Currently available antiarrhythmic drugs (AADs) prolong ERP by (1) prolonging the atrial action potential as in the case of delayed rectified potassium channel current (IKr) blockers such as d-sotalol, dofetilide, or ibutilide; (2) reducing excitability, thus promoting post-repolarization refractoriness (PRR), as in the case of sodium channel current (INa) blockers such as flecainide and propafenone, or (3) via both mechanism as in the case of multiple ion channels blockers such as amiodarone, dronedarone, ranolazine and vernakalant. The role of conduction slowing in anti-AF actions of INa blockers remains poorly understood. The present chapter describes our current understanding of anti-AF mechanisms of action of AADs.

Keywords

Atrial fibrillation • Antiarrhythmic drugs • Effective refractory period • Action potential • Reentry • Triggered activity

Introduction

The principal goal of antiarrhythmic therapy in the management of atrial fibrillation (AF) is to prolong the effective refractory period (ERP), which makes rapid activation of the atria impossible. Currently available antiarrhythmic drugs (AADs) prolong ERP by (1) prolonging the atrial action potential as in the case of delayed rectified potassium channel current (I_{Kr}) blockers such as d-sotalol, dofetilide, or ibutilide; (2) reducing excitability, thus promoting post-repolarization refractoriness (PRR), as in the case of sodium channel current (I_{Na}) blockers such as flecainide and propafenone, or (3) via both mechanism as in the case of multiple ion channels blockers such as amiodarone, dronedarone, ranolazine and vernakalant. The role of conduction slowing in anti-AF actions of I_{Na} blockers remains poorly understood. The present chapter describes our current understanding of anti-AF mechanisms of action of AADs.

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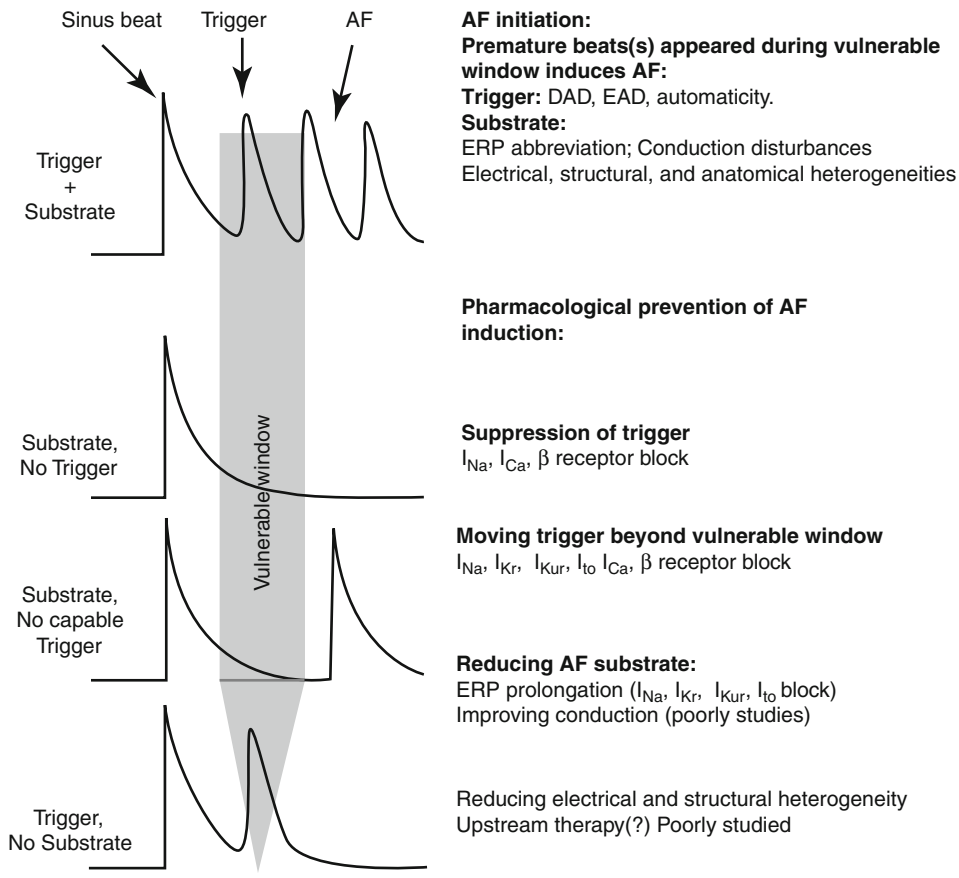

Figure 8–1. Factors involved in the initiation of atial fibrillation (*AF*) and pharmocological options to prevent the initiation of this arrhythmia

Electrophysiological Mechanisms of AF Generations

Understanding the anti-AF mechanisms of AADs requires a fundamental understanding of the electrophysiological mechanisms underlying the generation AF. Mechanisms of cardiac arrhythmias, including AF, are described in detailed in Chap. 6 of this book. In the current chapter, we briefly review the major principals of AF generation.

AF is commonly triggered by a focal mechanism (DAD- or EAD-induced triggered activity or automaticity) and maintained by a reentrant mechanism(s) [1]. Some forms of AF can be maintained by focal sources [2–5]. There are reentrant circuits that involve anatomical structures and those that can occur without anatomical obstacles (i.e., functional reentry). Unidirectional conduction block is a must for the initiation of all types of reentry except for spiral wave reentry. The initiation of spiral wave reentry results from wavebreak and can appear on the basis of temporal electrical heterogeneity without conduction block. Wavebreak commonly occur in conjunction with intrinsic (e.g., pectinate muscles, orifices, and blood vessels) or acquired (e.g., infarct scars, fibrosis) anatomical heterogeneities [6–9]. Curvature of tip of the wavefront determines the size of the core and the speed of revolution of the spiral wave. Circuits involving anatomical substrates are generally more stable than those based on functional electrical heterogeneities.

The initiation of AF commonly depends on the presence both triggers and substrates (Fig. 8.1). The trigger is may be in the form of one or more premature beats arising from the pulmonary veins or other structures within the atria (e.g., superior vena cava) as a consequence

of triggered activity or enhanced automaticity. The arrhythmogenic substrate is due to the development of electrical and structural heterogeneities, generally associated with an abbreviation of ERP. AF is said to beget AF, because the rapid activation of the atria leads to further abbreviation of the ERP within hours secondary to electrical remodeling [10]. Successful triggers are usually those originating from regions with relatively short ERP (e.g., pulmonary vein sleeves). Closely-coupled extrasystoles are better able to capture the vulnerable window for the initiation of AF (Fig. 8.1). The AF vulnerable window spans a period of time at the end of atrial repolarization, which is characterized by a high degree of spatiotemporal electrical heterogeneity. The vulnerable window is absent or very brief in healthy atria and is often significantly prolonged in remodeled atria.

The maintenance of AF is facilitated by the development of both electrical and structural remodeling [10–12]. The electrical remodeling abbreviates ERP due to abbreviation of APD and the structural remodeling contributes to development of conduction disturbances [13]. Structural remodeling in atria also develops with age and is associated with a number of diseases, including heart failure, ischemic heart disease and hypertension.

Although AF occurrence is often associated with ERP shortening, the initiation of AF may not always be associated with abbreviation of ERP. In many pathologies associated with AF, such as atrial dilatation, heart failure and hypotension, atrial ERP may not be altered or may even be prolonged [14–18]. Interestingly, although age is a major risk factor for development of AF, ERP prolongation is paradoxically associated with advancing age in both dogs and humans [19, 20]. It seems that in many patients experiencing AF or having a history of AF, a short ERP is largely a consequence of AF itself. Indeed, during on-going AF or frequent episodes of AF, ERP appears to become short in any pathology.

An important concept in the science of AF generation is that of "wavelength", defined as the product of ERP and conduction velocity. Simply defined, the wavelength is the length of the reentrant circuit occupied by the advancing wavefront and refractory to reexcitation. The value of the wavelength often predicts the probability of appearance/maintenance of AF (i.e., the shorter the wavelength the greater probability of AF induction and maintenance and vice versa) [21].

Anti-AF Pharmacology

The most common anti-AF approach involves prolongation of ERP, for both prevention initiation and termination of AF. Irrespective of the mechanisms underlying initiation or maintenance of AF, ERP prolongation is effective in slowing or terminating and/or preventing the re-induction of AF. Pharmacological prolongation of ERP can be achieved with prolongation of APD, induction of post-repolarization refractoriness (PRR), or by a combination of the two (Fig. 8.2). APD prolongation is largely due to inhibition of potassium current(s) and induction of PRR is exclusively due to block of the sodium channel responsible for peak I_{Na}. Multiple ion channel blockers can prolong ERP by both APD prolongation and induction of PRR.

The role of conduction alterations in anti-AF action of I_{Na} blockers remains controversial. Conduction slowing/disturbance by itself is expected to promote reentrant arrhythmias by reducing wavelength. This, however, may be the case for the ventricles but not for atria because the use of I_{Na} blockers is associated with the induction of ventricular but not atrial fibrillation. For instance, at the same concentration range, pilsicainide promotes ventricular spiral wave-mediated arrhythmias [22], but suppresses spiral wave-induced AF [23]. This distinction appears to be due to a greater effect of sodium channel blockers to reduce excitability in atria vs. ventricles (discussed below), thus producing a much greater prolongation of ERP in atria. Improvement or normalization of conduction may exert an anti-AF action in some AF pathologies (like heart failure) [24]. Improvement of conduction in atria, however, remains difficult to achieve. In most AF cases, conduction slowing in atria is due to structural remodeling. While connexin (principal gap junction subunits) is significantly altered in atria of congestive heart failure dogs, these changes do not contribute to atrial conduction disturbances and AF in this setting [25]. Upstream therapy may be useful in preventing

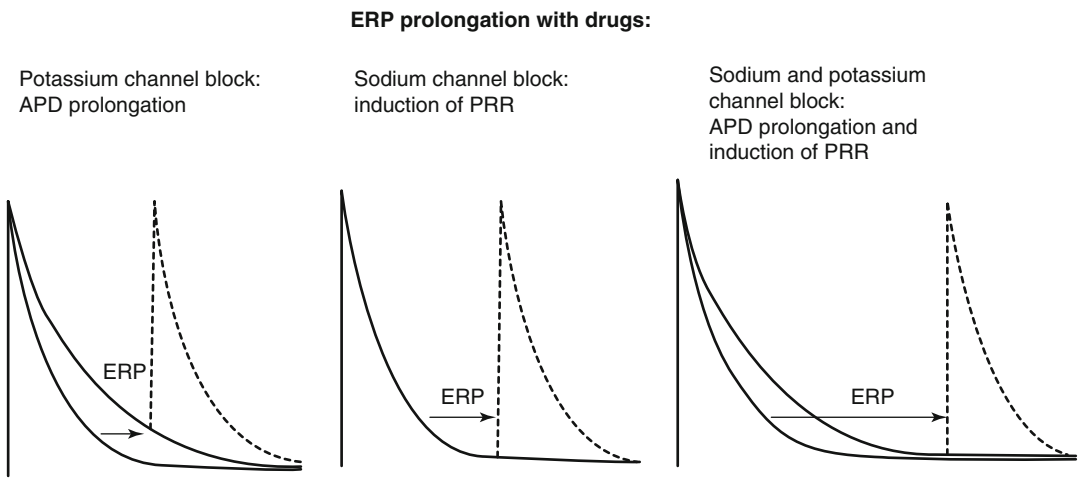

Figure 8–2. Prolongation of atrial effective refractory period (*ERP*) with potassium and/or sodium channel blockers. This schematic depicts the effects of sodium, potassium and mixed ion-channel blockers on action potential duration and effective refractory period (ERP, depicted by *arrows*). *APD* action potential duration, *PRR* post-repolarization refractoriness

or ameliorating atrial structural remodeling and, thus, atrial conduction disturbances, but this remains to be determined [26].

AF prevention can be achieved by suppressing the trigger and by reducing the arrhythmogenic substrate (see Fig. 8.1). The initiation of AF is often thought to be caused by intracellular calcium mediated triggered activity/automaticity [27, 28]. Dynamic interaction between sympathetic and parasympathetic systems play a critical role in the initiation of paroxysmal AF [29]. In this context, intracellular-mediated late phase 3 EADs [30, 31] have been proposed to play a prominent role in the induction of AF [29, 31]. Pharmacological reduction or normalization of elevated intracellular calcium activity can suppress the development of calcium-mediated triggered activity and AF. This can be achieved with sodium and calcium channel blockers and, theoretically, with a modulator of autonomic nervous system activity [29, 31, 32]. The AF trigger can be suppressed not only by a direct effect (e.g., by a reduction/normalization of intracellular calcium or blocking a specific ion current causing DAD/EAD/automaticity), but also by a prolongation of ERP (simply moving the trigger beyond the AF vulnerable window; see Fig. 8.1).

Although a reduction of electrical heterogeneity is expected to decrease AF vulnerability, pharmacological reduction of electrical heterogeneity in atria and its utility in anti-AF action are poorly defined. It is noteworthy that the antiarrhythmic value of pharmacologically reducing dispersion of repolarization and refractoriness in the ventricle has been demonstrated in many arrhythmogenic pathologies, including the long QT, short QT, early repolarization and Brugada syndromes, catecholaminergic polymorphic ventricular tachycardia, heart failure, ischemia and infarction [33–37].

The electrophysiological efficacy of AADs to prolong ERP at regular and rapid activation rates can vary significantly (i.e., I_{Na} blockers are use-dependent and I_{Kr} blockers are reverse-use dependent). These properties should be reflected in anti-AF efficacy of I_{Kr} and I_{Na} blockers to prevent and terminate AF. Both potassium and sodium channel blockers have been shown to more readily prevent AF initiation than to terminate on-going AF [38–40]. These results are consistent with the reverse-use dependence of I_{Kr} blockers, but at first glance appear to contradict the use-dependent effects of I_{Na} inhibitors.

Sodium Channel Block for AF

Many antiarrhythmic agents that block I_{Na} as their primary action are classified as Class IA, IB or IC based on their unbinding kinetics from

the sodium channel and their effect on APD in ventricular myocardium [41]. Class IB agents like lidocaine and mexiletine abbreviate APD and have rapidly unbinding kinetics from the sodium channel ($\tau < 1$ s). Class IA agents, like procainamide, quinidine and disopyramide, prolong APD (largely due to block of I_{Kr}) and have intermediate unbinding kinetics ($\tau > 1$ but <12 s). Class IC agents, like propafenone or flecainide, generally produce little to no effect on ventricular APD and manifest slow unbinding kinetics from the sodium channel ($\tau > 12$ s). However, recently it became evident that propafenone may cause atrial-selective APD prolongation [42], like many other agents that inhibit I_{Kr} (amiodarone, ranolazine, AZD1305, E-4031, etc.) [43, 44]. While amiodarone is classified as Class III antiarrhythmic agents (prolonging APD), this agent potently blocks early I_{Na} as well (predominantly in atria vs. ventricles; with a rapid kinetics, corresponding to Class IB) [45, 46], contributing to anti-AF properties of this drug. Most of the "novel" anti-AF AADs (ranolazine, vernakalant, AZD1305, AVE0118 etc.) potently block peak I_{Na} (in an atrial-selective manner [44]), but have not been classified under the Vaughan Williams classification scheme.

A critical and unique feature of I_{Na} blockers related to their antiarrhythmic actions is their ability to produce rate-dependent PRR, i.e., to prolong ERP without APD prolongation or to a greater extent than APD prolongation. The effectiveness of most I_{Na} blockers to inhibit I_{Na} is typically enhanced following acceleration of activation rate, a phenomenon termed "use-dependence" [46, 47]. This property has long been recognized to be very useful for suppression of rapid arrhythmias (including AF), while causing a relatively small effect at normal activation rates. The rate dependence of I_{Na} blocker is related to a generally higher affinity of I_{Na} blockers for the open and/or inactivated state of the sodium channels (i.e., during the action potential) than to the rested channels (i.e., during the diastolic interval, when net unbinding occurs). Acceleration of heart rate increases the proportion of time during which the sodium channels are in open and/or inactivated states vs. rested state. The efficacy of sodium channel blockade is normally augmented by depolarization of resting membrane potential (RMP), due to increases in the fraction of inactivated vs. rested sodium channels as well as slowed unbinding kinetics at these more positive potentials. APD shortening tends to reduce the efficacy of sodium channel blockade due to a relative decrease of the time during which the sodium channels remain in the inactivated state vs. rested state [45–47].

In the clinic, Class IC and Class IA (which also reduce I_{Kr}), but not Class 1B (relatively selective I_{Na} blockers) agents can effectively suppress paroxysmal AF. Class IA agents, however, are rarely used because of the risk of induction of TdP due to delayed ventricular repolarization [48]. Due to absence of "pure" I_{Na} blockers with slow/medium kinetics, it remains unknown whether a "pure" I_{Na} block is capable of effectively suppressing clinical AF. Note that pilsicainide, a purported highly selective early I_{Na} blocker with slow kinetics (a Class IC agent, used in Japan [49]) inhibits I_{Kr} and may cause long QT [50]. Very high (toxic) concentrations of "pure" I_{Na} blockers (lidocaine and TTX) can be very effective in preventing/terminating AF in experimental and theoretical settings because of their effects to reduce excitability at these high levels of I_{Na} inhibition [51, 52].

The antiarrhythmic mechanisms underlying anti-AF efficacy of I_{Na} blockers is multi-factorial, involving rate-dependent prolongation of ERP (largely due to PRR), depression of excitability, impaired impulse propagation, as well as non-I_{Na} -mediated influences, i.e., the prolongation of APD, due to inhibition of I_{Kr}. It is important to recognize that APD prolongation can significantly promote block of I_{Na}, secondary to reducing diastolic interval, during which much of the recovery from block occurs, and a more positive take-off potential, both resulting in reduced availability of sodium channels. The development of PRR secondary to reduced excitability appears to be the most important anti-AF action of I_{Na} blockers (Figs. 8.2 and 8.3) [39, 42, 43, 53, 54]. Inhibition of both peak and late I_{Na} also contributes to suppression of DADs, EADs, and automaticity, which contribute to the anti-AF action of the sodium channel blockers [55, 56].

It has long been appreciated that I_{Na} blockers (Class IC) can effectively prevent and terminate paroxysmal AF, but not persistent AF [57]. This

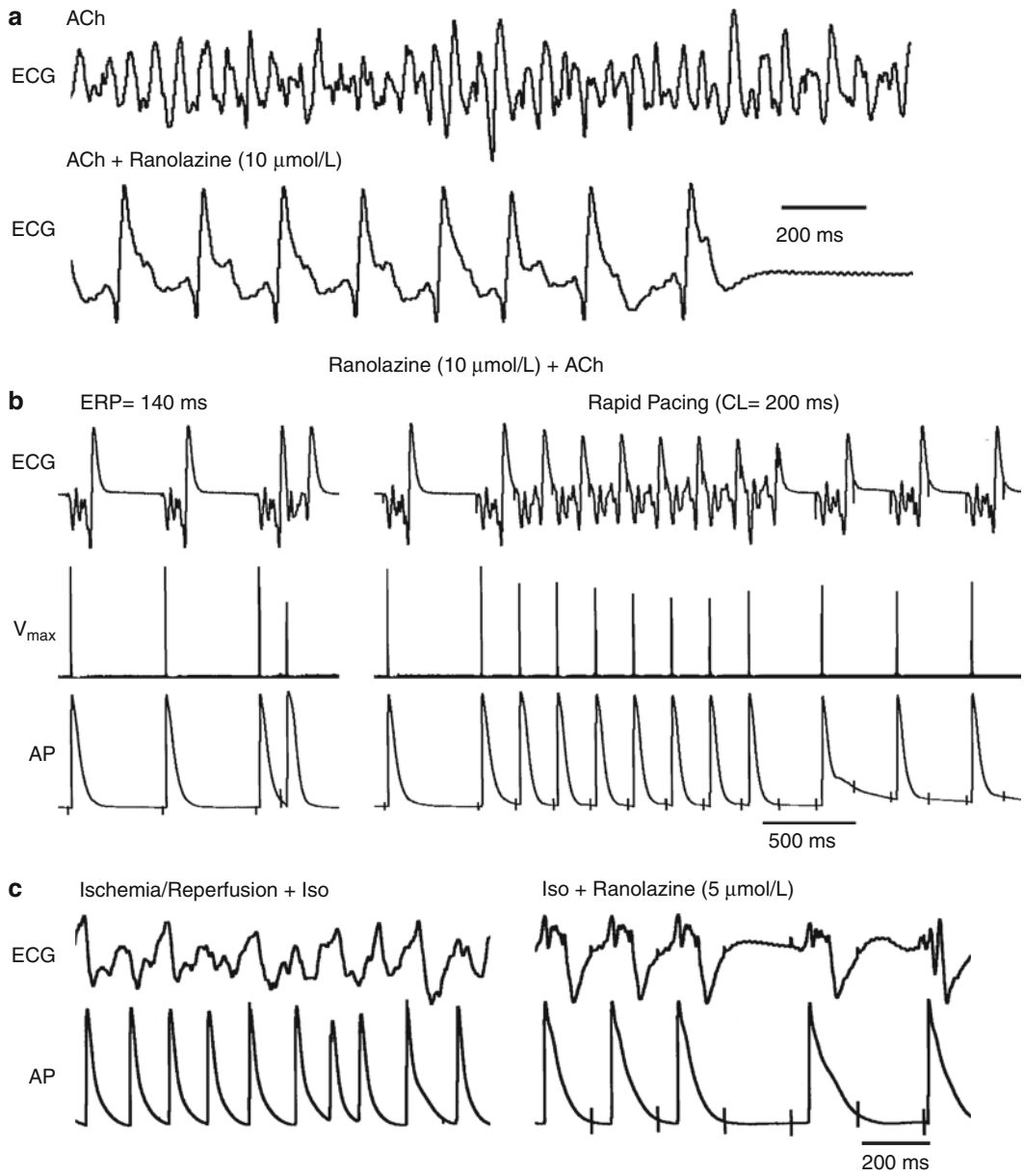

Figure 8–3. Ranolazine suppresses AF and/or prevents its induction in two experimental models involving isolated canine arterially-perfused right atria. (**a**) Persistent ACh (0.5 μM)-mediated AF is suppressed by ranolazine (10 μM). AF initially converts to flutter and then to sinus rhythm. (**b**): ERP measured at a CL of 500 ms is 140 ms. Attempts to re-induce AF fail because ranolazine-induced depression of excitability leads to 1:1 activation failure soon after CL is reduced from 500 to 200 ms (*right panel*). (**c**) Rapid-pacing induces non-sustained AF (48 s duration) following ischemia/reperfusion plus isoproterenol (Iso, 0.2 μmol/L) (*left panel*). Ranolazine (5 μM) prevents pacing-induced AF due to 1:1 activation failure (*right panel*). In both models, ranolazine causes prominent use-dependent induction of post-repolarization refractoriness (Reproduced with permission from Burashnikov et al. [39] with kind permission from Wolters Kluwer Health)

may be due to electrical remodeling causing atrial APD abbreviation as well as to significant structural remodeling, which is observed in patients with long-enduring AF [58]. Structural remodeling can contribute to conduction slowing/disturbances. It is unclear if the ability of I_{Na} blockers

to inhibit peak I_{Na} is reduced in persistent AF. The efficacy of Class IC agents to depress I_{Na} -mediated parameters remains well preserved in remodeled atria of goats [59, 60]. Class IC drug-induced prolongation of AF cycle length needed for termination of persistent AF becomes progressively longer with continuation of AF [59].

I_{Na} blockers that dissociate rapidly form the sodium channels (e.g., ranolazine, chronic amiodarone and AZD1305) have been shown to produce atrial-selective depression of sodium channel-dependent parameters and to effectively suppress AF in canine coronary-perfused atrial preparations at concentration that cause little or no effect in the ventricles [39, 43, 54, 61–63]. The atrial selectivity and anti-AF efficacy of ranolazine, AZD7009, and AZD1305 have been demonstrated in porcine and canine hearts *in vivo* and *in vitro* [54, 61, 62]. Vernakalant also appears to be an atrial-selective I_{Na} blocker since it slows conduction velocity in atria, but not in the ventricles and prolongs atrial ERP largely due to induction of PRR [64, 65].

Dronedarone, an amiodarone derivative, causes atrial but not ventricular PRR in canine and porcine hearts [40, 66]. The dronedarone-induced PRR is much less than that induced by amiodrone. Dronedarone's inhibition of peak I_{Na} is very voltage-dependent in guinea pig ventricular myocytes [66], which may contribute to dronedarone's atrial-selective induction of PRR, owing to the fact that the resting membrane potential of atrial cells is more depolarized than that of ventricular cells.

A number of factors underlie the atrial-selective effects of I_{Na} blockers, including a more negative steady-state inactivation relationship, a more positive resting membrane potential (RMP), and a more gradual phase 3 of the action potential in atrial vs. ventricular cells [39, 67–69]. The more negative half-inactivation voltage and more positive RMP importantly reduce the fraction of resting channels in atria vs. ventricles at RMP. Because recovery from sodium channel block occurs predominantly during the resting state of the channel, accumulation of sodium channel block is expected to be greater in atria vs. ventricles.

There is significant variability in the degree to which sodium channel blockers are atrial-selective [67, 68, 70]. Available data suggest that binding affinity of the I_{Na} blocker for a given state of the channel (i.e., open, inactivated, or resting) does not determine the drug's atrial-selectivity (for review see [67, 71]). The rate of dissociation of the drug from the sodium channel, however, appears to be key. I_{Na} blockers possessing rapid vs. slow unbinding kinetics tend to be highly atrial-selective (e.g., ranolazine, vernakalant, chronic amiodarone, but not propafenone) [42, 67, 69]. Agents like flecainide and propafenone, which dissociate relatively slowly from the sodium channel, allow for accumulation of block in both atria and ventricles at rapid rates, leading to absence of atrial-selectivity and in some cases ventricular-predominant effects [42, 72].

Atrial selective I_{Na} blockers have been shown to be effective in the management of AF in the clinic. A number of clinical studies have demonstrated the ability of ranolazine to prevent the induction of AF and terminate paroxysms of AF using a "pill-in-the-pocket" approach [73–76]. AZD7009 and AZD1305 have been reported to effectively suppress clinical AF [77–79]. Amiodarone is the best available agent for the long-term maintenance of sinus rhythm in AF patients. All of these agents are atrial-selective I_{Na} blockers (ranolazine, amiodarone, AZD7009, and AZD1305), that also inhibit other ion channels (particularly I_{Kr}), which is likely to importantly contribute to their atrial selectivity and anti-AF efficacy (discussed below). The degree to which the clinical efficacy of these AADs depends on block of I_{Na} remains to be determined. Of note, lidocaine, a "mild" atrial selective I_{Na} blocker [39] is not particularly effective against clinical AF, presumably because it is a relatively "pure" I_{Na} blocker.

Potassium Channel Block for AF

The common functional manifestation of block of potassium channels is the prolongation of APD_{90} and, thus, lengthening of ERP (see Fig. 8.2). The most prominent clinically- proven anti-AF potassium channel blockers are those that inhibit the rapidly activating delayed rectified potassium current (I_{Kr}; such as dofetilide, sotalol, ibutilide). Anti-arrhythmic

agents causing prolongation of APD are commonly classified as the Class III agents. Anti-AF efficacy of the slowly activating delayed rectified potassium current (I_{Ks}) has also been reported [80, 81], but remains poorly defined. It appears that the anti-AF effectiveness of I_{Ks} block is poor, but that it can enhance the anti-AF efficacy of I_{Kr} inhibition [81]. It is noteworthy that many multiple ion channel blockers inhibit I_{Ks} (e.g., amiodarone and quinidine).

There has been great interest in recent years in the anti-AF effectiveness of inhibition of atrial-specific potassium channels, including the channels that carry the ultra-rapid delayed rectifier potassium current (I_{Kur}), the acetylcholine-regulated inward rectifying potassium current ($I_{K\text{-}ACh}$), and the constitutively active $I_{K\text{-}ACh}$ (i.e., which does not require acetylcholine or muscarinic receptors for activation) [44, 82].

Block of $I_{K\text{-}ACh}$ or constitutively active $I_{K\text{-}ACh}$ may suppress vagally-mediated AF or AF in which vagal activity contribute to the initiation of paroxysmal AF. Clinical data indicate that vagal components contribute to the initiation of paroxysmal AF [32, 83]. Block of $I_{K\text{-}ACh}$ currents with tertiapin-Q prolongs atrial APD and suppresses AF in experimental models [84, 85]. Interestingly, CA-I_{KACh} is only marginally present in healthy non-fibrillating human or canine atria and is significantly increased in atria of chronic AF patients and canine tachycardia-remodeled atria [84, 86–88], suggesting that this current is a pathology-specific as well as atrial-specific target [89]. At present, a CA-I_{KACh}–selective blocker is not available.

I_{Kur} is the most investigated atrial-specific ion channel current and until recently was widely considered to be the most promising among atrial-specific targets for the treatment of AF [82, 90]. Enthusiasm for specific I_{Kur} blockers for the management of AF has diminished in recent years [67, 91–94]. Inhibition of I_{Kur} alone abbreviates APD and ERP in "healthy" atrial cells and produces only a minor ERP prolongation in remodeled atrial cells. Available data indicate that inhibition of I_{Kur} alone is ineffective against AF [91, 93, 94]. In fact, all prominent I_{kur} blockers (e.g., vernakalant, AZD1305 and AVE0118) inhibits peak I_{Na} and their atrial selective ERP prolongation is largely or exclusively due inhibition of peak I_{Na}, not I_{Kur} [44]. The apparent failure of pure I_{Kur} inhibition for suppression of AF may be explained by several factors. First, the contribution of I_{Kur} during AF is likely to be relatively small because I_{Kur} density is reduced with acceleration of activation rate [95]. Second, the contribution of I_{Kur} to atrial repolarization may be reduced in AF patients, since I_{Kur} density is reported to be decreased in cells isolated from atria of patients with chronic AF [96, 97].

Among the potassium currents, I_{Kr} block is the only one that has been proven to be widely effective against AF in the clinic. It is of some interest that all clinically-effective I_{Na} blockers also inhibit potassium currents (primarily I_{Kr}).

I_{Kr} blockers inhibit the channel in its open state, i.e., during phases 2 and 3 of the action potential. As a consequence, the inhibiting efficacy of I_{Kr} blockers is importantly determined by the pre-drug APD, i.e., the shorter pre-drug APD the smaller APD prolongation induced by I_{Kr} block and vice versa. The efficacy of I_{Kr} blockers to prolong APD is also rate-dependent, i.e., the faster the rate the weaker the efficacy. Thus, I_{Kr} block-induced APD prolongation is the smallest at rapid activation rates, where ERP prolongation is most desirable for AF suppression. This apparent disadvantage of I_{Kr} blockers for suppression of cardiac arrhythmias has been long recognized [98, 99].

Prolongation of ERP secondary to prolongation of AP is the primary anti-AF mechanism of I_{Kr} and other K channel blockers (see Fig. 8.2). Indeed, ERP prolongation has been associated with prevention and termination of AF in a vast majority cases with I_{Kr} blockers [100]. The ability of I_{Kr} blockers to prolong APD/ERP is decreased in remodeled atria in humans as well as in animal models with persistent AF (where APD is commonly short) [60, 101]. This may account for or contribute to a reduction of success rate of I_{Kr} inhibition to terminate persistent AF [57]. Still, I_{Kr} block using dofetilide or ibutilide remains the best available pharmacological approach for termination of persistent AF [57].

Multiple Channel Blockers for AF

With the exception of selective I_{Kr} blockers, such as dofetilide and sotalol, all clinically effective anti-AF agents inhibit multiple ion channels,

principally peak I_{Na} and I_{Kr} (e.g., amiodarone, flecainide and propafenone). Recent studies have highlighted the importance of the ability of I_{Na} blockers to also block I_{Kr} and prolong the atrial AP. I_{Kr} block produces atrial-predominant prolongation of ERP/APD [43, 102], which significantly promotes block of peak I_{Na} particularly at rapid activation rate (Fig. 8.4). This translates into a greater development of atrial-selective PRR. Atrial-selective ERP prolongation is a desirable property of anti-AF drugs, which reduces the probability of proarrhythmia in the ventricles [69, 103].

Prolongation of APD leads to a more depolarized take-off potential as well as loss of the diastolic interval at rapid rates of activation. Both effects contribute to reduced availability of sodium channels for activation. Prolongation of APD is especially effective in potentiating the effect of I_{Na} blockers that dissociate rapidly from the sodium channel, such as amiodarone and ranolazine (Fig. 8.4). This synergism of combined I_{Na} and I_{Kr} block forms the basis for the high anti-AF efficacy of these multi-ion channel inhibitors. Rapid dissociation of I_{Na} blockers from the sodium channel also contributes to their atrial selectivity, because ventricular tissues maintain their diastolic intervals at rapid rates, allowing dissociation of the drug before the next beat. The loss of the diastolic interval in atria slows drug dissociation, thereby allowing for the accumulation of block beat to beat [67].

The effect of I_{Na} inhibition can be potentiated by any mechanism that prolongs the atrial APD, including block of I_{Kur} and $I_{K\text{-}ACh}$. AVE0118 and vernakalant can do it by blocking I_{Kur}, whereas AVE0118 and the Chinese herbal medicine Wenxin Keli can do it via an anticholinergic effect [104].

Combinations of drugs may produce potent synergistic prolongation of ERP, providing a better anti-AF potential than single drug therapy. In a tachypacing-induced AF goat model, AVE0118 (an I_{Kur}/I_{to} and I_{Na} blocker) was shown to restore the ability of I_{Kr} blockers to prolong atrial ERP (this ability is significantly reduced in remodeled atria [60, 101]), greatly improving anti-AF efficacy [105]. With the continuation of persistent AF for 4–6 months, even the combination of AVE0118 and dofetilide gradually loses its ability to terminate AF (100 % failure when AF continues ≥4 month) [106].

Recent studies conducted in canine coronary-perfused atrial and ventricular preparations have shown a remarkable synergism when a relatively low concentration of ranolazine (5 μM) is combined with either chronic amiodarone or acute dronedarone, leading to atrial-selective depression of I_{Na}-dependent parameters and effective suppression of AF (Fig. 8.5) [40, 107]. Individually, dronedarone or a low concentration of ranolazine prevented the induction of AF in 17 and 29 % of preparations, respectively. In combination, the two drugs suppressed AF and triggered activity and prevented the induction of AF in 9 of 10 preparations (90 %) [40]. This drug combination produces a profound synergism in the ability to suppress peak I_{Na} and excitability in a use-dependent and atrial-selective manner so that the atria remain largely unaffected until rapid activation begins and the ventricles remain largely unaffected under all conditions. A Phase 2, Proof of Concept, Randomized, Placebo-Controlled, Parallel Group Study to Evaluate the Effect of Ranolazine and Dronedarone When Given Alone and in Combination on Atrial Fibrillation Burden in Subjects with Paroxysmal Atrial Fibrillation called ***Harmony*** is currently underway.

Conclusion

Safe and effective pharmacological management of AF remains one of the greatest unmet medical challenges facing our society today. With AF approaching epidemic proportions, the critical need to develop safe and effective drugs to suppress AF is undeniable. Recent years have witnessed important advance in our understanding of the mechanisms by which AF develops and the mechanisms by which drugs act to prevent its induction, yet our approach to pharmacologic therapy of AF remains empiric and largely disappointing. Drug-induced prolongation of ERP remains the most consistent factor associated with positive outcomes and future research should be directed at improving our ability to suppress excitability and prolong ERP in a use-dependent and atrial-selective manner so that the atria remain largely unaffected until rapid activation begins and the ventricles remain largely unaffected under all conditions. It appears clear that a multi-ion

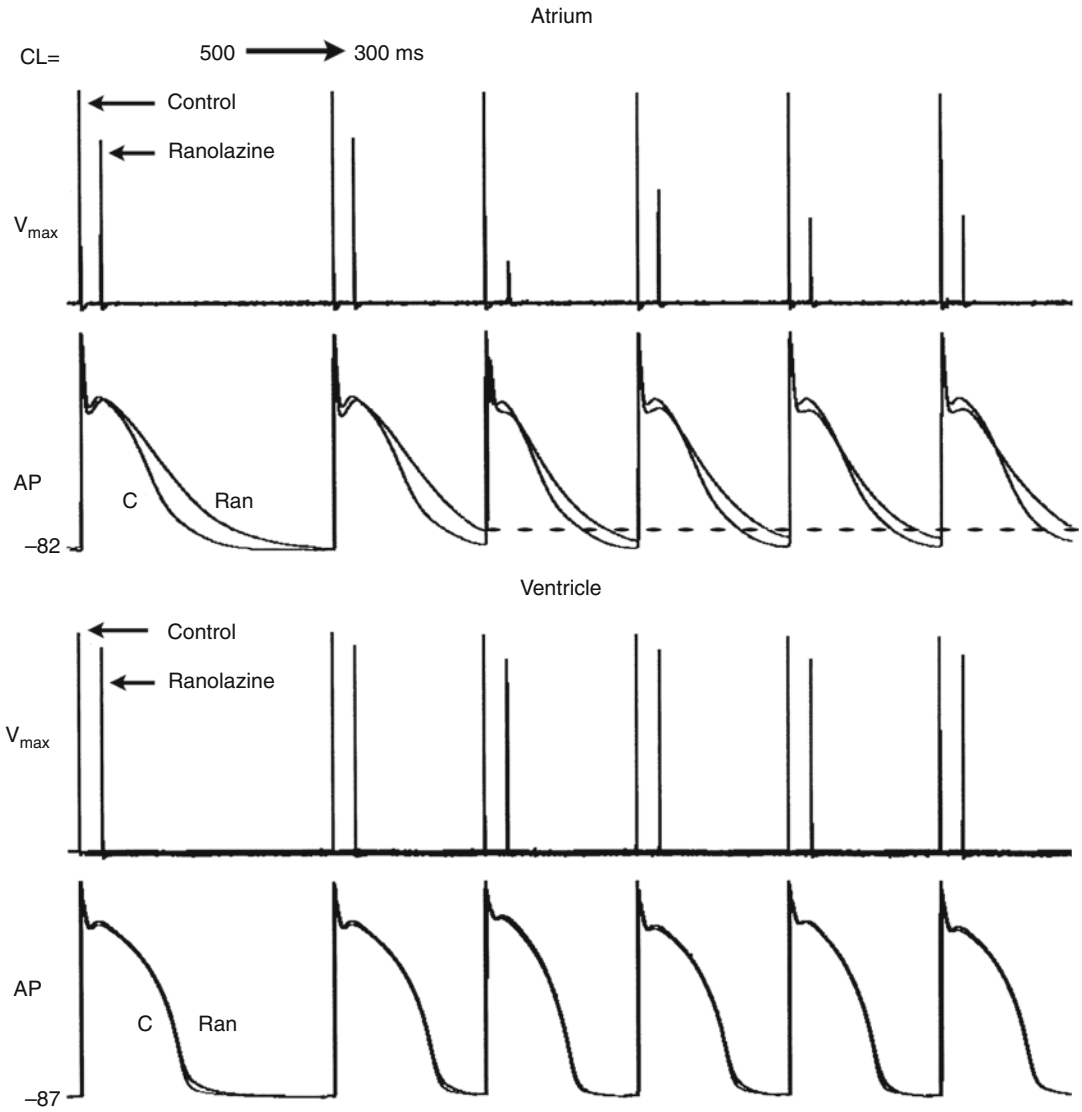

Figure 8–4. Ranolazine produces a much greater rate-dependent inhibition of the maximum action potential upstroke velocity (V_{max}) in atria than in ventricles. Shown are V_{max} and action potential (*AP*) recordings obtained from coronary-perfused canine right atrial and left ventricular preparations before (*C*) and after ranolazine (10 μM). Ranolazine prolongs AP duration in atria, but not the ventricles (due to I_{Kr} inhibition [43]). Acceleration of rate leads in the presence of ranolazine leads to a more positive take-off potential and elimination of the diastolic interval. Because much of the recovery from sodium channel block occurs during the diastolic interval, both effects lead to a reduced availability of sodium channels. Loss of the diastolic interval in atria but not in the ventricle accounts for the atrial selective depression of sodium channel current as signified by Vmax (Reproduced from Antzelevitch and Burashnikov [71], with kind permission from Elsevier)

channel approach is preferable to highly selective ion channel inhibition. Although not highlighted in this chapter, prevention via upstream therapy aimed at minimizing structural changes that promote AF should be an important goal of future research. Improvement of atrial conduction may be a useful anti-AF approach in some AF pathologies, but this approach remains poorly investigated. Better understanding of the mechanisms of AF generation and drug actions in different AF pathologies are needed. These insights will hopefully guide us to improved pharmacological management of patients with AF.

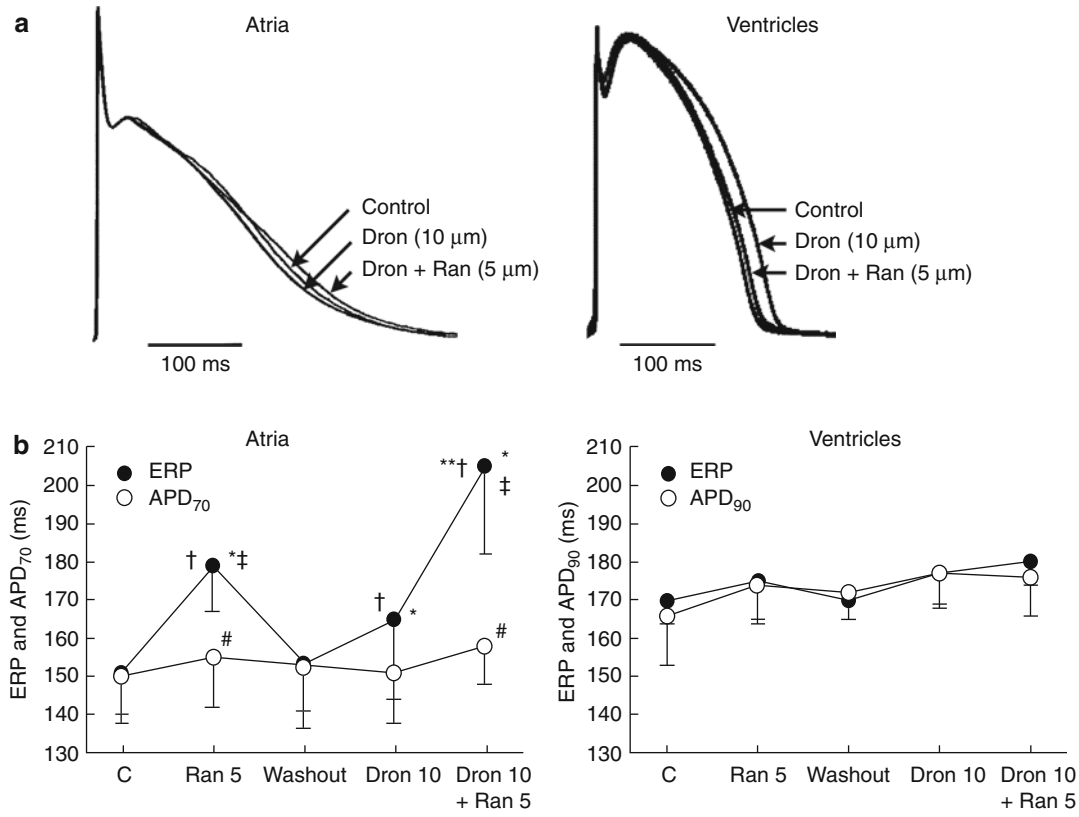

Figure 8–5. Atrial-selective induction of post-repolarization refractoriness (PRR) by ranolazine (*Ran*), dronedarone (*Dron*) alone and in combination (PRR) was approximated by the difference between effective refractory period (ERP) and action potential duration measured at 70 % repolarization (APD_{70}) in atria and between ERP and APD measured at 90 % repolarization (APD_{90}) in ventricles; ERP corresponds to $APD_{70–75}$ in atria and to APD_{90} in ventricles. (**a**) Shown are superimposed action potentials demonstrating relatively small changes with dronedarone and ranolazine and their combination. (**b**) Summary data of atrial-selective induction of PRR. Ventricular data were obtained from epicardium and atrial data from endocardial pectinate muscle (PM). n = 7–8. *p < 0.05 vs. respective control (C). †p < 0.05 vs. washout. ‡p < 0.05 vs. Dron 10. #p < 0.05 vs. respective ERP. **p < 0.05 – change in ERP induced by combination of Ran and Dron (from washout) vs. the sum of changes caused by Ran and Dron independently (both from washout). CL = 500 ms (Reproduced from Burashnikov et al. [40], with kind permission from Elsevier)

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9
Mechano-Electric Interactions and Their Role in Electrical Function of the Heart

J. Jeremy Rice and Peter Kohl

Abstract

The heart is an electrically controlled and chemically powered mechanical pump. There are complex interactions between cardiac structure and function, including electrophysiology, metabolism, and mechanics. These are based on a multitude of interdigitating regulatory loops with different inherent time-scales. This chapter will focus on the *acute cross-talk* between electrical and mechanical activity of the heart, and in particular its relevance for normal and abnormal heart rhythms.

Keywords

Excitation-contraction coupling • Mechano-electric coupling/mechano-electric feedback • Heart • Cardiomyocyte • Calcium handling • Stretch • Stretch-activated channel

Abbreviations

AP	Action potential
AT-II	Angiotensin II
ATP	Adenosine tri-phosphate
$[Ca^{2+}]_i$	Free cytosolic Ca^{2+} concentration
Ca^{2+}	Calcium
ECC	Excitation-contraction coupling
ET-1	Endothelin-1
ILCOR	International Liaison Committee on Resuscitation
LCC	L-type Ca^{2+} channel
MEC	Mechano-electric coupling
NCX	Na^+/Ca^{2+} exchanger
NHX	Na^+/H^+ exchanger
PT	Precordial thump
RyR	Ryanodine receptor
SAC, SAC_{NS}, SAC_K	Stretch activated channels of various ion selectivity

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SERCA	Sarcoplamsic/endoplasmic reticulum Ca^{2+}-ATPase
SL	Sarcomere length
SR	Sarcoplasmic reticulum
TnC, TnI, TnT	Troponin C, Troponin I, Troponin T
VF	Ventricular fibrillation
VT	Ventricular tachycardia

Integrated Cardiac Electro-Mechanics

Introduction

The heart is an electrically controlled and chemically powered mechanical pump. There are complex interactions between cardiac structure and function, including electrophysiology, metabolism, and mechanics. These are based on a multitude of interdigitating regulatory loops with different inherent time-scales. This chapter will focus on the *acute cross-talk* between electrical and mechanical activity of the heart, and in particular its relevance for normal and abnormal heart rhythms.

The cross-talk between cardiac electrics and mechanics can be viewed conceptually as a regulatory loop (Fig. 9.1). In this loop, electrical control of cardiac contraction is afforded by *Excitation-Contraction Coupling* (ECC) while, in turn, the mechanical environment affects cardiac electrical activity via *Mechano-Electric Feedback*, now commonly referred to as *Mechano-Electric Coupling* (MEC). This regulatory loop manifests itself at every level of structural integration, from whole heart to single cell. It supports the maintenance of steady-state cardiac performance, ensures adaptation to changes in circulatory demand (even in the de-innervated, transplanted, or artificially paced heart), and can contribute to the initiation, maintenance, and termination of heart rhythm disturbances [1].

Spatio-Temporal Considerations

The heart is a spatially heterogeneous organ, both in terms of structure (e.g. variations in regional tissue architecture, cell distribution, coupling, innervation, or blood supply) and function (active and passive tissue electro-mechanical properties). In addition, there are temporal gradients in key electro-mechanical behaviour (e.g. activation, repolarization, contraction, relaxation). Nonetheless, under physiological conditions the heart functions as a highly coordinated unit. This 'externally homogeneous' mechanical performance at the organ level arises from, and *necessarily requires*, structural and functional heterogeneity, from subcellular to whole organ levels [2].

While this appreciation has guided our thinking on structural and functional adaptation of the heart to (patho-)physiological developments, it leaves the question as to how an individual cardiomyocyte inside this complex organization 'knows' when and how to respond to beat-by-beat changes in electro-mechanical activity. The *when* is largely a function of the coordinated

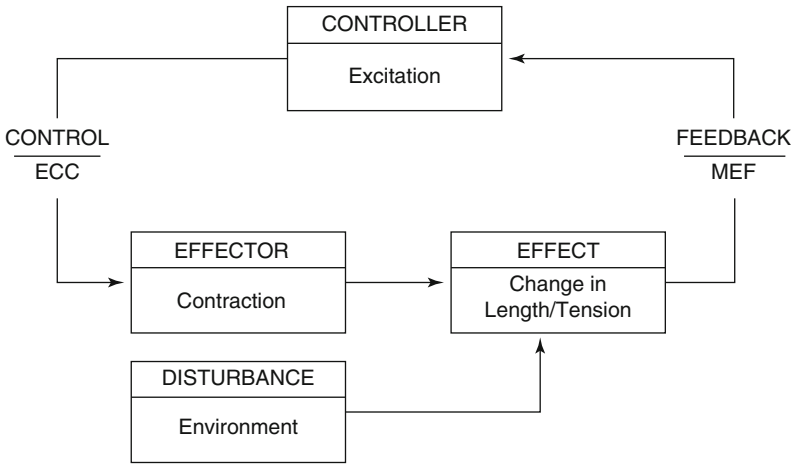

Figure 9–1. Conceptual scheme of cardiac electro-mechanical regulation. Electrical control of cardiac contraction is via *Excitation-Contraction Coupling* (*ECC*), while the mechanical environment affects cardiac electrical behaviour via *Mechano-Electric Feedback* (*MEF*) (From Kohl et al. [1]. Reprinted with kind permission from Elsevier)

spread of electrical excitation. The *how* requires sub-cellular regulatory pathways that match local mechanical performance to global mechanical demand; underlying processes are discussed next.

Excitation Contraction Coupling

Introduction

The process of ECC involves a transient rise in free cytosolic calcium concentration ($[Ca^{2+}]_i$), as the intermediate signal between electrical depolarization of the cell membrane and activation of contractile myofilaments. The sequence is initiated by calcium (Ca^{2+}) influx across the sarcolemma, which produces a secondary release of Ca^{2+} from the sarcoplasmic reticulum (SR, a specialized Ca^{2+}-storage compartment in mammalian cardiac myocytes). The SR can rapidly release Ca^{2+} in response to sarcolemmal Ca^{2+}-influx, a process termed 'Ca^{2+}-induced Ca^{2+}-release'. The released Ca^{2+} then binds to several intracellular Ca^{2+}-binding proteins, including troponin, which in turn activates the myofilaments. The majority of Ca^{2+} is re-sequestered back into the SR during each heartbeat, while the remainder (in steady-state this is an amount equivalent to the initial trans-sarcolemmal influx) is extruded across the membrane.

In reality, the events involved in ECC are intimately connected and cannot be decomposed into discrete sets of spatially or temporally defined stages. For example, SR Ca^{2+}-release occurs simultaneously with re-uptake, with the net effect depending on the relative balance of the competing processes at any given time. Also, SR Ca^{2+}-storage involves 'memory' mechanisms that render the amount of Ca^{2+} stored in, and released from, the SR strongly dependent on stimulation history. Thus, the system shows complex behavior within single beats and between multiple beats. The following sections outline separate steps in the activation and relaxation sequence of cardiac muscle. However, care must be taken to remember that this decomposition into discrete steps is highly artificial and underestimates the complex dynamics of ECC [3, 4].

From Action Potential to Calcium Release

Figure 9.2 shows a conceptual model of Ca^{2+}-handling during a typical heartbeat. The trigger events (Fig. 9.2a) involve sarcolemmal influx of Ca^{2+} through L-type Ca^{2+} channels (LCC; thick downward arrow), which produces a secondary and larger release of Ca^{2+} from the SR (upward thick arrow), released via ryanodine receptors

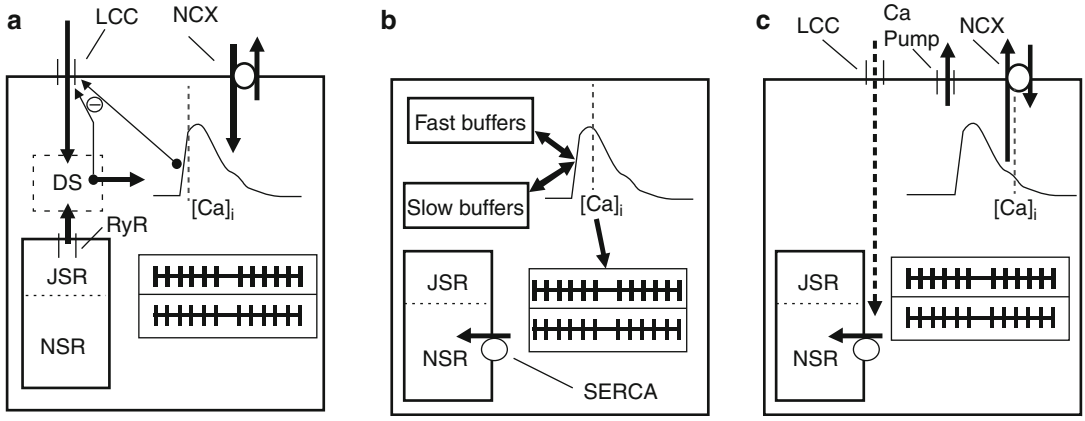

Figure 9–2. Schematic diagram of a cardiac cell, showing three conceptual steps in excitation-contraction coupling (ECC), with pseudo-representative timing relative to cytosolic free calcium-concentration ($[Ca^{2+}]_i$) dynamics indicated by grey dotted lines. (**a**) Ca^{2+}-induced Ca^{2+}-release; (**b**) Cytosolic Ca^{2+}-buffering and contraction changes; (**c**) Ca^{2+}-reuptake and -extrusion (for detail see text). *DS* dyadic space, *JSR* junctional sarcoplasmic reticulum, *LCC* L-type Ca^{2+} channel, *NCX* Na^+/Ca^{2+} exchanger, *NSR* network sarcoplasmic reticulum, *RyR* ryanodine receptor, *SERCA* sarcoplasmic/endoplasmatic reticulum Ca^{2+}-ATPase

(RyR) into the diadic space (dotted box labeled DS). An important negative feedback pathway exists in that the released Ca^{2+}, as well LCC influx itself, cause Ca^{2+}-induced inactivation of LCC, which reduces further influx (upward thin arrow with 'minus' sign). In addition, the cytoplasmic Ca^{2+} level can also produce inactivation of LLC. From the diadic space, Ca^{2+} diffuses into the myoplasm (thick rightward arrow) to increase $[Ca^{2+}]_i$ from approximately 0.1 to 1 μM (represented by the schematic Ca^{2+}-transient). The majority of this released Ca^{2+} binds to intracellular buffers, including troponin and calmodulin (not shown). Ca^{2+} can also enter the cell during the action potential (AP) upstroke and subsequent 'notch' (rapid partial repolarisation prior to the AP plateau) via the Na^+/Ca^{2+} exchanger (NCX), although this pathway is considered less important for ECC than Ca^{2+}-influx via LCC.

L-Type Ca^2+ Channel Influx

The LCC is the major influx pathway for Ca^{2+} during each heartbeat. This current has multiple roles in producing the Ca^{2+}-transient, both by directly increasing $[Ca^{2+}]_i$, and by triggering a larger secondary Ca^{2+}-release from the SR. Moreover this current contributes to AP morphology, especially in sustaining the AP plateau in spite of repolarizing K^+ currents. LCCs are activated by voltage and inactivated by both voltage and Ca^{2+}, but of the two, Ca^{2+}-based inactivation predominates in determining the amplitude and time-course of LCC currents [5, 6]. One potential role of this negative feedback (Fig. 9.2a, thin arrow) is to limit Ca^{2+}-influx after triggering Ca^{2+}-induced Ca^{2+}-release. However, longer-term roles in Ca^{2+}-homeostasis of both intracellular and SR Ca^{2+}-levels are also proposed [4]. Interestingly the LLC inactivates in response to both dyadic space Ca^{2+} and cytoplasmic Ca^{2+} (as shown schematically in Fig. 9.2), in spite of favored physical proximity and higher amplitude of the dyadic space Ca^{2+} signal. Recent findings at the molecular level have elucidated a temporally based mechanism that can separately sense both the high amplitude, Ca^{2+} impulses in dyadic space and the low amplitude, prolonged Ca^{2+} levels in the cytoplasm [6–8]. The Ca^{2+}-based inaction involves molecular interactions of the channel with a constitutively bound calmodulin, whose genetic manipulation to remove the negative feedback has surprisingly large effects on AP duration (lengthening it by four to five times) [5]. Thus, key electrophysiological parameters can be significantly more sensitive to Ca^{2+}-handling than customarily assumed.

SR Ca^2+ Release via RyR Channels

Ca^{2+}-release from the SR via RyR has been shown to be roughly proportional to the trigger influx of Ca^{2+} via LCC, although some variation exists, depending on how Ca^{2+}-flux is determined [8]. The system shows high gain, in that a small number of LLC will trigger release from a nearby cluster of RyR channels that open synchronously. As the discrete opening of LCC can modulate the number of activated RyR clusters, the large total Ca^{2+}-release tracks roughly linearly with total LCC Ca^{2+}-influx.

Another important property is that SR Ca^{2+}-release is a nonlinear, steeply increasing function of SR Ca^{2+}-load. Essentially no SR Ca^{2+}-release occurs below a threshold, after which SR rises steeply with SR load [8, 9]. Interestingly, there are indications that more than 100 % of the original SR Ca^{2+}-content can be released, suggesting that Ca^{2+} is recycled back into SR during the release process, and re-released again [8]. For a typical beat, though, Ca^{2+}-release fraction is estimated at 60–80 % [9]. Interestingly, there appears to be a set maximum SR Ca^{2+}-load beyond which spontaneous RyR Ca^{2+}-release occurs, with potential for arrhythmogenesis. In addition, spontaneous RyR Ca^{2+}-release appears to also play critical roles in determining resting $[Ca^{2+}]_i$.

From Calcium Release to Contraction

The translation of $[Ca^{2+}]_i$ to force generation occurs at the level of the sarcomere, i.e. the basic subcellular unit of the contractile apparatus (Fig. 9.3). The sarcomere is composed primarily of interdigitated thick myosin filaments that interact, when activated, with thinner actin filaments (for reviews see [11, 12]).

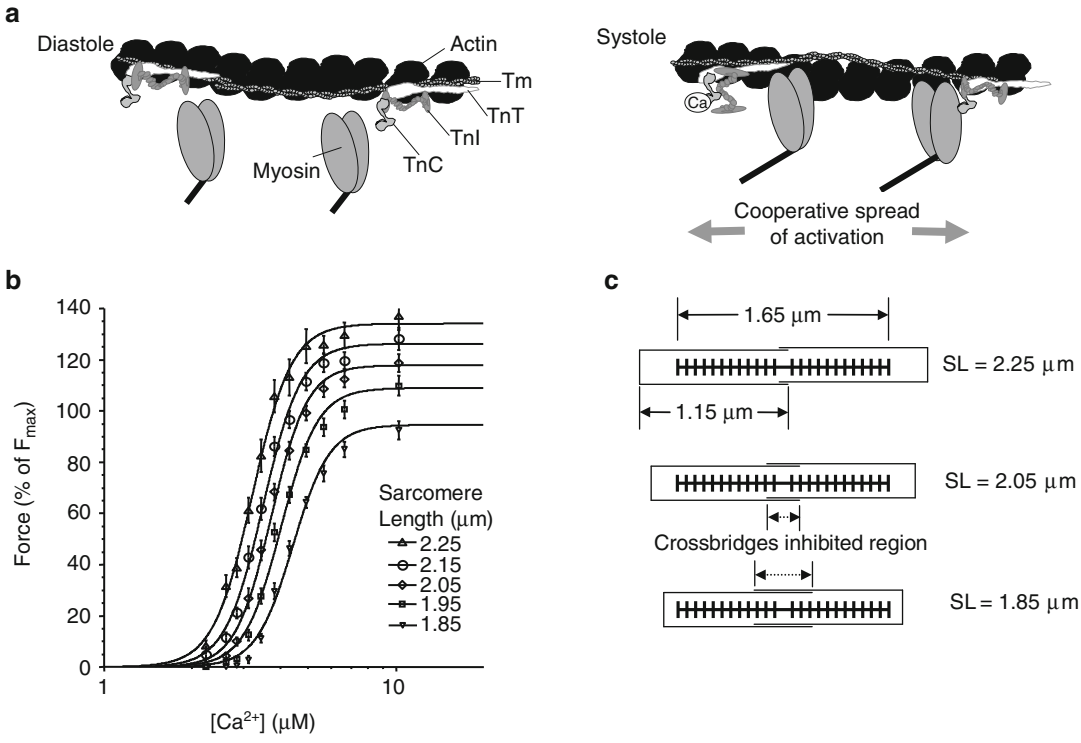

Figure 9–3. Contractile filaments and calcium. (**a**) Interrelation of thin actin filament with nearby myosin heads. During diastole (left), tropomyosin (Tm) and troponin subunits (TnC, TnT, and TnI) sterically block crossbridge formation. During systole, elevated $[Ca^{2+}]_i$ shifts the locations of tropomyosin/troponin to allow myosin head attachment and force generation. Note that cooperative activation can spread along the thin filament (see text for details) (From Bers [3]. Reprinted with permission from Springer). (**b**) Average force-Ca^{2+} relationships (pooled data, skinned rat cardiac trabeculae, n = 10) at five sarcomere lengths (*SL*); data are fitted to a Hill relationship. The level of cooperativity, assessed by Hill coefficient, is not affected by SL (From Dobesh et al. [10]. Reprinted with kind permission from The American Physiological Society). (**c**) Diagram showing expected sarcomere geometry for three of the SL shown. Thick filaments are schematically shown by the *thick lines* with protrusions to represent the myosin heads. Thin filaments are shown as *horizontal thin lines* that emanate from Z-disks, represented by the vertical lines. SL is defined as the spacing between two neighbouring Z-disks. Binding of crossbridges is assumed to be inhibited in the central region in which the thin filaments overlap, and hence the SL determines the degree of thin filament overlap and, ultimately, the number of recruitable crossbridges

While the basic interactions in the sarcomere are known, the underlying basis of several complex behavioral properties is still debated. For example, the myofilament system shows a high level of Ca^{2+}-sensitivity, as characterized by steep Force-Ca^{2+} relationships with a Hill coefficient equal to seven or more (Fig. 9.3b). Because each troponin binds a single Ca^{2+} ion on the regulatory site of Troponin C (TnC), a Hill coefficient of one is predicted; hence, the high Ca^{2+}-sensitivity is assumed to result from one or more cooperative mechanisms (for review see [13]).

Briefly, three types of cooperative interactions are most widely accepted. Attached crossbridges have been shown to increase the Ca^{2+}-affinity of TnC [14–16]. A second type of cooperativity among the regulatory proteins is thought to arise from nearest-neighbor interactions, produced by the overlap of adjacent tropomyosin units along the thin filament (Fig. 9.3a) [11, 12]. A third proposed mechanism is that the binding of one myosin head increases the binding rate of neighboring heads by holding the regulatory proteins in a more permissive conformation [17]. Alternatively, the binding of one crossbridge may

pull binding sites on a compliant thin filament into register with myosin heads that have an inherently different characteristic distance in their repeating structure [18].

Another complex phenomenon is the length-dependence of force-Ca^{2+} functions, often studied in isometric conditions (i.e. length is prescribed, while force is measured). Length-dependent effects can be separated into two main categories: changes in plateau force and changes in Ca^{2+}-sensitivity. As SL increases, changes in maximum Ca^{2+}-activated force may arise from changes in overlap of thin filaments, that increase the recruitable pool of crossbridges (Fig. 9.3c; note that the lengths of the thick and thin filaments differ from the accepted values for skeletal muscle [13, 19]). In support of this scheme, developed force and ATPase rate of maximally activated cardiac muscle are linear functions of SL [20]. The second category is a decrease in myofilament Ca^{2+}-sensitivity, as shown by the leftward shift in force-Ca^{2+} curves as SL increases (Fig. 9.3b). These length-dependent changes in Ca^{2+}-sensitivity are generally assumed to be the cellular basis of the Frank-Starling effect, although the biophysical basis is still under debate.

Another hypothesis suggests that SL changes alter the lattice spacing of the thick and thin filaments because the isovolumetric nature of cardiac cells produces a narrowing of cross-section with increasing cell length [21, 22]. Titin is a giant, infolded protein that runs the length of the thick filament and then connects across to the root of thin filaments and on to the lattice of proteins that comprise the Z-disc. Titin is ideally positioned, therefore, to sense SL (while titin is not shown in Fig. 9.3c, the span from the thick filament to the Z-disk distance is clearly a function of SL for the range shown). Indeed, selective removal of titin by mild treatment with trypsin (0.31 μg/mL for 10–15 min) affects both myofilament Ca^{2+} sensitivity and inter-filament spacing. Titin could modify crossbridge binding by modifying interfilament spacing [23], or could act by other mechanisms such as changing the angle that myosin heads emanate from the thick filament, a feature speculated to produce SL-dependent changes in Ca^{2+} sensitivity [22, 24]. In these schemes, the local concentration of myosin heads in the vicinity of the thin filament will modulate effective binding and cycling rates of crossbridges, ultimately modifying Ca^{2+}-sensitivity and developed force.

The inter-filament spacing hypothesis was proposed in the early 1980s [22] and is supported by several more recent reports [25]. However, other studies suggest that physiological changes in lattice spacing are insufficient to account for the observed changes in Ca^{2+}-sensitivity (for review see [26]). In addition to inter-filament spacing and titin effects, several other mechanisms have been proposed to explain the length-dependence of Ca^{2+}-sensitivity. Some mathematical models suggest changes in Ca^{2+}-sensitivity could result from cross-interactions of cooperative mechanisms with length-dependent changes in crossbridge recruitment [13, 19]. Clearly, more work is needed to elucidate the mechanism of this fundamental property of cardiac myofilament mechanics.

In addition to the immediate stretch-induced increase in cardiac force development, there is a secondary and more slowly occurring increase in force, which eventually reaches a plateau [27]. In contrast to the fast response, the slow force response involves an increase in Ca^{2+}-transient amplitude (Fig. 9.4) [28]. At the level of the myofilaments, there are no slow time-dependent changes in Ca^{2+}-sensitivity. In fact, length changes during the diastolic period alone are sufficient to generate a slow force response [29, 30], a finding that rules out the myofilament-based mechanisms discussed above. A full review of slow changes in force, and possible effects on membrane currents, is available elsewhere [31]. One obvious source of increased Ca^{2+} are mechanically modulated ion channels (discussed below), which either directly conduct Ca^{2+} ions, or allow Na^+ entry, which secondarily increases intracellular Ca^{2+}, for example via NCX.

More recently, contributions by second messenger systems have been proposed. Only a brief description is provided here, as more detailed reviews are available elsewhere [32]. It has been proposed that Na^+ influx occurs via the Na^+/H^+ exchanger (NHX) that may be activated by a stretch-induced increase in angiotensin II (AT-II) and endothelin-1 (ET-1) [33, 34]. More recently, several groups have proposed intermediate steps

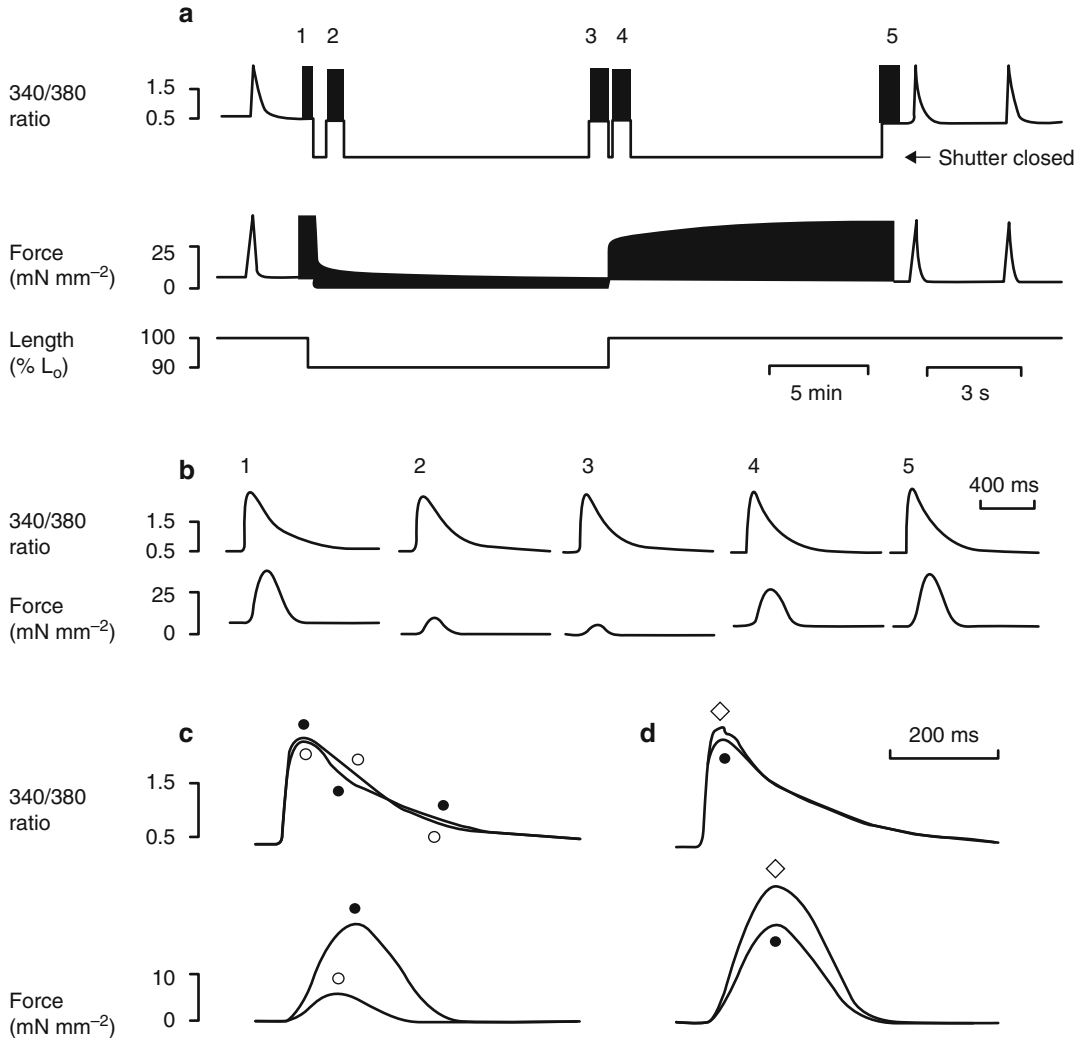

Figure 9–4. Illustration of immediate and slow changes in force after length changes. Records of the changes in fura2 fluorescence ratio and force produced by shortening a rat trabecula by 10 % for 15 min. (**a**) Records of 340 nm/380 nm fluorescence ratio and force, with a representation of the length change from the initial length (L_0). A shutter in the excitation light pathway was opened only for discrete 48 s recording periods (labelled 1–5) in order to avoid photobleaching of fura2. Note the slow changes in twitch force after the changes in muscle length. (**b**) Mean records (from 16 twitches) of fluorescence ratio and force measured during periods *1–5* in **a**. (**c**) Overlaid traces of the fluorescence ratio and force averaged during periods *3* (○) and *4* (●) to illustrate the rapid effects of the length increase. Resting forces have been subtracted from these traces. (**d**) Similar overlaid traces averaged during periods *4* (●) and *5* (◊) to illustrate the delayed effects of length increase. 24 °C, 1 mM external Ca^{2+}, 0.33 Hz stimulation rate (From Kentish and Wrzosek [28]. Reprinted with kind permission from John Wiley and Sons)

from AT-II/ET-1 activation to NCX. One pathway includes the generation of reactive oxidative species, which activate a cascade including extracellular signal-regulated kinases 1 and 2 and p90 ribosomal S6 kinase. These eventually lead to phosphorylation and activation of NHX [35]. An alternative pathways has intermediates that include phospholipase C and protein kinase C [36, 37], or cyclic adenosine monophosphate and protein kinase A [38]. Another proposed mechanism [39] involves generation of reactive oxidative species that, in turn, activate phospholipases C and A_2, which generate amphipaths (diacyl glycerol and arachidonic acid, respectively).

Amphipaths are thought to modify the local curvature of the membrane, and this can activate membrane-bound channels, including the transient receptor potential cation channel member 6 (TRPC-6), a cation non-specific stretch activated channel [40]. A complicated picture is emerging in which the second messenger systems are complex, perhaps partially redundant, and with the potential for substantial crosstalk between biophysical and biochemical properties of the cell.

Effects on the Ca^{2}+ Transient

While the exact mechanisms of length-dependent changes in Ca^{2+}-sensitivity are controversial, they may not be that important to understanding mechanical feedback on Ca^{2+}-transients. The amount of Ca^{2+} bound to the myofilaments is generally assumed to be affected by the number of attached crossbridges (instead of length itself) [14–16]. Hence, any feature that affects developed force can have secondary effects on Ca^{2+}-binding to troponin, and on the Ca^{2+}-transient.

For example, increasing muscle length by 10 % (see time-points 3 and 4 in Fig. 9.4) has a dramatic effect on developed force (see Force in Fig. 9.4b). This increase in developed force leads to greater Ca^{2+}-binding to TnC, initially lowering cytosolic $[Ca^{2+}]_i$. Later, during the Ca^{2+}-transient, $[Ca^{2+}]_i$ is slightly higher, as more Ca^{2+} is slowing coming off TnC (compared with the low force case). Similar effects on Ca^{2+}-transient have been seen elsewhere [14,28,29,41,42], although details depend on the nature of mechanical perturbations and Ca^{2+}-monitoring agents used. Taken together, the results suggest that changes in force-dependent Ca^{2+}-binding to troponin have a noticeable, but not dramatic, effect on $[Ca^{2+}]_i$ levels, which may restrict the role Ca^{2+}-buffering in mediating MEC effects in normal tissue. However, more profound MEC effects, leading to arrhythmic behavior, have been reported for pathological conditions, especially at the border between regions of strong and weak contraction, both in single cells [43] and trabeculae [44] (see section "Systolic Stretch Effects").

Finally, a few more general comments should be made about Ca^{2+}-buffering. The cytosol of the cardiomyocyte is heavily buffered, and the vast majority of Ca^{2+} ions are bound to several important buffers. For example, at diastolic $[Ca^{2+}]_i$ of 0.1 μM, only ~2.5 % of Ca^{2+} ions are 'free' in solution; this fraction increase to ~4.5 % when systolic $[Ca^{2+}]_i$ rises to 1 μM [3]. As shown in Fig. 9.3b, fast and slow buffering systems exist in addition to troponin and the SR. Several fast buffers have dissociation constants near the operating range of the Ca^{2+}-transient (0.1–1 μM), and these bind and release Ca^{2+} on each heart beat. This group includes calmodulin, adenosine triphosphate (ATP), creatine phosphate, and the phospholipids of the sarcolemma. Slow buffers have dissociation constants that are much lower than the operating range of $[Ca^{2+}]_i$, and hence Ca^{2+} remains largely bound to these buffers throughout the heartbeat. Slow buffers include myosin and two high-affinity, non-regulatory sites on TnC. In addition, the mitochondria comprise roughly 35 % of cytosolic volume and can potentially contain large amounts of Ca^{2+}. Their large volume, coupled with close proximity to myofilaments and RyR, suggest a potentially significant active role for mitochondria in ECC [3]. However, most findings suggest little net Ca^{2+} transit between mitochondria and cytosol on a beat-by-bet basis. In fact, mitochondrial Ca^{2+}-exchange may be an epiphenomenon of mechanisms that match ATP production to cellular consumption, using intracellular Ca^{2+} as a proxy for the energetic demand of a cell.

From Contraction to Relaxation

While 'contraction' tends to be at the focus of attention, the process of relaxation is just as important for cardiac performance, yet less well understood [45]. As diastolic filling occurs under low pressure, any residual force from incomplete relaxation can severely affect cardiac function. Relaxation is more than just elastic recoil of tissue, and complexities arise from the interplay of Ca^{2+}-release from the myofilaments, Ca^{2+}-reuptake into the SR, and crossbridge detachment (Fig. 9.2c).

Besides serving as a trigger, LCC Ca^{2+}-influx also serves to load the SR (Fig. 9.2c, dotted down-arrow). This influx of Ca^{2+} is thought mainly to influence the amount of Ca^{2+} releasable by the SR

on the subsequent beat (rather than the current beat). In steady-sate conditions, net loading is zero, as the amount of Ca^{2+} extruded by NCX matches that of LCC influx.

The majority of $[Ca^{2+}]_i$ (60–80 %, depending on species) is recycled back into SR via the 'sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase' (SERCA). Factors such as heart rate, inotrophic stimulation, or pathologies affect re-uptake rates (heart failure can decrease uptake by 50 %) [3, 46]. The major sarcolemmal efflux pathway is the NCX, extruding ~20–40 % of Ca^{2+} during the transient, whereas the sarcolemmal Ca^{2+}-ATPase and mitochondrial uptake are generally thought to contribute less [47].

With the decline of $[Ca^{2+}]_i$, Ca^{2+} ions unbind from TnC, starting the relaxation process. If TnC were a simple buffer, the process could be easily described. However, complexities arise from the presence of activated thin filaments and attached crossbridges, which increase TnC affinity for Ca^{2+} (as described previously in the section "From Calcium Release to Contraction"). Moreover, a small number of attached crossbridges are thought to be able to hold the thin filament in an activated state, even if Ca^{2+} has dissociated from neighbouring binding sites (Fig. 9.3a). Thus, attached crossbridges can slow relaxation both by increasing Ca^{2+}-affinity of TnC, and via holding the thin filament activated after Ca^{2+} has dissociated from a fraction of TnC.

Because of these features, contractions involving high levels of developed force (and many attached crossbridges) are slower to relax than those with lower force development. This affects especially final relaxation, past 50 % of maximum force [48, 49]. Thus, a larger force transient as is observed after application of stretch (see Fig. 9.4c, lower panel) is noticeably slower to relax than a low force transient (note that for the two runs shown in Fig. 9.4c, the activating Ca^{2+}-transients are very similar). Interestingly, increased force due to larger peak Ca^{2+}-transients also produces a slowed final relaxation, suggesting that the effect results from slow unbinding kinetics of crossbridges, not upstream Ca^{2+}-activation events [45, 49].

Finally, and as noted previously, complete relaxation is important for competent diastolic filling. Here, the steep Ca^{2+}-sensitivity of the myofilaments contributes to keeping developed force minimal at diastolic $[Ca^{2+}]_i$. Other features that may promote relaxation include the ability of myosin to detach under isometric or lengthening conditions (by quickly reversing the steps of attachment and head rotation) in a manner that retains most of the energy of ATP hydrolysis [13, 45]. In contrast, the typical forward cycling scheme requires ADP dissociation and new ATP binding for a crossbridge to detach (if only this slower detachment mechanism existed, relaxation rate would be greatly slowed). In fact, recent characterizations of isolated myofibrils suggest that relaxation is a non-uniform biphasic process [45], and while isolated myofibril behaviour may differ from intact muscle, the point is clear that relaxation is complex and is not just the reverse of activation.

Mechano-Electric Coupling

Diastolic Stretch Effects

As shown above, the heart is an exquisitely mechano-sensitive organ. This also applies to mechano-electric transduction, as will be obvious to anyone whose training involved the use of Langendorff-style heart preparations that can be stopped or started 'by the flick of a finger'. Mechanisms underlying these acute electrical responses to mechanical stimulation include stretch-activated ion channels (SAC), mechanical modulation of Ca^{2+}-handling, and effects mediated via communication with other cells.

As a rule, mechanical stimulation of resting cardiac tissue (during 'electrical diastole') causes membrane depolarization (Fig. 9.5) [50]. This behavior can be observed across the whole range of structural complexity, from whole organ to single cell, and it is understood to be largely mediated by depolarizing trans-membrane currents through SAC [51, 52].

SAC have been discovered in mammalian cardiomyocytes 25 years ago [53], and a number of channels with varying ion selectivity and gating properties have subsequently been described. Despite over two decades of research, a uniform SAC classification has yet to emerge.

Meanwhile, SAC can be distinguished based on ion selectivity. SAC that show little selectivity

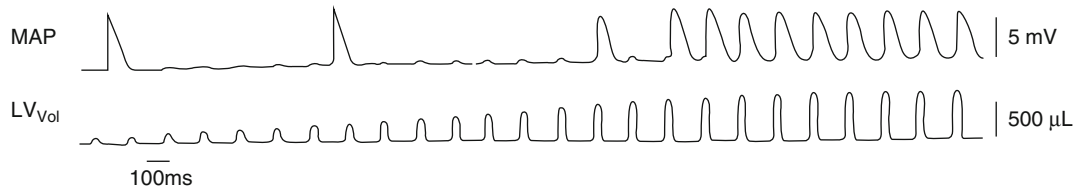

Figure 9–5. Diastolic stretch causes membrane depolarization. Monophasic action potential recordings (*MAP*) from an isolated rabbit heart (AV node disrupted) shows that injection of increasing amounts of fluid into a left-ventricular balloon (LV_{Vol}) causes matching diastolic depolarisations which, once threshold is reached, will mechanically pace the preparation (note: the first two action potentials are spontaneous escape beats not related to mechanical stimulation) (From Franz et al. [50]. Reprinted with kind permission from Wolters Kluwer Health)

for the various cations normally present in physiological solutions comprise the first group (SAC_{NS}; for Non-Selective). The second group preferentially conducts potassium (SAC_K). A third group of chloride-selective channels [54] has been described in settings that involve centrifugal membrane deformation (most commonly in the context of patho-physiologically increased cell volumes). The latter channels show significant lag-times between mechanical stimulus and increased channel open probability (1 min or more), rendering them less likely to underlie *acute* mechanical effects on heart rate and rhythm. (Mechano-sensitive chloride channels are likely to affect electrophysiology predominantly in settings such as ischaemia, reperfusion, or hypertrophy, where they are constituently activated; for further detail see [41]).

SAC_{NS} usually have reversal potentials between 0 and −20 mV. This is more positive than the membrane potential of resting cardiomyocytes, so that diastolic activation of SAC_{NS} will tend to depolarize cardiac cells. In working cardiomyocytes, SAC_{NS} activation may cause ectopic AP generation [51], while in pacemaker cells a positive chronotropic response can be observed [55].

SAC_K activation will tend to shift the membrane potential towards the potassium equilibrium potential (negative to −90 mV), so their activation will oppose depolarization, or aid repolarization.

Give that diastolic stretch tends to depolarize myocardium, SAC_{NS} are generally assumed to be the leading contributors to acute electrophysiological responses of normal cardiac cells to stretch. In support of this idea, block of SAC_{NS} prevents mechanically-induced extrasystoles [56]. In contrast, SAC_K appear to play secondary roles under physiological conditions, but they may well become important determinants of patho-physiological responses, such as in ischaemic tissue (see also section "Mechanical Cardioversion in Tachycardia and Fibrillation") [52].

Systolic Stretch Effects

The AP upstroke, whether triggered by mechanical or electrical stimulation, is firmly governed by the fast sodium current. Any additional stimuli, including mechanical, have little appreciable effect.

During the subsequent AP plateau, cardiomyocyte membranes are more positive than the reversal potentials of either SAC_{NS} or SAC_K, and stretch tends to have a repolarizing effect, which often leads to an overall reduction in AP duration [57]. However, cross-over of repolarization [58] and AP lengthening [59] have also been observed. In particular, stretch applied during late repolarization often prolongs the AP, potentially giving rise to early or delayed afterdepolarization-like behavior. These afterdepolarizations may trigger extra beats, such as seen in patients during balloon valvuloplasty [60]. Interestingly, similar responses could be involved in the initiation of certain arrhythmias, as (presumably Ca^{2+} overload-induced) *regional* after-contractions in a canine model of drug-induced long-QT1 syndrome were seen to precede depolarization in other parts of the heart and induction of torsades de pointes [61]. Also, in rat trabeculae, delayed afterdepolarization-induced arrhythmias (AP initiation occurred

before the applied pacing pulse) arose from the border zone of normal tissue and a centrally-positioned region of reduced contractility (by application of BDM or blebbistan [100 nmol/L]) in roughly two-thirds of preparations [44]. These arrhythmias were enhanced by addition of isoproterenol (200 nmol/L) or SCH00013 (30 nmol/L; a myofilament Ca^{2+} sensitizer), but they were unaffected by streptomycin (100 μmol/L) applied to block stretch-activated ion channels. Thus, the heart's own contractile activity may, in certain circumstances, give rise to mechanically-triggered ectopic excitation.

Even though mechanically-induced effects on systolic electrophysiology may be less apparent than those seen during diastole, MEC still affects electrical load, excitability and refractoriness of cardiac tissue. Not only can these responses be regionally and temporally heterogeneous, but they may occur on the background of an electro-mechanically highly heterogeneous substrate (during the latter part of systole), with implications for heart rhythm maintenance as illustrated below.

Mechanisms

Most acute electrophysiological responses to mechanical stimulation, observed in cardiac cells, can be reconciled with sarcolemmal SAC activation. That said, mechanically induced changes in Ca^{2+}-handling [31] and second messengers such as nitric oxid [62], are also likely to contribute, whether directly or indirectly. An interesting link between SAC and Ca^{2+}-handling is the possibility that certain non-sarcolemmal ion channels are manipulated by the mechanical environment. This could explain the observation that acute axial stretch increases the rate of Ca^{2+}-sparks (RyR-mediated Ca^{2+}-release events from the SR) in a directly mechano-mediated manner that was independent of trans-sarcolemmal ion fluxes [63].

Under normal conditions, though, large changes in developed force or length produce generally small changes in the cytosolic Ca^{2+}-transient (see discussion of Fig. 9.4). As such, mechanical perturbations alone are unlikely to produce large enough cell-wide perturbations in $[Ca^{2+}]_i$ to be pro-arrhythmic by activating Ca^{2+}-dependent inward currents (e.g., NCX), similar to that proposed in heart failure [64]. However, *in vitro* evidence suggests that stretch of locally weakened muscle regions can produce release of Ca^{2+} from SR near the boundary of strong and weak cells [65]. Mechanically inhomogeneous myocardium (whether locally weakened, or stiffened, say by scarring or fibrosis) may thus give rise to pro-arrhythmic Ca^{2+}-release.

Finally, mechano-sensitivity is not restricted to cardiomyocytes. Various mechano-sensitive cell populations affect cardiac electrical responses to mechanical stimulation, either by parakrine pathways (e.g. neurons, endothelial cells, connective tissue) [66], or through direct electrotonic interaction, either via connexin-based coupling (such as confirmed *in situ* for fibroblast-myocyte connections) [67] or possibly via connexin-free cytoplasmic links afforded by so-called tunneling nano-tubes [68]. Furthermore, several connexins have been found to exhibit mechanically modulated opening [69] which, if applicable to the heart, would have implications for signal propagation.

This dynamic area of research requires improved tools for projection from the sub-cellular to tissue and whole organ/individual levels. The identification, over a decade ago, of a highly selective peptide blocker of SAC [70] did raise significant hope for improved translational studies [71], but limited availability and high cost of the peptide have continued to pose obstacles to its wider use. Another approach towards linking systems level observation to molecular mechanisms may manifest itself from the application of optogenetic tools [72, 72a] to cardiac MEC research.

Relevance for Electrical Function of the Heart

Mechano-Sensitivity of the Normal Heart

Manifestations of both the Frank-Starling effect (mechanically induced positive inotropy) [73] and the Bainbridge effect (stretch-induced positive chronotropy) [74] can be observed in human heart, even after denervation (e.g. in the recently transplanted heart).

While the Frank-Starling effect is a well-established and efficient means of matching cardiac output to venous return, manifestations of the Bainbridge effect in humans are usually less evident. This is related to the fact that most interventions which temporarily raise venous return (e.g. tilt-table or orthostatic challenges) are associated with corresponding changes in arterial pressure. These, via reflex pathways, slow down (rather than accelerate) pacemaking. However, studies specifically targeting venous return in the absence of arterial pressure changes, such as using auto-transfusion (passive elevation of the legs), found that an isolated increase in venous return does indeed raise heart rate in human [75]. This response is believed to underlie the non-neural component of respiratory sinus arrhythmia (inspiration increases venous return which raises beating rate), or the sometimes observed phase-inverted sinus arrhythmia during positive pressure ventilation (when forced inspiration impedes venous return, which reduces beating rate).

Mechanical Pacing in Asystole and Bradycardia

Precordial percussion of the asystolic heart was among the first documented mechanical interventions for heart rhythm management. As reported in 1920, Stokes-Adams syndrome patients could be kept conscious during periods of ventricular standstill by pacing the heart using fist impacts to the chest [76].

Further applications include precordial thumps (PT) for the management of severe bradycardia, such as in the context of spinal anesthesia, or direct epicardial stimulation by 'finger tap', which is regularly employed by surgeons to reinstate rhythmic activity in hearts that are being weaned from cardiac bypass. The majority of case studies, conducted mainly in the 1970/1980s [77, 78], concluded that the fist is a suitable mechanical pacemaker, in particular in emergency situations where no alternative treatment modalities are available [79]. Of note, the impact energies required to trigger premature ventricular beats in humans are low, ranging from 0.04 to 1.5 J [80]. (For comparison: 1 J of impact energy is released by dropping a standard can of soda from 30 cm height).

Underlying mechanisms of mechanically-induced cardiac contractions are believed to be similar to those seen in isolated heart or tissue experiments, where diastolic mechanical stimulation causes cardiomyocyte membrane depolarization which, if large enough, triggers AP generation (Fig. 9.5) and subsequent ECC.

Of note, PT-induced ventricular contractions have significantly greater haemodynamic value than chest compressions, where blood is passively squeezed out of the heart (rather than actively ejected by the heart itself). In fact, the haemodynamic efficacy of mechanically induced beats matches that achieved with electrical pacing in patients [81]. The only prospective study into the outcomes of PT in out-of-hospital cardiac arrest victims found that PT had any positive effect only if applied to patients in primary asystole [82].

Mechanical Cardioversion in Tachycardia and Fibrillation

Case reports have shown that single PT can be successful as an emergency resuscitation measure to terminate ventricular tachycardia and fibrillation (VT and VF, respectively; see Fig. 9.6) [83, 85]. For cardioversion of tachycardic arrhythmias, PT is applied as a sharp impact to the lower half of the sternum, delivered from a height of 20–30 cm, using the ulnar edge of the tightly clinched fist, followed by active retraction after full impact (to emphasize the impulse-like nature of the stimulus) [86]. Energy levels involved in PT termination of tachyarrhythmia range from 2 to 8 J [86]. This can be sustained by the conscious patient, in contrast to external electrical defibrillation involving more than one order of magnitude higher energy levels.

Even though case reports have suggested that PT can be efficient in terminating witnessed cardiac arrest in about one third of cases, and that VT is more amenable to mechanical cardioversion than VF [85, 86], this has not been found in prospective studies [82, 87]. Beyond the 'publication bias' that is inherent to case reports (as opposed to prospective studies), severe pre-existing hypoxia may render PT less efficient [52]. That said, even in the acutely arrhythmic

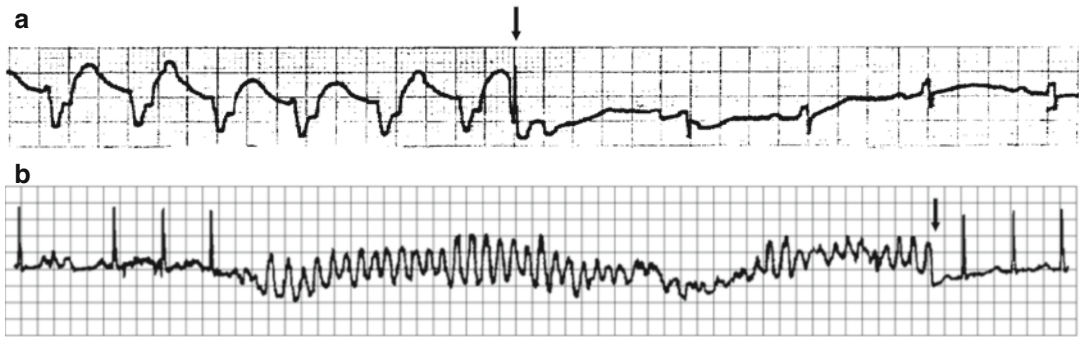

Figure 9–6. Tachyarrhythmia termination by precordial thump in man. Case reports showing termination of ventricular tachycardia (**a**) and early ventricular fibrillation (**b**) by application of a fist thump to the precordium (*arrows*) (From Pennington et al. [83] and Barrett [84]. Reprinted with kind permission from NEJM)

heart, PT is more efficient in treating asystole compared to VF [88].

Mechanisms underlying successful PT are believed to involve SAC_{NS} which, via depolarization of excitable gaps, may terminate re-entry. For VF, this would have to involve 'all' excitable gaps in the entire heart, which may be difficult to achieve in most cases. The reduced efficacy of mechanical interventions in severely hypoxic hearts has been linked to the fact that reduced ATP levels pre-activate at least some SAC_K [89]. This changes the overall electrophysiological response to mechanical stimulation, making it less efficient in depolarizing the excitable gap. In addition, the more pronounced AP shortening associated with increased contributions from SAC_K may potentially render PT arrhythmogenic in these circumstances.

Mechanical Induction of Arrhythmia

Both acute and sustained stretch have been linked to atrial and ventricular arrhythmogenesis [90, 91].

The specific contribution of *sustained stretch* to cardiac arrhythmogenesis is difficult to isolate with certainty, as pathologies that give rise to pressure- and/or volume-overload often carry an increased risk of heart rhythm disturbances via other mechanisms.

Interesting insight has been obtained, though, from the reverse conceptual approach, where volume-overload was temporarily eliminated by conducting the Valsalva maneuver. This caused a reduction in venous return and cardiac dimensions, with subsequent temporary (while cardiac dimensions were reduced) termination of ventricular and supra-ventricular tachyarrhythmias [91], even in heart transplant recipients [92], suggesting that maintained stretch is pro-arrhythmogenic.

Underlying mechanisms are likely to involve SAC, whose pharmacological block prevents overload-mediated atrial fibrillation in isolated heart [93]. Similarly, activation of SAC has been implied to contribute to reduced defibrillation efficacy during volume overload [94, 95].

Acute mechanical stimulation of the heart can cause a range of electrophysiological responses, from single ectopic beats, to conduction abnormalities, runs of VT, and VF [96]. In the context of non-penetrating extracorporeal impacts without cardiac structural damage, this is referred to as *Commotio cordis* [97]. Determinants of *C. cordis* outcome include impact type, location and energy [98], and timing [99].

The vulnerable window for mechanical induction of VF coincides, in a domestic pig model, with a 10–20 ms period prior to the peak of the ECG T-wave [99]. This vulnerable window is narrower than for electrical stimulation, and may explain why *C. cordis* is relatively rare.

Underlying mechanisms are likely to involve SAC activation, although the exact nature and individual contribution of subpopulations remains unresolved [100, 101]. At the cellular level, mechanical stimulation during the T-wave affects cardiomyocytes differently, depending on their actual state of AP repolarization at the

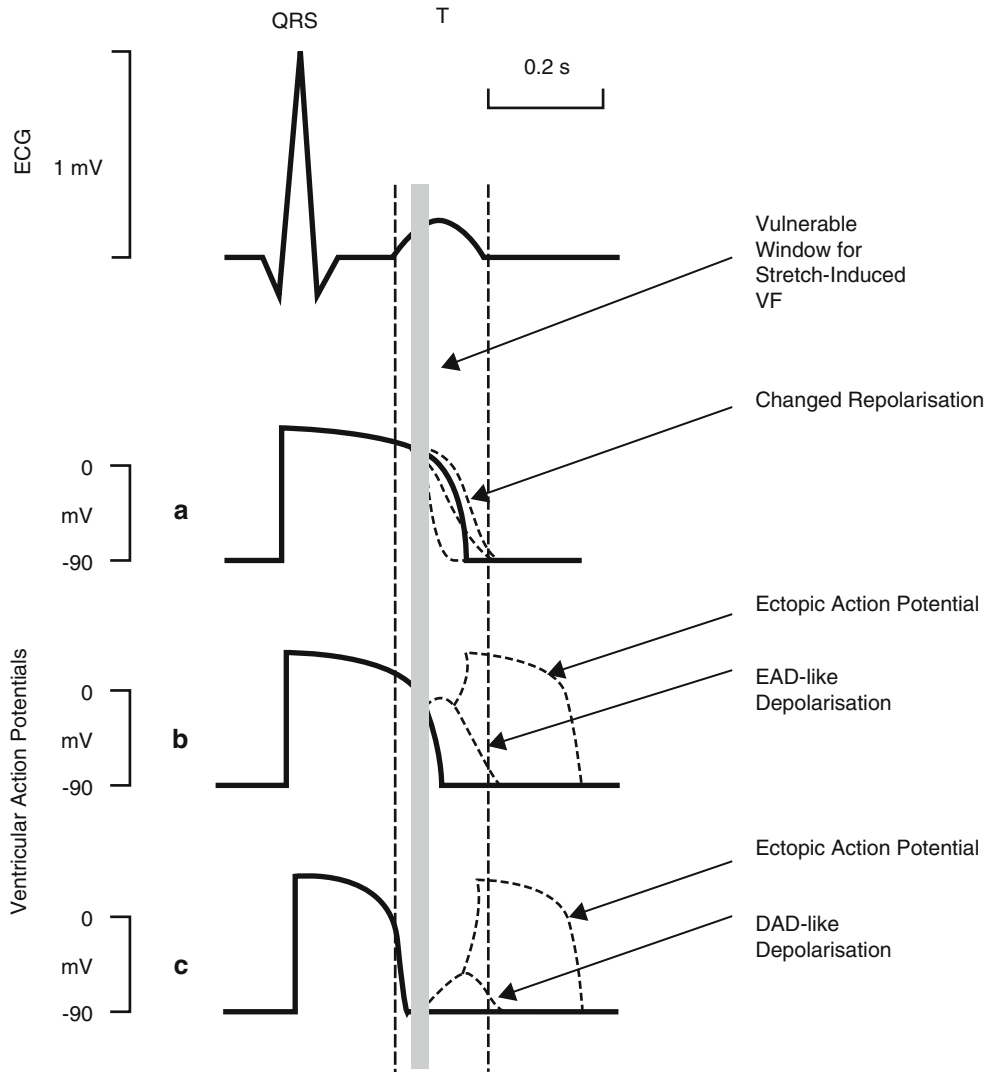

Figure 9–7. Schematic representation of cellular correlates of mechanical impacts during early repolarization (upstroke of the T-wave, see *grey band*). Effects depend on the actual membrane potential of cardiomyocytes in different regions of the heart (*panels* **a**–**c**) and include: (**a**) changes in action potential duration (shortening, prolongation, cross-over of repolarization); (**b**) early afterdepolarization (*EAD*) -like behaviour; or (**c**) delayed afterdepolarization (*DAD*) -like events, both of which may trigger extra beats (From Kohl et al. [100]. Reprinted with kind permission from Oxford University Press)

given point in time (panels a–c in Fig. 9.7). This may provide both trigger (ectopic AP) and sustaining mechanisms (heterogeneity of repolarization) for arrhythmogenesis. The most devastating effect of *C. cordis*, VT, requires that the mechanically stimulated myocardium overlaps with the 'trailing end' of an AP wave (i.e. the repolarisation wave) [102, 103]. This pre-requirement is met during a limited set of spatio-temporal combinations of impact site and underlying cardiac activity, which may explain why the vulnerable window for mechanical induction of VF is narrower than that of electrical stimulation.

Outlook

Cardiac mechanics should not only be seen as a key endpoint of heart rhythm management, but it is a rhythm-moderator in its own right. Mechanical interventions affect pace and force of cardiac contractions, and they may contribute

to the induction, sustenance, and termination of arrhythmias.

Many of the links between electro-mechanical cross-talk, tissue and organ structure, and temporal organization of cardiac function are still ill-understood. This ranges from basic science questions, for example regarding the modalities and mechanisms underlying mechano-sensing, to clinical issues, such as the effects of various pathological factors (e.g. regional inhomogeneities, hypertrophy, fibrosis) and treatment regimes (drugs, exercise) on mechanical arrhythmogenesis.

A better understanding of the cardiac electromechanical riddle will lead to more intelligent treatment strategies. This includes pharmacological leads [93], as well as device development/improvement opportunities ranging from the use of ultrasound for cardiac pacing [104], over cardiac assist technologies [105], biventricular pacing/resynchronization [106], and cardiac contractility modulating electrical stimulation [107], to application of electrical defibrillation during peak chest compression and mechanical cardioversion, to name but a few.

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10
Pathological Roles of the Cardiac Sodium Channel Late Current (Late I_{Na})

Sridharan Rajamani, John C. Shryock, and Luiz Belardinelli

Abstract

The causes, consequences, and potential therapeutic benefit of inhibiting cardiac late sodium current are reviewed. Myocardial sodium channels enable electrical excitability and impulse conduction in the heart. Depolarization induces sodium channel openings and a large inward Na^+ current that forms the upstroke of the cardiac action potential (AP). The cardiac AP is characterized by a long plateau phase during which Na^+ channels are inactivated. Disruption of the process of Na^+ channel inactivation, even when it affects only a small fraction of Na^+ channels, results in a late or persistent inward Na^+ current (late I_{Na}) that flows throughout the AP plateau. The magnitude of late I_{Na} is normally small but an increase can have pathological consequences. Both inherited (congenital) and acquired diseases may cause late I_{Na} to be enhanced. Mutations in genes encoding Na^+ channel alpha and beta subunits and channel-associated proteins are causes of an enhanced late I_{Na} and LQT3 syndrome. Ischemia, heart failure, oxidative stress, and increased activities of certain protein kinases are associated with an increase of late I_{Na}. The consequences of an increased late I_{Na} include prolongation of the duration of the AP and facilitation of early after-depolarizations, and increased loading of myocytes with Na^+. Myocyte Na^+ loading leads to Ca^{2+} loading via Na^+/Ca^{2+} exchange, delayed after-depolarizations, and activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). CaMKII activation is associated with phosphorylation of the Na^+ channel that further increases late I_{Na}, creating a potential positive feedback loop. Inhibition of late I_{Na} ameliorates electrical and mechanical dysfunction caused by LQT3 syndrome, ischemia, heart failure, and Na^+/Ca^{2+} overload. In these settings, the advantages of reducing an enhanced late I_{Na} may include: increased repolarization reserve associated with decreased AP duration and variability; decreased occurrences of early and delayed after-depolarizations and triggered arrhythmias; improvements of myocardial Ca^{2+} handling, ventricular diastolic relaxation, and contractile efficiency.

Keywords

Ischemia • Heart failure • Action potential • Arrhythmias • LQT3 syndrome • Ranolazine • Tetrodotoxin • CaMKII

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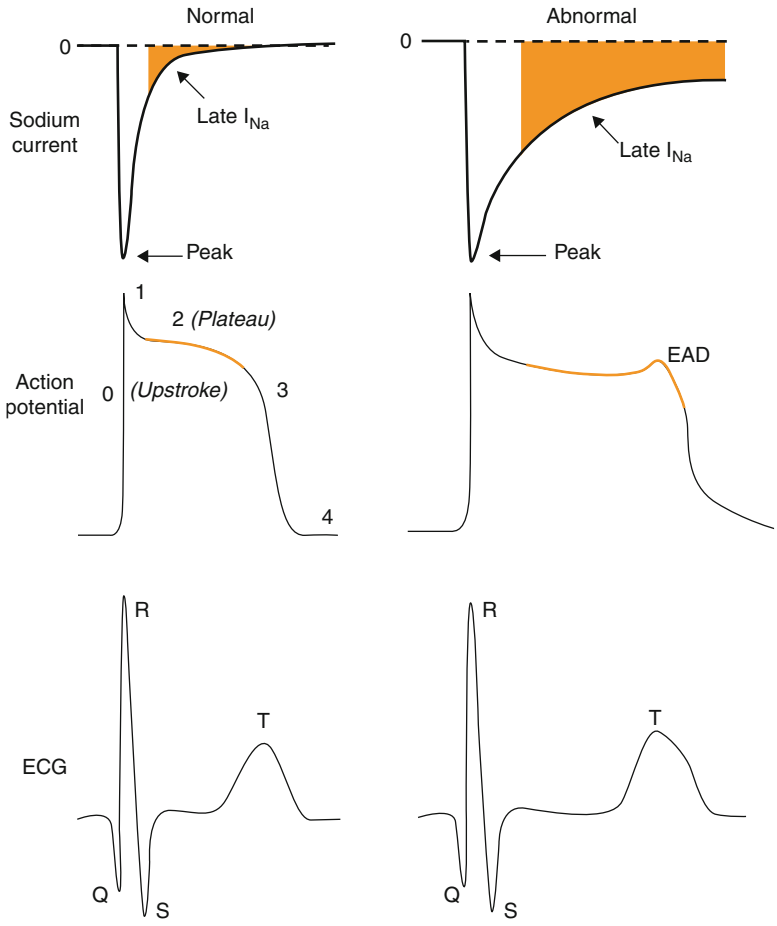

Figure 10–1. Effect of an abnormally increased late Na^+ current (*late* I_{Na}) to cause prolongation of the action potential duration and the QT interval in the electrocardiogram (*ECG*), and to cause early afterdepolarizations (*EAD*)

Abbreviations

AP	Action potential
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
DAD	Delayed afterdepolarization
EAD	Early afterdepolarization
RR	Repolarization reserve
RyR2	Ryanodine receptors subtype 2

Introduction

The magnitude of late Na^+ current (I_{Na}) is small and without apparent consequence in the normal heart. However, late I_{Na} is enhanced by both relatively common acquired (ischemic heart disease, arrhythmias, and heart failure) and rare congenital diseases [1–4]. This chapter focuses on the pathologic role of late I_{Na} as a mechanism to increase Na^+ and Ca^{2+} overloading of myocytes and its adverse consequences, including cardiac arrhythmias and slowing of relaxation of left ventricular contraction (Figs. 10.1 and 10.2). For additional reviews of the pathologic roles of cardiac late I_{Na}, see [4–19].

The Sodium Channel Late Current (Late I_{Na})

Voltage-gated sodium channels (VGSCs) are heteromultimeric membrane pore-forming proteins whose conductance of Na^+ is gated by changes in the membrane potential. VGSCs are required for the generation and propagation of action potentials (AP) in heart, nerve, and skeletal muscle. Sodium channel opening and peak I_{Na} are usually short in duration (often 1–2 ms) in these tissues, because Na^+ channels quickly

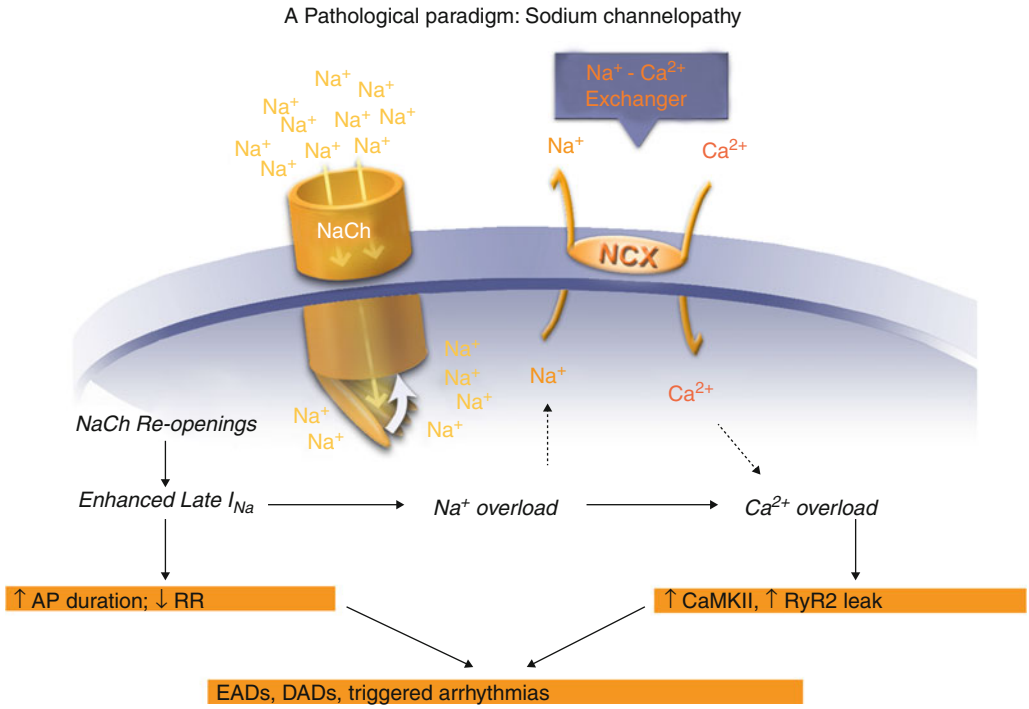

Figure 10–2. Pathological consequences of acquired and/or inherited Na^+ channelopathies

inactivate after opening. Sodium channels inactivate at depolarized potentials when the inner gate of the channel moves to block the pore. Upon membrane repolarization, Na^+ channels rapidly revert to the closed state. Presumably the high numbers of charged residues in the voltage sensing domains of the channel render the closed state very energetically stable (this may be important for survival of the organism). However, the inactivated state(s) of the Na^+ channel (which occur during prolonged depolarization) are not as stable, and Na^+ channel reopening from an inactivated state(s) may occur. A small I_{Na} (late or sustained I_{Na}) therefore persists during prolonged depolarization such as the plateau (phase 2) of the cardiac AP [20–26]. Unlike skeletal muscle and neuronal cells, heart cells undergo prolonged depolarization during the plateau (phase 2) of every AP, and thus cardiac Na^+ channels reside in inactivated states for approximately one-third of each cardiac cycle. Pathological conditions that lead to a failure of inactivation of Na^+ channels are thus likely to cause a large increase of late I_{Na} and Na^+ loading in the heart [27].

The functional relevance of cardiac late I_{Na} under physiological conditions is still unclear. Experimental [20, 28] and modeling studies [29] suggest that a relatively small persistent I_{Na} and few late openings of Na^+ channels can significantly increase AP duration in the heart. However, the presence of larger late I_{Na} (relative to peak I_{Na}) in Purkinje fiber and M cells than in ventricular epicardial myocytes may contribute to the longer AP duration [22, 30–33] and greater propensity to occurrences of early after-depolarizations (EADs) in the former cells [31–33]. The transmural distribution of late I_{Na} appears to be species-dependent [26, 31–35].

Several mechanisms have been proposed to explain the presence of late I_{Na}, including window current [21, 36, 37] and specialized populations of Na^+ channels (reviewed in [11, 15, 38]). An overlap of the voltage ranges wherein Na^+ channels activate and inactivate (i.e., approximately −70 to −50 mV) can lead to a steady-state Na^+ window current that will contribute to late I_{Na}. Steady-state TTX-sensitive Na^+ window current at 35–37 °C in sheep [21] and rabbit [36] Purkinje fibers was found to occur

from −70 to −20 mV, a range that is wider than predicted. The ratio of steady-state to peak I_{Na} was 0.06 % in rabbit Purkinje fibers [36]. One might expect window current to be most important during ischemia, and during phase 3 of the cardiac AP, when myocytes are depolarized to voltages at which window current is present. Because Na^+ channel inactivation and activation as a function of voltage are altered by channel mutations [39], and perhaps by other channel modifications such as phosphorylation, the voltage range for Na^+ channel "steady state" window current is not always clear. However, in the last 20 years, many researchers have recorded late I_{Na} and Na^+ channel openings that decrease with time and at voltages that are putatively outside the commonly-accepted potential range of the Na^+ window current (for example, voltages during the AP plateau are 0–20 mV, and outside the range for window current). The term "modal gating" (transient openings, scattered late openings and/or burst openings) has been applied to this form of Na^+ channel activity [23, 25, 26, 29, 31, 40–45]. Evidence from studies of cardiac $Na_v1.5$ currents [46, 47] suggests that late and peak I_{Na} are conducted by the same channels. However, neuronal Na^+ channel isoforms including $Na_v1.1$, $Na_v1.3$ and $Na_v1.6$ are also expressed in the heart [11, 48–53] and may contribute to late I_{Na}.

Causes of Increased Late I_{Na}: Inherited Na^+ Channelopathies Causing Long QT (LQT) Syndrome 3

Mutations in the cardiac Na^+ channel gene *SCN5A* cause LQT3. More than 80 LQT3 mutations have been identified to date [4]. Most are missense mutations and cause gain-of-function by increasing the probability that the cardiac channel $Na_V1.5$ will fail to inactivate properly or will more readily re-open from a closed state to generate increased late Na^+ current [37, 54–58]. A fivefold increase of late I_{Na} caused by the ΔKPQ mutation in $Na_V1.5$ was found to greatly increase loading of the myocyte with Na^+ in a modeling study by [27]. Many *SCN5A* mutations that enhance late I_{Na} are found in the DIII-DIV linker (including ΔKPQ), transmembrane segments DIVS4 and DIVS6, and the carboxy terminus of the channel, as befits their putative roles as "inactivation gate", the voltage sensor whose movement generates the signal for movement of the inactivation gate, the inactivation gate receptor, and the "latch" which keeps the inactivation gate closed, respectively [4, 56]. Some of these mutations occur at sites that are also known targets for phosphorylation by protein kinases [59, 60].

Four different β-subunits, $Na_{v\beta}$ (β_1 – β_4), have been identified to play a critical role for cell surface expression, modulation of voltage-dependence, and transduction from cell signaling molecules to the pore-forming α-subunit of cardiac Na^+ channels [61–67]. $Na_V1.5$ channel α subunits in ventricular myocytes were reported to co-localize with β2 and/or β4 subunits at intercalated discs [50].

The congenital mutation L179F in the β_4 subunit encoded by the gene *SCN4B* was reported to be responsible for the prolonged QT interval in a patient with 2:1 atrioventricular block [68]. Patch-clamp studies revealed a three- to eight-fold increase (gain-of-function) in late I_{Na} when L179F was co-expressed with an α-subunit. Recently, Ackerman and colleagues [69] reported a congenital mutation (V36M) in the β_3 subunit encoded by the gene *SCN3B* that was associated with SIDS. Patch-clamp studies revealed a marked decrease (loss-of-function) of peak I_{Na} but a marked increase (gain-of-function) in late I_{Na} when V36M was co-expressed with an α-subunit. Similarly, mutations in the gene *SCN1B* encoding the β1 subunit have been shown to cause febrile seizures [70].

Causes of Increased Late I_{Na}: Inherited Mutations in Non-Channel Proteins as Causes of Long QT (LQT) Syndromes and/or Enhanced Late I_{Na}

Caveolae are a special type of lipid raft that in which receptors and signal transducing molecules are organized [71]. In the heart,

caveolin-3 (encoded by the gene *Cav3*) is responsible for caveolae formation and is abundantly present in atrial, ventricular, and nodal cells [72]. Mutations in *Cav3* can lead to LQT9. It has been shown that $Na_v1.5$ is co-localized with caveolin-3 [73]. When the caveolin-3 mutations responsible for LQT9 in patients were heterologously expressed with $Na_v1.5$, a two- to three-fold increase in late I_{Na} was observed. This increase in late I_{Na} was suggested to be the principal mechanism for proarrhythmia in these patients [74]. Consistent with this interpretation is the finding that caveolin-3 mutations were identified in samples from a cohort of sudden infant death syndrome (SIDS) patients [75].

The *SNTA1*-encoded α1syntrophin (SNTA1) is a member of the dystrophin-associated multiprotein complex that interacts with the PDZ domain-binding motif of the cardiac Na^+ channel carboxyl terminus [76]. SNTA1 has been shown to interact with neuronal nitric oxide synthase (nNOS) and Ca^{2+}/calmodulin-dependent ATPase (PCMA4b) [77]. Nitric oxide (NO) has been shown to increase the amplitude of late I_{Na} in rat ventricular myocytes [78] and in the presence of STNA1, PMCA4b acts as a potent inhibitor of NO production by nNOS. Patients identified with *SNTA1* mutations [79–81] have been shown to have a prolonged QT interval (LQT12 syndrome) that may be associated with SIDS. Both peak and late I_{Na} were found to be increased when *SNTA1* congenital mutant proteins were co-expressed with $Na_V1.5$, nNOS and PMCA4b in cells.

Ankyrin-G is required for localization of $Na_V1.5$ to the intercalated discs of cardiac myocytes [82, 83]. The protein β_{IV}-spectrin binds to ankyrin-G and to CaMKII and thus targets CaMKII to the Na^+ channel, where it phosphorylates S571 [83]. Because the phosphorylation of $Na_V1.5$ by CaMKII leads to an increase of late I_{Na} [84–86], changes in the function of β_{IV}-spectrin may lead to changes in late I_{Na}. Recently, defects in other intracellular proteins that are known to interact with VGSCs have been shown to enhance late I_{Na}. An excellent review of protein interactions with Na^+ channels has been published recently [87].

Causes of Increased Late I_{Na}: The Acquired Na^+ Channelopathies in Myocardial Ischemia and Heart Failure

An imbalance between myocardial oxygen supply and demand, as occurs during ischemia, heart failure, or tachycardia, leads to increases of myocardial concentrations of Na^+ and Ca^{2+} [88, 89]. During ischemia and reperfusion following ischemia, amphiphilic lipids (e.g., palmitoyl-L-carnitine, lysophosphatidylcholine), glycolytic metabolites, reactive oxygen species (ROS) and catecholamines are increased, and intracellular concentrations of H^+, Na^+, and Ca^{2+} are elevated. Sodium-hydrogen exchange (NHE), reverse mode Na^+-Ca^{2+} exchange (NCX with Ca^{2+} entry), and CaMKII activities are increased. Hypoxia, palmitoyl-L-carnitine, lysophosphatidylcholine, glycolytic metabolites (2,3-diphosphoglycerate, glyceraldehyde phosphate), NO and H_2O_2 have all been reported to individually increase late I_{Na} and $[Na^+]_i$ in myocytes or myocardium [11, 28, 78, 90–101], although it is not clear whether they act directly on the Na^+ channel or indirectly through other targets. Na^+ channel blockers (e.g., tetrodotoxin, lidocaine, ranolazine, F15845) have been shown to reduce the rise of Na^+ in rat ventricular myocytes and isolated hearts during hypoxia and ischemia, respectively [16, 101–104]. This action of Na^+ channel blockers is associated with an improvement of contractile function and reduction of the hypoxia/ischemia-induced increase in the intracellular Ca^{2+} concentration [91, 93, 101, 103–111].

Late I_{Na} is increased in myocytes isolated from failing human and dog hearts [1, 2, 18, 45–47, 51, 112–116]. Inhibition of late I_{Na} in myocytes isolated from failing human and dog hearts by ranolazine leads to improvements of relaxation and reductions of afterdepolarizations and variability of duration of the AP [112–114]. These results indicate that late I_{Na} is a cause of both Ca^{2+} overload and reduced repolarization reserve in myocytes from failing hearts.

Late I_{Na} was also found to be increased in atrial myocytes isolated from hearts of rabbits with left ventricular hypertrophy. These atrial

myocytes had spontaneous EADs and automatic activity [117] that were abolished by tetrodotoxin. Late I_{Na}, automaticity, EADs, and delayed afterdepolarizations (DADs) could also be elicited in normal guinea pig atrial myocytes treated with ATX-II or H_2O_2, and they were abolished by ranolazine [118, 119].

Much progress has been made recently to understand the role of ROS in contributing to an increase of late I_{Na} and cardiac Na^+/Ca^{2+} overload. ROS are increased during reoxygenation (reperfusion) following myocardial ischemia [120, 121]. Nitric oxide has been shown to increase late I_{Na} in intact neurons and myocytes, and in excised membrane patches from both types of cells [8, 78]. Hydrogen peroxide increases late I_{Na} in isolated myocytes and induces arrhythmic activity of myocytes and intact hearts, including EADs, DADs and triggered activity [28, 98]. Arrhythmic activity induced by H_2O_2 is prevented by ranolazine [98, 119, 122, 123]. An increase of the intracellular Na^+ concentration has been shown to cause an NCX-mediated efflux of Ca^{2+} from mitochondria, reduction of the mitochondrial Ca^{2+} concentration, and reduction of the mitochondrial NADH/NAD^+ ratio [124]. The result is increased formation of ROS by mitochondria [125]. Paradoxically the effect of an increase of $[Na^+]_i$ is therefore to decrease mitochondrial Ca^{2+} but increase mitochondrial ROS production, and cytosolic Ca^{2+}.

Elevations of Ca^{2+}/calmodulin [126] and ROS [127–129] can cause activation of CaMKII. CaMKII can phosphorylate $Na_V1.5$, which leads to an increase of late I_{Na} [84, 130, 131]. Recently, enhancement of late I_{Na} has also been demonstrated to lead to activation of CaMKII; this effect appeared to be dependent on late I_{Na}-induced increases of intracellular Na^+ and Ca^{2+} concentrations [132]. CaMKII is upregulated in heart failure [133, 134] and in the infarct border zone [135]. The effects of CaMKII phosphorylations of the Na^+ channel to increase late I_{Na}, of the L-type Ca^{2+} channel to increase mode 2 gating, (late I_{Ca}) and of ryanodine receptors (RyR2) to increase Ca^{2+} leak and therefore reduce Ca^{2+} content of the sarcoplasmic reticulum are all factors contributing to a phenotype of arrhythmogenesis (early- and delayed afterdepolarizations) and contractile dysfunction in heart failure.

Other protein kinases (e.g., the tyrosine kinase Fyn, AMP-activated protein kinase, and protein kinases A and C) in addition to CaMKII are reported to phosphorylate cardiac Na^+ channels and increase late I_{Na} [59, 60, 136–138]. These kinases play critical roles in signal transduction and "metabolic sensing". However, their contributions to altered regulation of Na^+ channel inactivation and late I_{Na} in diseases states is not yet clear.

Electrophysiological Consequences of an Enhanced Late I_{Na}

The direct consequence of an enhancement of late I_{Na} is an increase of an inward current during the plateau and repolarization (phases 2 and 3) of the cardiac AP, which reduces repolarization reserve. The reduction of repolarization reserve increases the dispersion of repolarization and therefore of AP duration, both locally and across the wall of the heart [139]. An increased dispersion of repolarization creates a substrate for re-entrant activity, and is associated with electrical (T-wave) and mechanical alternans and proarrhythmia, and is predictive of torsade de pointes ventricular tachyarrhythmia [140, 141]. Prolongation of the duration of the AP provides additional time for membrane L-type Ca^{2+} channels to reactivate, and for the sarcoplasmic reticulum to fill and then release Ca^{2+} that increases cell membrane NCX (an inward current, I_{Ti}), either of which may contribute to an EAD [142]. A failure of Na^+ channel inactivation during phase 3 may lead to a larger late I_{Na} because the electrochemical driving force for Na^+ entry increases as repolarization proceeds. Thus both increased $I_{Ca,L}$ and increased I_{Na} contribute to EAD formation. EADs commonly occur at low heart rates (with longer AP durations) and can trigger torsade de pointes. The reverse rate dependence of drugs that block hERG K^+ current and cause torsade has been demonstrated to be increased by late I_{Na} and is reduced by blockers of late I_{Na} [143]. Enhancement of late I_{Na} may also lead to spontaneous depolarization of the resting membrane potential of a myocyte [119]. Finally, late I_{Na}, by its effects to destabilize repolarization and increase the heterogeneity of electrical activity in the myocardium, creates potential paths (substrates) for conduction of re-entrant activity in the heart.

The indirect consequences of an enhancement of late I_{Na} are an increase of Na^+ influx and of the intracellular Na^+ concentration, with subsequent effects on NCX and cellular Ca^{2+} handling [18]. The effect of an enhanced late I_{Na} to increase the concentration of intracellular Na^+ will decrease the electrochemical gradient available to extrude Ca^{2+} via NCX, and thereby contribute to Ca^{2+} loading of the cytoplasm and therefore of the sarcoplasmic reticulum. When the level of activation of CaMKII is also increased (by high heart rates, catecholamines, an increase of the cytosolic Ca^{2+} concentration, or ROS-mediated oxidation of the enzyme), CaMKII-mediated phosphorylation of RyR2 (sarcoplasmic reticulum Ca^{2+} release channels) will occur, and Ca^{2+} will be released from the sarcoplasmic reticulum during diastole. Phosphorylation of cardiac RyR2 by cAMP-dependent protein kinase A and by CaMKII sensitizes them to Ca^{2+} and facilitates rapid release of Ca^{2+} in response to calcium influx (i.e., calcium-induced calcium release) after excitation of the myocyte. However, it also increases the probability of spontaneous Ca^{2+} release during diastole. The released Ca^{2+} may exit the cell via NCX. The NCX-coupled exchange of three Na^+ for one Ca^{2+} causes a transient inward current (I_{ti}) and a DAD or EAD [144–146] that may initiate an AP [147] that can trigger arrhythmic activity [148, 149]. The spontaneous "unloading" of Ca^{2+} from the sarcoplasmic reticulum during diastole also causes a relative depletion of the Ca^{2+} store available for the following systolic contraction, thus reducing contractility. These events are more likely to occur in the presence of catecholamines and at high heart rates, and in failing hearts, where they lead to Ca^{2+} and electrical alternans [150, 151].

Late I_{Na} and Ca^{2+} Handling: A Pathological Partnership

As noted above, an increase of late I_{Na} causes both an increase of the intracellular Na^+ concentration and a prolongation of AP duration, increased activity of NCX in the reverse mode, and increased Ca^{2+} influx. This is exacerbated during prolonged ischemia, when reductions of cellular ATP and activity of the cell membrane Na^+/K^+ ATPase allow the intracellular Na^+ ($[Na^+]_i$) concentration to rise. There is general agreement that the cellular Ca^{2+} overload that occurs during ischemia and reperfusion is a result of a combination of decreased efflux of Ca^{2+} ions via the forward mode of NCX and increased influx of Ca^{2+} ions via the reverse mode of NCX. Direct evidence in support of the participation of reverse mode NCX in intracellular Ca^{2+} overload during reperfusion or reoxygenation after ischemia is derived from the observations that inhibitors of NCX [152, 153], antisense inhibition of NCX [154], and knockout of NCX [155] markedly decrease either contractile dysfunction or the rise in intracellular Ca^{2+} in myocardial cells in these settings. For review of NCX in heart failure, see [156].

An increase of the intracellular Ca^{2+} concentration will cause increased activation of CaMKII. Activation of CaMKII is followed by increased phosphorylation of phospholamban, L-type calcium channels, nitric oxide synthase (to increase formation of NO), RyR2, and Na^+ channels. Phosphorylation by CaMKII of both the Na^+ channel and the β-subunit of the L-type Ca^{2+} channel, $Ca_v1.2$, increase late I_{Na} [84–87], and mode 2 gating of the L-type Ca^{2+} channel (i.e., late I_{Ca} [157]), respectively. Increased formation of NO may also lead to enhancement of late I_{Na} [78]. Concomitant CaMKII-mediated increases of late I_{Na} and late I_{Ca} lead to greater entry of Ca^{2+} into myocytes but reduction of the Na^+ gradient for extrusion of Ca^{2+} via NCX, thereby facilitating Na^+/Ca^{2+} overload and cardiac dysfunction. The effect of late I_{Na} to reduce Ca^{2+} extrusion via NCX and thereby contribute to Ca^{2+} overloading and activation of CaMKII, when coupled to the effect of CaMKII to phosphorylate Na^+ and Ca^{2+} channels and increase late I_{Na} and late I_{Ca}, leads to a pathologic partnership between late I_{Na} and activation of CaMKII wherein one begets and amplifies the other.

The Therapeutic Potential of Decreasing Late I_{Na}

Selective inhibitors of cardiac late I_{Na} have therapeutic potential in the treatment of ischemic heart disease, arrhythmias and heart failure. Inhibitors of late I_{Na} are expected to be safe and effective because late I_{Na} is enhanced in

pathological settings such as ischemia, arrhythmias, and heart failure, but not in healthy myocardium where its inhibition is presumably without consequence. Inhibitors of late I_{Na} have been shown to attenuate ionic, metabolic, electrical and contractile dysfunction in preclinical models of hypoxia, ischemia, heart failure, or Na^+ overload, suggesting that an increase of late I_{Na} plays a critical role in the Na^+/Ca^{2+} overload pathology that underlies ischemia-induced damage to myocardium late I_{Na} is augmented. Late I_{Na} is augmented, the duration of the AP is prolonged, the dispersion of ventricular repolarization and beat-to-beat variability of AP duration (also referred to as instability) is increased, and EADs are common in ventricular myocytes from dogs and humans with chronic heart failure. The Na^+ channel blockers tetrodotoxin, saxitoxin, lidocaine and ranolazine have been shown to shorten the duration and variability of the AP and suppress EADs in ventricular myocytes from failing hearts. For review of the literature regarding the beneficial effects of inhibitors of late I_{Na} in models of ischemic heart disease, arrhythmias, and heart failure, see [13, 15–18].

Ranolazine is the most selective inhibitor of late I_{Na} in use in current clinical practice [15, 158–162]. It binds to the local anesthetic binding site of the Na^+ channel [163] and selectively reduces late relative to peak Na^+ current [112, 164]. It does not reduce heart rate, cardiac output, or blood pressure, and is not a systemic vasodilator. Ranolazine does not appear to be arrhythmogenic in spite of its secondary effect to reduce hERG K^+ current [162, 165–167], and decreases arrhythmic activity caused by other blockers of this current [168, 169] suggesting that reduction of late I_{Na} is sufficient to blunt the pro-arrhythmic actions of hERG blockers.

In summary, Na^+ and Na^+-induced Ca^{2+} overloading are characteristic of ischemic and failing hearts and contribute to arrhythmias and a slowing of relaxation (i.e., diastolic stiffness). An increase of late I_{Na} is a mechanism for Na^+ overloading during hypoxia/reperfusion and heart failure, in patients with gain-of-function *SCN5A* mutations and possibly in other cardiac diseases. Enhancers of late I_{Na} such as ATX-II, H_2O_2, and ischemic metabolites, and *SCN5A* mutations cause functional effects that mimic many but not all (e.g., ischemia-induced activation of $I_{K,ATP}$ and shortening of AP duration are not a result of increased late I_{Na}) of the effects of ischemia/reperfusion and hypoxia. Selective blockade of late I_{Na} reduces mechanical and electrical dysfunction caused by ischemia/reperfusion, arrhythmias, and heart failure, and is thus a rational therapeutic approach to treatment of these diseases.

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11
Sodium Ion Channelopathies

Yuka Mizusawa, Arthur A.M. Wilde, and Hanno L. Tan

Abstract

The voltage-gated cardiac sodium channel (Nav) is a multiple protein complex consisting of a pore-forming α-subunit, ancillary β-subunits and several regulatory proteins. Mutations in various sodium channel-related genes disrupt sodium channel function (gain-of-function or loss-of-function) and lead to diseases such as Brugada syndrome, long QT syndrome, progressive cardiac conduction disease, sick sinus syndrome or atrial fibrillation. Furthermore, sodium channelopathies may cause sudden infant death syndrome (SIDS). Sodium ion channelopathies were initially regarded as inherited diseases without structural abnormalities. However, emerging evidence indicates that *SCN5A* mutations may be associated with structural changes. For instance, *SCN5A* mutations are considered possibly causative in idiopathic dilated cardiomyopathy. Interestingly, various single SCN5A mutations may show different clinical phenotypes in single patients or families. These cases are called overlap syndromes. The pathophysiologic basis of these overlap syndromes has not been fully elucidated: in some cases, different functional effects of the mutation during different phases of the cardiac action potential may be involved, while, in others, various modifiers, including *SCN5A* and other gene mutations/polymorphism may be involved. On the other hand, some *SCN5A* mutations may be not causal but just a bystander of a sodium channelopathy. This chapter aims to summarize the current knowledge on the cardiac sodium channel, diseases caused by mutations in sodium channel encoding genes, and discuss the proposed underlying mechanisms.

Keywords

Sodium ion channelopathy • Cardiac sodium channel • α-subunit • β-subunit • Brugada syndrome • Long QT syndrome • Progressive cardiac conduction disease • Sick sinus syndrome • Atrial fibrillation • Sudden infant death syndrome • Dilated cardiomyopathy • Overlap syndrome

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Abbreviations

AF	Atrial fibrillation
ARVC	Arrhythmogenic right ventricular cardiomyopathy
BrS	Brugada syndrome
DCM	Dilated cardiomyopathy
LQTS	Long QT syndrome
LQT3	Long QT syndrome type 3
PCCD	Progressive cardiac conduction disease
SCD	Sudden cardiac death
SIDS	Sudden infant death syndrome
SSS	Sick sinus syndrome
VF	Ventricular fibrillation

Introduction

Sodium ion channelopathy is a collective term of diseases caused by a mutation in a gene which encodes subunits of the cardiac sodium channel or its regulatory proteins. Originally, this term was used for primary electrical diseases such as Brugada syndrome (BrS), long QT syndrome (LQTS), progressive cardiac conduction disease (PCCD) or sick sinus syndrome (SSS) with *SCN5A* mutations. Further research has revealed that other genes related to cardiac sodium channel function (e.g., ancillary β(beta)-subunits) may also be involved in the pathophysiology of sodium ion channelopathy. Moreover, it has been recognized that *SCN5A* mutations may also be involved in structural changes of the heart, as *SCN5A* mutations were found in idiopathic dilated cardiomyopathy (DCM), and structural changes were reported in BrS patients carrying a *SCN5A* mutation. Extensive research on sodium ion channelopathies has led us to the understanding of their nature, but, at the same time, revealed their complex mechanisms through the overlap syndrome. This chapter aims to provide an overview of current knowledge on the cardiac sodium channel (α(alpha)-subunit and ancillary β(Beta)-subunits) as well as sodium ion channelopathies related to *SCN5A* mutations, including unresolved issues.

Cardiac Sodium Channel

Voltage-gated cardiac sodium channels (Na_v) are transmembrane proteins responsible for the rapid upstroke of the cardiac action potential and impulse conduction through the heart. They are multiple protein complexes consisting of a pore-forming α(alpha)-subunit, ancillary β(Beta)-subunits (β(Beta)1-β(Beta)4 subunits) (Fig. 11.1), and several regulatory proteins [17].

The *SCN5A* gene encodes $Na_v1.5$, the α(alpha)-subunit of the cardiac sodium channel. $Na_v1.5$ consists of an intracellular N-terminus, four homologous domains (DI–DIV), intracellular linkers between these domains, and the C-terminus. Each domain consists of six transmembrane segments (S1–S6). Segment 5 (S5) and S6 are positioned close to the pore, so that they form a pore lumen where sodium ions can traverse the sarcolemma. S4 is positively charged and plays a role as the channel's voltage sensor. The P-loop between S5 and S6 lines the pore of the channel extracellularly.

At the normal resting membrane potential of working myocytes and myocytes of the specific conduction system (−90 mV), the sodium channel remains closed. When excitatory current from adjacent cells arrives and depolarizes the membrane potential positive to −75 mV, the sodium channel's activation threshold, all four S4 segments (the voltage sensors) move outward and the channels are activated, which leads to the opening of the channels. At the same time, the fast inactivation starts using the DIII-DIV linker as a 'lid' that occludes the pore intracellularly. The C-terminus helps to stabilize the fast inactivation process [18]. Since the inactivation process is slower than the activation process, channels remain open transiently during phase 0 of the action potential. Slow inactivation follows and, by the end of phase 1, virtually all sodium channels are inactivated. The remaining sodium current, the so-called 'late sodium current (I_{sus})' is still recorded during the repolarization phase of the action potential and slowly inactivates. The conformational change of the sodium channel during slow inactivation is less known, but

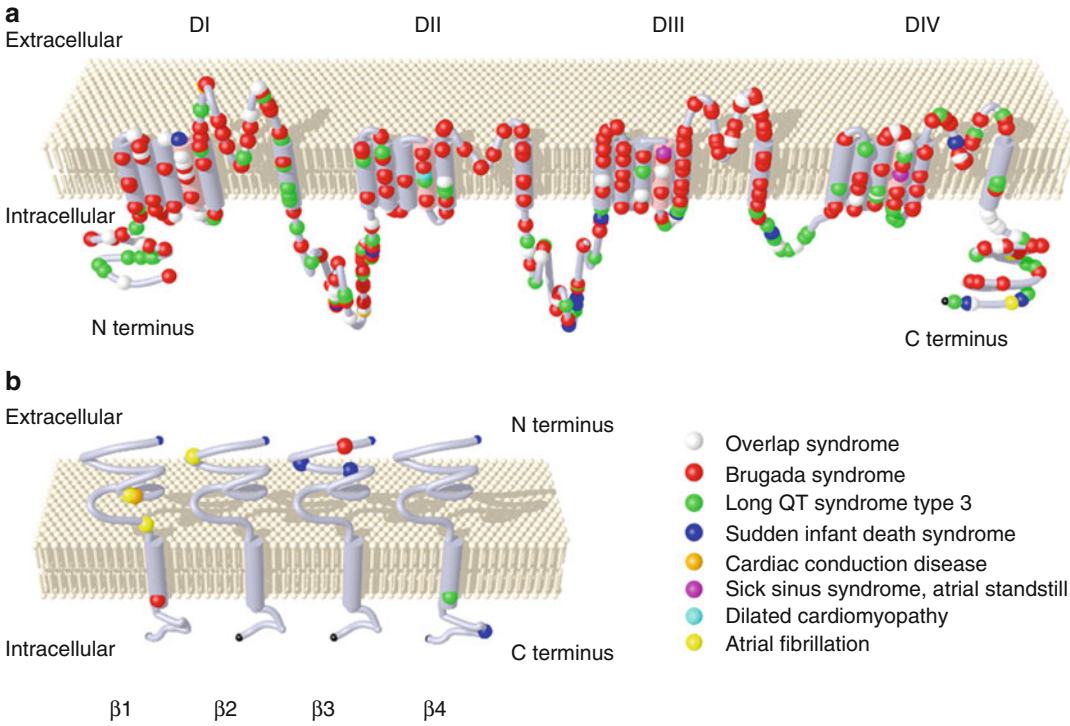

Figure 11–1. Schematic representation of the voltage gated Na^+ channel α(alpha)-subunit and β(Beta)-subunits. (**a**) The α(alpha)-subunit consists of four domains (D1–D4), each composed of six membrane-spanning segments (S1–S6) linked by intracellular and extracellular loops. The linkers between S5 and S6 control ion selectivity and permeation of the channel, while the positively charged segments S4 (in *pink*) act as a voltage sensor. Differently coloured circles display the location of mutations associated with Brugada syndrome, Long QT syndrome type 3, sudden infant death syndrome, cardiac conduction disease, sick sinus syndrome/atrial standstill, dilated cardiomyopathy, atrial fibrillation and overlap syndrome [1–12]. *DI* domain I, *DII* domain II, *DIII* domain III, *DIV* domain IV. (**b**) The β(Beta)-subunits consist of an extracellular N terminus, one transmembrane segment, and an intracellular C terminus. Note that the β(Beta)1-subunit has two isoforms. A mutation found in cardiac conduction disease was located in the β(Beta)1-subunit, whereas a mutation in Brugada syndrome was located in the β(Beta)1B subunit [10, 13–16] (The authors thank Dr. Andre Linnenbank for providing Fig. 11.1)

there are reports suggesting the involvement of the voltage sensors (S4), S5–S6 linkers, the S6 segments or the C-terminus [19–24]. After a few hundred milliseconds to a few seconds, the sodium channels are completely inactivated and wait for the next stimulation for activation.

$Na_v1.5$ itself can generate sodium currents when heterologously expressed alone, but β(Beta)-subunits play important roles in modulating current density and gating of the sodium channels. There are four β(Beta)-subunits (β(Beta)1-β(Beta)4) in the heart and they are encoded by the *SCN1B-4B* genes [25–27]. Beta-subunits consist of an extracellular N-terminus, a single transmembrane segment, and an intracellular C-terminus. Studies using both α(alpha)- and β(Beta)-subunits have shown that β(Beta)-subunits interact with the α-subunit and modify the expression of α(alpha)-subunit in the cell membrane or its gating process [28–31]. Besides β(Beta)-subunits, there are regulatory proteins, such as glycerol-3-phosphate dehydrogenase like protein (GPD1L), multicopy suppressor of *gsp*1 (MOG1), and caveolin-3 (*CAV3*), which are shown to interact with $Na_v1.5$ [17].

Table 11–1. Current changes in sodium ion channelopathies

Primary electric disease	Reported changes in I_{Na}
Brugada syndrome	↓
Long QT syndrome	↑
Progressive cardiac conduction disease	↓
Sick sinus syndrome	↓
Atrial fibrillation	↓,↑
Sudden infant death syndrome	↓,↑
Dilated cardiomyopathy	↓

↓ reduced net I_{Na}, ↑ increased net I_{Na}

Mutations in the α(alpha)-subunits, β(Beta)-subunits and some regulatory proteins of the cardiac sodium channel produce dysfunctional sodium channels and cause diseases, such as BrS, LQTS, PCCD, atrial standstill, SSS, and DCM through either gain-of-function or loss-of-function of the sodium channel (Table 11.1) [32].

Interestingly, most mutations reported in β(Beta)-subunits are located in the N-terminus, which suggests that this region is involved in the regulatory role of the β(Beta)-subunits in the sodium currents. Importantly, whether a mutation found in a patient is disease-causing or not needs careful interpretation, because putative causative mutations in the α(alpha)- and β(Beta)-subunits of the cardiac sodium channel are found mostly in a single index case without sound linkage data. Besides, rare *SCN5A* variants may be found in the general population at a proportion as high as 2–8 % [1, 2].

Sodium Ion Channelopathies

Brugada Syndrome (BrS)

BrS is an inherited disease which is characterized by coved type ST elevation in the right precordial leads of the ECG (Fig. 11.2), and sudden cardiac death (SCD) due to ventricular fibrillation (VF). VF predominantly occurs at rest or during sleep. Fatal arrhythmias have an age of onset in the 40s on average and predominantly occur in males [33]. In patients with BrS, 20–50 % have a family history of SCD. BrS has an autosomal dominant pattern of transmission with incomplete penetrance and variable expressivity [33]. *SCN5A* mutations are found in 20 % of BrS patients and, although rare, mutations in other genes have also been reported. These genes encode various calcium channels (*CACNA1C*, *CACNB2b*, *CACNA2D1*), β(Beta)-subunits of sodium channels (*SCN1B*, *SCN3B*), proteins which affect trafficking of sodium channels (*GPD1L*, *MOG1*), and the transient outward potassium current, I_{to} (*KCNE3*, *KCND3*, *KCNE5*) [34]. Risk stratification is

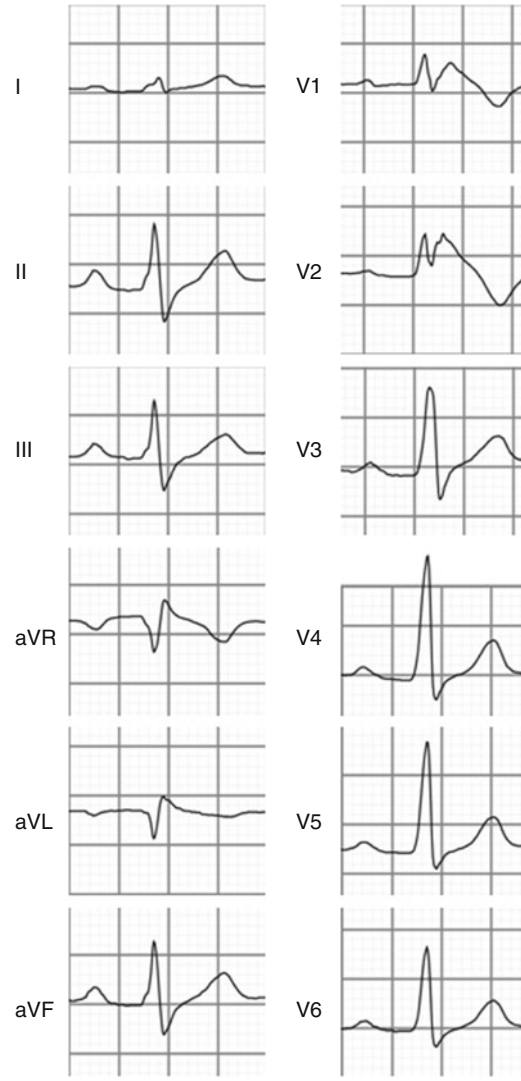

Figure 11–2. Diagnostic ECG of Brugada syndrome (type 1 ECG). Note that ST segment elevation with high take-off (J point) of ≥2 mm and a subsequent negative T wave is observed in the right precordial leads V1–V2

difficult and, although BrS is an inherited disease, a *SCN5A* mutation or family history of SCD has not been consistently found to be a risk marker for SCD [35–37].

More than 200 *SCN5A* mutations have been reported in BrS as a putative causal mutation so far [1]. A recent multicenter study by Kapplinger et al. analyzed 2,111 unrelated patients with variable ethnic backgrounds who were referred for genetic analysis of *SCN5A*. This study found 293 possibly causal mutations, of which 77 % were found only once. The most frequently found mutations in multiple cases were E1784K, F861WfsX90, D356N and G1408R. Two-thirds of mutations reported in the study were missense mutations, and the rest were frameshift mutations, nonsense mutations, splice-site mutations and in-frame insertions or deletions. Seventy-one percent of the mutations were located in one of the four transmembrane domains [1].

Experimental studies in heterologous expression systems have shown that BrS-related mutations cause loss-of-function of the sodium channel through decreased expression of sodium channels in the sarcolemma [38], expression of non-functional channels [3] or altered gating properties (delayed activation, earlier inactivation, faster inactivation, enhanced slow inactivation and/or delayed recovery from inactivation) (Table 11.2) [4, 39–42]. Of note, less than 10 % of the reported mutations found in BrS underwent functional analysis and the relationship between a mutation and clinical symptoms is often not well-understood. So far, there is only one study which suggested that truncated $Na_v1.5$ proteins (due to nonsense mutations, frameshift mutations or in-frame insertions or deletions) or missense mutations with >90 % peak I_{Na} reduction may lead to severe conduction disturbance or syncope [43], but it is presently too early to conclude if the findings are relevant or not.

Interestingly, not only putative causative *SCN5A* mutations have been reported to modify clinical phenotypes of BrS, but also *SCN5A* polymorphisms. H558R, a common *SCN5A* polymorphism, was shown to modify the electrophysiological property of a BrS-related mutant sodium channel as well as BrS type 1 ECG [44]. Possibly explaining, at least in part, the role of different ethnic background in the clinical effect of *SCN5A*, an *SCN5A* promoter polymorphism in a haplotype variant with a relatively high prevalence was reported to lead to difference in cardiac conduction parameters in individuals of Asian origin [45]. A study in two different strains of transgenic mice (129P2 and FVB/N) carrying the same *SCN5A* mutation (1798insD/+) showed that ventricles in 129P2 mice expressed low to undetectable sodium channel auxiliary subunit β(Beta)4, which led to slower conduction in the right ventricle in 129P2 mice compared to FVB/N mice [46].

Table 11.2 Reported biophysical mechanisms of increase in net sodium current (gain-of-function) and reduction in net sodium current (loss-of-function)

Gain of function
Persistent current (disruption of fast inactivation)
Changes in voltage dependence of activation (hyperpolarizing shift) and inactivation (depolarizing shift)
Faster recovery from inactivation
Slower inactivation
Loss of function
Reduction in current density
Reduced number of functional sodium channels in sorcolemma
Mutation located in ion-conducting pore
Truncated protein due to premature stop codon
Trafficking defect (channel protein retention in endoplasmic reticulum)
Gating changes
Changes in voltage dependence of activation (depolarizing shift) and inactivation (hyperpolarizing shift)
Slower recovery from inactivation
Enhanced intermediate inactivation
Enhanced closed-state inactivation

All these studies suggest that phenotypes related to *SCN5A* mutations and/or BrS may be affected by multiple factors. Importantly, one study on large *SCN5A* positive families with clinically affected BrS family members showed that, in the same family, there were individuals with BrS phenotype (type1 ECG) without the familial *SCN5A* mutation [47]. Therefore, a single *SCN5A* mutation may not play a major role to cause BrS.

Mutations in genes such as auxiliary β(Beta)-subunits of sodium channels (*SCN1B*, *SCN3B*) or *GPD1L* and *MOG1*, which affect trafficking of sodium channels, have been reported in relation to BrS [13, 14, 48–54]. Cellular electrophysiological studies have shown that

mutations in *SCN1B*, *SCN3B*, *GPD1L* or *MOG1* led to loss-of-function of I_{Na}. However, *GPD1L* is the only gene with sound genetic linkage so far and all others have been identified with candidate gene analysis used in single patients or in small families. Therefore, further study is needed to elucidate the pathophysiologic mechanism of BrS and the role of cardiac sodium channel-related genes or proteins [55].

Recent studies showed that structural abnormalities exist in some patients with BrS [56, 57]. As the derangements of both BrS and arrhythmogenic right ventricular cardiomyopathy (ARVC) are anatomically located predominantly in the right ventricle, overlap between BrS and ARVC has been suggested [58–61]. From a genetic point of view, there are few reports [62, 63]. One study has reported a *SCN5*A mutation in a single patient of ARVC with a drug-induced type-1 BrS-ECG [63]. Therefore, it is not clear at this point if these two conditions may share the same mechanism or not.

Long QT Syndrome (LQTS)

LQTS is characterized by QT prolongation on the baseline ECG and has a high risk of SCD due to *torsade de pointes* ventricular tachycardia, which often degenerates into VF. LQTS is classified into two forms: congenital or acquired. Acquired LQTS is precipitated by drug use and/or electrolyte imbalance such as hypokalemia, hypocalcemia or hypomagnesemia. Congenital LQTS is a hereditary disease and estimated to affect 1/5,000 individuals with either an autosomal-dominant (Romano-Ward syndrome) or autosomal-recessive inheritance pattern (Jervell and Lang-Nielsen syndrome) [64]. Thus far, 13 different types of LQTS, each based on a different causal gene, have been reported. Most types are related to cardiac potassium channels and a few are related to genes which encode the calcium channel or the sodium channel [65]. LQTS related to a mutation in the *SCN5A* gene is named LQTS type 3 (LQT3). The prevalence of LQT3 among genotyped LQTS patients is 13 %, whereas the prevalence of LQT1 (*KCNQ1* mutation) and LQT2 (*KCNH2* mutation) are 43 and 32 %, respectively [2]. The other subtypes are rare.

Patients with LQT3 experience significantly more severe arrhythmic events compared to LQT1 or LQT2 [66]. Events are related to bradycardia and occur at rest or during sleep [67]. Beta blockers appear to be effective in preventing cardiac events, but seem less effective than in LQT1 or LQT2 [67–69]. Other therapeutic approaches in LQT3 include antiarrhythmic drug therapy using mexiletine, flecainide or ranolazine [70–73], although the therapeutic efficacy of mexiletine may be mutation-specific and can lead to QT prolongation and arrhythmic events in other cases [74].

So far, more than 70 *SCN5A* mutations have been reported in LQT3 and most of them are missense mutations. Of note, whether a missense mutation is pathogenic depends on its location within the sodium channel [75]. Functional analysis in heterologous expression systems was performed in approximately half of the published mutations [76]. These studies have consistently shown that these mutations lead to gain-of-function of the sodium channel. This is due to the increase of I_{sus}, caused by disruption of the fast inactivation process [77], increasing window current [78, 79], slower inactivation [78, 80], faster recovery from inactivation [81, 82] or larger peak I_{Na} density (Table 11.2) [83]. In rare cases, mutations in genes such as *CAV3*, *SCN4B*, or α(alpha)1 syntrophin (*SNTA1*) have been also shown to cause LQTS through increased I_{sus} [15, 84–86].

Progressive Cardiac Conduction Disease (PCCD)

PCCD, also called Lenègre or Lev's disease, is originally characterized by progressive impairment of the conduction system with widening of the QRS complexes. No structural abnormality is observed and complete atrioventricular (AV) block may occur as a consequence, which necessitates pacemaker implantation. Histopathologic studies revealed a sclerodegenerative process in the His-Purkinje system [87, 88], and the disease was considered a primary degenerative disease or an exaggerated aging process. Patients are usually asymptomatic, but may experience syncope or SCD when advanced or complete AV block occurs.

From a genetic perspective, Schott et al. were the first to report a large family with cardiac conduction disease related to a *SCN5A* mutation [89]. Functional studies in families with cardiac conduction disease revealed loss-of-function of the sodium channel [90, 91]. As further research on *SCN5A* mutations was performed, it has been revealed that a single *SCN5A* mutation may show overlap of several phenotypes such as BrS, LQTS, atrial fibrillation, SSS, and DCM in combination with conduction disturbances [4–6, 92], (details discussed in the paragraph Overlap syndrome). Of note, the severity of clinical phenotypes varies and it is not known which factors other than *SCN5A* mutations cause more severe conduction disease with earlier disease onset or lead to different phenotypes. As the penetrance and phenotypic expression of a *SCN5A* mutation vary, further research to elucidate the mechanism of PCCD related to *SCN5A* mutations including (genetic) modifying factors is necessary.

Sick Sinus Syndrome (SSS)

SSS is a disease caused by sinoatrial node dysfunction and/or conduction disturbances between the sinoatrial node and surrounding atrial tissues. Clinically, sinus bradycardia, sinus arrest or Brady-Tachy syndrome is observed. It is usually diagnosed in the elderly and patients show symptoms such as shortness of breath, fatigue, palpitations or syncope. Patients with SSS are treated with pacemaker implantation. In hereditary SSS, loss-of-function *SCN5A* mutations have been reported. A study on congenital SSS cases has shown compound heterozygous mutations in *SCN5A* [93]. Loss-of-function mutations in *SCN5A* found in SSS may lead to overlap with other phenotypes, where some family members exhibit SSS, while others present with BrS or PCCD [6, 7, 92]. The mechanism of SSS due to loss-of-function mutations may be sinoatrial exit block by disruption of conduction into the surrounding atrium. On the other hand, bradycardia and sinus node dysfunction coexisting in LQT3 families is caused by gain-of-function of the sodium channel [94]. In this study, it was experimentally explained that the presence of I_{sus} (1–2 % of peak I_{Na}) and negative shift in voltage dependence of inactivation may reduce sinus rate by up to 10 %.

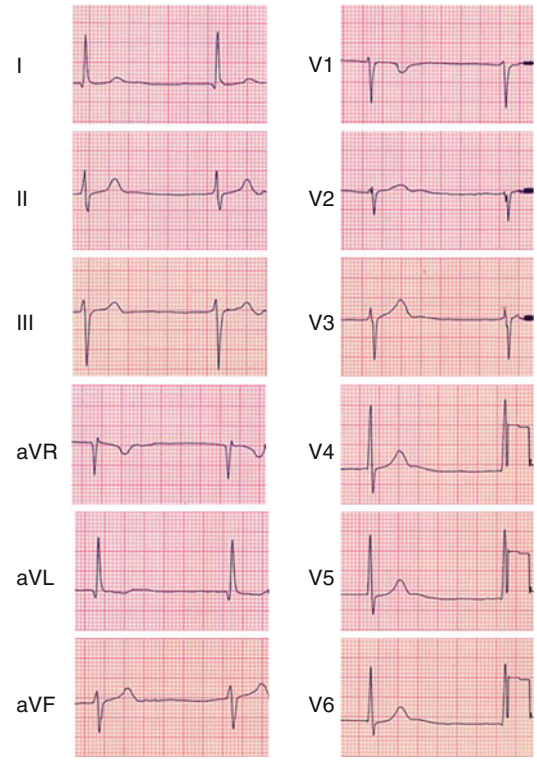

Figure 11–3. ECG of atrial standstill in a patient with *SCN5A* D1275N and a *Cx40* polymorphism. Junctional rhythm is observed

Atrial standstill has also been related to a *SCN5A* mutation. In this study, a loss-of-function *SCN5A* mutation in combination with a loss-of-function connexin-40 polymorphism were described as a cause of atrial standstill (Fig. 11.3) [95].

Of note, SSS has also been associated with mutations in *HCN4* [96–98]. *HCN4* encodes the pacemaker current, I_f, and the reported mutations resulted in loss-of-function of I_f. Thus, SSS is genetically heterogeneous, and *SCN5A* mutations are not the sole genetic cause of the disease.

Atrial Fibrillation (AF)

AF is recognized as the most common cardiac dysrhythmia and a major cause of morbidity and mortality. Predisposing conditions include aging, diabetes, obesity, heart failure, coronary and valvular disease and prior cardiac surgery [99]. In a minority of cases, AF occurs without

the aforementioned conditions. As a rare subtype, a familial form of AF has been recognized for many years. Heritable AF has been identified using linkage analysis [100, 101]. Mutations in potassium channels, sodium channels (α(alpha)- and β(Beta)-subunits), nucleoporin, connexin-40 and atrial natriuretic peptide have been reported in relation with AF [102]. Recent extensive research on ion channelopathies also revealed that AF was observed in BrS or LQTS [103–105].

Regarding the sodium channelopathies, mutations in the genes encoding either the α(alpha)-subunit or the β(Beta)-subunits of the cardiac sodium channel have been reported in AF, but appear to be rare [8, 16, 106, 107]. The first *SCN5A* mutations related to AF were found in DCM patients [9]. Subsequently, another *SCN5A* mutation was reported in a family with lone AF [8]. A common polymorphism (H558R), known as a modifier to reduce I_{Na} in heterologous expression systems, was also found more frequently in lone AF patients compared to controls [106]. These mutations are considered to create conduction delay and predispose to reentry in the atrium through loss-of-function of I_{Na}. Interestingly, gain-of-function type mutations were also reported in AF [5, 108]. These mutations have shown to delay inactivation, and therefore could lead to AF by increasing atrial excitability.

Sudden Infant Death Syndrome (SIDS)

SIDS is defined as the sudden death of an infant younger than 1 year of age, which remains unexplained after a thorough investigation, including a complete autopsy, clinical history, and death scene investigation. Risk factors identified by epidemiological studies include prone or side positions for infant sleep, soft bedding and sleep surfaces, and overheating [109].

Genetic factors have been also found to play a role in some patients with SIDS. Since 1970s, the association between congenital LQTS and SIDS has been pointed out [110, 111] and it is currently estimated that 5–20 % of SIDS are related to ion channelopathies [10, 112]. So far, 13 cardiac channelopathy susceptibility genes have been implicated in the pathogenesis of SIDS. These include potassium channel genes (*KCNQ1, KCNH2, KCNE1, KCNE2, KCNJ8*), sodium channel genes and related regulatory proteins (*SCN5A, SCN3B, SCN4B, GPDL1, CAV3, SNTA1*), the calcium release channel of the sarcoplasmic reticulum (*RYR2*), and other genes (*GJA1*) [31, 113–125]. The clinical syndromes associated with these mutations are LQTS, short QT syndrome, BrS, catecholaminergic polymorphic ventricular tachycardia or idiopathic ventricular fibrillation. Of these clinical phenotypes, LQTS is the most extensively studied in the newborn. In a prospective study of a large cohort of 34,000 neonates with an ECG performed on the third or fourth day of life, the authors demonstrated that 50 % of SIDS infants showed a prolonged QT interval [126]. The same group subsequently performed genetic testing which resulted in finding genes related to LQTS [127, 128]. More recently, they reported that the prevalence of LQTS disease-causing mutations in SIDS was 9.5 % [129].

Currently, SIDS related to mutations in cardiac sodium channel genes (*SCN5A, SCN3B, SCN4B*) constitutes 43 % of all mutations found and the majority are missense mutations [10]. Functional analysis of several mutations has revealed that some mutations cause gain-of-function, while others cause loss-of-function. Clinically, this leads to the LQT3 and BrS phenotypes, respectively. Another mutation found in *SCN3B* (V36M) showed both gain-of-function and loss-of-function phenotypes secondary to decreased peak I_{Na} and increased late $I_{Na.}$ The *SCN5A* polymorphism S1103Y, common in African Americans, has also been implicated as a possible modifier to increase susceptibility to SIDS by increasing late I_{Na} [130].

Although the mechanism of SIDS is not fully elucidated, some potential SIDS victims will certainly benefit from genetic analysis, considering that the prevalence of channelopathies in SIDS is up to 20 %. Genetic testing in SIDS victims with a channelopathy may also help to evaluate the variable expressivity in the ion channelopathies.

Dilated Cardiomyopathy (DCM)

DCM is diagnosed when there is evidence of dilatation and impaired contraction of the left ventricle or both ventricles. The disease is considered idiopathic if other causes (coronary artery disease, active myocarditis, hypertension,

cardiotoxic drugs such as doxorubicin, alcohol abuse) are excluded. Familial DCM accounts for >20 % of idiopathic DCM cases [131], but mutations are found only in 30–35 % [132]. Most of the genes reported in DCM encode sarcomeric proteins [132]. A recent study using a large multicenter DCM cohort has shown that 1.7 % of 338 DCM subjects (including 289 probands) carried a mutation in *SCN5A* [133]. Of 15 *SCN5A* mutation carriers in this study, 14 (93 %) manifested arrhythmias (supraventricular arrhythmia, SSS, ventricular tachycardia, and PCCD). Two-thirds of the reported *SCN5A* mutations (six of nine) were located in S3 and S4 transmembrane segments. This provides a clue to a shared mechanism of *SCN5A*-related DCM. The most frequently reported mutation in DCM so far is D1275N. It has been disputed whether DCM with *SCN5A* D1275N occurs as a consequence of atrial arrhythmias, because atrial arrhythmias frequently appear as the first clinical phenotype in the 20s to 30s, while DCM develops later in life [134]. To answer this question, Watanabe et al. studied *SCN5A*-D1275N using a mouse model and a heterologous expression system [135]. They showed that dysfunction of the conduction system, atrial and ventricular tachyarrhythmias, and DCM phenotype was observed in mice that carried the *SCN5A*-D1275N mutation. Yet, studies in the heterologous expression system did not reveal major differences between wild-type and D1275N. Accordingly, the true nature of SCN5A mutations may only be unmasked if different expression systems are used in experimental studies. For other *SCN5A* mutations found in DCM [9, 133], further studies are awaited to prove if they are causal for DCM or not.

Overlap Syndrome

In overlap syndrome, a single *SCN5A* mutation is associated with multiple phenotypes in a single patient, in a family sharing the same mutation or in different families having the same mutation. For example, loss-of-function mutations in *SCN5A* have been shown to present multiple phenotypes, such as BrS, SSS or PCCD [3, 6, 92, 94]. More remarkably, some *SCN5A* mutations are associated with multiple phenotypes which are associated both with loss-of-function of the sodium channel (BrS) and gain-of-function (LQTS) [4, 5, 7]. This appears to be contradictory from a functional point of view. Patch-clamp studies of *SCN5A* 1795insD showed that this may be explained by concurrent gating changes: enhanced late sodium current leads to gain-of-function and LQT3, whereas faster intermediate inactivation causes loss-of-function and, as a consequence, BrS. A subsequent study showed that a mutation of the same residue to a histidine (Y1795H) or cysteine (Y1795C) also showed distinct phenotypes: BrS and LQT3, respectively [136].

Why other single *SCN5A* mutations may lead to different clinical phenotypes is not fully understood. However, some studies suggested that the existence of modifier genes affects the phenotype. For example, the D1275N mutation leads to atrial standstill when combined with a connexin-40 polymorphism, but to DCM and conduction disturbances when such a polymorphism is absent [95, 137, 138]. A common *SCN5A* polymorphism, H558R, was also reported to modify the expression of a *SCN5A* mutation in an experimental study, or the clinical severity of conduction disease or BrS [44, 139, 140]. Different clinical expressivity of a *SCN5A* mutation in different ethnic groups may be also explained by different genetic backgrounds. Bezzina et al. reported that a promoter haplotype variant of the sodium channel was commonly found in Asian populations, but not in Caucasians. This variant led to conduction disturbance represented by ECG parameters, such as longer PR and QRS duration. Gender also plays a role in different phenotypic expressivity. For example, in BrS, it is known that men are predominantly clinically affected. One of the reasons is that action potential properties are different between men and women. I_{to} is more abundant in men, whereas I_{CaL} is more expressed in epicardium of women [141–143]. Testosterone is another modifier that has been reported to affect BrS type 1 ECG [144]. Variants in *KCNE5*, which is located in the X chromosome, have been recently reported to be implicated as a modifier in BrS or idiopathic ventricular fibrillation [54].

Overlap syndrome suggests that there are several modifiers including gene mutations/variants affecting the expressivity of phenotypes related to *SCN5A* mutations. Further study is awaited to

reveal the mechanisms leading to different clinical phenotypes.

Summary

In the past 20 years, extensive research on cardiac sodium channels and dysrhythmias due to mutations in sodium channel encoding genes has expanded our knowledge on sodium ion channelopathies. The α(alpha)-subunit of the sodium channel as well as ancillary β(Beta)-subunits and regulatory proteins have shown to play an important role in generating sodium current and, furthermore, in sodium channelopathies. *SCN5A* mutations lead not only to inherited sodium channelopathies without structural abnormality, but may also lead to structural changes or dilated cardiomyopathy. Conversely, genotype-phenotype relations caused by a single *SCN5A* mutation still appear to be complex. Although we have learned that several modifying factors including polymorphisms in genes affect phenotypes, our current knowledge is not sufficient yet to fully understand which factors contribute to particular phenotypes. As *SCN5A* mutations are found in 2–8 % of the healthy populations, some mutations may actually be just a bystander. Our journey to unveil the mechanism of sodium channelopathies continues through both clinical and experimental studies.

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L-Type Calcium Channel Disease

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Abstract

The discovery of the role of voltage-gated L-type calcium channels (LTCC) as a cause of cardiac channelopathies is one of the most relevant advancements in the field of arrhythmology. LTCC defects have been identified with delay as compared with mutation in other potassium or sodium channels. A possible explanation relies on the complex genetic architecture of LTCC, which made difficult to plan and perform detailed investigations. The contribution of different gene/proteins is required in order to recapitulate a fully functional calcium conducting multimerical complex and mutations have been identified in different channel subunits. Functionally, LTCC have a key role in cardiac excitability, therefore it is not surprising that they may cause multiple and often severe clinical phenotypes: Timothy syndrome, Brugada syndrome, short QT syndrome, early repolarization and J wave syndromes.

In this chapter we present an overview of the structure, physiology and pathophysiology of the cardiac Cav1.2 encoded by the CACNA1c gene with a specific focus on Timothy syndrome.

Keywords

Inherited arrhythmias • Genetics • Cardiac channelopathies • Sudden cardiac death • Ion channels • Ventricular fibrillation • Calcium handling

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Among the most important recent advancements of knowledge in the field of inherited arrhythmogenic diseases, the identification of the role voltage-gated L-type calcium channels (LTCC) represents an important step. LTCC dysfunction can result from structural aberrations causing periodic paralysis and malignant hyperthermia sensitivity (Cav1.1), incomplete congenital stationary night blindness (Cav1.4) and inherited arrhythmias (CaV1.2) [1].

CaV1.2 (the alpha subunit of the L-type cardiac calcium channel) has a central role in cardiac excitability and therefore it is not surprising that, when it is affected by genetic defects, it can cause multiple and often severe clinical entities: Timothy syndrome, Brugada syndrome, Short QT syndrome, early repolarization and J wave syndromes [2–5].

In this chapter we present an overview of the structure, physiology and pathophysiology of the cardiac Cav1.2 encoded by the *CACNA1c* gene with a specific focus on TS since the other CaV1.2 phenotypes are the focus of additional chapters in this book.

L-Type Calcium Channel

Structure of Cardiac Cav1.2 Channel

Cav1.2 constitutes the pore forming protein (alpha-1 subunit) responsible for voltage-dependent L-type Ca^{2+} channel in the heart. The pore forming protein requires a number of additional peptides in order to work properly. The channel is indeed a macromolecular complex, made up of α1, α2/δ and β subunits. Alpha-1 subunit (the pore) is a protein of about 2,000 amino acidic residues and consists of four homologous domains (I to IV), each formed by six transmembrane spanning segments (S1–S6), and a membrane-associated loop between S5 and S6 (Fig. 12.1) [6]. The α1 subunit forms the ion-selective pore, the voltage sensor, the gating

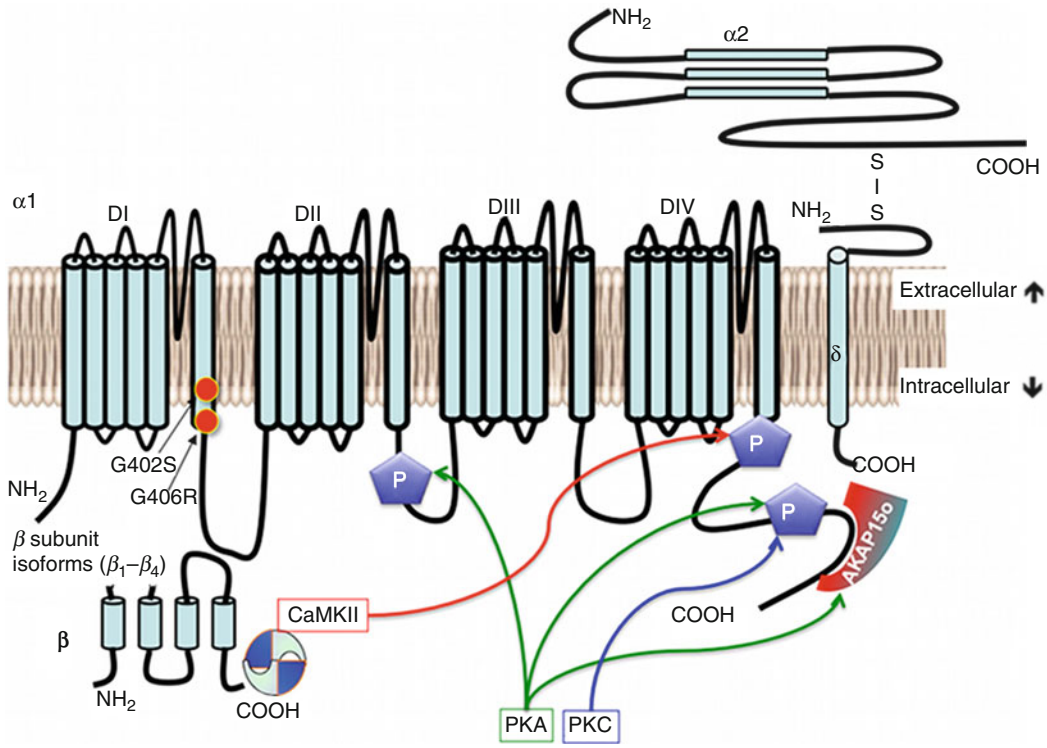

Figure 12–1. Predicted topology of Cav1.2 and its resulting proteins related to TS pathophysiology. Mutations found in Timothy syndrome (*red dots*) interact with channel phosphorylation leading to a sort of hyperphosphorylation state. This process is thought to be mediated by CaMKII (through beta 2 subunit) and by AKAP150, which targets protein kinases to phosphorylation sites (such as Serine 808, 1535 – putative CaMKII site – and 1928)

machinery, and the binding sites for channel-modulating drugs. The positively charged S4 of each domain serves as the voltage sensors for channel activation. It is thought that the S4 move outward and rotate under the influence of the electric field so to induce a conformational change that opens the pore [7]. The pore is asymmetric: the outside pore is constructed by the pore loop, which contains highly conserved glutamate residues for calcium ion selectivity [8]. The inside pore is composed of the S6 segments, which include the receptor sites for L-type Ca^{2+} channel antagonist drugs [6].

Beta, alpha2 and delta subunits have a regulatory role. Interestingly, α2 and δ subunits are encoded by a single gene that is translated as a precursor polypeptide and is post-translationally cleaved into the two subunits. The transmembrane δ subunit anchors the α2 protein to the membrane via a single putative transmembrane segment. This association is mediated by disulfide bridges. A range of functional effects has been identified for the associated subunits, including ligand binding, increasing peak currents, modulation of activation and inactivation (increasing the rate of both voltage and Ca^{2+} dependent inactivation) rates [9, 10].

Calcium Channel Function

Cav1.2 is the most abundantly expressed calcium channel in the ventricular myocytes. It produces a voltage-dependent inward Ca^{2+} current (I_{Ca}) that activates upon depolarization and it is a crucial player in the maintenance of the plateau of cardiac action potential; thus the I_{Ca} modulation substantially affects action potential duration. Furthermore, Ca^{2+} ions play an important role in excitation-contraction coupling, since I_{Ca} triggers the release of calcium from the sarcoplasmic reticulum thereby elevating the cytoplasmic Ca^{2+} to initiate contraction.

Regulation and Tissue Distribution of Cardiac Cav1.2 Channel

The regulation of cardiac Cav1.2 channel activity involves several pathways. Protein kinase A (PKA), Protein kinase C (PKC) and Ca^{2+}-binding protein calmodulin constitute key mechanisms for controlling Ca^{2+} influx [11–14]. Furthermore, relevant to Timothy syndrome pathophysiology (see below), Cav1.2 channel activity is also sensitive to calcium concentration, catecholamine [15] and Ca^{2+}-Calmodulin-dependent protein kinase II (CaMKII) [16, 17].

Beside cardiac muscle, Cav1.2 is expressed in several tissues including, peripheral and central nervous system, liver, testis, spleen, connective tissue, and bone marrow. It follows that with such a widespread distribution CaV1.2 abnormalities may perturb the function of several organs and cause complex phenotypes.

Another level of complexity of calcium channel physiology is represented by the fact that *CACNA1c* gene (the gene encoding for Cav1.2) undergoes extensive alternative splicing. More than 20 splice variants have been described, producing expressed peptides with distinct electrophysiological and pharmacological properties [18]. Cell-selective expression of Cav1.2 channels containing specific alternatively spliced exons increases the functional variability for specific cellular activities in response to changing physiological signals. This complex regulation emphasizes the difficulty to predict the clinical manifestation of mutants Cav1.2 proteins. Of clinical relevance, it is unclear why gain of function mutations cause the complex phenotype of Timothy syndrome with multi-organ involvements (see below) while loss of function mutations have been associated with Brugada/short QT phenotype with no signs of extra-cardiac disorders [2].

Timothy Syndrome

Historical Notes

In 1992, Reichenbach reported and defined it as a novel clinical entity, a case of a male infant born at the 36th week of gestation by cesarean section (because of intrauterine bradycardia) who died suddenly at age 5 months. Second degree atrioventricular block, QT interval prolongation and syndactyly were observed [19]. Subsequently Marks et al. reported three additional cases of LQTS and syndactyly [20], confirming the typical features of this disorder.

The first systematic description of the disease was jointly reported in a collaborative study by

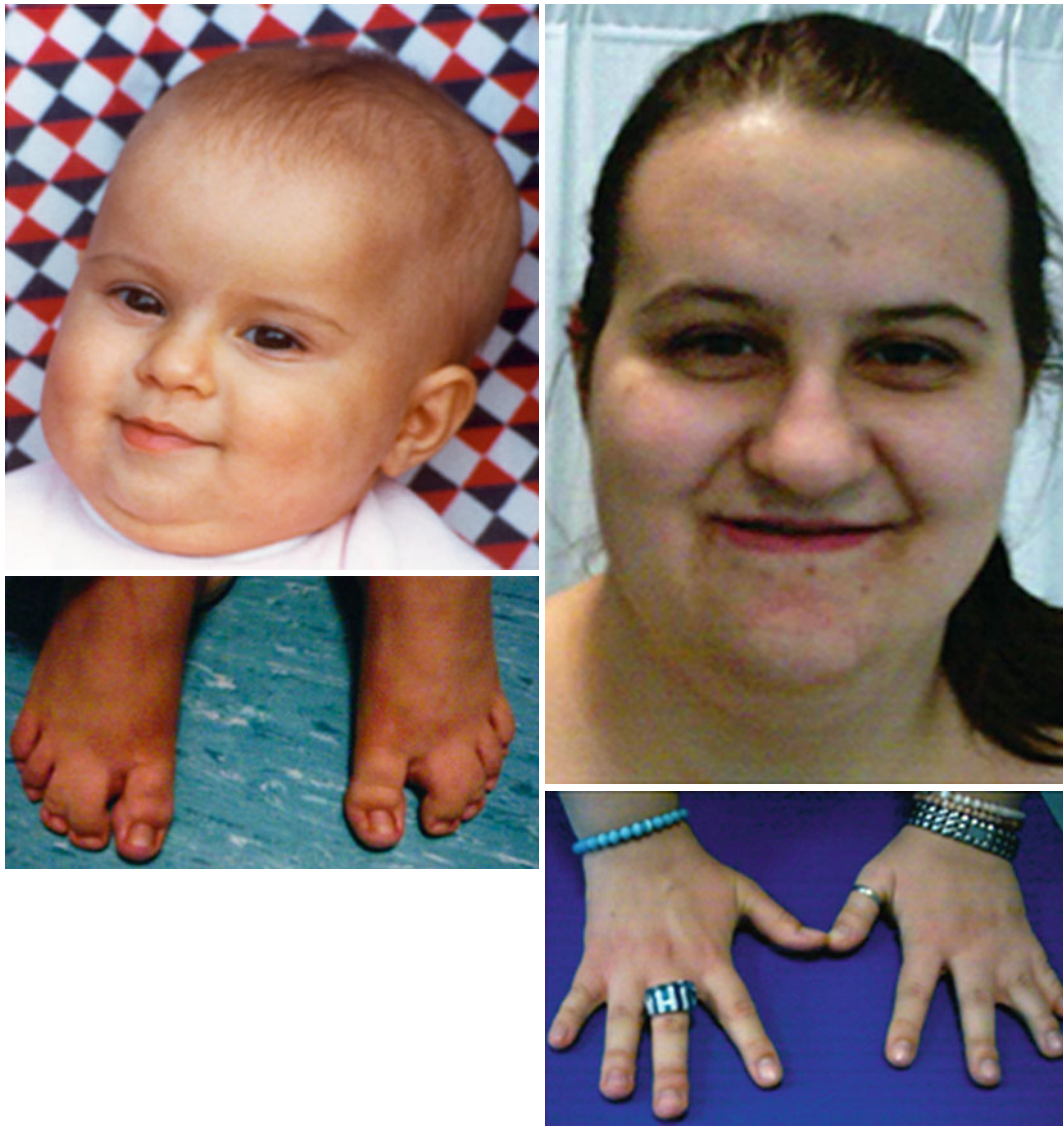

Figure 12–2. Syndactyly of feet (*left panel*) and hands (*right panel*) in patients with TS

the groups of Mark Keating and our group. In this study we described 13 cases of this distinctive form of LQTS, presenting with a complex disorder (Fig. 12.2) (see below) with multiorgan involvement and defined the disease as Timothy syndrome (MIM:601005) [5]. Few tens of TS cases have been identified after the initial formal description of the disease, thus suggesting that this condition is extremely rare, possibly because most of the patients die before reproductive age.

Phenotype and Natural History

The first signs of TS may manifest during gestation with fetal bradycardia and 2:1 atrioventricular block but diagnosis is often made within the first few days of life due to abnormal ventricular repolarization and soft tissue syndactyly of hands and toes. With few exceptions (possibly related to a different genetic substrate, see below), syndactyly has been observed in the vast majority of cases.

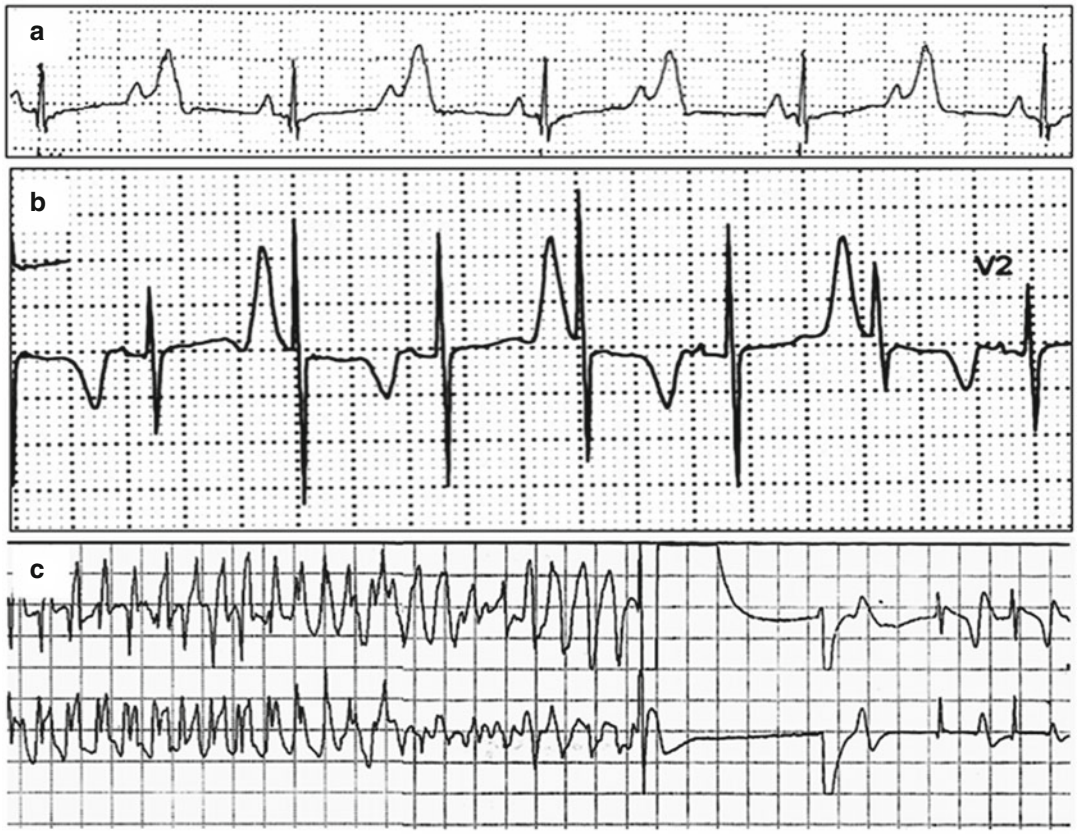

Figure 12–3. ECG recordings in a TS: (**a**) long QT and 2:1 atrioventricular block. P = atrial activation, (P wave) (**b**) T wave alternans. (**c**) Ventricular fibrillation detected and terminated by ICD shock in a 3 years-old TS patient

The QT interval is markedly prolonged in TS (mean value 600 ms); such extreme QT prolongation can cause 2:1 functional AV block. Remarkable abnormalities of T wave morphology consisting of long and straight ST segment, negative T wave and macroscopic T wave alternans, are also evident.

The most frightful manifestations of the disease is represented by cardiac tachyarrhythmia (VT or VF) that occur in 79 % of patients and is the most frequent cause of death (Fig. 12.3). Mortality is in the range of 60 % and the mean age of death is of 2.5 years. However, several additional pathologic (cardiac and extra-cardiac) phenotypes contribute to the TS phenotype:

- congenital heart disease (patent doctus arteriosus, ventricular septal defect, patent foramen ovalis, Tetralogy of Fallot) (55 % of cases).
- hypertrophic cardiomyopathy, cardiomegaly and ventricular systolic dysfunction (30 % of cases).
- facial dysmorphisms (91 % of cases)
- predisposition to sepsis (50 % of cases)
- metabolic (severe hypoglycaemia) and immunologic (recurrent infections) disturbances (40 % of cases).
- neuropsychiatric involvement (autism and autism spectrum disorder, seizures, psychological developments delays) (83 %).

Genetics of Timothy Syndrome

The ST-T wave morphology in TS patients resembles that of LQTS patients with sodium channel mutations, suggesting the involvement of an inward current active during the plateau

phase of the cardiac action potential. However the screening of the *SCN5A* gene (as well as that of the other known LQTS genes) was negative. Thus *CACNA1c* was considered a plausible candidate. In 2004, Splawski et al. identified G1216A transition in exon 8A (an alternatively spliced exon), which caused the G406R amino acid transition in DI/S6 in TS patients [5]. Subsequently, Splawski et al. reported two individuals with a severe variant of TS but without syndactyly, they named it as TS2 [21]. Genetic analyses show G1216A and G1204A in exon 8, which caused G406R and G402S amino acid transition respectively (Fig. 12.1).

By means of immunostaining experiments it was shown that exon 8A is expressed in the central nervous system, including hippocampus, cerebellum, and amygdala. Abnormalities of these brain regions have been implicated in autism, a typical feature of TS. Thus, TS may represent the first evidence for a genetic predisposition to behavioral disorders. Interestingly, the clinical relevance of transmembrane Ca^{2+} current alterations and behavioral abnormalities has been further supported by the association between allelic variants in the *CACNA1h* gene (CaV3.2, T-type calcium channel) in patients with autism spectrum disorder [22]. Although not directly related with TS pathogenesis these data strengthen the concept that Ca^{2+} dysfunction may be involved in severe neuropsychological disorders and indirectly the direct the pathogenetic role of Cav1.2 in autism.

Exon 8A of *CACNA1c* is also expressed throughout the heart and the vascular system, brain, in developing digits and teeth. Thus, the expression pattern of exon 8A is consistent with the phenotypic abnormalities associated with TS [5].

Exons 8 and 8A are mutually exclusive as they encode the same structural domain (DI/S6), but one of the two must be present to encode a functional channel. Experimental data show that 22.8 % of Cav1.2 proteins contain exon 8A, and 77.2 % contain exon 8 [5]. In the brain, 23.2 % contain exon 8A, and 76.8 % contain exon 8. Consistent with the expression, TS2 patients having mutation in exon 8 appear to have a longer QTc than TS1, and a more severe pattern of arrhythmias.

Interestingly the TS2 patient reported so far do not show syndactyly, possibly because of the differential tissue expression of exons 8 and 8A [21]. On the basis of this evidence some work has been done to identify the causes of phenotypic variability in TS. The mechanism of choice between the two exon 8 transcripts is regulated by the polypyrimidine tract binding protein (PTB). This peptide mediates a switch from exon 8 to 8A splicing in a tissue-specific manner and may be responsible for part of variable expressivity [23]. Furthermore somatic mosaicism in the transmission of G406R mutation was demonstrated in the original publication by Splawski et al. [5]. More recently, Etheridge et al. reported that somatic mosaicism contributes to phenotypic variation in TS, suggesting careful genotyping of parental tissue other than peripheral blood lymphocytes [24]. Indeed the possibility for normal parents with no previous family history of TS to generate affected children because of mosaicism has a relevant impact for genetic counseling.

Mechanism of Arrhythmogenesis

Splawski et al. [5] provided the initial biophysical characterization of TS mutations showing that G406R channels display an abnormal behavior of current inactivation. While inactivation of WT channel current was nearly complete in 300 ms, G406R channels only slightly inactivated during the same time frame. In the voltage dependence of inactivation curves, WT channel inactivation was complete at +20 mV. In contrast, inactivation was only 56 % for the G406R channels (Fig. 12.4). Thus, loss of voltage-dependent inactivation is the main macroscopic current defect leading to action potential prolongation [5, 21]. Such action potential prolongation leads to delayed afterdepolarizations and triggered activity.

At single channel level, Erxleben et al. [25] observed a decrease in unitary channel conductance and an increase in spontaneous "mode 2 gating" (which is characterized by increased open probability and longer channel activations). Such mode 2 gating could produce the apparent loss of macroscopic current inactivation at the whole cell level. Furthermore, the

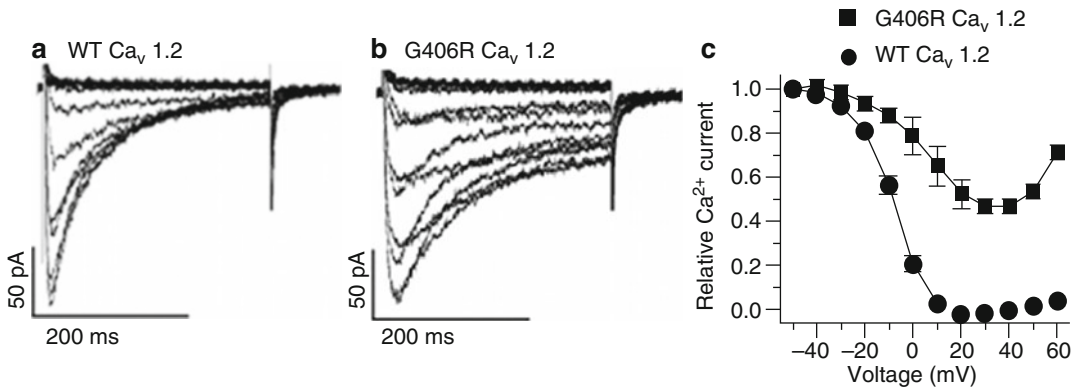

Figure 12–4. Wild-type (**a**) and G406R (**b**) Cav1.2 channel currents recorded from CHO cells in response to voltage pulses applied in 10 mV increments from −40 to +60 mV. (**c**) Voltage dependence of Ca^{2+} current inactivation for WT and G406R channels (From Splawski et al. [5]. Reprinted with kind permission from Elsevier)

presence of the TS mutation in rabbit Cav1.2 makes the protein much more sensitive to CaMKII (Ca^{2+}-Calmodulin-dependent protein kinase II) and the channel is less easily de-phosphorylated. Thus, the mutation is likely to a kind of hyper-phosphorylated state that. The role of CaMKII has been confirmed in another study by lentiviral infection of rat myocytes [26]. In this study TS Ca(V)1.2-expressing ventricular myocytes exhibited increased CaMKII activity and a proarrhythmic phenotype that included action potential prolongation, increased I(Ca) facilitation, and afterdepolarizations.

More recently, a transgenic mouse model of TS has been developed and allows to dissect arrhythmogenesis of TS [27]. Cheng et al. reported that TS mouse model show QT prolongation and spontaneous severe ventricular tachycardia. Isolated ventricular myocytes presented arrhythmogenic early and delayed afterdepolarizations. The underlying mechanisms may be associated with the reactivation of Cav1.2 and Ca^{2+} overload, which is mediated by a CaV1.2 interacting peptide, AKAP150. Mutant TS channels are indeed abnormally coupled with this AKAP150, which promotes channel activity [27]. In the same paper and altered sub-cellular localization of CaV1.2 was observed (increased expression in the intercalated discs and multiple clusters in the sarcolemma and near the nuclear envelope). Interestingly a different TS mouse model also recapitulates some of the autism spectrum disorder phenotype of the disease: repetitive and preservation behavior, reduced social memory, and increased contextual fear memory [28].

The presence of prolonged action potential was also demonstrated in reprogrammed human skin cells (IPS cells) from TS patients differentiated these cells into cardiac myocytes: electrophysiological recording and calcium (Ca^{2+}) imaging studies of these cells revealed irregular contraction (which may explain cardiac hypertrophy of some TS cases), excess Ca^{2+} influx, prolonged action potentials [29].

Therapy for Timothy Syndrome

As pointed out previously, ventricular tachyarrhythmias (VT or VF) are the leading cause of death in TS. In general, the lack of large cohorts of patients prevents any systematic clinical assessment of therapy effectiveness in TS. Beta-blockers are considered a generally effective in patients with congenital long QT syndromes and are also indicated in TS. Mexiletine and calcium channel blockers have been proposed in the attempt of shortening ventricular repolarization, restoring 1:1 conduction, and reduce the risk of arrhythmias. A recent case report suggests the effectiveness of ranolazine both to shorten QT (JT) interval and to prevent arrhythmias [30].

Implantable Cardioverter Defibrillator (ICD) is an important tool to prevent sudden cardiac death in TS patients. The implant should be

considered in all patients with confirmed diagnosis, even if asymptomatic, as soon as the body weight allows the procedure.

Despite cardiac arrhythmia prevention is the primary goal, it is very important to remark that TS patients may die for other causes: (1) Severe infections, probably the consequence of altered immune responses, can lead to death despite aggressive antibiotic therapy. (2) Intractable hypoglycemia has also been reported (Priori SG, 2005 personal communication). A close monitoring of glucose levels especially in patients treated with beta-blockers is required since these drugs may mask the hypoglycemic symptoms. (3) Severe ventricular arrhythmias may arise during induction of anesthesia.

Conclusion

TS is a genetic channelopathy with complex clinical presentation. Mutations in Cav1.2 cause LQTS associated with dysfunction in multiple organ systems, including congenital heart disease, syndactyly, immune deficiency, hypoglycemia and autism. Impaired gating caused by mutation is supposed to be the underlying mechanism. Implantable cardioverter defibrillator and beta-blockers are the recommended treatments.

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Nerve Sprouting, Defibrillation and Calcium Waves

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Abstract

Zoll et al. first reported the successful termination of ventricular fibrillation (VF) by externally applied electrical countershocks. In the same seminal report, the authors also discovered that recurrent VF may occur shortly after successful ventricular defibrillation. Due to the general availability of the implantable cardioverter-defibrillator (ICD), patients may survive the initial VF episodes but suffer from multiple recurrent VF and defibrillation shocks within a short period of time. The clustering of recurrent VF episodes (electrical storm) after initial successful defibrillation suggests that the first episode of VF begets subsequent episodes of VF. The mechanisms by which VF begets VF remains poorly understood. In this chapter we will discuss the mechanisms of neural remodeling after myocardial infarction (MI). We propose that nerve sprouting and sympathetic hyperinnervation occur after MI. The increased sympathetic nerve densities in the heart is highly heterogeneous, with portions of the heart showing increased nerve densities while the remaining portions of the heart showing denervation. During sympathetic nerve activation, there is increased heart rate and augmented intracellular calcium (Ca_i) concentration. In addition, we found that the action potential duration (APD) is abbreviated after a fibrillation-defibrillation episode in failing ventricles. The shortened APD and the elevated

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Ca_i promotes late phase 3 early afterdepolarization (EAD) and Ca^{2+}-transient triggered firing (CTTF), leading to recurrent cardiac fibrillation. We also propose that the mechanisms of APD shortening after fibrillation-defibrillation episodes in the failing (but not in the normal) ventricles are due to the upregulation of small conductance Ca^{2+} activated potassium (SK) currents. We hope that these discussions will help the readers better understand the relevance of cardiac neural remodeling and electrical remodeling in the mechanisms of arrhythmogenesis.

Keywords
Defibrillation • Ventricular fibrillation • Calcium dynamics • Optical mapping • Afterdepolarization • Triggered activity • Small conductance calcium activated potassium channels • Apamin • Heart failure • Sudden death

Introduction

Zoll et al. [1] first reported the successful termination of ventricular fibrillation (VF) by externally applied electrical countershocks. In the same seminal report, the authors also discovered that recurrent VF may occur shortly after successful ventricular defibrillation. Due to the general availability of the implantable cardioverter-defibrillator (ICD), patients may survive the initial VF episodes but suffer from multiple recurrent VF and defibrillation shocks within a short period of time [2–7]. The clustering of recurrent VF episodes (electrical storm) after initial successful defibrillation suggests that the first episode of VF begets subsequent episodes of VF. The mechanisms by which VF begets VF remains poorly understood. In this chapter we will discuss the mechanisms of neural remodeling after myocardial infarction (MI). We propose that nerve sprouting and sympathetic hyperinnervation occur after MI. The increased sympathetic nerve densities in the heart is highly heterogeneous, with portions of the heart showing increased nerve densities while the remaining portions of the heart showing denervation. During sympathetic nerve activation, there is increased heart rate and augmented intracellular calcium (Ca_i) concentration. In addition, we found that the action potential duration (APD) is abbreviated after a fibrillation-defibrillation episode in failing ventricles. The shortened APD and the elevated Ca_i promotes late phase 3 early afterdepolarization (EAD) [8, 9] and Ca^{2+}-transient triggered firing (CTTF) [10, 11], leading to recurrent cardiac fibrillation. We also propose that the mechanisms of APD shortening after fibrillation-defibrillation episodes in the failing (but not in the normal) ventricles are due to the upregulation of small conductance Ca^{2+} activated potassium (SK) currents. We hope that these discussions will help the readers better understand the relevance of cardiac neural remodeling and electrical remodeling in the mechanisms of arrhythmogenesis.

Cardiac Nerve Sprouting

Cardiac innervation comes from multiple different sources. The extrinsic sympathetic innervation arises from the stellate ganglia and paravertebral sympathetic ganglia while the vagal nerves came directly from the brain. In addition, there is also an extensive intrinsic cardiac nervous system [12] in the heart. These intrinsic nerves form ganglionated plexuses (or "ganglionated plexi", or GP). Both adrenergic and cholinergic neurons are present in these ganglionated plexi [13]. The cardiac nerves are highly plastic and can undergo significant remodeling in response to cardiac injury. The initial response is denervation and denervation supersensitivity, the latter can contribute to cardiac arrhythmogenesis [14]. However, in response to injury and denervation, cardiac nerves undergo nerve sprouting. The cells in the heart releases and synthesizes the nerve growth factor (NGF) and other neurotrophic factors after MI [15, 16]. These neurotrophic factors were transported retrogradely through the axons to the cell body within the stellate ganglion, causing the nerves to sprout and heterogeneously hyperinnervate the myocardium [17, 18]. There are

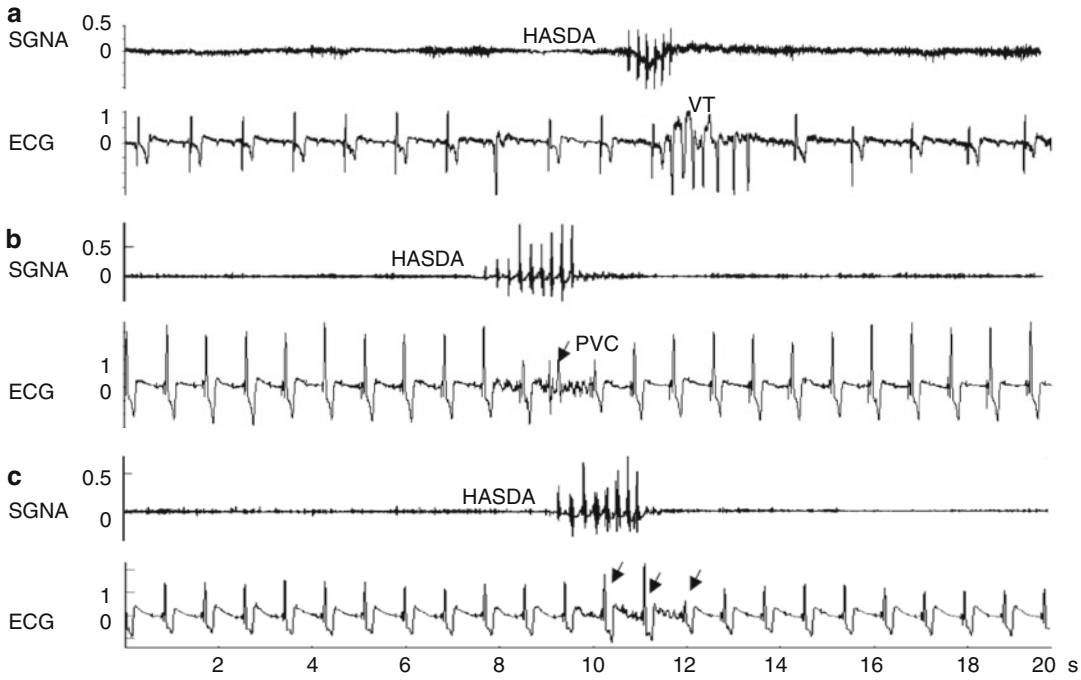

Figure 13–1. High amplitude spike discharge activity (*HASDA*) induced ventricular tachycardia (*VT*) (*Panel* **a**), premature ventricular contraction (*PVC, arrow*) (*Panel* **b**) and QRST morphology change (*arrows*, *Panel* **c**) in dogs with MI, atrioventricular block and NGF infusion to the left stellate ganglion (From Zhou et al. [21] with permission)

potential benefits of cardiac reinnervation, as has been shown in patients with orthotopic cardiac transplantation [19]. However, a combined sympathetic hyperinnervation and electrical remodeling can also trigger spontaneous cardiac arrhythmias and negatively affect the prognosis.

Sympathetic Nerve Activity and Cardiac Arrhythmia

To demonstrate a direct relationship between cardiac reinnervation and arrhythmia, we developed a technique to directly record sympathetic nerve discharges in ambulatory dogs [20]. We also developed a canine model of ventricular tachycardia (VT), VF and sudden cardiac death (SCD) by creating MI, atrioventricular block and infusing NGF into the left stellate ganglion [18]. We found that there are two different types of sympathetic nerve activities in the stellate ganglion. The first one is the high amplitude spike discharge activities (HASDA), which averaged about seven spikes per episode, with amplitude averaging around 0.9 mV. The frequency between the spikes is more or less regular, with a period (cycle length) of about 150 ms. Each episode lasts about 1 s. These high amplitude discharges are high arrhythmogenic, and are often followed by VT, premature ventricular contractions or changes of QRS morphology (Fig. 13.1) [21]. While HASDA is highly arrhythmogenic, they are rare occurrences. The vast majority of nerve activity patterns are of much lower amplitude and longer duration. These are low amplitude burst discharge activities (LABDA), which gradually increases prior to the onset of VT. Persistent LABDA was seen to precede episodes of VF and SCD (Fig. 13.2).

Defibrillation and Intracellular Ca^{2+} Dynamics

As shown in Fig. 13.2, there is increased sympathetic discharges both preceding and following the onset of VF. The sympathetic nerve activity releases norepinephrine, which increases the intracellular Ca^{2+}. Abnormally large Ca^{2+}

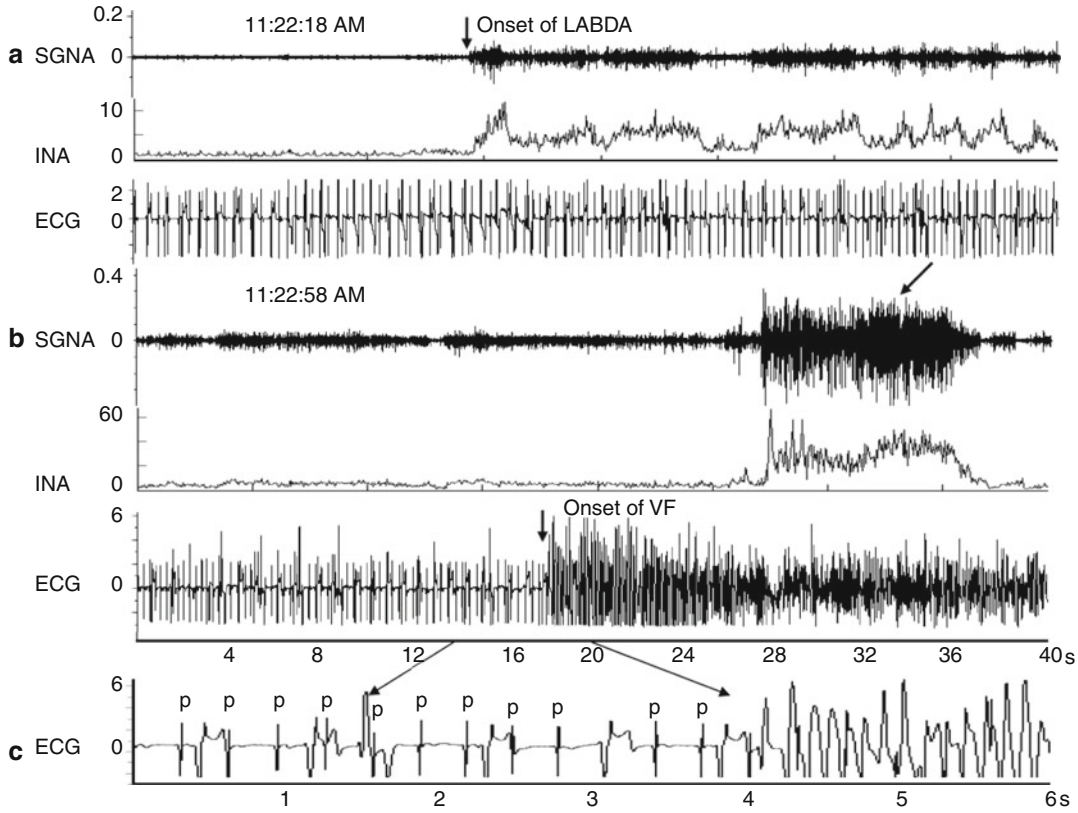

Figure 13–2. An example of increased left stellate ganglion nerve activity (*SGNA*) preceding VF and SCD. *Panel* **a** shows increased LABDA resulted in accelerated idioventricular rhythm. *Panel* **b** shows that VF occurred approximately 40 s later. *Panels* **a** and **b** are continuous. *Panel* **c** shows a view of 6 s recording from *panel* **b**. P, P wave, which is dissociated from the ventricular activation due to complete atrioventricular block. The *arrow* on ECG tracing points to the onset of VF. The arrows on the SGNA tracing point to massive sympathetic discharges after the onset of VF. *INA* integrated nerve activity. The unit for INA in this and all other figures is μV (From Zhou et al. [21] with permission)

transient is known to be a mechanism of cardiac arrhythmia [22]. Zoll et al. [1] showed that electrical defibrillation is effective in terminating VF. However, in the same manuscript, the authors showed VF may also recur spontaneously soon after electrical defibrillation. The mechanisms by which electrical shocks terminate VF remain unclear, so are the mechanisms by which VF may spontaneously recur after VF-defibrillation episodes. Since the strong electrical shocks alter the transmembrane potentials by creating a voltage gradient field, defibrillation was thought to be achieved exclusively by the changes of membrane potential through electrical current. However, recent experimental data have demonstrated that defibrillation shocks also change Ca_i, which may be of great importance to the outcome of the defibrillation.

Mechanisms of Defibrillation

To understand the mechanisms of defibrillation, it is necessary to also understand the mechanisms of defibrillation failure. The mechanisms of failed defibrillation depend on the strength of defibrillation shocks [23, 24]. At weak strength shocks (typically >100 V lower than the shock strength with 50 % probability of successful defibrillation (DFT_{50})), reentrant activation immediately after the shock contributes to the

defibrillation failure by maintaining continuous VF. Optical mapping studies have reported that there was no post-shock interval at weak shock strengths and more phase singularities remained after delivery of the weaker shocks [23, 24]. To achieve defibrillation, the virtual electrode polarizations (VEPs) created by the shock-induced electric field need to have sufficient amplitude to halt the activation wavefronts of VF. However, this is not the only reason of defibrillation failure at weak shock strengths. Even if the VEPs extinguish all existing VF activation wavefronts, it is not sufficient to ensure successful defibrillation, because the VEPs can themselves generate new activation wavefronts that reinitiate VF. Since VF consists of unsynchronized rapid activations, some portions of the ventricular myocardium are in their vulnerable period no matter when the shock is delivered. When a critical value of the potential gradient encounters a critical degree of refractoriness of the tissue during the vulnerable period, reentry is formed at the critical point, which reinitiates VF [25, 26]. Stronger electric shocks prevent the formation of the critical point and the re-initiation of VF, because of the presence of an upper limit to the strength of shocks given during the vulnerable period that could induce VF (i.e. the upper limit of vulnerability) [27, 28]. Another type of the critical point has been reported in a study by Efimov et al. [29]. In this pattern, the critical point is created during the action potential plateau by shock-induced VEPs of depolarization and hyperpolarization. The hyperpolarization caused by virtual anode de-excites the myocardium during the plateau of the action potential, leading to recovery of the excitability at the hyperpolarized region. This allows the adjacent depolarized region caused by virtual cathode to activate the hyperpolarized region, giving rise to a new activation wavefront and initiation of reentry (i.e. the virtual electrode-induced phase singularity) [29]. Electric shocks at a sufficiently strong strength also prevent the virtual electrode-induced phase singularity, because an increase in the shock strength enhances the degree of depolarization and hyperpolarization at the virtual cathode and anode, respectively, which accelerates the conduction velocity of the activation wavefront across the boundary of each VEP [30]. The electric shock can evoke an action potential in the excitable segment of the ventricular myocardium during VF. However, the weak strength shocks can exert little effects on the myocardium during a refractory phase of the action potential, which leave the ventricular repolarization unsynchronized. Therefore, the critical point of either mechanism induced by electric shocks can induce reentry in the tissue with heterogeneous repolarization at weak shock strengths.

On the other hand, the mechanism of defibrillation failure differs at stronger shock strengths near defibrillation threshold (DFT) (shock strength within 50 V of DFT_{50}). It has been reported that the near DFT strength shock applied during the refractory period is capable of extending the APD and the effective refractory period [31]. The degree of the APD extension depends on the shock coupling interval, thereby causing the myocardium to repolarize at a constant time regardless of the pattern of the pre-shock electrical activities [32]. This synchronized repolarization elicited by the strong electric shock prevents the genesis of the shock-induced critical point and reentry. The optical mapping study has identified that the number of post-shock phase singularities decreases continuously from pre-shock values to zero as the shock strength increases [24]. Importantly, even though reentry was not observed immediately after the near DFT strength shock, defibrillation does not necessarily succeed. In other words, immediate post-shock reentry is not responsible for defibrillation failure at near DFT shock strengths. In that case, activation is not recorded in both electrical and optical mapping studies for a period of 50–90 ms following the near DFT shocks (i.e. the isoelectric window), after which earliest activation always propagates centrifugally away from the early site in a focal pattern [23, 33–35]. If the shock fails to defibrillate, these foci arise rapidly and repetitively for several cycles, resulting that the activation wavefronts degenerates back into VF. If the shock successfully defibrillates, no foci are observed or only a few

focal activations last until they stop spontaneously. One question is whether the shock extinguishes all VF activations during isoelectric window. Many electrical and optical studies mapped epicardial surfaces; hence one may assume that activation wavefronts are still present intramurally or in the Purkinje fibers during isoelectric window, and the focal pattern of the epicardial activation for the first post-shock beat represents epicardial breakthrough from the deeper layer of the ventricle. A simulation study [36] demonstrated that, during the isoelectric window, an activation wavefronts induced by VEPs within the deep ventricular wall propagated fully intramurally through an excitable tunnel, until it emerged onto the epicardium, forming the focal pattern of the first post-shock epicardial activation. However, three-dimensional whole-heart mapping studies with plunge electrodes showed no evidence of wavefront propagation or reentry during the isoelectric window, and a new activation wavefront emerged focally following the isoelectric window [33, 35]. It is possible that plunge needle electrodes could not detect slowly propagated graded responses or were too few recordings to demonstrate activations in the intramural regions or the Purkinje system. However, we found that the isoelectric window followed by a focal discharge was still observed using an optical mapping technique in rabbit hearts with endocardial cryoablation in which only a thin layer (≈0.5-mm) of surviving epicardial tissues [37]. Therefore, the experimental evidence available supports the idea that the near DFT shocks eliminate all VF activations. A new wavefront of the focal mechanism rather than reentry is re-induced by the defibrillation shock in failed defibrillation.

Role of Intracellular Ca^{2+} in the Generation of Postshock Focal Activation

We simultaneously mapped epicardial membrane voltage (V_m) and Ca_i during attempted defibrillation at a near DFT shock strength in Langendorff-perfused rabbit ventricles [37]. We found that the heterogeneous post-shock distribution of Ca_i plays a key role in defibrillation failure. A focal activation following the isoelectric window in unsuccessful defibrillation always originates from the regions of low Ca_i surrounded by elevated Ca_i (i.e. Ca_i sinkholes) (Fig. 13.3a). No Ca_i sinkholes are present after type A successful defibrillation. We also determined the relationship between the Ca_i sinkholes and vulnerability to VF, since re-induction of VF by a defibrillation shock itself is responsible for defibrillation failure. The Ca_i sinkholes were also present after a shock on T-wave that induced VF (Fig. 13.3b) [37, 38]. What is the mechanism by which the first post-shock activation consistently arises from the Ca_i sinkhole? The first post-shock activation following the isoelectric window is focal, suggesting triggered activity as a potential mechanism. In some episodes of unsuccessful defibrillation, an earlier rise in Ca_i than V_m are observed at the early site for the post-shock focal activation. The ratio map shows that a rise in Ca_i precedes a rise in V_m at the Ca_i sinkhole site (i.e. Ca_i prefluorescence, arrow in Fig. 13.4). It is possible that the Ca_i prefluorescence might be attributable to spontaneous (voltage-independent) Ca^{2+} release from the sarcoplasmic reticulum (SR) that induces triggered activity by the reverse excitation-contraction coupling [39]. The low Ca_i in the sinkhole reflects more complete SR Ca^{2+} uptake. With the SR more Ca^{2+} loaded and more fully recovered, it may be more susceptible to spontaneous Ca^{2+} release from the SR, compared with regions with persistently high Ca_i. Consistent with this hypothesis, suppression of SR function by ryanodine and thapsigargin significantly reduced the upper limit of vulnerability and DFT [37]. Alternatively, EAD might be another explanation for the triggered activity. At the Ca_i sinkhole site, the Ca_i continues to decline, which promotes the recovery of $I_{Ca,L}$ from inactivation. Reactivation of $I_{Ca,L}$ increases both Ca_i and V_m, leading to EAD and triggered activity. Another possible mechanism is the electrotonic interaction with surrounding cells. In the Ca_i sinkholes, APD is shorter than in the surrounding sites, possibly because the reduced Ca_i level in the sinkholes may shorten the APD by the

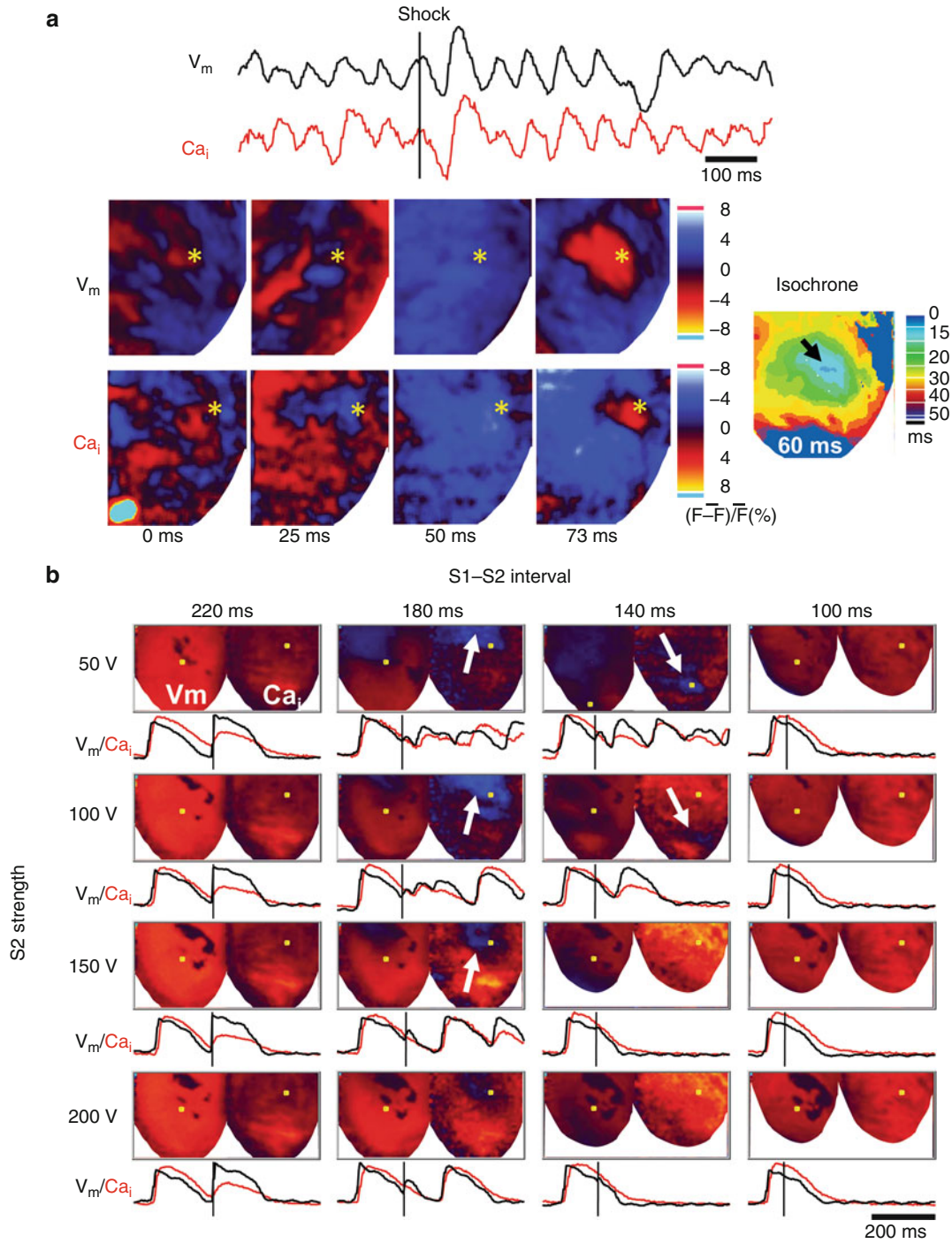

Figure 13–3. Ca_i sinkhole in unsuccessful defibrillation (**a**) and when shock was given during the vulnerable period (**b**) in a Langendorff-perfused rabbit ventricle. (**a**) Epicardial V_m (*black line*) and Ca_i (*red line*) optical signals from the site marked by *asterisk* in snapshots of ratio maps. Electric shock at near DFT strength was applied at time zero (*vertical line*). After the shock, the Ca_i map showed an area with continued decline of Ca_i, resulting in a *blue* region on the Ca_i map (marked by *asterisks* in the frame 25–50 ms). This *blue* region had a lower Ca_i level than the surrounding tissues (Ca_i sinkhole). Thereafter, the entire V_m map showed repolarization coded with *blue* (frame 50 ms), consistent with the isoelectric window. Note that a focal discharge started at the Ca_i sinkhole site. (**b**) Shock on T wave with different S1–S2 coupling intervals and S2 shock strength. Note that all episodes associated with induction of VF or repetitive responses had the Ca_i sinkhole (*white arrow*) after the shock (From Hwang et al. [37] with permission)

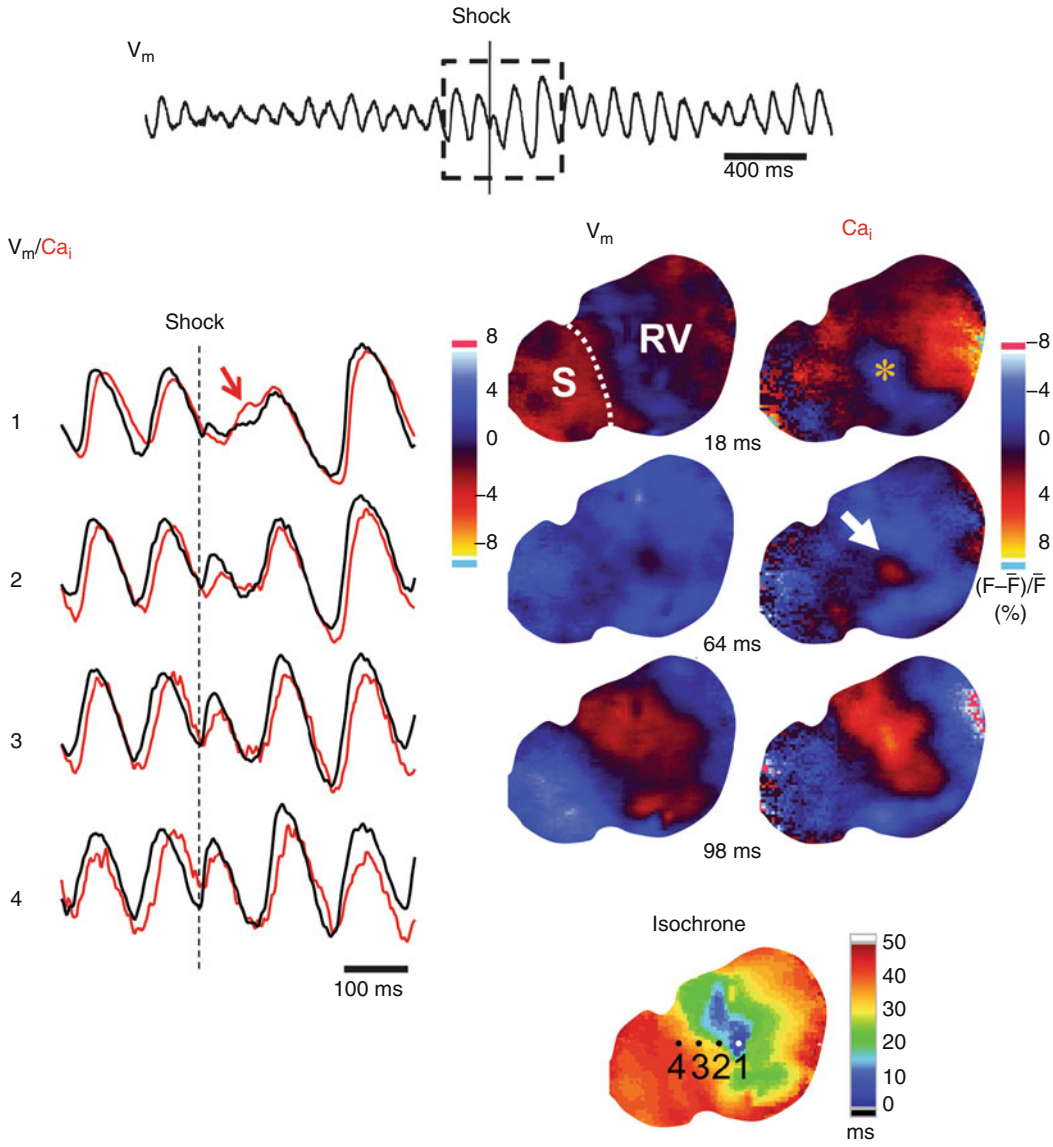

Figure 13–4. Ca_i prefluorescence at the Ca_i sinkhole after unsuccessful defibrillation in the endocardial surface of a Langendorff-perfused rabbit ventricle. Local V_m (*black lines*) and Ca_i (*red lines*) optical signals were obtained at sites indicated in the isochronal map for the post-shock first activation. The Ca_i sinkhole (*asterisk*) was observed (frame 18 ms) after shock at a near DFT strength. Note a rise in Ca_i (*white arrow*, frame 64 ms) while V_m map was still in a quiescent period (Ca_i prefluorescence), followed by a focal post-shock activation. At this site (site 1), a local Ca_i elevation (*red* region in frame 64 ms) preceded a rise in V_m. *S* interventricular septum, *RV* right ventricle

bidirectional coupling between Ca_i and V_m [40, 41]. Electrotonic currents from the surrounding depolarized regions might assist impulse formation in the sinkhole region in the similar way to phase 2 reentry or reflection [42].

Biphasic Defibrillation

The heterogeneity of Ca_i as well as that of V_m immediately after shock seems important in the mechanisms of defibrillation and vulnerability,

because it facilitates the formation of Ca_i sinkholes. It is known that biphasic waveform shock is more effective than monophasic waveform shock in achieving successful defibrillation. We demonstrated that the greater efficacy of biphasic waveform is directly related to less heterogeneous post-shock Ca_i distribution, thus reducing the probability of Ca_i sinkhole formation and VF re-initiation [43]. The electric field created by defibrillation shock alters the V_m but it also significantly changes the Ca_i [44, 45]. Transient hyperpolarization at virtual anodes is expected to increase the driving force for Ca^{2+} entry through $I_{Ca,L}$, potentiating SR Ca^{2+} release, while transient depolarization at virtual cathodes is not expected to exert this effect [46]. It follows that a shock-induced APD prolongation is more accentuated at the virtual anode sites than at the virtual cathode sites, since the increased Ca_i prolonged APD by potentiating inward Na^+-Ca^{2+} exchange current (I_{NCX}), consistent with positive Ca_i-APD coupling [43, 47]. During VF, the effects of VEPs on Ca_i are variable, because effects of shocks on Ca_i depend on the timing of shock application relative to the local repolarization state [43, 45]. Thus, the sites of the Ca_i sinkhole are not predictable by distribution of the VEPs, but the heterogeneous effects of VEPs on Ca_i would promote the Ca_i sinkhole formation. This may account for the more reduction in the Ca_i sinkholes by the biphasic waveform shocks which have a neutralizing effect on the VEPs.

Intracellular Ca^{2+} Dynamics and Immediate Recurrences of VF After Successful Defibrillation

Defibrillation failure can be viewed as an immediate recurrence of VF after the shock halts all VF activations. This type of VF recurrence generally occurs 50–100 ms after the shocks [23, 24, 37]. However, as documented by Zoll et al. in their first paper on electrical defibrillation [1], VF can also recur seconds after an electrical countershock that was initially successful. More recent studies show that spontaneous recurrences of VF can occur 1 s to 2 min after defibrillation for long-duration VF with resultant ischemia [48]. Also, defibrillation during global ischemia is often followed by immediate VF recurrences (<10 s after shock) [49]. Importantly, when the VF recurrences occur during ECG saturation caused by the strong electric shock, it masquerades as defibrillation failure. This implies that a shock-resistant VF, which is often observed in the out-of-hospital sudden cardiac arrest [50], may include this type of the pseudo-defibrillation failure. Furthermore, it has been reported that recurrent VFs in patients with a normal structural heart frequently arise from the distal Purkinje system [51]. In canine electrical mapping studies, Purkinje fibers are highly active and the earliest post-shock activation always originates from the Purkinje fibers after long-duration VF [48]. We recently found that triggered activity due to delayed afterdepolarization (DAD) is the mechanism of post-shock ventricular arrhythmias originating from the Purkinje fibers which have a higher Ca_i-V_m coupling gain due to a smaller I_{K1} [52].

Upregulation of I_{KAS} in Heart Failure and Electrical Storm

Recurrent spontaneous VFs after successful defibrillation are observed frequently in patients with heart failure [53]. We recently developed a rabbit model with pacing-induced heart failure which shows recurrent episodes of spontaneous VF [54, 55]. Simultaneous mapping of V_m and Ca_i in the failing hearts identified that marked APD shortening and disproportionally persistent Ca_i transient after successful defibrillation induce afterdepolarizations during late phase 3 of the action potential possibly by promoting the forward mode of I_{NCX} (i.e. CCTF or late phase 3 EAD) [9–11], which causes triggered activity and re-initiation of VF. The importance of intracellular Ca^{2+} handling in electrical storm is also supported by the study by Tsuji et al. [56]. In that study, the authors

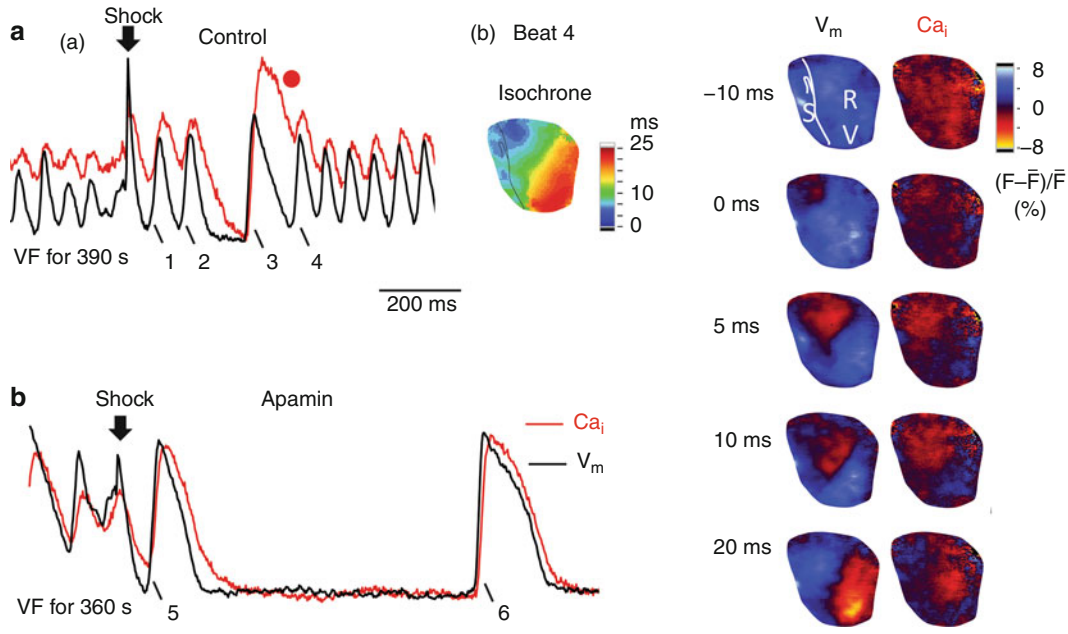

Figure 13–5. Immediate recurrence of VF and the effects of apamin. The *black* and *red lines* indicate optical tracings for V_m and Ca_i, respectively, obtained from endocardial surface in a failing ventricle. **A**(*a*), Before apamin, a defibrillation shock (*arrow*) was followed by two postshock beats (1 and 2). Optical maps (not shown) confirmed complete cessation of wavefronts after beat 2, consistent with type-B successful defibrillation. After a short pause, there was a large Ca_i transient (*red dot*), short APD (beat 3) and first beat (beat 4) of SVF arising from late phase 3. This mode of VF reinitiation is consistent with CTTF and late phase 3 EAD mechanisms observed in atrial preparations and pulmonary veins [8, 10, 11]. **A**(*b*), Snapshots of Ca_i and V_m ratio maps at times from 10 ms before to 20 ms after the onset of beat 4. Note Ca_i remained elevated throughout the mapped field while V_m has already repolarized. The beat 4 arose during persistently high Ca_i and initiated the SVF. (**b**) After apamin (1 μM) infusion, the postshock beats (5 and 6) had longer APD than the beats 1–4 in **A**. The APD and Ca_iTD were approximately the same, and SVF episodes were completely prevented. *PM* papillary muscle, *S* interventricular septum, *RV* right ventricle (From Chua et al. [55] with permission)

found abnormal protein phosphorylation may affect the Ca^{2+} handling and contribute to the electrical storm in rabbits with complete heart block. In addition to abnormal Ca_i accumulation, shortened APD is also a major factor that promotes electrical storm. We discovered that in heart failure, there is a significant upregulation of apamin-sensitive potassium current (I_{KAS}) conducted through the SK channels. Because of the Ca_i accumulation during VF, this channel is activated to conduct I_{KAS} and shorten the APD. Inhibiting I_{KAS} by apamin can suppress recurrent VF in our model (Fig. 13.5). Patch clamp studies confirmed the upregulation of I_{KAS} in failing rabbit ventricles (Fig. 13.6). Our recent studies documented that SK channel is upregulated in failing human ventricles [57]. Further studies of I_{KAS} may contribute to the better understanding of the mechanisms by which VF begets VF.

Conclusions

Nerve sprouting and sympathetic hyperinnervation occur after MI and other forms of cardiac injury. Increased sympathetic discharges directly trigger spontaneous VT and VF, in part through the increased Ca^{2+} transients. Abnormal Ca_i handling can also activate I_{KAS} in failing

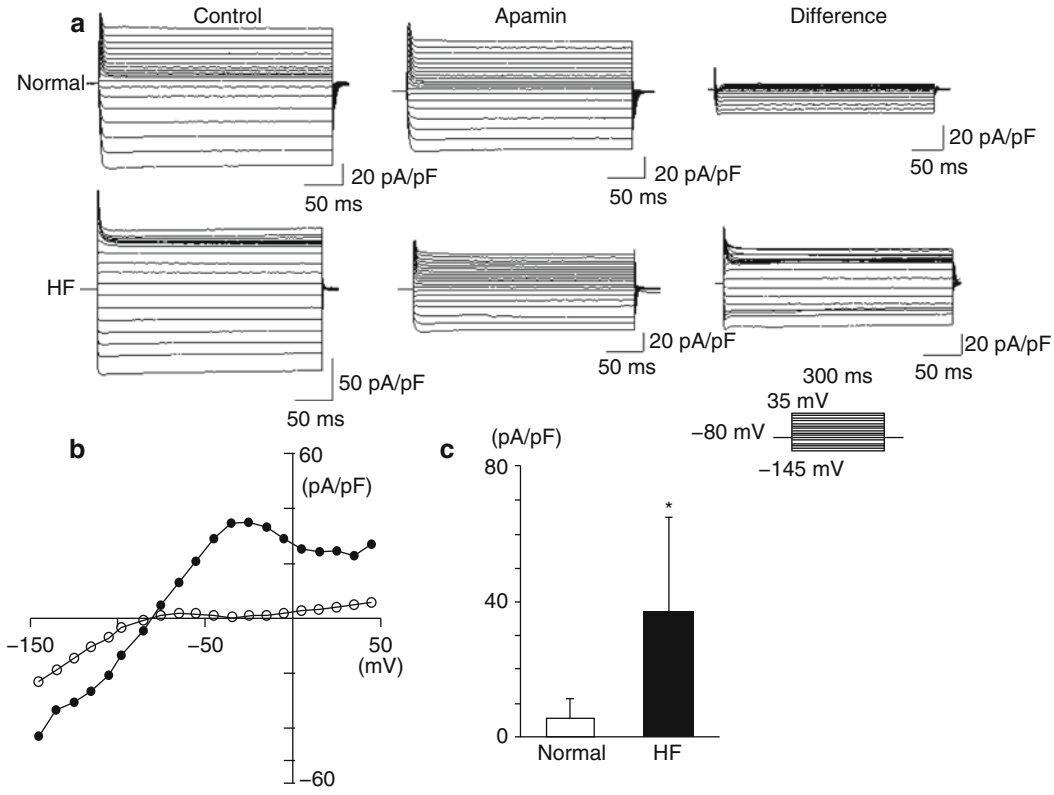

Figure 13–6. Apamin-sensitive currents (I_{KAS}) in normal and failing ventricles. (**a**) Representative superimposed current traces obtained by a step-pulse protocol (indicated in *inset*) in a cardiomyocyte of normal rabbit (*upper panels*) and HF rabbit (*lower panels*). Control depicts current traces in the absence of apamin ($I_{control}$); Apamin, current traces in the presence of 1 μmol/L apamin (I_{apamin}); Difference, I_{KAS} calculated as $I_{control} - I_{apamin}$. (**b**) Current–voltage (I–V) relationship of the I_{KAS} obtained in *panel* **a**. Current density was measured at the end of step pulse. ( ) normal rabbit, ( ) HF model. (**c**) I_{KAS} density at a membrane potential of 0 mV in normal (n = 7 cells from 4 rabbits) and HF ventricles (n = 6 cells from 3 rabbits). Error bars represent S.D. *$p < 0.05$ (From Chua et al. [55] with permission)

ventricles and contribute to postshock APD shortening and the occurrence of electrical storm. Better understanding of neural remodeling and the consequences of abnormal Ca_i handling is important to the understanding of the mechanisms of fibrillation and defibrillation.

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14
K^+ Channelopathies (I_{Ks}, I_{Kr}, and I_{to})

Kevin J. Sampson and Robert S. Kass

Abstract

Voltage gated K^+ channels are comprised of a pore-forming α-subunit and one or more accessory subunits and allow for K^+ ions to flow along their electrochemical gradient, typically out of the cell, in response to changes in membrane potential. Voltage-gated K^+ channels act to help set the plateau potential in cardiac cells as well as to repolarize the membrane during an action potential. In cardiac myocytes, they are the primary class of channels responsible for controlling the duration of the action potential, alterations in which can have profound arrhythmic effects. Defects in K^+ channel function have been linked to a number of heritable and acquired conditions leading to arrhythmia in affected patients. As we continue to characterize channel function biophysically, our mechanistic understanding of these conditions grows. Hopefully, this will continue to lead to novel therapeutic strategies not only for the relatively rare inherited channelopathies, but more widespread arrhythmias commonly encountered in clinical practice.

Keywords

Ion channels • Long QT syndrome • Atrial fibrillation • Electrophysiology • Arrhythmia

K^+ Channels

Voltage gated K^+ channels are comprised of a pore-forming α-subunit and one or more accessory subunits and allow for K^+ ions to flow along their electrochemical gradient, typically out of the cell, in response to changes in membrane potential. Voltage-gated K^+ channels act to help set the plateau potential in cardiac cells as well as to repolarize the membrane during an action potential. In cardiac myocytes, they are the primary class of channels responsible for controlling the duration of the action potential, alterations in which can have profound arrhythmic effects.

Early on, the understanding of cellular excitation and outward currents was limited to model systems and the work of Hodgkin, Huxley and Cole, who first described the ionic basis of the electrical activity in the giant squid axon [1–4]. These investigators described the interplay of inward and outward currents in controlling the repolarization of an action potential and made

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remarkable predictions about the nature of ion channels from macroscopic recordings. The first cloning of an α-subunit of the acetylcholine receptor in 1982 marked the beginning of a new era in which molecular tools allowed the discovery of many physiologically crucial channels [5, 6]. As K^+ channels were cloned, investigation in heterologous systems allowed for an improved understanding of the functional properties of channels [7, 8].

The crystal structure of a bacterial potassium channel was solved in 1998 and helped provide a structural correlate for the functional behavior of ion channels [9]. An improved understanding of the structure-function relationship in K^+ channels has identified critical regions in the channel responsible for voltage sensing and ion selectivity [10]. Further investigations have revealed that K^+ channels are part of larger macromolecular complexes and assemble with a number of partner proteins that can alter their trafficking, phosphorylation state, voltage sensing, and gating.

Acquired Versus Inherited K^+ Channel Dysfunction

Channelopathies are commonly divided into two primary clinical categories: inherited and acquired. The inherited form involves the mutation of an ion channel and for K^+ channels; the most common inherited cardiac disorder is the Long QT syndrome (LQTS). LQTS takes its name from prolongation of a patient's QT interval on their ECG that results from mutation of genes that encode ion channels or proteins that interact with them to alter their contribution to repolarization (see Chap. 30) [11].

The acquired form of K^+ channel dysfunction results from drug therapy, certain disease states, or electrolyte disorders. Any of a number of compounds including antihistaminics, antipsychotics, antibiotics and antiarrhythmics can lead to acquired LQTS and predispose patients to lethal arrhythmias by blocking the rapidly activating component of the delayed rectifier K^+ current (I_{Kr}) [12, 13]. Fatal arrhythmias resulting from block of I_{Kr} have led to the removal of many drugs from the market by the FDA and a requirement for new drugs to be tested for changes in the QT interval.

K^+ Channelopathies

This chapter focuses on three voltage-gated K^+ channels and the role of their dysfunction in disease: The transient outward K^+ current, I_{to}, that serves to end the depolarizing spike of the action potential upstroke and set the plateau potential; and the slowly (I_{Ks}) and rapidly (I_{Kr}) activating components of the delayed rectifier K^+ current that largely control action potential repolarization. Mutation in the genes encoding the pore forming α-subunit, or one of the many proteins that interact with the macromolecular complexes that K^+ currents are a part of, can lead to either prolongation or abbreviation of action potentials. Such alterations have been shown to trigger cardiac arrhythmias and even lead to sudden death Fig. 14.1.

Pathologies due to mutation in ion channels were described clinically many years before an understanding of the underlying mechanisms. The Romano-ward and the Jervell and Lange-Nielsen syndromes, both congenital long QT syndromes, were first described clinically roughly 50 years ago [14, 15]. However, it wasn't until 1995 that the link between K^+ channels and action potential prolongation was made [16, 17]. As a result of these reports and the functional and structural knowledge of K^+ channels, an understanding began of why certain drugs used to prevent cardiac arrhythmia could infact be pro-arrhythmic themselves [12, 18]. Recently the same K^+ channels implicated for Long QT have been found to be involved in familial atrial fibrillation (FAF) and the short QT syndrome(SQTS) [19, 20]. Further, mutation of I_{to} has now been implicated in the Brugada syndrome (BrS) Table 14.1.

Voltage-Gated K^+ Channels

The functional diversity among the primary voltage-gated K^+ channels in the human heart can be classified in two broad classes. That of transient outward (I_{to}) and delayed-rectifying (I_{Kr} and I_{Ks})

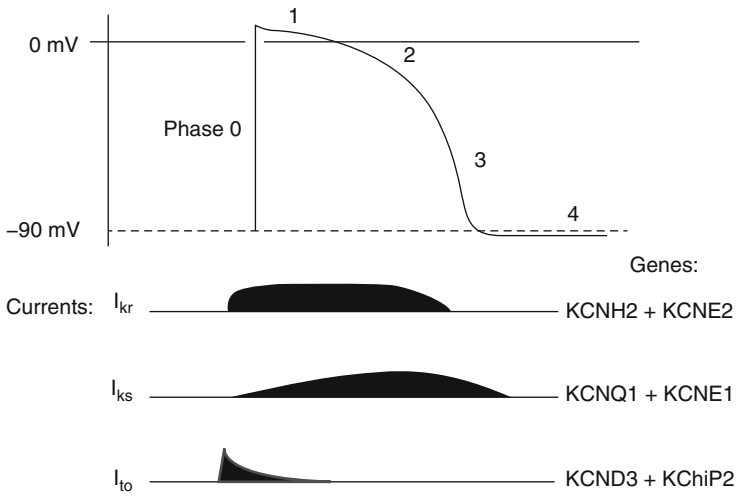

Figure 14–1. I_{Kr}, I_{Ks}, and I_{to} their respective genes and their role in the repolarization of the ventricular action potential

Table 14–1. K^+ channel heritable arrhythmia genes and their associated currents and inherited diseases

Gene	KCNQ1	KCNH2	KCNE1	KCNE2	KCNE3	KCND3
Function	α-subunit	α-subunit	β-subunit	β-subunit	β-subunit	α-subunit
Current	I_{Ks}	I_{Kr}	I_{Ks}	I_{Kr}		I_{to}
Inherited disorder	LQT1	LQT2	LQTS	LQT6	BrS	BrS
	FAF2	FAF1				
	SQT2	SQT1				

K^+ channels. Between atrial and ventricular tissues, and throughout the specialized conduction system these K^+ channels are present at variable levels leading to a diversity in the morphology and duration of action potentials [21]. These different action potentials in various cardiac cell types are required to perform their specialized roles in the heart and typically are spatially arranged in a manner that is protective from arrhythmia. Dysfunction in the normal heterogeneity of action potentials brought on by K^+ channel dysfunction is a potent pro-arrhythmic substrate.

Molecular Determinants of Voltage-Gated K^+ Channels

Most of what is known about the biophysical basis of voltage-gated K^+ channel gating is derived from studies of Shaker ($K_{v1.1}$) channels [22]. Voltage-gated K^+ channels are typically composed of two primary subunits: The α-subunits are the pore-forming subunits that also contain a voltage sensing domain and a selectivity filter (see Fig. 14.2a). β-subunits are accessory subunits that affect the channel gating properties. In addition to these two canonical subunits, K^+ channels can interact with a plethora of intracellular proteins including A-kinase anchoring proteins that can play a key role in their roles in cardiac physiology.

α-subunits are composed of six transmembrane segments (S1 – S6) and functional voltage-gated K^+ channels form by tetramerization of α-subunits with a central ion-conducting pore (see Fig. 14.2b). The first four transmembrane segments are primarily responsible for the channels response to voltage, with charged residues in S4 sensing changes in membrane potential [23, 24]. A conserved motif in the pore loop, between domains S5 and S6, of K^+ channels is responsible for ion selectivity.

The voltage-gated K^+ channel accessory subunits are a diverse set of proteins. For I_{Kr} and I_{Ks}, they are largely modulated by the KCNE family of β-subunits that are small (~100–150 amino acids) single transmembrane proteins. Another

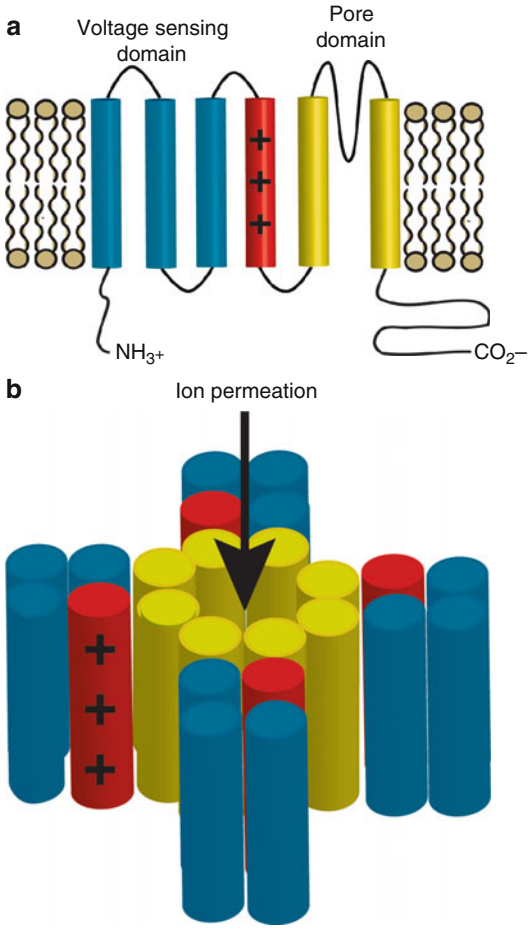

Figure 14–2. Topology of voltage-gated K^+ channels. (*Panel* **a**) α-subunit consist of six transmembrane helix with the first four largely responsible for voltage sensing and the last two forming part of the channel pore. (*Panel* **b**) Voltage-gated K^+ channels are tetramers that assemble to make function channels with a central ion-conducting pore

class of K^+ channel subunits often co-assembling with faster opening and inactivating channels is typically larger intracellular proteins including KChaP, Kvβ1–3, and KChiP2.

Delayed Rectifying K^+ Currents

Delayed-rectifying current was first described in sheep Purkinje fibers [25, 26]. Following those initial observations, characterization of the same current was made in atrial and ventricular cells as well as in pacemaker cells from various species [21].

The two prominent components first described in atrial and ventricular guinea pig myocytes based on their differences in time- and voltage-dependent properties were I_{Ks} ($I_{K,slow}$) and I_{Kr} ($I_{K,rapid}$) [27–29]. I_{Kr} is known to activate and inactivate rapidly and displays a marked inward rectification wherein ion conduction is favored inwardly. This rectification is a function of the channels recovering from inactivation faster than they deactivate. On the contrary, I_{Ks} does not display any inward rectification, minimally inactivates, and activates and deactivates slowly [28, 29]. These currents can be found in atrial and ventricular myocytes cells in most large mammals, including human, as well as in the purkinje fiber system [21]. In rodents, there are little if any delayed-rectifying channels as the AP is very brief and repolarized by rapidly opening K^+ channels [30].

Congenital Channelopathies

In the following section, we will focus on congenital K^+ channelopathies. Since the discovery that inherited QT prolongation could be caused by mutation in the K^+ channel responsible for the rapidly and the slowly activating component of the delayed rectifier K^+ current (I_{Kr} and I_{Ks} respectively), other congenital cardiac K^+ channelopathies have been described, such as the short QT syndrome (SQTS, see Chap. 33), familial atrial fibrillation (AF, see Chap. 35), and the Brugada Syndrome (BrS, see Chap. 28).

Long QT Syndrome

The Long QT syndrome is a collection of cardiac disorders characterized by a prolongation of the QT interval on the ECG that affects an estimated 1 in 5,000–10,000 (see Fig. 14.3 and Chap. 27).

The autosomal dominant inherited form of LQTS, originally called the Romano-Ward syndrome, is a collection of hundreds of mutations distributed over more than a dozen genes [31]. Modern nomenclature refers to these mutations as LQT1-LQT13. Another rare form of

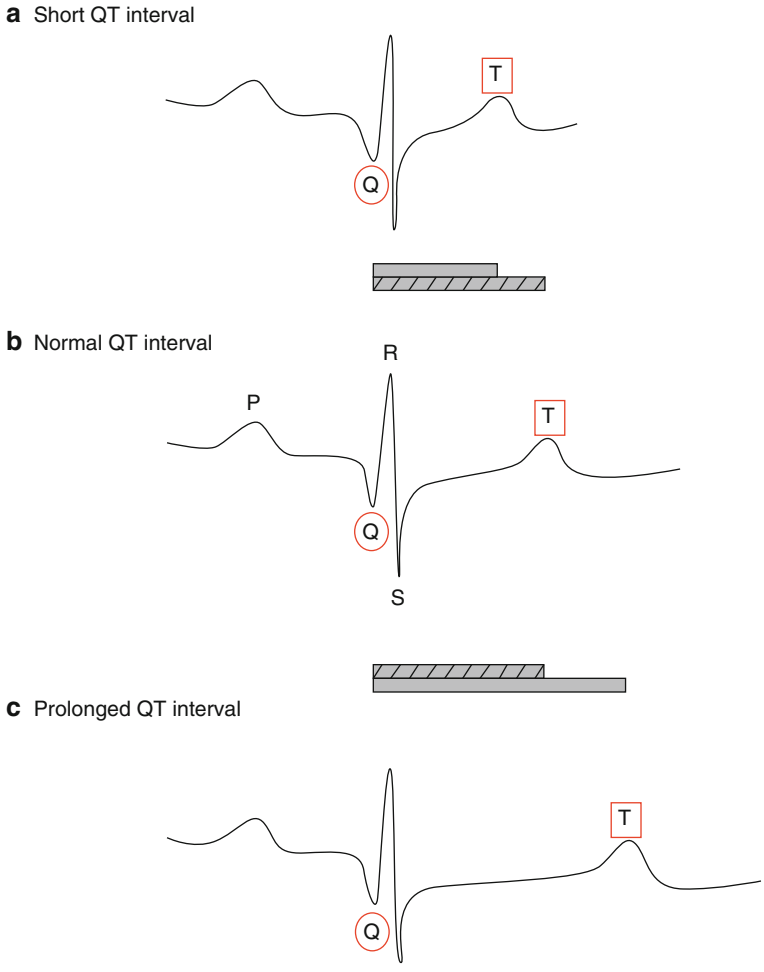

Figure 14–3. Schematic representations of normal (**b**), short (**a**) and long (**c**) QT interval durations

inherited LQTS is autosomal recessive and called the Jervell and Lange-Nielsen syndrome. In this syndrome, mutation in the genes responsible for I_{Ks} lead to cardiac arrhythmia and congenital deafness owing to these channels also being present in the inner ear [32]. To date five genes, KCNQ1, KCNE1, KCNH2, KCNE2, and AKAP9, that are directly responsible for the delayed rectifier K^+ current (I_{Ks}) and the rapidly activating component of the delayed rectifier K^+ current (I_{Kr}), have been implicated in LQTS.

I_{Ks}

I_{Ks} is a major contributor to cardiac action potential repolarization (phases 2 and 3, see Fig. 14.1). KCNQ1, like other voltage-gated K^+ channels, forms a homotetramer to make function channels (see Fig. 14.2b) [17]. Expression of the alpha subunit, KCNQ1, alone in heterologous systems results in a K^+ current that rapidly activates and partially inactivates(see Fig. 14.4a, 1) [33, 34]. The characteristic slowly activating, and slowly deactivating, current known as I_{Ks} is only observed with the coexpression of KCNE1 [33, 34]. At lower heart rates, I_{Ks} slowly activated over the course of an action potential and eventually leads to terminal repolarization. As rate increases and diastolic recovery times shorten, I_{Ks} fails to completely deactivate between beats and as a result a steady accumulation of open channels allows for critical rate-dependent shortening of the action potential [11]. This critical role of the channel allows for adequate diastolic filling time for the heart.

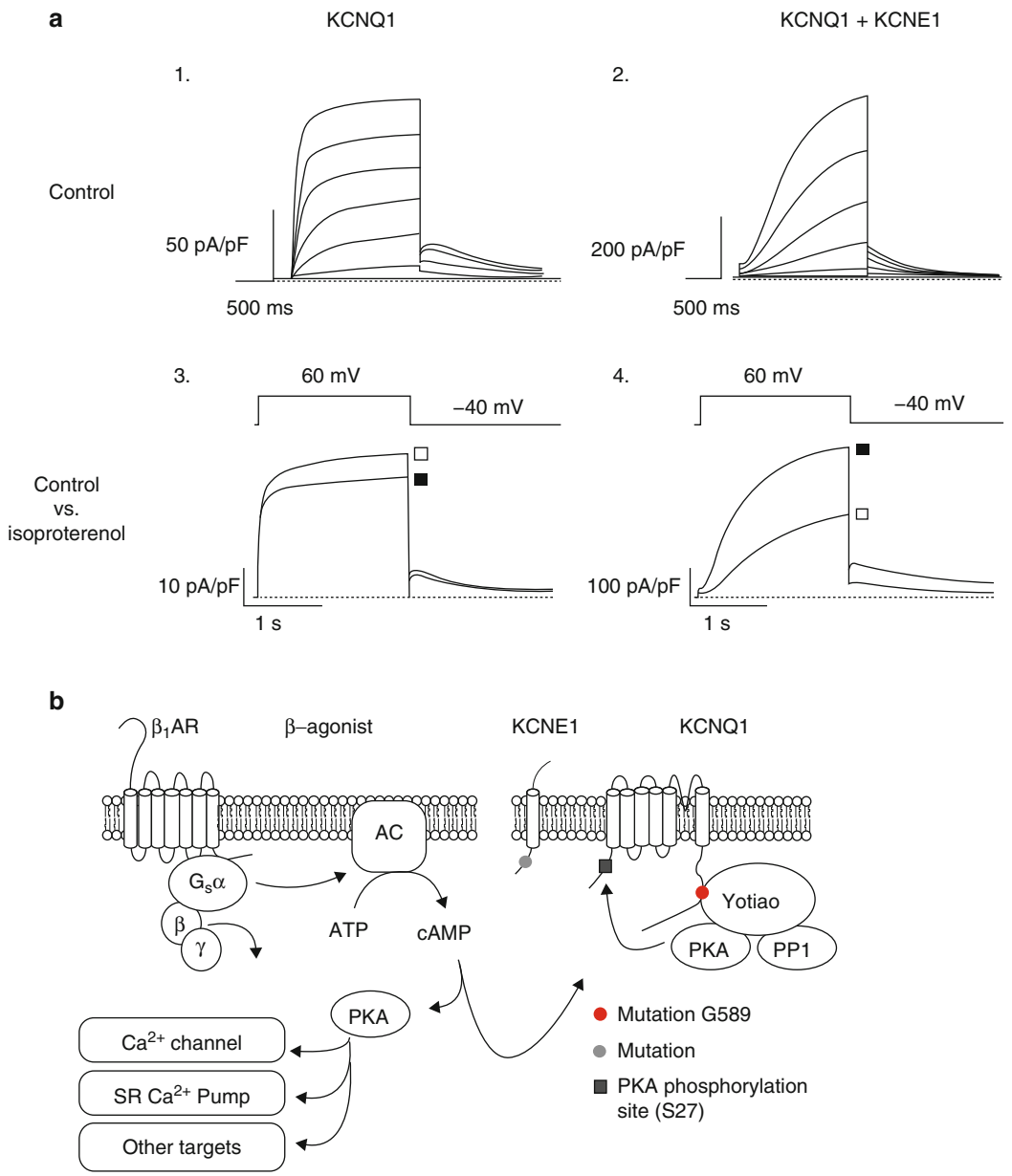

Figure 14–4. (**a**) Currents recorded from KCNQ1 alone (A1) and when coexpressed with KCNE1 (A2) in Chinese hampster ovary cells. The effect of β-adrenergic stimulation on channel function (■) requires co-expression of both subunits, KCNQ1 and KCNE1 (A3 and A4), (□) control conditions. (**b**) Schematic of the β-adrenergic stimulation pathway, briefly binding of agonist to β-adrenergic receptors begins a cascade of actions that leads to protein kinase A (PKA) phosphorylating various cardiac targets, including KCNQ1 at serine 27 (S27)

Sympathetic Nervous Stimulation of I_{Ks}

Binding of epinephrine to G protein coupled β-adrenergic receptors (β-AR), leads to activation of adenylyl cyclase. Adenylyl cyclase subsequently synthesizes cyclic adenosine monophosphate (cAMP) from adenosine trisphosphate (ATP), which leads to an increased activity of the cAMP-dependent protein kinase A (PKA). In cardiac myocytes, PKA has a large number of downstream targets including I_{Ks}. Following β-AR stimulation, I_{Ks} amplitude increases, activation speeds, and deactivation slows (see Fig. 14.4). Interestingly, in heterologous systems, co-expression of KCNQ1 and

KCNE1 alone is not capable of reproducing this behavior. The functional response of the channel to cAMP requires the presences of a third protein, A kinase anchoring protein 9 (AKAP9), also known as Yotiao [7]. AKAP9 tightly controls the posttranslational state of the channel by recruiting PKA, protein phosphatase 1, and phosphodiesterase PDED3. This highly evolved channel complex controlling the phosphorylation state is critical to the normal function of the heart, as changes in I_{Ks} function directly affect repolarization in the heart and its ability to respond appropriately to changes in rate.

I_{Ks} Related LQTs

Three variants of the Long QT syndrome are directly linked to defects in I_{Ks} function that lead to a prolonged cardiac action potential. These mutations decrease the total amount of K^+ crossing the membrane during repolarization either by decreasing the number of channels on the cell surface, altering the channel's voltage dependence and/or kinetics in such a way that I_{Ks} channel activity is reduced, or blunting the channel's response to adrenergic stimulation.

Long QT syndrome variant 1 (LQT1) results from mutation of KCNQ1 and is the most common form of clinically observed inherited Long QT syndrome. Over 240 different mutations in KCNQ1 have been identified from patients presenting with Long QT. Long QT syndrome variant 5 (LQT5) results from mutation in KCNE1 and is a rarely occurring variant. More recently mutation in AKAP9 was found to underlie Long QT in a patient implicating a role for basal phosphorylation of the channel in cardiac myocytes [35].

I_{Kr}

The importance of hERG, standing for human ether-a-go-go-related gene, in normal human cardiac electrical activity became obvious when inherited mutation in KCNH2 was found to cause LQTS variant 2 [16, 36]. hERG (KCNH2) homotetramers form the channel pore and co-assemble with KCNE2 to generate cardiac I_{Kr} [37]. A physiological role of KCNE2 was elucidated when variants were identified as being associated with congenital and drug-induced LQT [31, 38, 39]. I_{Kr} kinetics are distinct from I_{Ks} in that despite both contributing to repolarization, they achieve this result via different mechanisms. For I_{Kr}, at depolarized potentials the channels open slowly and rapidly inactivate; however, during repolarization the channels recover from inactivation faster than they deactivate, leading to an increase in current critical to membrane repolarization in cardiac myocytes. I_{Kr} inactivation is a critical part of the channel function and is achieved by a C-type inactivation mechanism wherein a collapse of the selectivity filter reduces the ability of the channel to conduct K^+ [12] (Fig. 14.5)

I_{Kr} Related LQTs

Like in I_{Ks}, mutation in both the α and β subunits of the I_{Kr} channel have been identified as causally linked to LQT2 and six respectively. LQT2 is the second most commonly observed LQT variant, behind LQT1, and is characterized by a reduction in I_{Kr} during repolarization that leads to prolongation of the action potential. This reduction in I_{Kr} is typically a result of trafficking defects that reduce the number of channels in the membrane but can also be the result of defects in activation, increased activation, or changes in ion conduction/selectivity [40, 41]. LQT6, which results from mutation in the β subunit KCNE2 also acts to reduce cardiac I_{Kr} and is much less commonly observed clinically.

Recently, small molecule activators of I_{Kr} have been identified that have the ability to increase current either through attenuation of inactivation or through a slowing of deactivation that can cause the channel to accumulate in the open state [42, 43]. This may have the potential to open a novel avenue for pharmacological intervention in some subclasses of LQTS.

Short QT Syndrome

Another rare inherited disease, the short QT syndrome (SQTS) [44], was identified in 2000 as coupled with paroxysmal AF. Further characterization showed that in one family, SQTS was linked to a history of ventricular fibrillation and sudden death [45].

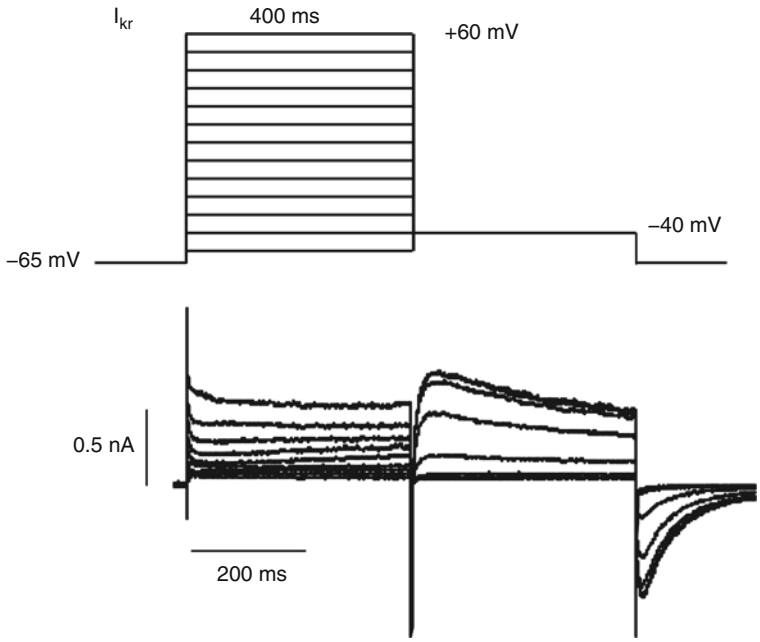

Figure 14–5. The protocol used to record a current-voltage relationship of I_{kr} and the resultant currents. Cells are first depolarized from a resting potential of −65 mV to different test potentials ranging from −60 mV to +60 mV for 400 ms and repolarized to −40 mV. Upon repolarization, recovery from inactivation procedes channel deactivation leading to larger currents

Not surprisingly, for K^+ channel SQTS, it was shown that mutations which increase K^+ current led to the phenotype. Mutations have been identified in three different genes encoding subunits of K^+ channels. The first mutations (SQT1) described were found in the gene KCNH2 where two different missense mutations, resulting in the same amino acid change (N588K), in the S5-P loop region of the cardiac I_{Kr} channel were identified. The mutation dramatically increases I_{Kr}, and reduces the affinity of the channel to I_{Kr} blockers [20]. Subsequently, a mutation has been found in a patient suffering from idiopathic ventricular fibrillation and a short QT interval. The mutation was a substitution of a valine by leucine at position 307 (V307L) in KCNQ1 (SQT2) and led to increased K^+ current through I_{Ks} in a heterologous expression system [46]. SQT3 has most recently been observed as mutation in the gene KCNJ2 (D172N) that encodes for a K^+ inward rectifier channel Kir2.1, the predominant component of cardiac I_{K1}. Again, the phenotype is increased K^+ channel activity that leads to shorter APDs that underlie the clinically observed short QT interval. In SQTS, patients are noted to have a high incidence of arrhythmia, particularly atrial fibrillation [45, 47].

Atrial Fibrillation

Atrial fibrillation (AF) is a common cardiac arrhythmia, affecting millions in the US and leading to a twofold increase in mortality and a two to sixfold increase in stroke risk [48]. The vast majority of clinically observed AF is age-related. AF is characterized by a rapid and irregular atrial activation.

Mutation in KCNQ1 and KCNE2 have both recently been implicated in familial AF. In January 2003, Chen et al. reported for the first time a KCNQ1 mutation in a family with atrial fibrillation in the absence of structural cardiac disease [19]. In this particular case a missense mutation at position 140 (S140G) led to increased I_{Ks}, initially attributed to constitutively open channels. Representative current-voltage relationships of normal and S140G mutant channels expressed with or without KCNE1 are shown in Fig. 14.6. When S140G is co-expressed with KCNE1 (Fig. 14.6b), channels are instantaneously open (left arrow) and upon repolarization deactivation is severely slowed (right arrow). More recent studies have shown that this deactivation defect is the mechanism for channel accumulation in the open state and if held at sufficiently negative potentials, the channel can

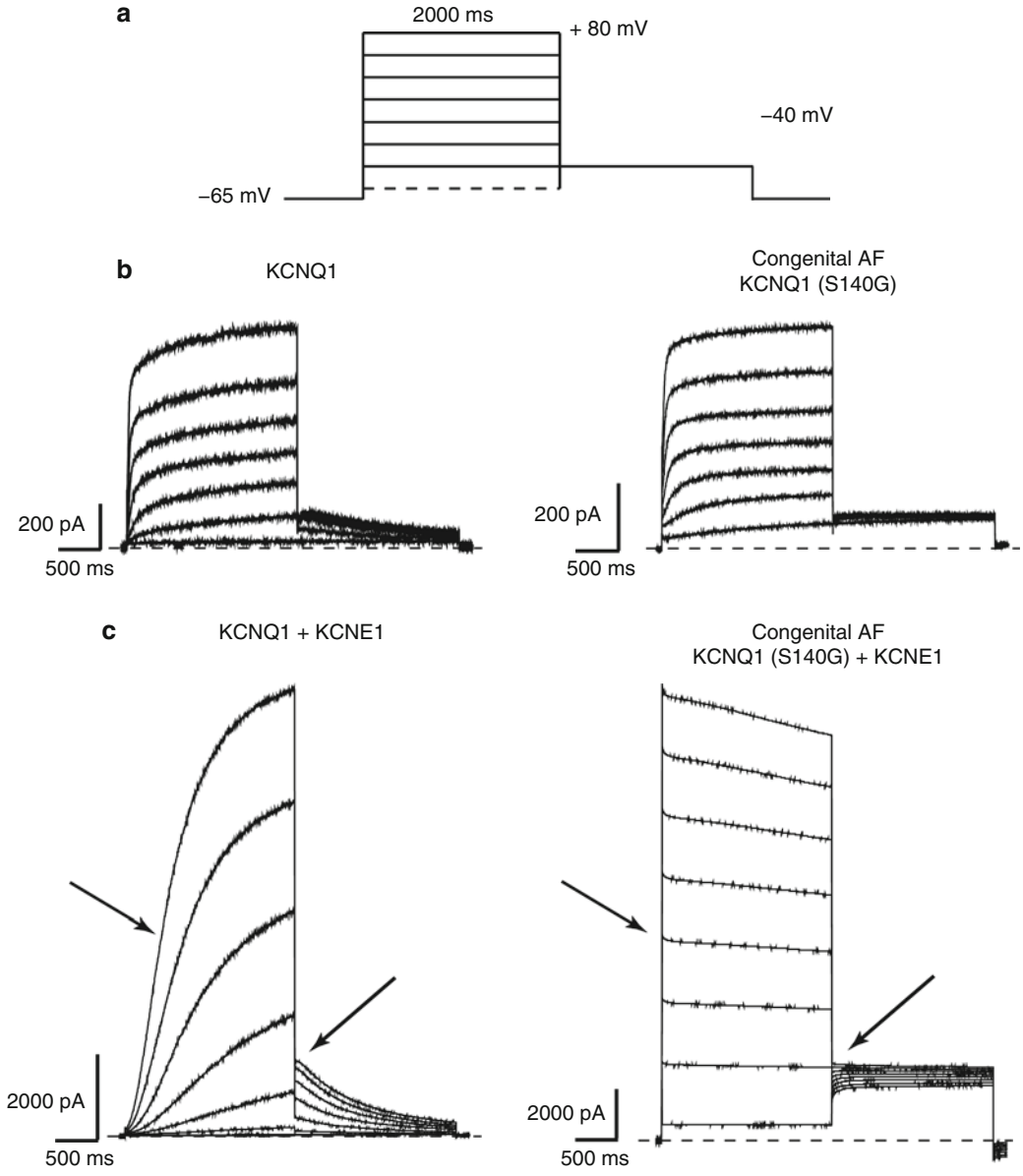

Figure 14–6. Familial atrial fibrillation mutant in KCNQ1. (**a**) Cells were depolarized from a resting potential of −65 mV to test potentials from −60 mV to +80 mV, for 2 s and repolarized to −40 mV for an additional 2 s. (**b**) Currents recorded for KCNQ1 (*left*) and S140G (*right*) expressed alone in heterologous systems. (**c**) Currents recorded when KCNE1 is co-expressed showing normal slow activation and deactivation in wild-type channels (*left*) and instantaneously open channels with no sign of deactivation in S140G (*right*)

close and when closed opens at a rate similar to wild-type channels [49].

In November 2004 Yang et al. reported the results of the molecular screening of 28 unrelated patients with AF [50]. They analyzed eight genes encoding K^+ channels and reported an arginine to cysteine mutation at position 27 (R27C) in KCNE2. When examined in heterologous expression, currents from KCNH2/KCNE2 were not impaired. However, when mutant KCNE2 is coexpressed with KCNQ1, (WT KCNQ1/KCNE2 is known to produce a resting membrane potential stabilizing background K^+ current [51]) the mutation increased K^+ current.

Brugada Syndrome

The Brugada Syndrome is a genetic disease, characterized by ST elevation in the right precordial leads of the ECG that predisposes patients to sudden cardiac death (see Chaps. 28 and 29). Originally linked to Na^+ and Ca^{2+} channel dysfunction, a broader set of genes have now been identified to underlie this disease. To date, the vast majority of Brugada Syndrome cases have not been linked to a specific genetic defect. Recently, mutation in an α subunit (KCND3) and a β subunit(KCNE3) of the fast-opening, transient outward K^+ current, I_{to}, have been implicated in patients presenting with Brugada Syndrome [52, 53].

I_{to} is encoded by an α subunit from the KCND family as well as an accessory β subunit to form a fast opening and fast inactivating voltage gated K^+ current. In all three observed cases of Brugada linked to mutation in I_{to}, an increase in peak K^+ current is observed and thought to alter the naturally occurring transmural variation in action potentials across the right ventricular wall. This is thought to lead to spatial heterogeneity in repolarization that presents as ST elevation on the right precordial ECG leads.

Non Inherited Channelopathy

K^+ channel dysfunction can also occur in the absence of genetic alterations as a result of pharmacological intervention, hypokalemia, hypomagnesmia, or abnormal posttranslational modification often resulting from a disease state. An array of drugs have been shown to prolong QT interval and predispose to arrhythmia including some antihistamines, antipsychotics, antibiotics, and drugs designed as antiarrhythmics [13].

It has long been observed that common medications can prolong the QT interval leading to symptoms similar to those of patients suffering from inherited LQTs. For example, drug-induced Torsade de Pointes (TdP) by the antiarrhythmic drug quinidine is observed in up to 1.5 % of patients [12]. Similarly, in a study of 35 children with gastroesophageal reflux disease, administration of the motility agent cisapride was observed to prolong QT in 11 % of patients and TdP was observed in 5.7 % [54]. These examples represent the most common form of acquired LQTS which results from direct interaction and block of cardiac I_{Kr} [55]. As a result of the block of I_{Kr}, a number of drugs have been taken of the market including, but not limited to cisapride, sertindole, grepafloxacin, terfenadine and astemizole [56].

In several disease states, including heart failure and chronic atrial fibrillation, electrical remodeling takes place wherein the relative amounts of the proteins that make up cardiac ion channels are up- or down-regulated. As there is evidence of possible variation in stoichiometry of α and β subunits [57, 58] that lead to channels with different kinetics, remodeling has the capability to affect K^+ current. This change in function may compound, or correct, the disease phenotype. Another manner in which genetically normal K^+ channels may predispose to arrhythmia is through hyperphosphorylation, as can occur in heart failure [59]. As phosphorylation speeds activation and slows deactivation of I_{Ks}, hyperphosphorylated I_{Ks} may help provide the substrate for chronic arrhythmias in heart failure in the same manner as it does in FAF [60].

Conclusions

Defects in K^+ channel function have been linked to a number of heritable and acquired conditions leading to arrhythmia in affected patients. As we continue to characterize channel function biophysically, our mechanistic understanding of these conditions grows. Hopefully, this will continue to lead to novel therapeutic strategies not only for the relatively rare inherited channelopathies, but more widespread arrhythmias commonly encountered in clinical practice.

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15
Cardiac ATP-Sensitive Potassium Channels and Associated Channelopathies

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Abstract

ATP-sensitive K^+ (K_{ATP}) channels integrate cellular energy signals with membrane electrical activity. Heteromultimers of *KCNJ11*-encoded inwardly rectifying potassium Kir6.2 channels and *ABCC9*-encoded ATP-binding cassette SUR2A proteins, sarcolemmal K_{ATP} channels are vital in heart energy homeostasis. Knockout of the Kir6.2 pore compromises cardioprotection afforded by ischemic preconditioning, impairs tolerance to sympathetic surge, and aggravates the impact of endurance challenge or hemodynamic load precipitating heart failure under stress. In human cardiovascular medicine, mutations in the regulatory SUR2A subunit have been linked to K_{ATP} channelopathy-associated electrical and cardiomyopathic disorders, including syndromes of adrenergic atrial fibrillation and dilated cardiomyopathy with tachycardia. In clinical heart failure, a common polymorphism in the Kir6.2 subunit has been identified as a biomarker for impaired performance in stress-test. The Kir6.2 K23 allele, present in over half the population, has been further pinpointed as a risk factor for susceptibility to maladaptive cardiac remodeling in hypertension. Cardiovascular disorders associated with genetic variation in K_{ATP} channel genes also include myocardial infarction and ventricular fibrillation. Thus, advances in molecular medicine have enabled a growing understanding of K_{ATP} channel function in health and disease, underscoring the impact on individual and public cardiovascular wellness.

Keywords

Heart Failure • Arrhythmias • Cardiomyopathy • Genetics of cardiovascular disease • Clinical genetics

Deconvolution of corrupted biological pathways in disease and translation of patient specific molecular mechanisms into tailored management algorithms have begun to extend the reach of individualized medicine from principles to practice. A case in point is the emergent deciphering of the pathobiology underlying life threatening human diseases caused by dysfunction in adenosine triphosphate sensitive potassium (K_{ATP}) channels [1]. K_{ATP} channels are vital biosensors that regulate membrane potential

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dependent functions to match the body's energy demands [2]. Unique within the ion channel superfamily, K_{ATP} channels are gated by intracellular ATP/ADP and thereby couple cellular metabolism with membrane electrical activity [3, 4]. Expressed widely in excitable tissues, such as the heart, K_{ATP} channels are prime coordinators of metabolic well being [5]. The functionality of cardiac K_{ATP} channels in the plasma membrane, the cellular compartment where these channels are best understood, is provided by the co-assembly of distinctive heteromultimeric complexes formed by a potassium selective channel pore and a regulatory ATP binding cassette transporter subunit [6]. The octameric K_{ATP} channel stoichiometry is enforced by quality control checkpoints that harmonize channel biogenesis by stopping incomplete complexes from reaching the plasma membrane. Functional K_{ATP} channel complexes endow a cardioprotective phenotype [7, 8]. In contrast, K_{ATP} channel deficient ventricles exhibit a remodeled proteome exposing markers predicting cardiovascular disease susceptibility [9–11]. Overexpression of channel subunits typically generates a protective phenotype at cellular and organ levels [12–14]. Only recently have human K_{ATP} channelopathies become recognized as bona fide entities in molecular medicine [15–19]. Indeed, over the past few years, molecular genetic investigations have linked K_{ATP} channel mutations to human cardiac arrhythmia including adrenergic atrial fibrillation, and heritable dilated cardiomyopathy in which a weakened and enlarged heart fails to pump blood effectively, leading to heart failure [20, 21]. In this regard, use of humanized models to recapitulate a pathogenic K_{ATP} channel mutation and pinpoint tissue restricted lesions that stratify the consequences of genetic variation on disease traits have been particularly useful. Even more recently, a common polymorphism in the Kir6.2 pore has been diagnosed as a risk factor for maladaptive cardiac remodeling in the population at large and linked to abnormal cardiopulmonary performance in patients with heart failure [22, 23]. From the very initial report of K_{ATP} channel activity detected in heart muscle plasma membrane [24] to breakthroughs in the molecular medicine of K_{ATP} channelopathies, there has been a remarkable advance across the spectrum defining K_{ATP} channel based knowledge.

K_{ATP} Channel Primer

K_{ATP} channel-like function has been reported in a number of subcellular locales [24–26]. Plasmalemmal K_{ATP} channels, the topic of this chapter, assemble as heteromultimers of inward-rectifier K^+ (Kir6) channels and ATP-binding-cassette (ABC) sulfonylurea receptor (SUR) proteins [27–32]. Tissue diversity, cell distribution, and regulatory specificity are ensured by assortment of subunit combinations. Human Kir6.1 and Kir6.2 genes – *KCNJ8* and *KCNJ11* – map to chromosome 12p11.23 and 11p15.1, while SUR genes map to locus 11p15.1 and 12p12.1 for *SUR1* (*ABCC8*) and *SUR2* (*ABBC9*), respectively. Alternative splicing gives rise to SUR2 protein variants, SUR2A and SUR2B. Biophysical properties are largely shared among plasmalemmal K_{ATP} channels, and include potassium selectivity and inward rectification imparted by the Kir protein, while response to cellular energetic signals is conferred by nucleotide interaction with both Kir and SUR channel subunits [33].

Kir6.2 is integral in the make-up of myocellular K_{ATP} channels, and targeted disruption of *KCNJ11* generates a Kir6.2-deficient state characterized by lack of functional K_{ATP} channels in ventricular myocytes [34]. Sarcolemmal K_{ATP} channels in the ventricle are composed primarily through co-assembly of Kir6.2 and SUR2A subunits (Fig. 15.1). Recombinant Kir6.2/SUR2A octamers match the features of native ventricular K_{ATP} channels [35]. The molecular architecture may however exhibit plasticity, in particular in response to metabolic challenge. Kir6.1 mRNA expression, for example, is upregulated by ischemia or hypoxia [36]. A disparate structure of atrial compared to ventricular K_{ATP} channels has been proposed, implicating SUR1-based channels in atrial-specific functions [37, 38].

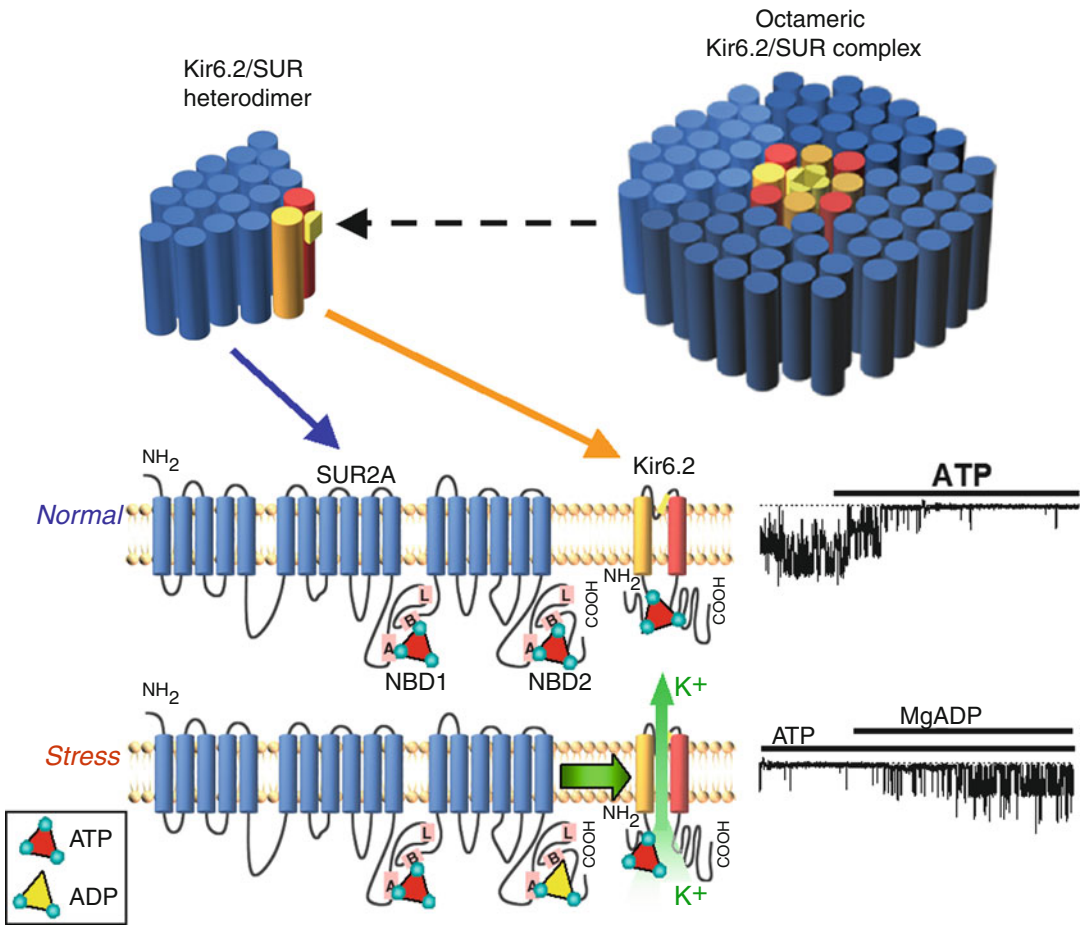

Figure 15–1. Structure and function of ventricular sarcolemmal K_{ATP} channel complexes. The pore-forming Kir6.2 subunit assembles with the regulatory SUR2A protein (containing nucleotide-binding domains NBD1/NBD2 with Walker A/B motifs and linker L region) to form hetero-multimeric K_{ATP} channels abundantly expressed in the ventricular sarcolemma. The defining feature of K_{ATP} channel operation is adenine nucleotide-dependent gating, ensuring high-fidelity coupling between the cellular energetic state and membrane electrical activity. Intracellular ATP keeps K_{ATP} channels closed under normal conditions, while ADP promotes channel opening in response to metabolic challenge. Stress-induced build-up of MgADP at NBD2 of SUR2A antagonizes ATP-induced channel inhibition, promoting K^+ efflux. K_{ATP} channel opening translates into shortening of the cardiac action potential and accelerated repolarization

Crosstalk of cardiac K_{ATP} channels with myocellular energetics is facilitated by associations with phosphotransfer and glycolytic enzymes [39]. Metabolism-related proteins with established links to cardiac K_{ATP} channels include creatine kinase, adenylate kinase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, long chain acyl-CoA dehydrogenase, pyruvate kinase, and triosephosphate isomerase [40–45]. To date, >100 K_{ATP} channel-dependent proteins, demonstrating a prioritized metabolic theme, have been identified by deconvolution of the K_{ATP} channel-deficient heart proteome [9–11]. ATP/ADP modulation of channel function is a defining property of K_{ATP} channel complexes as metabolic sensors (see Fig. 15.1) [5, 8, 33, 46]. The interface between Kir6.2 subunits is critical for ATP-mediated pore inhibition [47].

Nucleotide binding domains (NBD1 and NBD2) of SUR2A harbor intrinsic ATPase activity, endowing this regulatory K_{ATP} channel subunit with the ability to modulate ATP-induced Kir6.2 pore inhibition and thereby K^+ efflux [48–52]. By relying on the intactness of cooperative NBD1/2 interaction, a stabilized ADP-bound post-hydrolytic conformation at NBD2 of SUR in the presence of Mg^{2+}, promotes K_{ATP} channel opening [1, 53–55]. Nucleotide-bound conformations of SUR mediate regulation of K_{ATP} channel activity by pharmacological agents, such as sulfonylurea inhibitors and potassium channel openers [33, 55].

K_{ATP} Channel-Dependent (Patho) Physiology

K_{ATP} channels operate as an energy-sparing system limiting muscle energy expenditure during propagation of action potentials [7, 8, 33, 56]. Defined as the most densely expressed K^+ channels in the myocardium, K_{ATP} channels are critical in cardiac energy homeostasis and electrical stability across a spectrum of stress conditions, from acute ischemia to chronic exertion and heart failure [12, 19, 57].

In ischemia, K_{ATP} channel opening shortens cardiac action potential duration and controls Ca^{2+} influx [58, 59]. Sarcolemmal K_{ATP} channel activation is responsible for the electrical current that underlies ST-segment elevation of transmural ischemic injury, and has been implicated in protection afforded by ischemic preconditioning [12]. Specifically, in the Kir6.2-knockout mouse following transmural anterior myocardial infarction, absence of significant and sustained ST-segment change contrasts the wild-type counterpart that demonstrates prompt and pronounced ST-segment elevation following ischemic injury [60]. Moreover, K_{ATP} channels have been implicated in the ischemic preconditioning mechanism by which exposure to brief ischemia preceding a sustained ischemic insult reduces subsequent infarct size [61]. Analogous to ischemic preconditioning, pharmacologic activation by K_{ATP} channel openers has also protective benefit [62, 63]. Both ischemic and pharmacologic preconditioning are abolished in the absence of Kir6.2-containing K_{ATP} channels [64, 65]. Absence of sarcolemmal K_{ATP} channel activity has negative effects on cardiac relaxation and contractility under acute ischemic stress. In parallel, knockout of Kir6.2 negates protection afforded by ischemic preconditioning on myocardial energy generation, transfer and utilization [66]. Total ATP turnover, a global parameter of energy demand, fails to increase in the ischemic-preconditioned K_{ATP} channel knockout as opposed to the wild-type, correlating with failure of preconditioned hearts lacking K_{ATP} channels to functionally recover [66]. The K_{ATP} channel-dependent cardioprotective potential is underscored by improved myocardial function during post-ischemic reperfusion following introduction of the SUR2A transgene into the SUR2 null cardiomyocyte [67].

Beyond protection against ischemia, K_{ATP} channels appear central to cardiac participation in the general adaptation syndrome [57]. In the "fight-or-flight" response, metabolic adaptations in bodily functions achieve a superior level of performance necessary to cope with the demands of imposed stress. The sustenance of augmented performance requires energy-controlling mechanisms, such as the K_{ATP} channel, ensuring that the reaction to stress itself does not become harmful [57]. A common trigger of the general adaptation syndrome is systemic sympathetic stimulation that augments cardiac contractility and heart rate, and thereby provides the necessary higher cardiac output to meet demand. Enhanced cardiac output imposes a significant metabolic load on the heart. A compensatory increase in outward K^+ current activates when the sustained augmentation of heart muscle performance competes with the ability of cellular energetics to maintain contractile and electrical stability. The K_{ATP} channel-mediated action potential shortening limits actomyosin ATP consumption by reducing Ca^{2+} influx, restraining energy utilization to ensure functional and structural cellular integrity [68]. Under sympathetic distress, hearts without K_{ATP} channels lack stress-induced cardiac action potential shortening predisposing to cytosolic Ca^{2+} overload associated with contractile dysfunction, and possibly death [68]. On autopsy, contraction bands, pathognomonic of cytosolic Ca^{2+} loading, are

visible throughout the myocardium of the Kir6.2-knockout but not wild-type counterpart [68]. Measurement of oxygen consumption has revealed that increased workloads produce only moderate elevation of energy expenditure, in line with K_{ATP} channel-dependent shortening of action potential duration [56]. Conversely, absence of K_{ATP} channel-driven action potential shortening in K_{ATP} channel-deficient hearts precipitates significant elevation in oxygen consumption [56].

Under catecholamine challenge, action potential prolongation remains uncompensated in the absence of K_{ATP} channel function, predisposing the myocardium to early afterdepolarizations [69]. This deficit in repolarization reserve translates into a high risk for induction of triggered activity and ventricular dysrhythmia [69]. Proarrhythmogenic features and lack of adaptation to stress in transgenic mice with cardiac myocyte-specific ablation of K_{ATP} channels verifies that these features are intrinsic to the myocardium [70]. Moreover, K_{ATP} channels may provide a vital feedback element for cardiovascular tolerance in sepsis, where the systemic inflammatory response to infection imposes a high demand for bodily adaptation [71]. The exaggerated susceptibility provoked by disruption of Kir6.1-containing channels was linked to progressive deterioration in cardiac activity, ischemic myocardial damage, and contractile dysfunction [71]. K_{ATP} channels, harnessing the ability to recognize alterations in the cellular energy state and to translate this information into changes in membrane excitability, therefore provide a necessary link for maintaining myocardial well being in the face of stress-induced energy demanding augmentation in performance.

Exercise training elicits an array of metabolic responses that underlie fitness. Mice lacking K_{ATP} channels when challenged with a regimented training protocol failed to manifest improved exercise capacity [72]. Repetitive exercise-stress unmasks a survival disadvantage in the Kir6.2-knockout associated with cardiac damage, implicating K_{ATP} channel activity in achieving physiologic benefits of exercise training without accumulating deficits [72]. Exercise intolerance has also been documented in the setting of ablation of the regulatory SUR2 subunit [73], underscoring the role of K_{ATP} channels in ensuring optimal performance. Beyond physical exercise, in distinct hyperadrenergic states exemplified by cocaine abuse, K_{ATP} channel deletion amplifies poor cardiovascular outcome while promotion of channel activity by potassium channel opening drugs improves survival [74, 75].

Intact K_{ATP} channels prevent transition from a state of disease risk to that of overt organ failure. In hypertension induced by volume overload, knockout of Kir6.2 K_{ATP} channels predisposes to heart failure and death [76]. Defective decoding of hypertension-induced metabolic distress signals in the K_{ATP} channel knockout sets in motion pathological Ca^{2+} overload and aggravates cardiac remodeling through a calcium/calcineurin-dependent cyclosporine-sensitive pathway [76]. Similarly, in models of pressure overload, such as that imposed by transverse aortic constriction, compromised K_{ATP} channel function renders the heart vulnerable to poor outcome [77]. The constricted K_{ATP} channel knockout displays biventricular congestive heart failure, characterized by exercise intolerance, cardiac contractile dysfunction, hepatopulmonary congestion and death. Surviving K_{ATP} channel knockouts develop sequelae, including exaggerated fibrotic myocardial hypertrophy associated with nuclear upregulation of calcium-dependent pro-remodeling MEF2 and NF-AT pathways, precipitating chamber dilatation [77]. Moreover, it has been documented that disease-induced K_{ATP} channel metabolic dysregulation, even in the absence of channel gene defect, is a contributor to the pathobiology of heart failure, illustrating a mechanism for acquired channelopathy [78].

Genetically-determined K_{ATP} channel malfunction was originally linked to insulin secretory disorders, namely congenital hyperinsulinism and neonatal diabetes [4, 17–19, 79, 80]. Beyond isolated failure of pancreatic β-cells, K_{ATP} channel mutations are also pathogenic in the DEND syndrome, characterized by varying degrees of delayed speech/motor development, epilepsy, neonatal diabetes, muscle hypotonia, and balance issues [17–19]. An even broader role in disease pathogenesis has been realized with the discovery of K_{ATP} channel malfunction in human skeletal myopathies [81, 82]. In cardiovascular medicine, K_{ATP} channelopathies have

been associated with atrial fibrillation and dilated cardiomyopathy with tachycardia, as well as phenotypic modifiers of preclinical and overt heart disease [1, 19].

Human Adrenergic Atrial Fibrillation: A K_{ATP} Channelopathy

Atrial fibrillation is increasingly recognized as having genetic underpinnings [83, 84]. A case in point is the early onset cases in a subset of patients attributable to monogenic defects. The paradigm of a heritable basis for atrial fibrillation is exemplified by reports of familial disease attributed to gain-of-function or loss-of-function mutations in ion channel genes predicted to accelerate or slow repolarization. In these cases, channel malfunction creates an arrhythmogenic substrate of re-entry or triggered activity caused by reduced electrical refractoriness or after-depolarization, respectively. Initially, channelopathy-based atrial fibrillation predicted shortening of the action potential duration and proarrhythmogenic reduction in refractory period as mechanisms of arrhythmia [85, 86]. An alternative mechanism for atrial fibrillation, namely increased propensity for prolongation of action potential duration and triggered activity in the human atrium, was identified for a loss-of-function mutation in *KCNA5*, encoding the voltage-dependent Kv1.5 channel [87]. A possibly equivalent mechanism has been reported in the case of a K_{ATP} channel mutation conferring risk for adrenergic atrial fibrillation originating from the vein of Marshall [20]. The mutation was identified in a middle-aged patient who, in the absence of identifiable risk factors, presented with long-standing atrial fibrillation precipitated by activity and refractory to medical therapy. In this patient with early-onset atrial fibrillation and an overtly normal heart, adrenergic stress as a possible trigger was investigated using a candidate gene approach and invasive electrophysiologic testing under sympathomimetic challenge [20]. The focal source of rapidly firing electrical activity was mapped to the vein of Marshall, a remnant of the left superior vena cava rich in sympathetic fibers and a recognized source for adrenergic atrial fibrillation. Although this potentially arrhythmogenic veno-atrial interface is present in the population at large, it does not trigger arrhythmia in the majority of individuals despite comparable environmental stress exposure. It was postulated that the patient was vulnerable to adrenergic atrial fibrillation due to an inherent defect in electrical stability [20].

Molecular genetic investigation demonstrated a missense mutation in *ABCC9*, encoding the regulatory subunit of cardiac K_{ATP} channels [20]. Identified in exon 38, specific for the cardiac splice variant of SUR2A, this heterozygous c.4640C > T transition caused substitution of the threonine residue at amino acid position 1547 with isoleucine (T1547I). Protein alignments revealed that the missense substitution altered the amino acid sequence of the evolutionarily conserved carboxy-terminal tail. Homology modeling mapped the defect adjacent to the signature Walker motifs of the nucleotide binding domain, required for coordination of adenine nucleotides in the nucleotide binding pocket. Removal of the polar threonine (T1547) and replacement with the larger aliphatic and highly hydrophobic isoleucine, as would occur in this patient, predicted compromised nucleotide-dependent K_{ATP} channel gating [20].

Patch-clamp recording demonstrated that the T1547I substitution compromised adenine nucleotide-dependent induction of K_{ATP} channel current [20]. Mutant T1547I SUR2A, co-expressed with the *KCNJ*11-encoded Kir6.2 pore, generated an aberrant channel that retained ATP-induced inhibition of potassium current, but demonstrated a blunted response to ADP. A deficit in nucleotide gating, resulting from the T1547I mutation, would compromise the homeostatic role of the K_{ATP} channel required for proper readout of cellular distress and maintenance of electrical stability.

The pathogenic link between channel malfunction and adrenergic atrial fibrillation was verified, at the whole organism level, in a murine knockout model deprived of operational K_{ATP} channels. Compared with the normal atrium, resistant to arrhythmia under adrenergic provocation, vulnerability to atrial fibrillation was recapitulated in the setting of a K_{ATP} channel deficit [20]. Thus, a lack of intact K_{ATP} channels, either due to a naturally occurring mutation affecting channel regulation or a targeted

disruption of the channel complex, is a substrate for atrial electrical instability under stress, and a molecular risk factor for adrenergic atrial fibrillation.

Once the vein of Marshall had been isolated by radiofrequency ablation, atrial fibrillation could no longer be provoked by programmed electrical stimulation and burst pacing with or without isoproterenol infusion [20]. This case demonstrates that vulnerability to arrhythmia can be caused by an inability of mutant K_{ATP} channels to safeguard against adrenergic stress-induced ectopy. The apparently curative outcome was achieved by disrupting the gene-environment substrate for arrhythmia conferred by the underlying K_{ATP} channelopathy [19].

K_{ATP} Channel Dysfunction: Risk Factor for Electrical Instability

While the case underscores heritable channel dysfunction in lone atrial fibrillation, K_{ATP} channel deficit could play a broader role in the pathogenesis of electrical instability. Gene expression and electrophysiological studies in patients with atrial fibrillation demonstrate altered atrial ion channel mRNA transcription and post-translational activity, including downregulation of the K_{ATP} channel pore and associated current [88, 89]. Moreover, structural heart disease and/or atrial dilation may compromise metabolic and mechanosensitive gating of K_{ATP} channels [90–92], precipitating a suboptimal repolarization reserve and providing a substrate for the more common acquired form of atrial fibrillation.

K_{ATP} channel alteration may also impact predisposition towards ventricular vulnerability to arrhythmia. In this regard, a case of ventricular fibrillation with prominent early repolarization was recently reported in a young patient who was resuscitated following an episode of sudden death. Subsequent, unrelenting ventricular fibrillation was unresponsive to several classes of antiarrhythmics prior to rhythm restoration with quinidine [93]. Myopathic and coronary heart disease were excluded, and a K_{ATP} channel subunit amino acid substitution, namely the S422L variant of the *KCNJ8* gene encoding Kir6.1, identified [93]. The gain-of-function K_{ATP} channel variant was further linked to the pathogenic substrate of pleiotropic J-wave abnormalities, in single Brugada syndrome and early repolarization syndrome cases [94].

Dilated Cardiomyopathy Associated with K_{ATP} Channel Mutation: CMD10 Syndrome

Beyond isolated arrhythmias, K_{ATP} channelopathy has been implicated in a syndrome of cardiomyopathy with ventricular arrhythmia. The ontological spectrum of cardiomyopathy-associated mutant gene products has encompassed the fundamental components of excitation-contraction coupling such as contractile, cytoskeletal, and myocellular ion regulatory proteins [95]. Human molecular genetic studies have also linked K_{ATP} channel defects and aberrant homeostatic stress response in the pathogenesis of dilated cardiomyopathy [19]. These defects, identified in the regulatory K_{ATP} channel subunit, impair channel-dependent decoding of cellular metabolic state, establishing a previously unrecognized mechanism in human heart failure [18].

The cardiomyopathic-arrhythmia syndrome characterized by the triad of dilated cardiomyopathy, ventricular arrhythmia, and *ABCC9* K_{ATP} channel mutations has been designated CMD1O (OMIM #608569) [21], and salient phenotypic traits were reproduced by K_{ATP} channel knockout under imposed stress [77]. Clinically, this entity was reported in middle-aged patients with marked left ventricular enlargement, severe systolic dysfunction, and ventricular tachycardia. In these patients, heterozygous mutations were identified in exon 38 of *ABCC9*, which encodes the C-terminal domain of the SUR2A channel subunit, specific to the cardiac splice variant. DNA sequencing of a mutated allele identified a 3-bp deletion and 4-bp insertion mutation (c.4570-4572delTTAinsAAAT), causing a frameshift at L1524 and introducing four anomalous terminal residues followed by a premature stop codon (fs1524) [21]. Another mutated allele harbored a missense mutation (c.4537G>A) causing the amino acid substitution A1513T. The identified frameshift and missense mutations occurred in evolutionarily conserved domains of

SUR2A, and neither mutation was present in unrelated control individuals [21].

These mutations were mapped to domains bordering the catalytic ATPase pocket within SUR2A. Structural molecular dynamics simulation showed that residues A1513 and L1524 flank the C-terminal β-strand in close proximity to the signature Walker A motif, required for coordination of nucleotides in the catalytic pocket of ATP-binding cassette proteins [21]. Replacement of A1513 with a sterically larger and more hydrophilic threonine residue or truncation of the C-terminus caused by the fs1524 mutation would disrupt folding of the C-terminal β-strand and, thus, the tertiary organization of the adjacent second nucleotide binding domain (NBD2) pocket in SUR2A. Indeed, ATP-induced K_{ATP} channel gating was aberrant in channel mutants, suggesting that structural alterations induced by the mutations A1513T and fs1524 of SUR2A distorted ATP-dependent pore regulation [21]. Thus, the mutations A1513T and fs1524 compromise ATP hydrolysis at SUR2A NBD2, generating distinct reaction kinetic defects. Aberrant catalytic properties in the A1513T and fs1524 mutants translated into abnormal interconversion of discrete conformations in the NBD2 ATPase cycle. Alterations in hydrolysis-driven SUR2A conformational probability induced by A1513T and fs1524 perturbed intrinsic catalytic properties of the SUR2A ATPase, compromising proper translation of cellular energetic signals into K_{ATP} channel-mediated membrane electrical events. Traditionally linked to defects in ligand interaction, subunit trafficking or pore conductance, human cardiac K_{ATP} channel dysfunction provoked by alterations in the catalytic module of the channel complex establishes a new mechanism for channelopathy.

Risk Factor for Cardiac Remodeling in the Population

Susceptibility or resistance to heart failure, despite apparently similar risk load, is attributable to individual variation in homeostatic reserve. Following identification of mutations within a K_{ATP} channel gene in patients with dilated cardiomyopathy [21], the relationship between the common Kir6.2 E23K polymorphism (rs5219) and subclinical heart disease was investigated [23]. A community-based cross-sectional cohort of 2,031 predominantly Caucasian adults was utilized, for which detailed clinical and prospective echocardiographic data were available. Genotype frequencies were in Hardy–Weinberg equilibrium (EE = 44 %; EK = 47 %; KK = 9 %) and similar to previously reported control populations. In the group at large, there was no significant association between genotypes and measures of cardiac structure/function (left ventricular dimensions, mass, and ejection fraction), electrical instability (atrial and ventricular arrhythmias), or metabolism (fasting glucose, diabetes, and body mass index) at enrollment. However, among individuals with documented hypertension at the time of echocardiography ($n = 1{,}187$), the KK genotype was significantly associated with greater left ventricular dimension and volume in both diastole and systole [23]. A synergistic effect on left ventricular size of KK genotype and left ventricular mass, a marker of chronic cardiac stress load, further validated the impact of Kir6.2 E23K on cardiac structure in hypertension. From a public health perspective, hypertension is the most common risk factor for congestive heart failure, and left ventricular enlargement is an established precursor of symptomatic ventricular dysfunction. The Kir6.2 K23 allele, present in over half the population, is thus implicated as a risk factor for transition from hypertensive stress load to subclinical maladaptive cardiac remodeling [23]. These findings, consistent with previous human and animal studies [19, 76], uncover an interactive K_{ATP} channel gene-environment substrate that confers cardiac disease risk. Determining the overall impact of Kir6.2 E23K across ethnic groups and on long-term clinical outcome, i.e., progression to left ventricular enlargement and clinical heart failure, will require further study.

Clinical Biomarker for Impaired Stress Performance

The translational significance of the Kir6.2 E23K polymorphism in human cardiac physiology was more recently explored in a cohort of patients with heart failure who underwent comprehensive exercise stress testing [22]. The frequency of the minor K23 allele was found over-represented in the 115 subjects with congestive heart failure compared to the 2,031 community-based controls described above (69 vs. 56 %, $P < 0.001$). Moreover, the KK genotype, present in 18 % of heart failure patients, was associated with abnormal cardiopulmonary exercise stress test results [22]. In spite of similar baseline heart rates at rest among genotypic subgroups, subjects with the KK genotype had a significantly reduced heart rate increase at matched workloads. Molecular modeling of the tetrameric Kir6.2 pore structure revealed the E23 residue within the functionally relevant intracellular slide helix region [22]. Substitution of the wild-type E residue with an oppositely charged, bulkier K residue would potentially result in a significant structural rearrangement and disrupted interactions with neighboring Kir6.2 subunits, providing a basis for altered high-fidelity K_{ATP} channel gating, particularly in the homozygous state. Blunted heart rate response during exercise is a risk factor for mortality in patients with heart failure, establishing the clinical relevance of Kir6.2 E23K as a biomarker for impaired performance under exercise stress underscoring the essential role of K_{ATP} channels in human cardiac physiology [22].

Association with Myocardial Infarction

Experimental evidence has also suggested that K_{ATP} channels could be involved in the pathogenesis of coronary vasomotor dysfunction and ischemic heart disease [96, 97]. The potential clinical significance of such a premise was documented in a cohort of patients with myocardial infarction at an early age, whereby a rare missense variant V734I in the *ABCC9* SUR2A K_{ATP} channel subunit was found overrepresented [98]. Statistical significance was demonstrated after controlling for multiple established risk factors for coronary artery disease [98].

Overview

Deficient cellular energetics set by aberrant K_{ATP} channel function is increasingly implicated in a spectrum of conditions underlying metabolic imbalance and electrical instability [5]. Indeed, cardiac K_{ATP} channelopathies are emerging as a recognized disease entity underlying heart failure and arrhythmia [1, 19]. Understanding the molecular structure and function of K_{ATP} channel subunits [8], and their relationship to cellular metabolic signaling [99], has been instrumental in interpreting the pathophysiology of channel malfunction associated with heart disease predisposition [12]. From individual patients to populations, variants in K_{ATP} channel genes have now been documented in human dilated cardiomyopathy [21], atrial fibrillation [20], and as risk factors for electrical instability [93, 94], adverse cardiac remodeling [23], impaired performance under stress [22] or myocardial infarction [98]. Beyond the initial deciphering of genotype-phenotype relationships, development and application of high-throughput platforms to screen for disrupted coding and/or regulatory sequences in cardioprotective K_{ATP} channel genes, as well as diagnosing corrupted interactions within the cellular milieu, would advance current knowledge regarding this homeostatic channel complex and its implications in cardiovascular medicine. In particular, deconvolution of altered metabolic pathways and signaling cascades associated with pathogenic K_{ATP} channel mutation may offer unique opportunities to pinpoint lesions that stratify the consequences of genetic variation on disease traits [18]. In this regard, it can be anticipated that systems biology and network medicine strategies will be increasingly deployed to resolve the K_{ATP} channel interactome [11]. Mapping of the systems integration of molecules

and their respective biological networks in health *versus* disease will, in turn, guide the judicious development of prognostic discriminators of disease variability and selection of treatment response predictors [100–102]. Advances in the molecular medicine of K_{ATP} channelopathies are thus poised to offer new perspectives in the diagnosis and therapy of individuals and populations [103–110].

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16
Cardiac K_{ATP} Channels in Health and Diseases

Hai Xia Zhang, Jonathan R. Silva, and Colin G. Nichols

Abstract

Adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels were discovered in the heart almost 30 years ago. They are present in multiple tissues and link membrane excitability to the metabolic state of the cell. Under physiological conditions, cardiac K_{ATP} channels are predominantly closed, but they may open during exertion, stress, and ischemia. Experimental and modeling studies have shown that activation of sarcolemmal K_{ATP} channels causes dramatic action potential shortening in vitro, which can be cardioprotective. Conversely, there is emerging evidence that K_{ATP} channel mutations are linked to heart diseases, including congestive heart failure and arrhythmias. The debate regarding the role, and even the very existence, of mitochondrial K_{ATP} channels is still ongoing. Filling these knowledge gaps will require further study, and integration of results from basic cellular electrophysiology, to animal models and clinical disease. This chapter will address the current understanding of cardiac K_{ATP} channels regarding molecular composition, regulation of channel activity, and physiological and pathophysiological roles.

Keywords

ATP • Potassium channel • Sulfonylurea receptor • Kir6.1 • Kir6.2 • SUR1 • SUR2A • Ischemia • Metabolism

Introduction

Adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels link membrane excitability to the metabolic state of the cell by opening and closing in response to changes in intracellular nucleotide concentrations. Under physiological conditions, where millimolar ATP levels persist, channels should be predominantly closed, implying a limited role for cardiac K_{ATP} channels under normal conditions in healthy myocardium. Certain conditions may cause K_{ATP} channel opening, such as exertion [1, 2], stress [3],

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severe ischemia, and gain-of-function genetic mutations. Opening of K_{ATP} channels in response to stress and ischemia can be cardioprotective [4], yet gain-of-function mutations are associated with several pathologies including congestive heart failure [5] and Early Repolarization Syndrome [6–8]. In combination, these findings suggest a complex physiological role for cardiac K_{ATP} channels.

There are several reviews that cover the classical physiology of the cardiac K_{ATP} channel [4, 9–14]. Much has been recently learned from genetically modified animals, where the different subunits that compose K_{ATP} can be genetically manipulated. This chapter will address the current state of cardiac K_{ATP} channel knowledge regarding its molecular composition, molecular regulation of channel activity, and its physiological and pathophysiological roles.

Molecular Composition of Cardiac K_{ATP} Channels

Subunit Composition and Structural Basis of K_{ATP} Channels

In 1995, the cloning of the two types of K_{ATP} channel subunits-a novel inwardly rectifying K^+ channel (Kir6x) [15, 16] and the ATP binding cassette (ABC) family-sulfonylurea receptor (SURx) [17] introduced a structural paradigm for the K_{ATP} channel family (Fig. 16.1). The pore-forming subunits of the channel, Kir6.x, are genetically encoded by *KCNJ8* (Kir6.1) or *KCNJ11* (Kir6.2), while the regulatory SURx subunits are encoded by *ABCC8* (SUR1) and *ABCC9* (SUR2). Two major distinct isoforms of the SUR2 subunits, SUR2A and SUR2B, are generated by alternative RNA splicing [18, 19]. As indicated in Fig. 16.1d, the Kir6.2 and SUR1 genes are located adjacent to each other on human chromosome 11p15.1 [15]; as are Kir6.1 and SUR2 genes on chromosome 12p12.1 [20, 21], implying an evolutionary duplication event. Functional K_{ATP} channels form as heteroctamers in which four Kir6.x subunits form a central pore with each subunit associated with a single SUR subunit, producing a 4:4 stoichiometry (see Fig. 16.1b) [22, 23]. The Kir6.x subunit consists of two transmembrane helices (TM1 and TM2), and cytoplasmic NH_2 and COOH termini (see Fig. 16.1a, c). The two transmembrane helices and the pore-forming domain are similar to the pore-forming S5-S6 membrane segment of voltage-gated K^+ channels. Kir6.x also contains the K^+ channel signature sequence-TVGY/FG, which confers K^+ selectivity [24]. SURx subunits are atypical members of the ABC family. Typically, ABC family members have two transmembrane domains (TMD1 and TMD2), each containing six transmembrane helices that function as transmembrane transporters, while SUR subunits do not appear to function as transporters. SURx subunits contain an additional transmembrane domain (TMD0) consisting of five transmembrane helices, concatenated to the N-terminus of TMD1 by an extended cytoplasmic linker (L0) (see Fig. 16.1a). Both TMD0 and L0 physically interact with the N-terminus of Kir6.x to modulate channel trafficking and gating [25–28]. Different subunit composition provides distinct channel properties in different tissues. For example, channels formed with Kir6.1 have a small single-channel conductance compared to those that contain Kir6.2 (~35 *vs.* ~80 ps in 150 mMK^+ solutions) [15, 29–31], and SUR subunits determine the pharmacological profiles of the channels (channels with SUR1 open in response to diazoxide, while those with SUR2A open with pinacidil) [10].

Chamber-Specificity of Cardiac K_{ATP} Channels

For many years, there was a consensus that cardiac K_{ATP} channels were essentially exclusively formed by Kir6.2/SUR2A complexes. Heterologously expressed Kir6.2 and SUR2A recapitulate essential cardiac ventricular K_{ATP} channel properties [29, 32], and Kir6.2 and SUR2A functionally [32] and physically [33] interact. There is also an absence of K_{ATP} channel activity in the ventricles of Kir6.2 knockout (Kir6.2$^{-/-}$) mice [34] and disruption of the SUR2 gene greatly reduces sarcolemmal K_{ATP} channel activity [35, 36]. Normal activity is reported in ventricular myocytes from both Kir6.1 knockout (Kir6.1$^{-/-}$) [37] mice and SUR1 knockout (SUR1$^{-/-}$) mice [38].

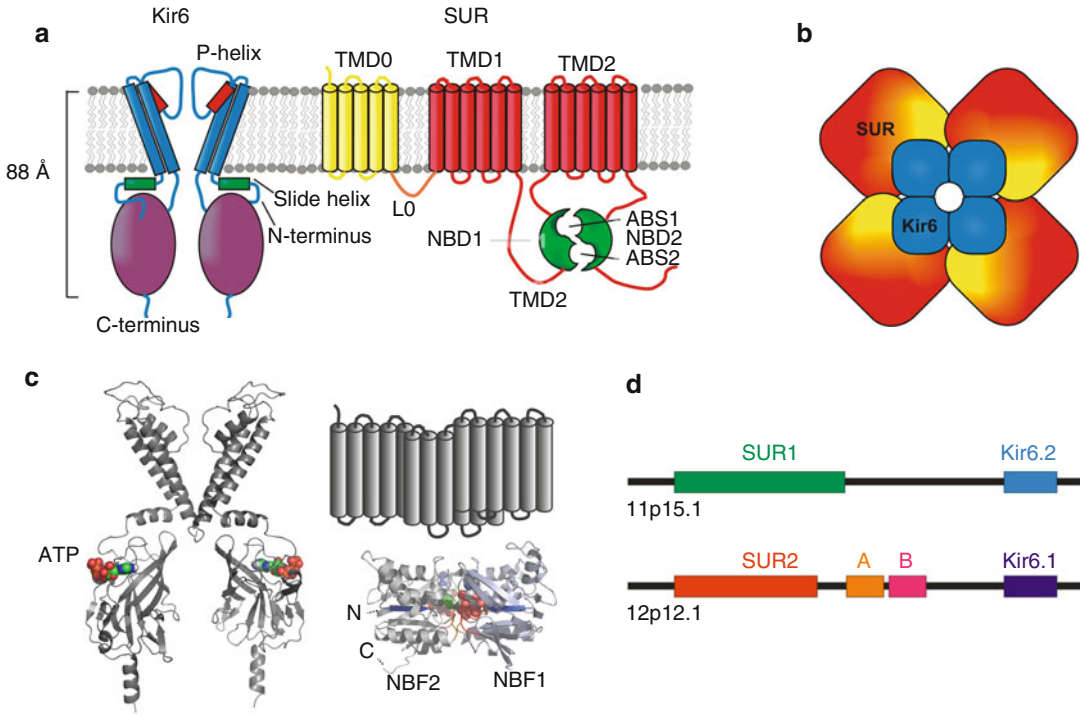

Figure 16–1. The structural basis of K_{ATP} channels. (**a**) K_{ATP} channels are formed from Kir6 (*left*) and SUR (*right*) subunits. Kir6 subunits each consist of two transmembrane helices (M1, M2), a pore-forming region (including the P-helix and selectivity filter), and cytoplasmic NH2 terminus (including the amphipathic slide helix) and COOH terminus. SUR consists of the TM0 and L0 regions that interact with and modulate gating of Kir6, followed by two additional 6-helix TM regions (TM1 and TM2), each followed by a nucleotide binding fold (*NBF*). The two nucleotide binding folds together generate two ATP/ADP binding sites (ABS1 and ABS2) at their interface. (**b**) The K_{ATP} channel is a heteroctamer of four Kir6 subunits which form the central pore with each Kir6 associated with one SUR subunit. (**c**) Homology models, based on crystal structures of related proteins, predict one inhibitory ATP binding site at each of the Kir6.x interfaces and Mg-dependent ATP/ADP binding sites (ABSs) in the dimeric nucleotide binding folds (*NBF*) of SUR. (**d**) Human SUR and Kir6 gene structures indicate that each pair is located adjacent to each other on two chromosomes

However, there is also evidence that all Kir subunits (Kir6.1 and Kir6.2) and SUR subunits (SUR1 and SUR2A/B) are expressed in the heart and play a functional role [15, 16, 18, 29, 39–41]. Results from heterologous expression systems show that more than one type of Kir6.x or SURx subunit can exist within a single functional channel [42–46], suggesting that the native composition of each subunit might be heterogeneous. The first hint of a role for Kir6.1 in cardiomyocytes was that a dominant negative Kir6.1 suppressed cardiac K_{ATP} [40], although others have not seen a comparable effect [47]. Additionally, a gain-of-function mutation in Kir6.1 has been linked to Early Repolarization Syndrome [6–8], although the specific cell type in which the mutation might be acting is unknown.

Confirming a role for SUR1 in the heart, a recent study has shown that K_{ATP} currents are abolished in atrial myocytes from $SUR1^{-/-}$ mice while remaining unchanged in ventricles [38].

Pharmacological studies show that the atrial K_{ATP} channel is more sensitive to diazoxide than pinacidil, indicating that the Kir6.2/SUR1 complex is present. Conversely, the ventricular K_{ATP} channel is more sensitive to pinacidil than diazoxide implying the presence of the Kir6.2/SUR2A complex [38]. This notion is further supported by a subsequent study on intact hearts [48] in which only diazoxide reduces action potential duration (APD) in atria while only pinacidil reduces

APD in ventricles. Moreover, the diazoxide effect is abolished in atria from SUR1$^{-/-}$ mice while pinacidil action is unaffected in ventricles [48]. These studies demonstrate a previously unappreciated chamber-specific K_{ATP} channel heterogeneity in the heart. Though the functional consequences of having different SUR subunits in atria and ventricles is not yet well understood, the presence of the SUR1 subunit in atria may have clinical consequences since SUR1 is the most sensitive subtype to changes in nucleotide concentrations [49], as well as a differential pharmacological sensitivity [50, 51].

K_{ATP} Channels in the Cardiac Conduction System

K_{ATP} channels are also present in the cardiac conduction system. Electrophysiological studies have detected K_{ATP} channels in rabbit sinoatrial node (SAN) cells [52], atrioventicular node (AVN) cells [53], and Purkinje cells [54]. The reported properties of these K_{ATP} channels in the conduction system are distinct from those in ventricular myocytes, suggesting a different K_{ATP} channel composition. Specifically, these channels have a smaller single-channel conductance, when recorded in rabbit SAN cells (50 ps) [52] and in Purkinje cells (60 ps) [54] as well as in mouse cardiac conductance system (57 ps) [55] than that in ventricular myocytes (80 ps), which would point to Kir6.1 with its smaller conductance. A recent study revealed Kir6.1, Kir6.2, and SUR2 RNA messages in mouse SAN and AVN tissue as well as atria and ventricles [56]. However, K_{ATP} currents are abolished in SAN cells from Kir6.2$^{-/-}$ mice, which indicates that Kir6.2 is an essential subunit in the SAN [57]. In the same study, sarcolemmal K_{ATP} channel activation inhibited SAN automaticity during hypoxia which indicates a potential role for sarcolemmal K_{ATP} channels in regulating heart rhythm during pathological conditions. Glibenclamide, a selective K_{ATP} channel antagonist, prevents cardiac conduction delay caused by low-flow ischemia in perfused mouse hearts [55], indicating a role of K_{ATP} channels in modulating cardiac conduction under metabolic stress. Computer simulations predict that activation of K_{ATP} channels by 1–5 mM cytoplasmic ATP will cause significant shortening of the Purkinje cell action potential [54], suggesting that K_{ATP} channels may have an important influence on atrioventricular conduction in response to changes in heart metabolism.

In conclusion, cardiac K_{ATP} channels are not exclusively generated by Kir6.2/SUR2A complexes nor are the different isoforms uniformly distributed through the heart. At least in mice, atrial K_{ATP} channels are formed by the Kir6.2/SUR1 complex. K_{ATP} composition in other species has not yet been systematically studied, but pharmacological studies in human hearts suggest that SUR1 and SUR2A may be both present, more interestingly, remodeling in molecular composition may happen in heart disease condition [58]. In addition, Kir6.1 may play a critical role in the cardiac conduction system. This diversity of subunit composition within the heart may allow K_{ATP} channels to function in distinct functional roles depending on the cell type in which they are expressed.

Regulation of Cardiac K_{ATP} Channels

Regulation by Nucleotides

Cytoplasmic ATP inhibits cardiac K_{ATP} channels with a half-maximal inhibition ($K_{1/2}$) of ~10–50 μM [59–63]. Recognition of the heteromultimeric architecture of the channel and molecular cloning of the cDNA encoding each subunit allowed the identification of amino acid residues that determine ATP inhibition. Pore-forming Kir6.2 subunits alone, without regulatory SUR subunits do not effectively traffic to the plasma membrane [64–67]. However, truncated Kir6.2, lacking the C-terminal RKR endoplasmic reticulum retention signal, can reach the membrane [64]. These truncated Kir6.2 generated channels retain ATP-induced inhibition, implying that the determinants are contained within the Kir6 subunit [64]. The cytoplasmic N- and C-termini of Kir6 form an ATP binding pocket with a total of four binding pockets per channel [68–72] at the interface of adjacent subunits (see Fig. 16.1c). Phosphorylation is not necessary for channel inhibition because nonhydrolyzable analogues of ATP are still effective [60, 73, 74]. In the absence of Mg^{2+}, reducing the number of phosphate groups from three to two

(ADP) or one (AMP) also blocks channel activity but with reduced affinity [59, 60, 75–77], suggesting an important role of the γ-phosphate for ATP binding and K_{ATP} channel inhibition.

In the presence of Mg^{2+}, both ATP and ADP stimulate channel activity [14, 60, 63, 78–81]. Mg-nucleotide stimulation and responsiveness to diazoxide and sulfonylurea require expression with SUR subunits [15, 29, 51, 64, 82–86], indicating that the structural elements responsible for MgADP stimulation and other K_{ATP} modulators are on the SUR subunit. Mutations of key SUR subunit residues disrupt the response of K_{ATP} channels to MgATP and MgADP [87, 88], as well as K_{ATP} channel openers [82, 89]. The nucleotide binding domains-NBD1 and NBD2, located between the eleventh and twelfth transmembrane regions and the C-terminus of SUR subunit, are essential structures for nucleotide binding and stimulation [82, 89–92] (see Fig. 16.1a, c). Mutations in NBD1 prevent ATP binding at both NBDs and interfere K_{ATP} channel openers acting on NBD2 [82, 88, 89, 93, 94]. SUR1 and 2A have intrinsic Mg^{2+}-dependent ATPase activity which hydrolyzes ATP at the NBDs, in particular NBD2 [95–97], and evokes a "power stroke" which overcomes ATP inhibition of Kir6 [97, 98]. Experimentally, MgADP stimulation is more effective than MgATP [14, 60, 63, 78–81] suggesting that binding of MgADP at the NBDs maintains an activated state of SUR, reducing Kir6.2 sensitivity to ATP inhibition [14, 82, 98, 99]. Binding of MgADP at NBD2 may also promote ATP binding at NBD1 [91, 98, 100]. Although there are currently no crystal structures of SUR subunits or domains, related bacterial NBDs crystallize as head-to-tail dimmers [101], with two ATP binding sites (ABSs) formed at the dimer interface. A recent mutagenesis study indicates that both NBDs form as a heterodimer and mutations at the dimer interface disrupt stimulation by MgADP and diazoxide, suggesting an important role for the NBDs in SUR-dependent regulation [101].

Regulation by Anionic Lipids

Membrane phosphoinositides potently stimulate K_{ATP} activity by interaction with the Kir6.2 subunit [102–106]. Application of phosphatidylinositol 4,5-bisphosphate (PIP_2) to the cytoplasmic membrane increases channel open probability and decreases ATP sensitivity [102–104, 107], demonstrating that ATP inhibition is a dynamic property that is dependent on membrane composition. PIP_2 and ATP bind competitively to K_{ATP} channels [105, 108]. However, this apparent competition may be revealing allosteric regulation instead of competitive ligands [72, 109]. Following the activation of K_{ATP} channels during metabolic inhibition, a secondary decrease in PIP and PIP_2 causes the channel activity to decline [110]. PIP_2 likely binds to a pocket formed by the N- and C- termini of two adjacent Kir6.2 subunits [72, 111], directly interacting with positively charged residues in the slide helix near the lipid-cytoplasm interface to stabilize channel opening [109, 112–114]. The negatively charged head group and phospholipid tail of PIP_2 are critical for channel activation by PIP_2 because the PIP_2 cleavage products-IP3 and diacylglycerol have limited effect [102].

Another class of anionic lipids, long chain fatty acyl-coenzyme A (LC-CoA) esters (e.g. oleoyl-CoA and palmityl-CoA), also potently activate K_{ATP} channels [115–121]. Long-chain acyl-CoA esters are β-oxidation products of fatty acids and the principal metabolic substrates of the heart which are thought to link K_{ATP} channel activity to the cellular metabolism of fatty acids. Through the same mechanism of phosphoinositides stimulation on K_{ATP} channels, LC-CoA esters increase K_{ATP} channel activity by decreasing ATP sensitivity and reducing channel rundown [117–120]. Acyl-CoA esters bind at Kir6.2 subunit with different binding sites from those for MgADP [116], but similar residues for PIP_2 binding are probably involved [122] and an acyl-CoA ester binding motif reportedly locates in the C-terminus of Kir6.2 subunit [123].

Regulation by Other Metabolic Signals

Dynamically, K_{ATP} channel regulation is predominantly a balance between ATP-induced inhibition and MgADP-induced activation [12, 59, 124]. At saturating concentrations, MgADP shifts the IC_{50} for ATP inhibition from ~30 to

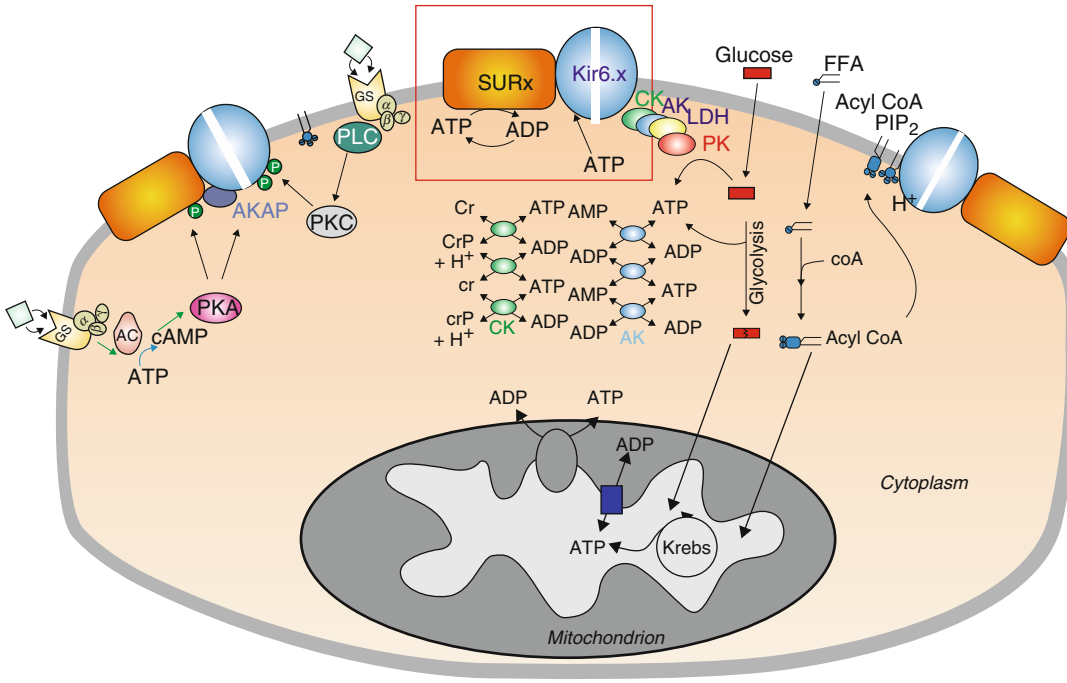

Figure 16–2. The molecular regulation of cardiac K_{ATP} channels. The ratio of ATP to ADP levels is the major direct determinant of channel activity (*box*). Metabolic enzymes, including adenylate kinase (*AK*), creatine kinase (*CK*), and lactate dehydrogenase (*LDH*), in the cytoplasm and physically associated with the channel may serve to amplify metabolic changes, or locally buffer and control ATP/ADP levels, thereby fine-tuning channel activity. Non-nucleotide ligands, including PIP_2, acyl-CoA, and H^+, may also play a key role. PIP_2 and acyl-CoAs have powerful stimulatory effects that are antagonistic to ATP inhibition. In addition, hormone receptor activation can lead to protein phosphorylation (*P*) with both stimulatory and inhibitory effects on the channel. However, none of these molecules acts in isolation, and the resultant K_{ATP} channel activity is an integrated response to a myriad of interrelated metabolic signals

~300 μM, which is still below the normal bulk cytosolic ATP concentration. K_{ATP} channels may respond to sub-sarcolemmal rather than bulk cytosol ATP concentration [125, 126]. Glycolysis has been reported to effectively inhibit channel activity, and functional glycolytic enzymes are associated with K_{ATP} channels [127], which suggests that glycolytic enzymes located in the membrane or adjacent cytoskeleton near the channels may be important in maintaining a high local cytosolic ATP/ADP ratio near K_{ATP} channels to inhibit channel activity [126]. In addition, phosphotransfer enzymes are recognized in amplifying small changes in bulk cytosolic ATP concentration, thereby regulating channel activity (Fig. 16.2). For example, the adenylate kinase (AK) phosphotransfer system facilitates conversion of ATP to ADP causing channels to open, whereas creatine kinase (CK) and protein kinase (PK)/glycolytic systems promote conversion of ADP to ATP and channel closure [61, 125, 128–130]. Competitive regulation between AK, CK, and PK regulates the conversion between ATP and ADP, which results in local changes of the ATP/ADP ratio in the vicinity of K_{ATP} channels, thereby regulating channel activity. Protein kinase A (PKA) [131, 132] and protein kinase C (PKC) [133] also increase channel activity and decrease ATP sensitivity through direct phosphorylation of K_{ATP} channels. Interestingly, AK [134], CK [135], lactate dehydrogenase (LDH) [135], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [136] and PK [137] all physically interact with K_{ATP} channel subunits and this channel-enzyme complex may play an integral role in regulating the nucleotide concentration in the microenvironment surrounding the channel, and fine-tuning the response of K_{ATP} to the energetic state of the cell (Fig. 16.2).

The emerging picture of K_{ATP} regulation in the heart is complex. While ATP is a primary inhibitor, it is now clear that K_{ATP} activation is the result of dynamic regulation by multiple factors, which may account for the highly variable ATP sensitivity [62], the high variance of the latency to channel activation during anoxia [138], and the metabolic state-dependent inhibition by K_{ATP} channel blockers [139–141].

Cardiac K_{ATP} Channel Function

Cardiac K_{ATP} Channels and Cardioprotection

Severe metabolic stresses such as anoxia, metabolic inhibition and global ischemia cause marked channel opening, but under normal metabolic conditions, sarcolemmal K_{ATP} channels are predominantly closed, and therefore not expected to contribute significantly to cell excitability. However, due to the higher density of K_{ATP} compared to other sarcolemmal K^+ channels, opening of as few as 1 % of K_{ATP} channels is expected to shorten cardiac action potential by about 50 % [142–145]. In so doing, K_{ATP} activation will reduce calcium entry and cause myocyte contraction to fail [146] and the energy stores that would otherwise be depleted in the contracting cell are preserved, providing cardioprotection.

In accordance with this cardioprotection concept, K_{ATP} channel openers enhance the preservation of ATP [147] and produce anti-ischemic effects by causing action potential shortening [148–150]. In parallel, glibenclamide prevents cardioprotective action potential shortening [151–153]. Renewed evidence for the cardioprotection by sarcolemmal K_{ATP} channels has been obtained through genetic manipulation. Moderate overexpression of SUR2A reportedly increases sarcolemmal K_{ATP} density and protects the heart against metabolic stress including hypoxia and ischemia/reperfusion [154]. Consistently, ischemic protection is abolished in rat cardiac myocytes transfected with a dominant-negative fragment of SUR2A which causes reduced sarcolemmal K_{ATP} expression [155]. Additionally, SUR1 overexpressing mice and Kir6.2$^{-/-}$ mice exhibit a disruption of K_{ATP} activity and impairment of the cardiac response to systolic overload following chronic transverse aortic constriction [156, 157].

However, in contrast to the accepted notion that increased K_{ATP} channel activity will produce cardioprotection, two recent studies have shown that neither SUR2$^{-/-}$ mice nor SUR1$^{-/-}$ mice have impaired response to ischemia reperfusion [158, 159]. SUR2$^{-/-}$ mice may actually be resistant to cardiovascular stress caused by global ischemia, due to reduced infarct size [158], contrary to the expected phenotype of impaired protection. However, these SUR2$^{-/-}$ mice were generated by disruption of NBD1, which might allow translation of the preceding TMD0 and TMD1 or following TMD2 of SUR2, and novel short form SUR2 proteins have been identified in both WT and SUR2$^{-/-}$ hearts [40]. The cardioprotection observed in these SUR2$^{-/-}$ mice may be due somehow to the presence of these short form proteins, but may also be related to abolition of the SUR2B component of vascular K_{ATP} channels [158]. Similar cardioprotection was also observed in SUR1$^{-/-}$ mice [159]. In an *in vivo* model of myocardial ischemia/reperfusion injury, SUR1$^{-/-}$ mice exhibit increased protection and reduced infarct size as well as preservation of left ventricular function [159], presenting the first potential pathological role for SUR1 in the heart. Again the mechanism is unknown, but may be related to abolition of SUR1, and hence abolition of K_{ATP} in the atria, where SUR1 is an essential subunit [38, 48]. While it is not clear how SUR knockout mice produce enhanced cardioprotection, clearly reduction of either SUR1 or SUR2 expression can modulate cardioprotection, and hence these subunits may be useful cardioprotective targets.

Cardiac K_{ATP} Channels and Ischemic Preconditioning

Cardioprotective functions of the K_{ATP} channel have been best characterized during ischemic preconditioning. First recognized in 1986, this type of preconditioning arises from brief ischemic challenges that precede a prolonged insult, improving recovery of contractile function and reducing infarct size that results from more severe metabolic insult [160]. Among many endogenous ligands, such as adenosine and

acetylcholine, and associated signaling proteins, such as protein kinase C and MAP or JUN kinases, K_{ATP} channels may be the common end-target in ischemic preconditioning [161]. Preconditioning can be mimicked by adenosine, as well as by synthetic K_{ATP} channel openers such as pinacidil and cromakalim [150]. Additionally, glibenclamide inhibits ischemic as well as adenosine- and acetylcholine-mediated preconditioning [150, 162]. Ischemic preconditioning is also abolished in hearts from Kir6.2$^{-/-}$ mice [156], in hearts from Kir6.2 gain-of-function mutation mice which show decreased K_{ATP} density [163], and in rat cardiac myocytes transfected with a dominant-negative fragment of SUR2A [155], strongly suggesting a role for sarcolemmal K_{ATP} channel as a preconditioning mediator.

In 1991, K_{ATP} channels were also reported in the mitochondria [164]. A succession of pharmacological studies that followed argued that mitochondrial rather than sarcolemmal K_{ATP} channels are primarily involved in preconditioning. First, action potential shortening cannot account for the cardioprotective effects of K_{ATP} channel openers [165–167]. Second, diazoxide, reportedly without effect on sarcolemmal K_{ATP} channels, can mimic ischemic preconditioning [168–170]. Third, 5-hydroxydecanoic acid (5-HD), reportedly a specific mitochondrial K_{ATP} channel blocker, effectively abolishes ischemic preconditioning and the anti-ischemic effects of K_{ATP} channel openers without inhibiting action potential shortening [171–173].

Several studies show that diazoxide activates sarcolemmal K_{ATP} channels [174, 175] and 5-HD suppresses APD shortening in ischemic hearts [152, 176], suggesting diazoxide and 5-HD actually target the sarcolemmal K_{ATP} channel. Moreover, additional K_{ATP}-independent targets are recognized as being sensitive to diazoxide and 5-HD: diazoxide inhibits succinate oxidation and succinate dehydrogenase activity; 5-HD serves as substrate for acyl-CoA synthetase [177]. Therefore, the use of the above pharmacological tools as probes for mitochondrial K_{ATP} requires reassessment.

Genetic manipulation has made it possible to circumvent some of the limitations of pharmacology by examining gene function directly. The molecular structure of the mitochondrial K_{ATP} channel is presently unknown, but Kir6.2, the primary pore-forming subunit of sarcolemmal K_{ATP} channels, does not appear to form mitochondrial K_{ATP} channels [178]. One study, using pharmacological tools and transfection systems suggests that current through the Kir6.1/SUR1 complex mimics the properties of mitochondrial K_{ATP} channels [50]. In this study, Kir6.1/SUR1 channels expressed heterologously in HEK293 cells were activated by diazoxide but not P-1075, and were inhibited by 5-HD but not HMR1098. The pharmacological properties of Kir6.1/SUR1 channels are similar to those reported for mitochondrial K_{ATP} channels [50], suggesting a Kir6.1/SUR1 composition in mitochondria. Therefore, genetically controlling Kir6.2 may be a direct means of assessing the role of K_{ATP} channels in the sarcolemma rather than in the mitochondria. Kir6.2$^{-/-}$ mice lose ischemia preconditioning [156] as well as diazoxide induced preconditioning [179]. Preconditioning is also abolished in cardiac Kir6.2 gain-of-function mice in which cardiac basal K_{ATP} activity is increased but overall activity is dramatically decreased [163, 180]. These studies are therefore more consistent with sarcolemmal rather than mitochondrial K_{ATP} channels mediating preconditioning. Ultimately, the existence of conventional K_{ATP} channels in mitochondria may be questionable: both Kir6.1 and SUR1 subunits may be expressed strongly at the sarcolemma of ventricular myocytes [41]. Even efforts to determine the molecular composition of mitochondrial K_{ATP} from immunohistological experiments may be unreliable given the still considerable difficulties in isolation of mitochondrial membrane proteins [181].

Cardiac K_{ATP} Channels and Arrhythmia

Lethal ventricular arrhythmia remains a leading cause of sudden death in patients with ischemic heart disease; 80 % result from ventricular fibrillation accompanied by myocardial ischemia and infarction [182]. The development of malignant arrhythmias during ischemia may be primarily due to the opening of K_{ATP} channels which cause rapid external potassium accumulation and reduction in action potential duration and therefore reduction in the refractory

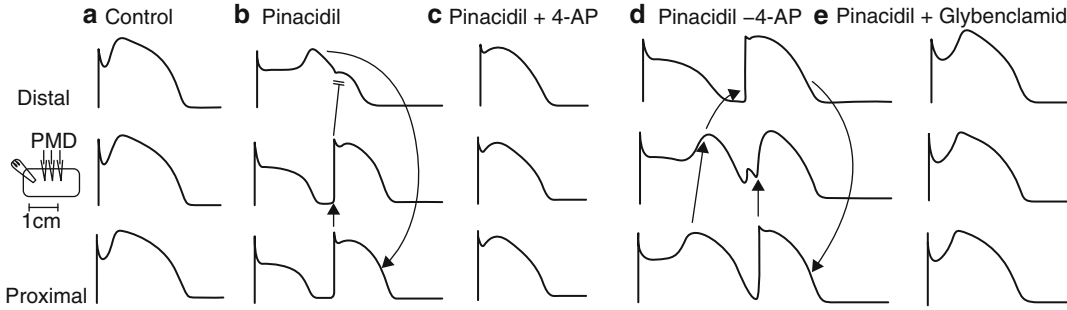

Figure 16–3. K_{ATP} opening increases dispersion of repolarization. Action potential traces were recorded simultaneously from three sites along a canine epicardial preparation, *P* proximal, *M* middle, *D* distal. (**a**) Control traces recorded without any treatment. (**b**) Recorded after exposure to pinacidil shows the development of electrical inhomogeneity with loss of the dome at the two proximal sites but not at the distal site. The marked difference in repolarization times results in reentrant excitation at the proximal and middle sites. (**c**) Shows the effects of 4-aminopyridine (*4-AP*), a transient outward current blocker, in the continued presence of pinacidil. Inhibition of the transient outward current restores electrical homogeneity to the preparation, thus abolishing all arrhythmic activity. (**d**) Washout of *4-AP* is attended by reappearance of electrical inhomogeneity and reentrant activity. (**e**) Illustrates the effects of glybenclamide, an inhibitor of the ATP-sensitive potassium current, in the continued presence of pinacidil. The effects of pinacidil were completely reversed, electrical homogeneity was restored, and all reentrant activity was abolished. The presumed circus movement pathways are indicated by the arrows (From Di Diego and Antzelevitch [186]. Reprinted with permission from Wolters Kluwer Health)

period in the ischemic myocardium [183, 184]. Additionally, dispersion of action potential duration may result from K_{ATP} activation: epicardial cells may be preferentially affected by K_{ATP} opening due to an action potential notch caused by the transient outward current (I_{to}) [185]. Depression of the membrane potential by I_{to} in conjunction with K_{ATP} channel opening will tend to prematurely deactivate Ca^{2+} channels, leading to early action potential termination. Selectively shortened action potentials in epicardium by K_{ATP} opening will then result in a transmural dispersion of repolarization, causing ST-segment elevation in the ECG and giving rise to phase 2 re-entry, which can precipitate ventricular tachycardia and ventricular fibrillation (VF) [185, 186]. In accord with this idea, Di Diego and Antzelevitch were able to show reentry in isolated ventricular myocardium after adding the K_{ATP} opener pinacidil, electrical homogeneity was restored and arrhythmias were abolished by blocking I_{to} (4-aminopyridine, 4-AP) or K_{ATP} (glibencamide) (Fig. 16.3) [186].

While K_{ATP} channel activation is potentially proarrhythmic and may predispose to lethal reentrant arrhythmias, the opening of K_{ATP} channels during ischemia is also expected to stabilize the resting membrane potential and reduce ectopic pacemaker activity, i.e. an antiarrhythmic effect. Indeed, pharmacological studies provide two contrasting effects of K_{ATP} channels with respect to arrhythmias. Glibenclamide decreases the incidence of sustained tachycardia and ventricular fibrillation during ischemia-reperfusion [187–189], but increases the occurrence of tachycardia [190]. Similarly, some have observed that K_{ATP} channel openers promote tachycardia and fibrillation [58, 187, 190], while, others see a reduction in the overall incidence of arrhythmias [149, 191]. Grover et al. [149] reported opposite effects of the K_{ATP} opener cromakalim in different species: inhibition of fibrillation in anesthetized dogs but induction of fibrillation in isolated rat hearts, implying that the arrhythmic effects of K_{ATP} channels may be a function of the experimental model.

The significance of K_{ATP} channel composition in arrhythmia induction has been further explored through genetic manipulation. $Kir6.2^{-/-}$ mice exhibit high risk for induction of triggered activity and ventricular arrhythmia under catecholamine challenge, due to defective action potential shortening and predisposition to early afterdepolarizations [192]. Thus, sarcolemmal K_{ATP} is required for tolerance against triggered catecholamine-induced ventricular arrhythmia. Evidence for a proarrhythmic effect of sarcolemmal K_{ATP} channels comes from double transgenic

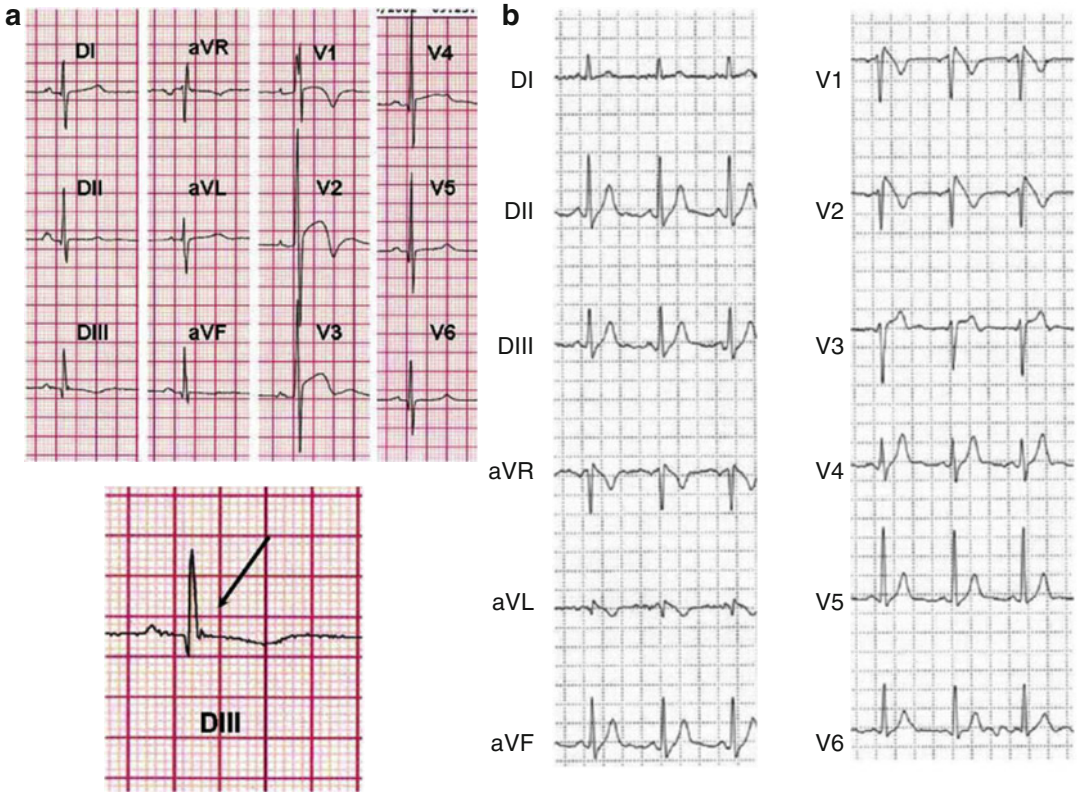

Figure 16–4. ECGs in Kir6.1-associated J-wave syndrome. ECG recordings of one early repolarization syndrome case and one Brugada syndrome case associated with S422L mutation in Kir6.1 gene. (**a**) Twelve-lead ECGs of case 1 showing intraventricular conduction delay (V1), repolarization abnormalities in leads V1–V4 (*left*), and intermittent J waves in the inferior leads. Magnification of the J wave observed in lead DIII is shown at the bottom. (**b**) Spontaneous type 1 Brugada ECG pattern recorded during 24-h Holter monitoring in case 2 (From Medeiros-Domingo et al. [7]. Reprinted with permission from Elsevier Limited)

(DTG) mice which overexpress both SUR1 and an ATP-insensitive Kir6.2 in the heart [193]. These SUR1-expressing DTG mice develop several different types of arrhythmias including AV block, atrial and ventricular tachyarrhythmia, indicating a proarrhythmic effect of K_{ATP} channels comprised of SUR1 and ATP-insensitive Kir6.2. In marked contrast, SUR2A-expressing DTG mice exhibit no change in arrhythmia incidence [193]. In addition, single transgenic mice which overexpress SUR1 but not SUR2A exhibit PR prolongation, suggesting delayed conduction in the AV nodal or diffuse conduction system. These results may reflect the higher sensitivity of SUR1-based K_{ATP} channels to metabolic activation [49], and indicate that expression of hyperactive K_{ATP} in the heart is likely to be proarrhythmic.

Also of interest are K_{ATP} mutations that confer risk for arrhythmia. The SUR2 T1547I mutation was associated with adrenergic atrial fibrillation originating from the vein of Marshall in a patient with paroxysmal atrial fibrillation [194]. More recent studies also report association of the S422L mutation in Kir6.1 with J-wave syndromes, which include Early Repolarization Syndrome and Brugada Syndrome (Fig. 16.4) [6–8]. As discussed above, the existence and distribution of Kir6.1 in the heart are not yet well established, nor can the consequences of mutations in other tissues be excluded, but these findings will certainly cause a re-evaluation of cardiac K_{ATP} channel function.

A role for sarcolemmal K_{ATP} channels during arrhythmias may be masked by the potential that mitochondrial K_{ATP} inhibition influences

the incidence of arrhythmia. 5-HD suppressed the incidence of ventricular fibrillation induced by coronary ligation in rats [195], suggesting an antiarrhythmic effect for mitochondrial K_{ATP} inhibition. On the other hand, the antiarrhythmic effects of both Nicorandil and Minoxidil in rabbits were abolished by 5-HD but not by HMAR 1883, suggesting a proarrhythmic effect of mitochondrial K_{ATP} inhibition. The role of 5-HD in arrhythmias became more confusing when it was reported that 5-HD was not antiarrhythmic in dogs [196] or in rats [197]. Conversely, HMR 1883 decreased the incidence of ischemia-induced irreversible ventricular fibrillation and improved survival during acute myocardial infarction in conscious rats as well as during reperfusion after brief myocardial ischemia in anesthetized rats [198], while in the same study, 5-HD did not protect hearts in these arrhythmia models [198], suggesting arrhythmias during ischemia and ischemia/reperfusion are the result of opening sarcolemmal K_{ATP} channels rather than mitochondrial K_{ATP} channels.

Cardiac K_{ATP} Channels and Cardiomyopathy

Cardiac hypertrophy can be an adaptive response but prolonged hypertrophy can progress into heart failure and sudden death, which may result from consequently disturbed electrical activity [199]. Decreased K_{ATP} channel responsiveness to ATP inhibition has been reported in hypertrophied cardiomyocytes suggesting hyper-activity [200, 201]. However, impaired K_{ATP} channel activation has also been reported in hypertrophic myocytes and suggested as a potential explanation for reduced tolerance of hypertrophied hearts to ischemia [202]. Interestingly, $SUR2^{-/-}$ mice exhibit greater ventricular mass and relative heart size compared to control cohorts, evidence of hypertrophy [158]. $Kir6.2^{-/-}$ mice also show increased mortality and exaggerated hypertrophy in response to pressure overload [157, 203] and to mineralocorticoid/salt challenges [204], suggesting that disrupting K_{ATP} channel actually predisposes hearts to hypertrophy under stress.

K_{ATP} channels appear to be less sensitive to ATP inhibition and more sensitive to K_{ATP} channel openers in failing human hearts, which may indicate that K_{ATP} remodeling may favor a beneficial effect in failing hearts [58,205]. Optical mapping of failing hearts reveals increased APD shortening in response to diazoxide (Fig. 16.5). A heart failure model induced by transgenic expression of cytokine tumor necrosis factor alpha (TNFα) shows a disturbed link between the metabolic state of the cell and K_{ATP} channel activity and increased cardiomyocyte vulnerability to stress [206]. In this case, application of K_{ATP} channel openers improved stress tolerance [206]. The same group has reported linkage of human heart failure to K_{ATP} channel mutations. Mutations in SUR2 that cause decreased catalytic activity of NBD1 and disruption of K_{ATP} channel gating were identified in patients with dilated cardiomyopathy [9, 124]. Also, the Kir6.2 E23K polymorphism, which causes enhanced channel activity and is associated with type 2 diabetes susceptibility [207] also appears to be linked with subclinical maladaptive cardiac remodeling among individuals with increased stress load due to hypertension [208] and is over-represented in patients with congestive heart failure [5]. More recently, an in-frame deletion (E332del) and a missense mutation (V346I) were identified in two nonrelated sudden infant death cases. Both mutations lead to loss-of-function of Kir6.1 channels which may predispose a maladaptive cardiac response to systemic metabolic stressors [209].

Cardiac K_{ATP} Channels in Adaption to Physiological Stress

Potential protective effects of K_{ATP} are not limited to an extreme metabolic insult, such as global ischemia. A physiological role for cardiac K_{ATP} channels has been recognized during adaptation to physiological stress [1–3]. Short-term exercise up-regulates cardiac sarcolemmal K_{ATP} channel expression and enhances APD shortening which in turn reduces cardiac energy consumption in response to heart rate acceleration [1]. In addition to the lack of protection during ischemia-reperfusion, $Kir6.2^{-/-}$ mice exhibit intolerance to vigorous exercise in treadmill test and impaired survival in response to isoproterenol treatment compared

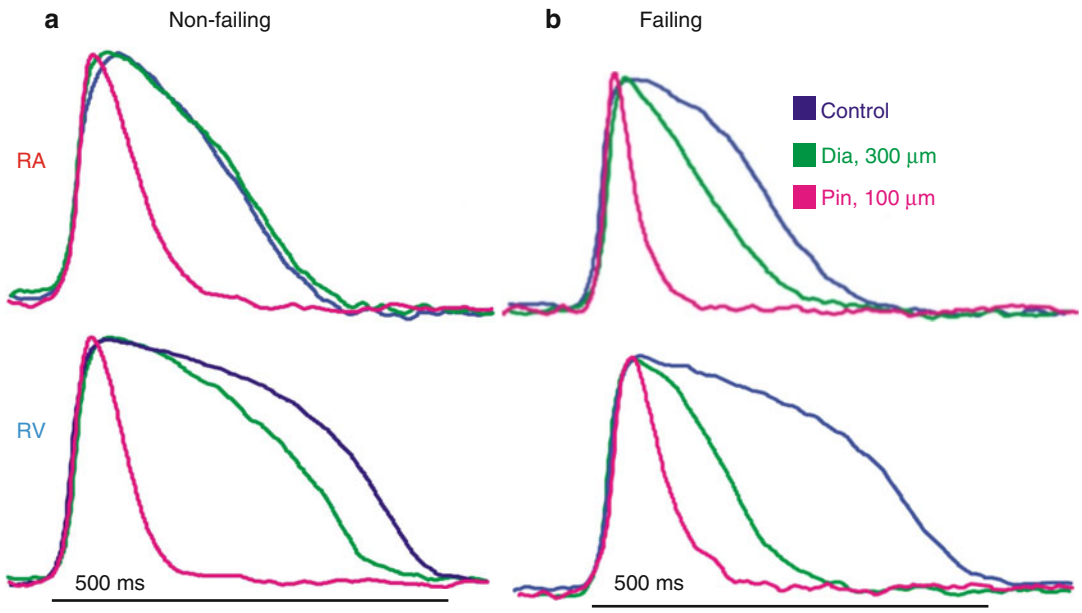

Figure 16–5. Pathologic changes in human K_{ATP} channel opener responses. Optically mapped action potentials of human right atrial–ventricular preparation from a non-failing heart (**a**) and a congestive failing heart (**b**). A, In the non-failing heart, action potential in right atria (*RA*) is shortened by diazoxide but not pinacidil, while action potential in right ventricles (*RV*) is shortened by both diazoxide and pinacidil. (**b**) Shows diazoxide-induced action potential shortening in *RA* and a greater shortening in *RV* in the congestive failing heart, implying remodeling of K_{ATP} channel in failing hearts (From Fedorov et al. [58]. Reprinted with permission from Elsevier Limited)

with age- and gender-matched WT controls [3]. The Kir6.2$^{-/-}$ mice show less action potential shortening and diastolic calcium overload associated with contractile dysfunction which may explain the decreased adaption to stress [3]. Bulk adenine nucleotide content was not changed under the exercise and isoproterenol challenge, suggesting K_{ATP} channel activation may be caused by metabolic fluctuations in subsarcolemmal compartments. In addition, during chronic stress induced by long-term swimming training, Kir6.2$^{-/-}$ mice develop calcium-dependent structural damage and impaired contractile function, with a significant reduction in left ventricular fractional shortening and impaired cardiac output [2]. The Kir6.2$^{-/-}$ animals lack K_{ATP} in multiple tissues and further studies are warranted. However, selective disruption of K_{ATP} channels in heart (loss-of-function mutation of pore-forming subunit Kir6.1 [αMHC-Kir6.1AAA]) abolishes the enhanced APD shortening in response to the workload imposed by heart rate acceleration and causes increased cardiac energy consumption [1], strongly suggesting that K_{ATP} channels may play a more widespread role in cardiac physiology than generally appreciated, and are not limited to roles in pathological states.

Conclusions

Much has been learned about cardiac K_{ATP} channel since its discovery, but there are still significant gaps in our understanding. Recently, it has been recognized that cardiac K_{ATP} channel is chamber specific in mouse heart, but it is not clear whether this is universal for all species, whether there is any transmural heterogeneity or whether K_{ATP} channel composition differs in the conduction system. Many K_{ATP} channel mutations have now been identified as causal in human neonatal diabetes mellitus (NDM) and hyperinsulinism of infancy [210–212], but these patients have no reported cardiac abnormalities. Experimental and modeling studies have shown that activation of sarcolemmal K_{ATP} channels causes dramatic action potential shortening *in vitro*, but the function of cardiac K_{ATP} channels *in vivo* is not well established, even

though there is emerging evidence that K_{ATP} channel mutations are linked to heart diseases. The debate regarding the role, and even the very existence, of mitochondrial K_{ATP} channels is still ongoing. Filling these knowledge gaps will require further study, and integration of results from basic cellular electrophysiology, to animal models and clinical disease.

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17
Ca^{2+} Release Channels (Ryanodine Receptors) and Arrhythmogenesis

Sameer Ather and Xander H.T. Wehrens

Abstract

Intracellular calcium (Ca^{2+}) release channels (ryanodine receptors type 2, RyR2) are present on the sarcoplasmic reticulum (SR) in cardiomyocytes and are required for excitation-contraction coupling in cardiac muscle. RyR2s are macromolecular channel complexes associated with regulatory proteins that modulate RyR2 function in response to extracellular signals. Recent studies have provided new mechanistic insight into the role of diastolic SR Ca^{2+} leak through RyR2 as a trigger for a wide range of cardiac arrhythmias. RyR2 has been implicated to play a central role in arrhythmias associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), heart failure (HF) and drug-induced arrhythmias. At present, there is no FDA approved medication that selectively targets RyR2. However, novel therapeutic approaches are being evaluated to correct defective RyR2 Ca^{2+} release based on recent advances in the understanding of the cellular mechanisms underlying arrhythmias in HF and CPVT.

Keywords

Ryanodine receptor channel • Ca^{2+} release channel • Arrhythmia • Sarcoplasmic reticulum • Delayed after-depolarization • Catecholaminergic polymorphic ventricular tachycardia • Heart failure

Introduction

Intracellular calcium (Ca^{2+}) release channels (ryanodine receptors, RyR) are present on the sarcoplasmic reticulum (SR) in cardiomyocytes and are required for excitation-contraction (EC) coupling in cardiac muscle. Each RyR channel contains four pore-forming subunits that contain large cytoplasmic domains, which serve as scaffolds for proteins that regulate the activity of the channel. An important regulatory protein is calstabin2 (FKBP12.6), a subunit that stabilizes the closed state of the channel to prevent aberrant Ca^{2+} leak from the SR [1]. Direct targeting of several protein kinases and phosphatases

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to the type 2 cardiac RyR channel (RyR2) allows for rapid and localized modulation of SR Ca^{2+} release in response to extracellular signals [2].

Recent studies have provided new mechanistic insight into the role of diastolic SR Ca^{2+} leak through RyR2 as a trigger of cardiac arrhythmias. For example, inherited mutations in the RyR2 gene have been linked to cardiac arrhythmia syndromes including catecholaminergic polymorphic ventricular tachycardia (see also chap. 31) [3]. Moreover, defective regulation of RyR2 may contribute to arrhythmogenesis in heart failure (HF) [4–9]. These findings have initiated the development of a new class of drugs called 'RyR stabilizers' that specifically target these molecular defects, improve cardiac function and prevent arrhythmias in relevant animal models [10–19].

Excitation-Contraction Coupling in the Heart

The process of EC coupling comprises a sequence of events that translates electrical membrane depolarization into contraction of the cardiomyocyte [20]. During cardiac muscle contraction (systole), Ca^{2+} is released from a network of intracellular Ca^{2+} stores referred to as the SR (Fig. 17.1). When the heart subsequently relaxes to fill with blood (diastole), the cytoplasmic Ca^{2+} is actively pumped back into the SR. Cyclic release of Ca^{2+} from the SR Ca^{2+} store and Ca^{2+} uptake from the cytosol are thus critical for rhythmic contraction and relaxation of the heart [22].

Depolarization of the cardiomyocyte membrane leads to activation of voltage-gated L-type Ca^{2+} channels (LTCC) located in plasma membrane invaginations called transverse or T-tubules (see Fig. 17.1). Entry of Ca^{2+} through LTCCs triggers a much greater release of Ca^{2+} from the SR via the RyR2 intracellular Ca^{2+} release channels, which is known as Ca^{2+}-induced Ca^{2+} release (CICR) [23]. For relaxation to occur during diastole, Ca^{2+} is extruded from the cytoplasm via a specialized Ca^{2+} pump known as the SR Ca^{2+} ATPase (SERCA2a). SERCA2a activity is regulated by phospholamban, which inhibits SR Ca^{2+} uptake in its non-phosphorylated form. Finally, cytosolic Ca^{2+} is also extruded from the cardiomyocyte via the electrogenic plasma membrane Na^+/Ca^{2+} exchanger (NCX) [22, 24].

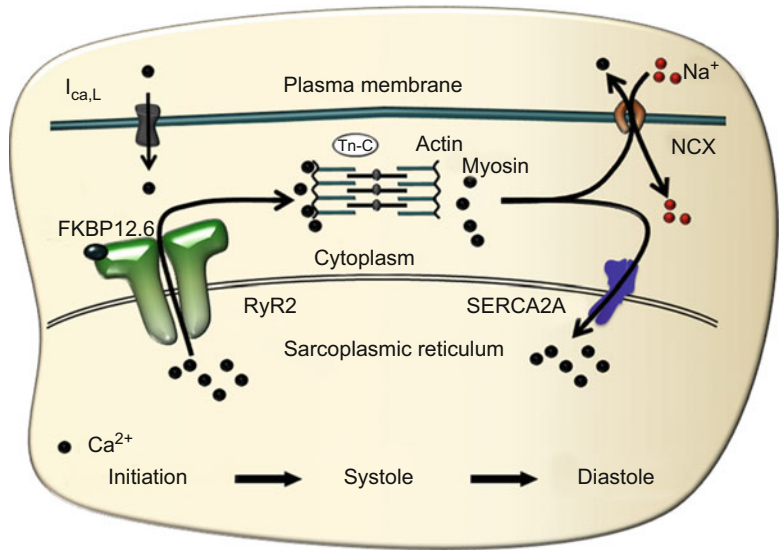

Figure 17–1. Excitation-contraction coupling in the heart. With the advent of an action potential, L-ype Ca^{2+} channels are activated resulting in the influx of Ca^{2+}. This influx of Ca^{2+} activates the adjacent RyR2 resulting in outpouring of Ca^{2+} stored in the sarcoplasmic reticulum (SR) into the cytosol. The release of Ca^{2+} activates the contractile proteins initiating systole. The increased cytosolic Ca^{2+} inactivates L-type Ca^{2+} channels, activates NCX (causing efflux of Ca^{2+}) and activates SERCA2a (causing reuptake of Ca^{2+} into SR). This results in the termination of systole and the onset of diastolic phase. $I_{Ca,L}$ L-type Ca^{2+} current, *NCX* Na^+/Ca^{2+}-exchanger, *PLN* Phospholamban, *RyR2* Ryanodine receptor type 2, *SERCA2a* Sarco/endoplasmic reticulum Ca^{2+}-ATPase, *TnC* Troponin C (Modified from Wehrens et al. [21]. With kind permission from Future Medicine Ltd)

Advanced imaging techniques have revealed that cytosolic Ca^{2+} transients in cardiomyocytes represent the summation of 10^4 up to 10^6 localized, subcellular Ca^{2+} release events called Ca^{2+} sparks [25]. The CICR process amplifies and scales the intracellular Ca^{2+} signal via summation of functionally independent Ca^{2+} sparks [26]. Moreover, Ca^{2+} sparks are characterized by local refractoriness, suggesting that local SR Ca^{2+} release is independent of the duration of the LTCC-mediated Ca^{2+} influx [27]. It is estimated that about 100 or more RyR2 channels may become functionally coupled in a Ca^{2+} release unit in order to open and close simultaneously by the process of coupled gating [28]. On the other hand, Ca^{2+} release from the SR also results in local Ca^{2+} depletion in subcompartments of the SR Ca^{2+} stores [29]. Therefore, the intrinsic (e.g. channel composition) and extrinsic (e.g. regulation by extracellular signals) properties of RyR2 channel gating are an important determinant of the net Ca^{2+} concentration in the cytosol and the SR at any given time point during the cardiac contraction-relaxation cycle.

Cardiac Ryanodine Receptors

RyRs are comprised of four 560-kDa pore-forming subunits, which form tetrameric ion channels located on the SR membranes [30]. RyR2 is the predominant isoform in the heart and functions as the principal intracellular Ca^{2+} release channel during EC coupling [31]. Apart from a relatively small C-terminal pore-forming domain, approximately 90 % of the RyR2 N-terminal sequence forms the electron-dense cytosolic foot structure in the junctional space between the T-tubule and the SR (Fig. 17.2). This

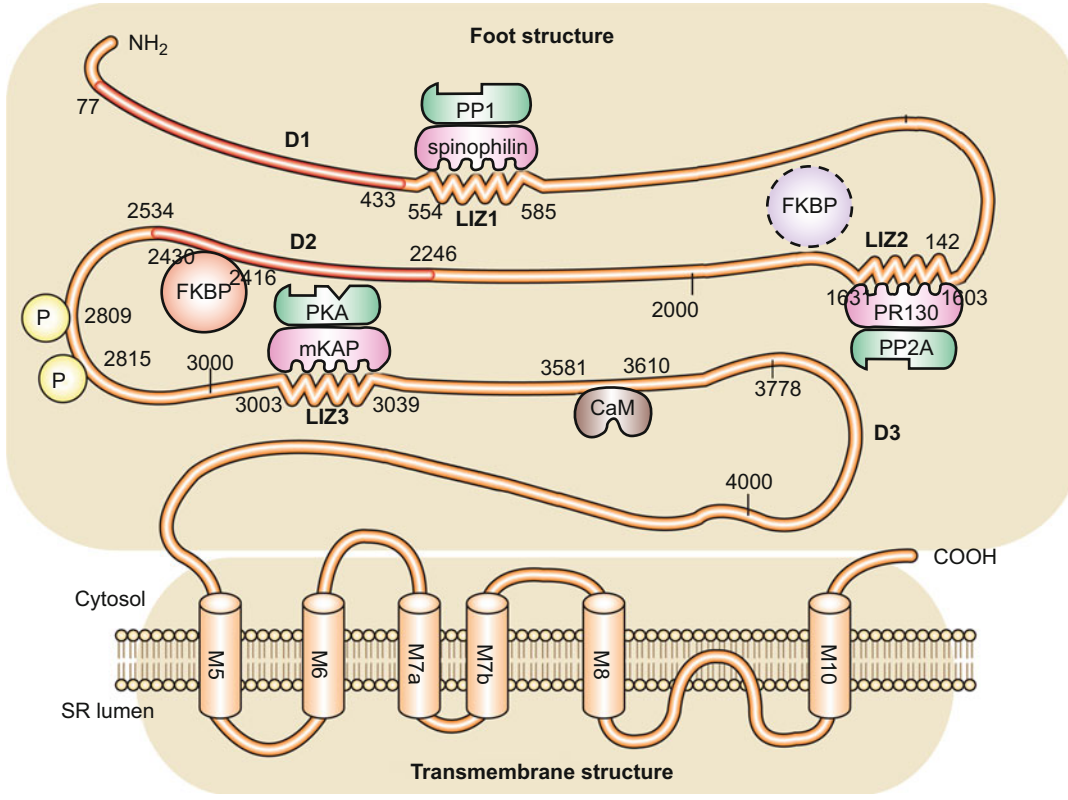

Figure 17–2. Primary structure of the cardiac ryanodine receptor macromolecular complex. The primary structure of a cardiac ryanodine receptor subunit, with the binding domains of protein kinase A (*PKA*), protein phosphatases 1 and 2A (*PP1*, *PP2A*), calmodulin and FKBP12.6 indicated. PKA and PP1/PP2A are bound to RyR2 via their specific adaptor proteins. The domains highlighted in red (77–433, 1724–2534) as well as the transmembrane domains correspond to the three CPVT mutation hotspot regions (*D1*, *D2* and *D3*). *CaM* calmodulin, *FKBP* FKBP12.6, *LIZ* leucine-isoleucine zipper, *PKA* protein kinase A, *PP* protein phosphatases, *SR* sarcoplasmic reticulum (From Macmillan Publishers Ltd: Yano et al. [32]. Reproduced with kind permission from Nature Publishing Group)

cytoplasmic RyR2 domain functions as a scaffold for modulating proteins, which regulate gating of the transmembrane Ca^{2+}-conducting pore [24, 33, 34].

The Ryanodine Receptor Macromolecular Complex

A major regulatory subunit interacting with RyR2 Ca^{2+} release channels is the 12.6 kDa cytosolic FK506-binding protein (FKBP12.6), also known as calstabin2 [35, 36]. FKBP12.6, a peptidyl-prolyl *cis*–trans isomerase that tightly associates with RyR2 monomers, stabilizes the closed conformational state of RyR2 enabling the channel to close completely [37, 38]. Therefore, in the heart, an important physiologic role of FKBP12.6 appears to be inhibiting the RyR2 channel at low intracellular Ca^{2+} concentrations to ensure diastolic muscle relaxation and preventing detrimental effects of intracellular Ca^{2+} leak during diastole [13, 38]. In addition, FKBP12.6 plays a role in the orchestration of simultaneous RyR2 gating in Ca^{2+} release units comprised of functionally linked RyR2 [28, 39].

Other modulators associated with the large cytosolic RyR2 domain include calmodulin (CaM) [40], sorcin [41], the protein kinases A (PKA) [5], Ca^{2+}/CaM-dependent protein kinase II (CaMKII) [42, 43], and the phosphatases, PP1 and PPA2A [2]. These kinases and phosphatases are believed to bind to RyR2 via specific anchoring proteins mAKAP, spinophilin and PR130, respectively (see Fig. 17.2). Conserved leucine/isoleucine zipper (LIZ) motifs in the targeting proteins correspond to complementary LIZ domains in RyR2, allowing for highly localized RyR2 regulation.

RyRs also associate with proteins at the luminal surface within the SR membrane. Junctin and triadin are most likely involved in anchoring RyR2 to the SR membrane [44, 45]. Calsequestrin (CSQ) is the major Ca^{2+} binding protein in the SR and provides a high capacity intra-SR Ca^{2+} buffer [46]. Recent studies suggest that Ca^{2+}-dependent conformational changes in CSQ modulate RyR2 channel activity and that dysfunctional luminal regulation of RyR2 may lead to cardiac arrhythmias (see below) [47]. Finally, we recently demonstrated that junctophilin-2 (JPH2) binds to RyR2 and mediates both physical and functional coupling between RyR2 and voltage-gated LTCC on the plasmalemma [48].

Regulation of RyR2

Phosphorylation

The maximal cardiac force development during systole (inotropy) can be enhanced by increasing the amplitude of the intracellular Ca^{2+} transient, which is dependent on the amount of intracellular Ca^{2+} released via RyR2 [49]. Upregulation of SR Ca^{2+} cycling can be achieved by phosphorylation of proteins involved in SR Ca^{2+} release and Ca^{2+} uptake pathways by cAMP-dependent PKA [5], CaMKII [43] and protein kinase C (PKC) [50]. A potent mechanism to increase cardiac contractility rapidly is mediated by stress-mediated activation of β(Beta)-adrenergic receptors by catecholamines resulting in increased intracellular cAMP synthesis and activation of PKA [51]. PKA phosphorylates both RyR2 to increase SR Ca^{2+} release,[2] as well as phospholamban to enhance the activity of SERCA2A [52]. The net effect of β(Beta)-adrenergic receptor activation is amplification of the EC coupling gain such that for any given LTCC Ca^{2+} current more SR Ca^{2+} release is triggered [53].

Some studies have shown that phosphorylation by PKA increases RyR2 channel open probability by increasing the sensitivity to Ca^{2+}-dependent activation [5, 38, 54, 55], although some studies indicate that PKA phosphorylation might not directly alter RyR2 open probability [56–58]. Although some studies suggest PKA might phosphorylate an additional residue on RyR2 *in vitro* [59], PKA phosphorylation of RyR2 is completely prevented in RyR2-S2808A knockin mice, in which serine 2808 is replaced by an alanine [60]. The transient nature of RyR2 activation by PKA phosphorylation in cells suggests powerful negative feedback mechanisms that prevent uncontrolled intracellular Ca^{2+} release. Indeed, RyR2 phosphorylation is locally regulated by protein phosphatases, which are targeted to the channel complex [2, 61]. Moreover, it was

shown that the phosphodiesterase PDE4D3 associates with RyR2 limiting excessive phosphorylation of the channel, which has been shown to detrimentally affect the cardiac function in disease states of the heart [62].

RyR2 channels can also be phosphorylated by CaMKII, which phosphorylates a nearby site (serine 2814) on RyR2 and does not decrease the binding affinity of FKBP12.6 [43]. We demonstrated that genetic inhibition of S2814 in RyR2-S2814A mice inhibits the increase in RyR2 open probability following CaMKII phosphorylation of the channel [48]. CaMKII phosphorylation of RyR2 is believed to play an important role in the positive force-frequency relationship, as S2814A knock-in mice did not develop increased cardiac output at higher heart rates [63].

Redox Modulation

The open probability of RyR2 is not only modulated by phosphorylation but also by redox signaling [64–66]. RyR2 contains around 90 cysteine residues per monomer of which approximately 20 are in a reduced state and are potential targets for various redox modifications, including S-nitrosylation, S-glutathionylation, and disulfide crosslinking [67]. Experimental evidence suggests that redox modulation of RyR2 by NO, H_2O_2, nitroxyl, and hydroxyl radicals alter its activity *in vitro* [68, 69]. In general, oxidizing conditions increase the open probability of RyR2, whereas reducing reagents such as dithiothreitol reverse these effects [70–72]. However, prolonged exposure to high doses of oxidizing agents may cause irreversible and detrimental RyR activation [72, 73].

Although the effect of redox modulation of RyR2 is well established, the physiological role of endogenously produced oxidants is not. S-nitrosylation is an important reversible biological reaction involving nitric oxide in which nitrosyl groups are added to thiol residues. Out of the three known nitric oxide synthase (NOS) isoforms, cardiomyocytes express two constitutive NOS enzymes, neuronal NOS (nNOS) and endothelial NOS (eNOS). eNOS localizes to caveolae [74–76], where compartmentalization with β-adrenergic receptors and L-type Ca^{2+} channels [77] allows NO to inhibit β (beta)-adrenergic-induced inotropy [76, 78]. nNOS, however, is targeted to cardiac SR [79]. NO stimulation of SR Ca^{2+} release via RyR *in vitro* suggests that nNOS has an opposite, facilitative effect on contractility [67, 80]. This opposite effect is further evidenced by the observation that nNOS deficiency reduces inotropic response in contrast to eNOS deficiency that enhances contractility due to corresponding changes in SR Ca^{2+} release [81]. In addition to redox modulation by nitrosylation, cardiac SR is reported to have NADH oxidase as well as NOX2 oxidase (that utilizes NADPH instead of NADH) that regulate the RyR2 complex under physiological conditions [82–84].

Growing evidence suggests that redox modification of RyR2 may contribute to abnormal Ca^{2+} handling in disease states. S-nitrosylation (>3 sites per monomer) leads to *reversible* RyR activation [67]. In contrast, oxidation does not affect channel function up to a certain threshold (5–6 thiols per monomer), beyond which it leads to *irreversible* activation [67]. Moreover, oxidation of reactive thiols induces cross-linking between the subunits of RyR resulting in channel activation, which can be reversed by S-nitrosylation [65]. In addition, nNOS deficiency leads to an increase in reactive oxygen species (ROS) that may lead to oxidation of reactive thiols on RyR2 [85]. Thus, whereas S-nitrosylation seems to control the basal redox state of the channel, oxidation is a pathological process found in disease/stress states like HF [64].

Role of RyR2 in Arrhythmogenesis

Arrhythmias have a complex pathogenesis that include disorders of impulse formation or conduction or combinations thereof. Among the disorders of impulse formation, triggered activity in the form of early or delayed afterdepolarizations (EAD and DAD, respectively) is the underlying mechanisms for most ventricular arrhythmias. It is believed that diastolic opening of RyR2 may lead to the release of Ca^{2+} from the SR, which in turn activates the Na^+/Ca^{2+}-exchanger (NCX). Activation of NCX removes Ca^{2+} from the cytosol but also concurrently results in an inward Na^+ current, which – if large enough – can trigger a DAD, extrasystole or even ventricular arrhythmia (Fig. 17.3) [90]. Such

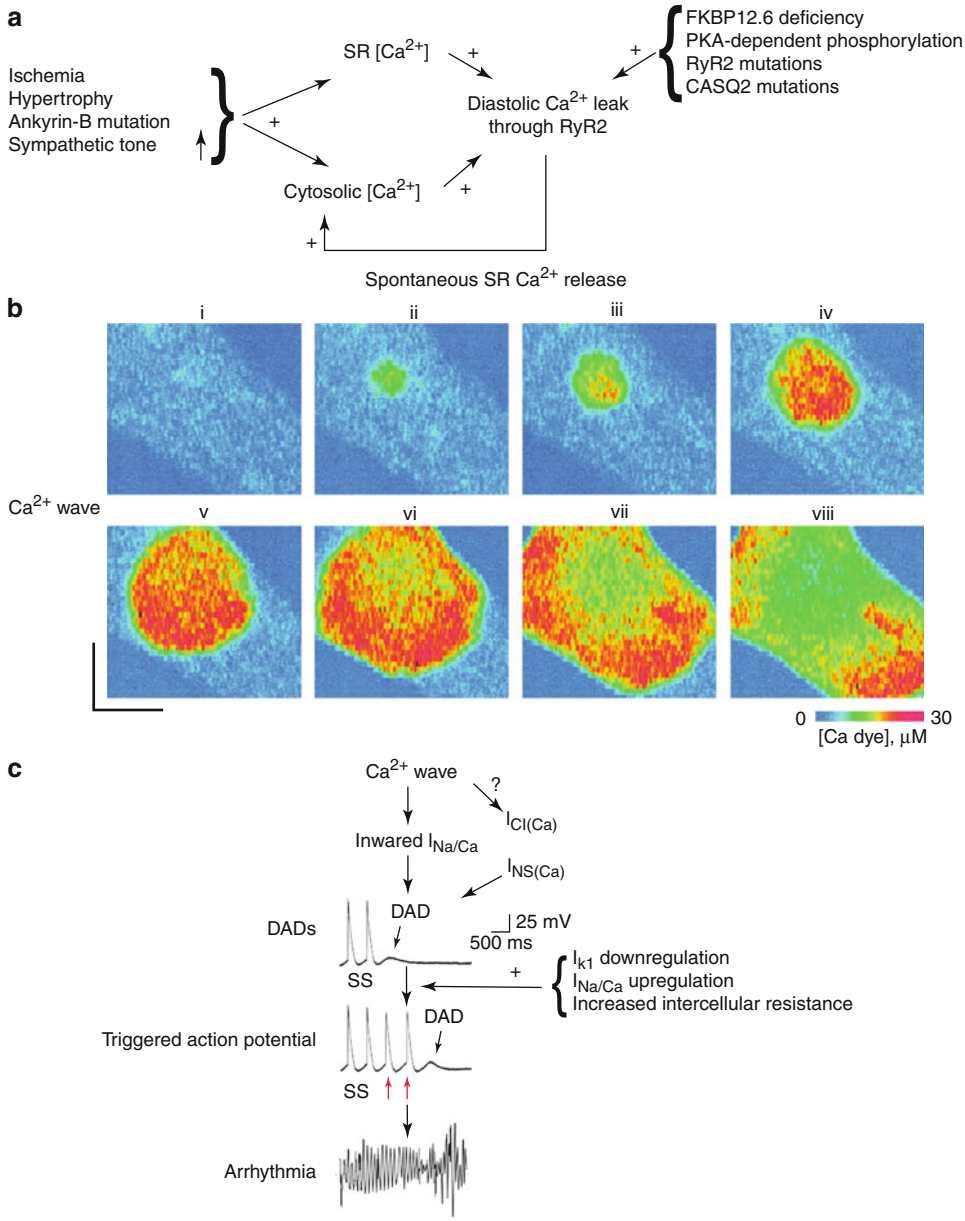

Figure 17–3. Proposed scheme of events leading to delayed after-depolarizations (DAD) and triggered tachyarrhythmia. (**a**) Congenital (e.g., ankyrin-B mutation) and/or acquired factors (e.g., ischemia, hypertrophy, increased sympathetic tone) may cause a diastolic Ca^{2+} leak through ryanodine receptor channels type 2 (*RyR2*), resulting in localized and transient increases in $[Ca^{2+}]_i$ in cardiomyocytes. (**b**) Representative series of images showing changes in $[Ca^{2+}]_i$ during a Ca^{2+} wave in a single cardiomyocyte loaded with a Ca^{2+}-sensitive fluorescent dye. Intracytosolic Ca^{2+} (*i*), focally elevated Ca^{2+} (*ii*) diffuses to adjacent junctional sarcoplasmic reticulum (*SR*), where it initiates more Ca^{2+} release events, resulting in a propagating Ca^{2+} wave (*iii–viii*). (**c**) The Ca^{2+} wave, through activation of Ca^{2+}-sensitive inward currents, will depolarize the cardiomyocyte (*DAD*). In cardiomyocytes, the inward $I(Na^+/Ca^{2+})$ is the major candidate for the transient inward current underlying DADs, although the role of the Ca^{2+}-activated Cl^- current $[I(Cl^-(Ca^{2+}))]$ and a Ca^{2+}-sensitive nonspecific cation current $[I(NS(Ca^{2+}))]$ cannot be excluded. If of sufficient magnitude, the DAD will depolarize the cardiomyocyte above threshold resulting in a single or repetitive premature heartbeat (*red arrows*), which can trigger an arrhythmia. Downregulation of the inward rectifier potassium current (I_{K1}), upregulation of $I(Na^+/Ca^{2+})$, or a slight increase in intercellular electrical resistance can promote the generation of DAD-triggered action potentials. S, stimulus (Modified from Rubart and Zipes [86], Mohler et al. [87], Qin et al. [88], Subramanian et al. [89]. With kind permission from American Society for Clinical Investigation)

DAD-induced arrhythmias are found in patients with genetic mutations in RyR2 in the absence of structural heart disease (e.g., catecholaminergic polymorphic ventricular tachycardia, CPVT). In addition, conditions that cause $[Ca^{2+}]_i$ overload such as HF and high digoxin levels, are also associated with DAD mediated ventricular arrhythmias [5, 6, 8, 91–94].

Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is an inherited disorder characterized by exercise or stress-induced ventricular arrhythmias in patients with structurally normal heart (see also the CPVT chapter) [95]. The most common form is the autosomal-dominant CPVT type 1 (CPVT1), which is caused by mutations in the *RYR2* gene. A less common form is CPVT type 2, which is autosomal-recessive and is caused by mutations in the *CSQ2* gene, which encode CSQ isoform 2 (CSQ2) [3].

Over the past 10 years, >140 CPVT-linked mutations have been identified in the *RYR2* gene (www.fsm.it/cardmoc). It is believed that *RYR2* mutations cluster into three definable regions that are highly conserved across species [3]. These domains are often designated as the N-terminal domain (amino acids 1–600), the central domain (amino acids 2000–2500), and the C-terminal pore-forming domain (mutations clustered between amino acids 3800–4000 and 4500–5000) [3]. The vast majority of *RYR2* mutations identified so far are missense mutations with less than 5 % being caused by small insertions/deletions [3].

Most studies have revealed that the functional consequence of RyR2 mutations is an increased open probability of the Ca^{2+} release channel [34]. The increased open probability of RyR2 is due to increased sensitivity of RyR2 to either luminal or cytosolic Ca^{2+} or both, such that minimal increases in either of the two Ca^{2+} stores lead to diastolic Ca^{2+} leak [38, 47]. Other studies have demonstrated that the gain-of-function channel phenotype can be unmasked following PKA phosphorylation [38]. These experimental findings correspond to the clinical observation that CPVT patients develop ventricular tachycardia in response to exercise and/or adrenergic stimulation [95–97]. Together, these observations support the view that the arrhythmias in CPVT1 need two substrates, a leaky RyR2 channel and a precipitant in the form of beta-adrenergic stimulation and/or increased luminal/cytosolic overload. This is because an increased diastolic Ca^{2+} leak at baseline will lead to depletion of SR Ca^{2+} stores thus restoring the Ca^{2+} release/leak to near-normal levels [98]. However, under conditions of beta-adrenergic stimulation and Ca^{2+} overload, enhanced SR Ca^{2+} loading will promote diastolic Ca^{2+} releases via mutant RyR2 channels leading to DADs and ventricular tachycardia [14].

Several mechanisms have been proposed to explain the gain-of-function defects found in CPVT mutant RyR2 channels. RyR2 has N-terminal and central domains that interact with each other to act as a switch that opens and closes the channel [99]. Zipping of the interacting domains facilitates channel closing, whereas unzipping promotes RyR2 opening. The presence of a CPVT mutation in either domain might weaken the interdomain interaction leading to domain unzipping and erroneous activation of RyR2 and diastolic Ca^{2+} leak [99]. This was evidenced in a mouse model with central domain CPVT1 mutation R2474S that showed aberrant unzipping of the domain switch regions, lowering of the threshold of luminal $[Ca^{2+}]$ for channel activation, sensitizing the channel to PKA-dependent phosphorylation, and CPVT [100]. In addition to the domain switch, central domain seems to have subdomain zipping too. A knock-in mouse model of mutation S2246L in the central domain revealed that an abnormally tight local subdomain-subdomain interaction might lead to defective interaction between the N-terminal and central domains [101].

Other studies have demonstrated that RyR2 mutations may impair the interaction between RyR2 and FKBP12.6, a channel-stabilizing subunit [38]. Several mutations in the N-terminal and central domain have been shown to lower FKBP12.6-RyR2 binding affinity [38, 90]. However, some studies have challenged this mechanism as some of these mutations were shown not to alter FKBP12.6 binding to RyR2 [47, 102]. On the other hand, a recent study linked domain unzipping and FKBP12.6 unbinding

wherein PKA mediated hyperphosphorylation and dissociation of FKBP12.6 was associated with domain unzipping in failing hearts [103]. Although, the relative importance of the two mechanisms remains uncertain, but both domain unzipping and FKBP12.6 dissociation appear to promote pathogenic RyR2 gain-of-function associated with ventricular arrhythmias.

Although the pathogenic mechanisms underlying CSQ2 dysfunction in patients with CPVT-2 are beyond the scope of this chapter, it is thought that mutations in CSQ2 may lead to destabilization of the CICR mechanism by reducing effective Ca^{2+} buffering inside the SR [46], or causing altered interactions with the RyR2 channel complex leading to impaired RyR2 regulation by luminal Ca^{2+} [104]. Moreover, it has been suggested that RyR2 mutations might cause arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) [105–107]. However, this association is generally doubted by most scientists in the field, and therefore, we will not discuss this condition in this chapter.

Drug-Induced Arrhythmias

It is well recognized that several drugs, in particular, some inotropic agents, can increase $[Ca^{2+}]_i$ either by enhancing RyR2 activity or by inhibiting Na^+/K^+-ATPase [91, 92]. Among these, digoxin is the prototypical drug associated with $[Ca^{2+}]_i$ overload-induced arrhythmias. Digitalis glycosides, including digoxin were the mainstay of HF therapy until the mid-1990s when the 'Digitalis Investigation Group' (DIG) trial showed a lack of reduction in mortality, and post-hoc analyses showing a possible increase in mortality in a subgroup of patients [108]. This increase in mortality is due to the narrow therapeutic range of digoxin. Because digoxin inhibits Na^+/K^+-ATPase, an excessive increase in $[Na^+]_i$ can promote Ca^{2+} entry via the Na^+/Ca^{2+} exchanger, which can eventually lead to SR Ca^{2+} overload [91]. Whereas increased SR Ca^{2+} content leads to enhanced systolic Ca^{2+} release and positive inotropy, diastolic SR Ca^{2+} leak might be detrimental due to the increase risk of ventricular arrhythmias as discussed above [91].

Other inotropes that include β (beta)1 agonist (epinephrine) activate the β (beta)-adrenoceptor/adenylylcyclase/PKA cascade [92]. This results in production of cAMP and activation of intracellular PKA [92]. PKA in turn phosphorylates many proteins, including RyR2, which may promote diastolic Ca^{2+} leak, an increase in $[Ca^{2+}]_i$, and possibly activation of CaMKII, another important kinase that phosphorylates and activates RyR2 [5, 38, 43, 48, 54, 55]. Thus, most inotropic agents available till date have the potential to promote RyR2-mediated SR Ca^{2+} leak either directly by RyR2 phosphorylation or by increasing $[Ca^{2+}]_i$, setting the stage for increased arrhythmogenesis [91, 92].

Ventricular Arrhythmias in Failing Hearts

HF is a progressive disorder in which the heart is unable to pump blood commensurate to the needs of the body. HF is a leading cause of death with approximately half of the patients eventually dying due to lethal ventricular arrhythmias [109, 110]. HF is characterized by reduced cardiac contractility, which at the cellular level is related to reduced intracellular Ca^{2+} transients [8, 24, 111]. This reduction in Ca^{2+} transients has been attributed to both an increased SR Ca^{2+} leak (via RyR2) and reduction in SR Ca^{2+} loading (due to downregulation of SERCA2a activity) [5, 8, 111]. In early stages of HF, RyR2 phosphorylation due to β(Beta)-adrenergic receptor activation is believed to help maintain the systolic Ca^{2+} transient [5]. At more advanced stages of HF, however, chronic hyperphosphorylation of RyR2 will promote maladaptive remodeling characterized by sustained RyR2 leakiness associated with reduced SR Ca^{2+} stores and decreased systolic Ca^{2+} release [5, 6, 8, 93, 94].

Moreover, enhanced diastolic SR Ca^{2+} leak via RyR2 may induce arrhythmias via EADs or DADs [9, 112, 113]. As SR Ca^{2+} release depends on SR Ca^{2+} store loading, increased SR Ca^{2+} leak and reduced SR Ca^{2+} loading in HF loading has been predicted to reset the SR Ca^{2+} stores to a lower level that might intuitively reduce the diastolic Ca^{2+} leak [114, 115]. However, studies have revealed that in spite of reduced SR Ca^{2+} content, SR Ca^{2+} leak continues unabated in HF ('Ca^{2+} overload paradox') [8]. This increased diastolic Ca^{2+} leak in the presence of reduced SR Ca^{2+}

content has been attributed to increased open probability of RyR2 due to either post-translational modification of the protein or changes in the composition of RyR2 macromolecular complex [8]. One of the most important modifiers of RyR2 function in HF is its increased phosphorylation by PKA and CaMKII, and reduced PP1 and PP2a activity [5, 6]. Activation of PKA and CaMKII can occur by many pathways, including chronic hyperadrenergic state found in patients with HF (PKA activation) [116], increased cytosolic Ca^{2+} (CaMKII activation) [6], and increased oxidative stress [8, 117, 118]. Increased oxidative stress can lead to oxidation of thiol residues on RyR2 or increase RyR2 phosphorylation by oxidative activation of CaMKII [64, 85]. Our recent studies have shown that inhibition of CaMKII phosphorylation of RyR2 reduces diastolic Ca^{2+} leak and ventricular arrhythmias in animal model of HF [48]. Thus, abnormal RyR2 function may contribute to ventricular arrhythmias in HF and could serve as a potential target for preventing sudden cardiac death in such patients [4–9, 48].

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy with an incidence of 1 in 3,500 male births [119]. Overall, a quarter of patients die from cardiac causes, half of which are due to sudden cardiac death [119]. The *dystrophin* gene codes for dystrophin protein and dystrophin-associated glycoproteins provide a structural link between the myocytes cytoskeleton and extracellular matrix [120]. In DMD, due to the absence of dystrophin, there is fragility of cell membrane, which results in abnormal stress-induced entry of Ca^{2+} into the cells leading to diastolic SR Ca^{2+} leak via RyR2 [121]. This was further confirmed in animal studies wherein diastolic Ca^{2+} release events and VT could be suppressed by pharmacological inhibition of RyR2 [16, 121]. DMD is one of many cardiac conditions that do not have a primary RyR2 disorder but have a higher RyR2-mediated SR Ca^{2+} leak. This highlights the central role of RyR2 in arrhythmogenesis not only in DMD but in other types of cardiomyopathies.

Ryanodine Receptors as a Therapeutic Target

Based on the discussion above, RyR2 plays an important role in the pathogenesis of atrial and ventricular arrhythmias arising from both genetically and acquired cardiac conditions [48, 122–126]. At present, there is no FDA approved medication that selectively target RyR2. Class II anti-arrhythmic agents (β (beta)-blockers) affect RyR2 function indirectly by reducing the sympathetic drive that drives RyR2 phosphorylation [116]. This class of drugs is also modestly effective in patients with CPVT, who typically still require ICD implantation once they have experienced syncope [95, 96]. There are efforts ongoing to develop drugs that block RyR2 specifically for the treatment of cardiac arrhythmias, some of which will be discussed below.

JTV519 and Related Compounds

JTV519 (also referred to as K201) is a 1,4-benzothiazepine derivative, that in addition to being a non-specific multiple channel blocker, also reduces diastolic SR Ca^{2+} leak by stabilizing RyR2 (Fig. 17.4) [128, 129]. Although, JTV519 has been shown to reduce diastolic SR Ca^{2+} leak, the exact mechanisms remain contentious [12, 130]. As per the current school of thought, JTV519 stabilizes the closed state of RyR2 by increasing its affinity for FKBP12.6 [12]. This is supported by studies showing that HF and CPVT are characterized by diastolic Ca^{2+} leak via RyR2 and depletion of FKBP12.6 and vice versa FKBP12.6 deficiency leads to Ca^{2+} leak and ventricular arrhythmias indicating the importance of FKBP12.6 in arrhythmogenesis [5, 10, 38]. JTV519 has been shown to inhibit both exercise and epinephrine-induced arrhythmias only in mice that have lower FKBP12.6 levels but not in mice that are completely deficient in FKBP12.6 providing indirect evidence that the effect of JTV519 on RyR2 requires rebinding of FKBP12.6 [12]. This is supported by another study that showed that JTV519 prevents pacing induced HF and restores the stoichiometry of FKBP12.6-RyR2 to normal levels [10]. On the other hand, few studies have shown that FKBP12.6 is not

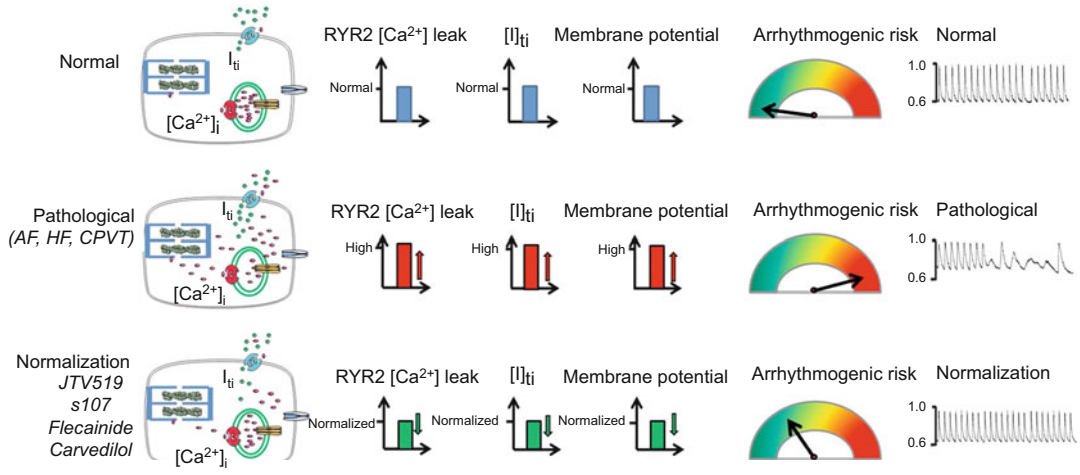

Figure 17–4. Blockers and stabilizers of ryanodine receptors type 2 (*RyR2*) prevent arrhythmia. Under normal conditions, RyR2 rarely opens in diastole. Spontaneous opening leads to leaky RyR2 in various pathophysiological situations, generating aberrant Ca^{2+} sparks and Ca^{2+} waves that activate inward depolarizing I_{ti} currents via Na^+/Ca^{2+} Exchanger (NCX), that in turn generate delayed after-depolarizations (DADs) and arrhythmia. Compounds that block or stabilize RyR2 prevent Ca^{2+} leakage and arrhythmogenic risk (Modified from Thiereau et al. [127] with kind permission from Elsevier)

required for an effect of JTV519 on RyR2 [130, 131] and that the effect of JTV519 has been attributed to reversal of domain unzipping to produce a zipped state which reduces SR-mediated Ca^{2+} leak in failing hearts [103]. Still, even in CPVT animal models that do not have apparent dissociation of FKBP12.6 from RyR2, JTV519 reduces ventricular arrhythmias once challenged with increased Ca^{2+} load [14, 15]. Thus, JTV519 remains an important investigational drug that has a potential to prevent and/or treat arrhythmias with the added advantage of improvement in cardiac function if used in patients with HF induced arrhythmias [10, 13]. However, its off-target effects on other ion channels, including I_{Kr} can lead to prolongation of QTc interval that requires further studies to exclude its proarrhythmic potential [132].

S107 is a derivative of JTV519 that was developed because of its specificity for RyR2 and its favorable drug properties, including drug stability and oral bioavailability [133]. Like JTV519, S107 has been shown to increase the binding of FKBP12.6 to RyR2 and reduce the diastolic SR Ca^{2+} leak and ventricular arrhythmias in an animal model of CPVT (RyR2-R2474S knock-in mice) [90]. Duchenne muscular dystrophy is also characterized by enhanced diastolic SR Ca^{2+} leak and ventricular arrhythmias that can be reversed by S107 in animal model [16]. Although S107 seems to be a better drug than JTV519 [133], its applicability might be limited to arrhythmias that are caused by diastolic SR Ca^{2+} leak.

Flecainide

Flecainide is a Class Ic anti-arrhythmic agent that is approved by FDA for the treatment of ventricular and supraventricular arrhythmias [134–136]. Its predominant mechanism of action is inhibition of fast Na^+ channels resulting in slowing of atrial and ventricular conduction [134]. However, recent work has shown that flecainide also acts as an open state RyR2 blocker [17, 137]. Studies have shown that flecainide reduces diastolic SR Ca^{2+} leak and ventricular arrhythmias in an animal model of CPVT and also in three patients with this disease [17, 18]. Despite the promising results, there are several limitations in the use of this drug. Flecainide causes significant electrical heterogeneity that can both suppress and provoke arrhythmias [138]. The drug also depresses cardiac function and is contraindicated in patients with moderate to severe cardiac failure [139]. Lastly, it suppresses sinus node function and cardiac

conduction and is contraindicated in patients with sinus node dysfunction and conduction blocks [134]. The Cardiac Arrhythmia Suppression Trial (CAST) showed that flecainide increases all-cause and arrhythmic deaths in patients with recent myocardial infarction [140]. Despite all the limitations, flecainide provides an exciting opportunity to treat CPVT patients with ventricular arrhythmias due to abnormal SR diastolic Ca^{2+} leak in the absence of structural heart disease and is currently being evaluated in clinical trials with promising results [141]. In addition, further modification of this drug to increase its specificity of action on RyR2 may eliminate its proarrhythmic properties making it a powerful tool in the treatment of RyR2 leak mediated arrhythmias.

Carvedilol Derivatives

Carvedilol is a non-selective β (beta)-blocker that has additional antioxidant and α (alpha)-adrenergic blocking properties [142]. It is currently indicated for patients with HF and has shown significant survival benefit in such patients owing partly to reduction in sudden cardiac death [143, 144]. Although β (beta)-blockers in general reduce sudden cardiac death in HF patients, carvedilol use leads to a higher reduction in sudden cardiac death, in comparison with β (beta)1-specific blockers like metoprolol [144]. This additional protection by carvedilol has previously been attributed to stabilization of RyR2 channels via its antioxidant properties or stabilization of FKBP12.6 [11, 145]. Recent work has shown that carvedilol can block RyR2 in its open state [19]. Further, the RyR2 blocking properties of carvedilol have been shown to be separate from its β (beta)-blocking properties evidenced in a study wherein a carvedilol analog could block RyR2 and prevent ventricular arrhythmias in a susceptible animal model without significant β (beta)-blockade [19]. This discovery provides exciting new opportunities to stabilize RyR2 without the blood pressure and heart rate lowering effects of carvedilol, which limit its titration in many patients. Future studies will be required to evaluate whether such derivatives have any significant side effects including reduction of cardiac contractility. This is less of a concern with carvedilol and flecainide as both are open state blockers (compared to closed state blockers like tetracaine) [17, 137]. Together, the discovery of the antiarrhythmic effects of JTV519, flecainide and carvedilol derivatives herald a new approach in treating arrhythmias by normalizing Ca^{2+} handling in cardiomyocytes via stabilization of RyR2 (see Fig. 17.4).

Summary and Conclusions

RyR2 Ca^{2+} channels on the SR are required for EC coupling in cardiac muscle. RyRs are macromolecular channel complexes associated with regulatory proteins that modulate RyR2 function in response to extracellular signals. Cardiac arrhythmia is an important cause of death in patients with HF and inherited arrhythmia syndromes, such as CPVT. Alterations in RyR2 function in CPVT cause diastolic Ca^{2+} leak from the SR, which may lead to DADs and triggered cardiac arrhythmias. Novel therapeutic approaches, based on recent advances in the understanding of the cellular mechanisms underlying arrhythmias in HF and CPVT, are currently being evaluated that correct defective RyR2 Ca^{2+} release specifically in these lethal syndromes.

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18
Caveolae and Arrhythmogenesis

Matteo Vatta

Abstract

Caveolae are flask-shaped invaginations of the plasma membrane measuring approximately 50–100 nm in diameter. Caveolae are particularly rich in cholesterol and glycosphingolipids, and they are involved in a wide variety of biological processes such as membrane endocytosis, cholesterol homeostasis, tumorigenesis and cellular signaling. In the last several years, these microdomains, greatly abundant in the cardiovascular system and in particular the myocardium, were investigated for their capacity to concentrate and compartmentalize various signaling molecules such as adrenergic receptors, which have been also involved in the modulation of ion channels function. In addition, various ion channels were identified have been found to clustered to caveolae or been physically linked to caveolins, the major determinants of caveolae. Nevertheless, the relationship between the metabolism of caveolae and the ion channels function remains poorly understood. However, recent advances in the molecular genetics of caveolins revealed a tight connection between these microdomains and the susceptibility to arrhythmogenesis in humans suggesting an increasingly important role of these plasmalemmal organelles in trafficking and regulation of ion channel function.

Keywords

Caveolae • Caveolin-1 • Caveolin-2 • Caveolin-3 • Ion channel • Cardiomyocytes • Cholesterol • Cell trafficking • Kv2.1 • Nav1.5 • K1.5 • Cav1.2

Summary

The plasma membrane is a semi-permeable barrier constituted by a lipid bi-layer, which defines the boundaries between the intracellular and extracellular space. However, unlike the historical model of the "fluid mosaic" for the plasma membrane, in which integral membrane proteins are evenly distributed and free to diffuse, the current knowledge suggests a more heterogeneous view of the plasma membrane in which proteins and lipids are clustered within specialized vesicular microdomains termed lipid rafts. This novel scenario for the cell surface has dramatic implication in cardiomyocytes, where the plasma membrane contributes to the correct exchange of electrolytes and ions essential for membrane

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depolarization and excitation-contraction coupling (ECC), which are at the basis for normal cardiac function. All major ion channels implicated in the regulation of the cardiac action potential mainly localize at the cell surface and the cellular membrane grants these specialize proteins a formidable interface capable of regulating the cellular response and the modulation of ion channel function upon various stimuli from the extracellular and intracellular environment. The structural support, protein turnover, and functional regulation of ion channels on the plasma membrane occur through self renewal and molecular adaptation, which represents the molecular plasticity of the plasmalemma and its ability to maintain an adequate composition to ensure an adequate cellular performance and response.

Cell trafficking through the endoplasmic reticulum (ER) and the Golgi apparatus, which represent a source of bi-lipid vesicles and post-translationally modified proteins, is the mechanism that modifies the cell surface arrangement, and inter-talks with the main sub-cellular compartments.

Caveolae (little caves in Latin) are plasmalemmal organelles, deeply involved in vesicular transport from the ER and Golgi compartments to the cell surface. Caveolae are characterized by a peculiar "flask-like" shape and they are virtually ubiquitous, but particularly abundant in cells of the cardiovascular system, including endothelial cells, smooth muscle cells, macrophages cardiomyocytes and fibroblasts plasma membrane microdomains. In addition, many channels instrumental for ion homeostasis and regulation of the cardiac action potential have been co-localized within caveolae, suggesting an increasingly important role of these plasmalemmal organelles in trafficking and regulation of ion channel function.

In this chapter, we will discuss the overall function of caveolae and the relationship between caveolae and ion channel function as the base of their involvement in arrhythmogenesis. Caveolae and their major component caveolins may represent novel molecular machinery implicated in ion channels function.

Caveolae: The Discovery

The tale of caveolae started back in 1953, when at the dawn of biological application of electron microscopy (EM) to investigate the cellular ultrastructure George Palade observed a large numbers of narrow-necked small plasma membrane invaginations in endothelial cells of the heart [1]. Palade named these invaginations "plasmalemmal vesicles". Two years later, in 1955 Yamada confirmed Palade's observation, identifying 50–100 nm "flask shaped" invaginations of the plasma membrane in the gall bladder [2]. Yamada proposed the name caveolae, which in Latin means 'little caves' to describe these morphologically structure [2]. Since that initial finding, further EM studies have identified caveolae in most cell types, especially endothelial cells and adipocytes, but absent in red blood cells, platelets, lymphocytes, neuronal tissues, and CaCO-2 human fibroblasts [3–5]. All these ultrastructural investigations achieved a detailed morphological definition of caveolae.

In fact, caveolae have been defined as flask-shaped invaginations of the plasma membrane regular in shape and size distinct from the larger electron-dense clathrin-coated vesicles involved in various phenomena of endocytosis.

Caveolae: Tissue Distribution

Caveolae have been identified in most tissues and cell types, although at different surface density. In particular, caveolae are intensely abundant in endothelial cells, adipocytes, type I pneumocytes, which are the major constituents of lung alveoli [6]. However, distinction can occur within the same cellular type, as it has been observed in continuous endothelium, with higher density of caveolae in contrast to the fenestrated endothelium with a more modest caveolae number [7]. Although the absolute number of caveolae measured in the aforementioned endothelial cells has been challenged by studies using different tissue preparation, the ratio of caveolae in the two endothelial cell types appears conserved [8–10].

Ultrastructural analysis has demonstrated that the adipose tissue is the prevalent source of caveolae with up to 20 % of the adipocyte plasma membrane occupied by caveolae [11]. Endothelial and pneumocyte cells of the lung are the second major source of lipid raft vesicles with a relatively high abundance of these plasma membrane microdomains [6]. Thus caveolae can greatly increase the surface area of numerous cell types, an observation that lends credence to the original speculation that caveolae are involved in macromolecular transport and mechanotransduction events.

In addition, caveolae are also abundant in the skeletal muscle and in the cardiovascular system. In particular, caveolae are particularly abundant in the endothelial and smooth muscle cells of the vasculature as well as in the myocardium [12].

Caveolae: Structure and Composition

The caveolae are smooth uncoated plasma membrane microdomains of 50- to 100 nm in diameter easily distinguishable from the clathrin-coated vesicles. Caveolae can be occur isolated or in clusters with a peculiar rosette formation deriving from the fusion of individual caveolae. In muscle cells, caveolae occur mostly individually, although clusters have been also reported [13, 14]. In particular, EM analysis of myocardial and skeletal muscle caveolae demonstrated that when they cluster, caveolae occur in ordered linear arrays suggesting a possible association with the cytoskeletal structure [15–20]. This observation was confirmed by studying caveolae in cell migration. In fact, caveolae are preferentially distributed to the retracting edge of the migrating cell [21–23]. Although extensive investigation revealed the various morphological subsets of caveolae, it is still unclear what the overall function of the archetypal caveolae organelle is, and in particular, if different subset of caveolae represent each a specialized function.

The first clue to understand what caveolae are and what their biological significance is comes from biochemical studies about caveolae composition.

Caveolae can be distinguished from the plasma membrane thanks to their peculiar composition, extremely rich in proteins and lipids such as cholesterol, ceramide, and diacylglycerol (DAG), sphingomyelin, and *cis*-unsaturated phospholipids such as phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol bi-phosphate [24]. Due to their high content in specific lipids caveolae have been also defined as lipids rafts. Caveolae are formed by the aggregation of glycosphingolipids and sphingomyelin in the Golgi apparatus to be transported to the plasma membrane as concentrated units [6]. There are multiple types of rafts categories depending on the presence of specific marker proteins, ultrastructure data and varying lipid composition [25]. Caveolae are considered one subpopulation of lipid rafts for their lipid constituents and biochemical characteristics (Table 18.1). However, their specific morphology and the presence of the scaffolding protein such as caveolin, the principal marker of the caveolae, distinguish them from other raft types [26].

Caveolins: The Markers for Caveolae

Caveolins not only are the main elements of caveolae, but they are the "corner-stone" of these lipid rafts.

Caveolins were identified quite fortuitously at the end of 1980s, when investigators studying chicken embryonic fibroblast transformed with Rous Sarcoma Virus (RSV) purified several phospho-tyrosine containing proteins, one of those being resistant to nonionic detergents extraction [27]. This 22-kDa protein, later identified in caveolae by EM analysis and thus termed caveolin, showed an immunohistochemical pattern consistent with parallel arrays of individual vesicles along the actin stress fibers, and with altered localization after cellular transformation [28]. The latter observation supported the hypothesis that the phosphorylation of caveolin occurred upon transformation with v-Src, suggesting the hypothesis of a possible role of caveolin and caveolae in oncogenesis [28].

In 1992, studies on cellular trafficking identified caveolin (also called VIP21) to localize

Table 18–1. Principal lipids and protein component of caveolae

Lipids	Cholesterol
	Ceramide
	Sphingomyelin
	Diacylglycerol (DAG)
	Cis-unsaturated phospholipids
	Phosphatidylserine
	Phosphatidylethanolamine
	Phosphatidylinositol
	Phosphatidylinositol bi-phosphate (PIP2)
Protein	Caveolins (-1, -2, and -3)
	G-proteins
	G-alpha, -G-beta
	Adrenergic receptor (AR)
	Beta-1, beta-2, beta-3?
	Cytokine receptors
	Epidermal growth factor receptor (EGFR)
	Platelet-derived growth factor receptor (PDGFR)
	Insulin receptor
	Bradykinin receptor
	Endothelin receptor
	GRB-2–growth factor receptor bound protein 2
	SOS–son of sevenless
	Signal tranduction
	Protein kinase C (PKC) alpha subunit
	Phosphatidylinositol 3-kinase (PI3K)
	Mitogen-activated protein kinase (MAPK)
	eNOS, nNOS
	Calmodulin
	Cytoskeletal
	Actin
	Myosin
	Ezrin

to the Golgi apparatus, the plasma membrane, and membrane-bound vesicles [29].

Caveolin Genes and Their Products

Caveolin-1 and -2

Caveolins is the general term to define the three members of the three caveolin (*CAV1-3*) gene family identified so far, each encoded by a separate gene (Fig. 18.1). The gene coding for caveolin-1 (*CAV1*), is the first gene to be identified, and is composed of three exons that are highly conserved in sequence and structure across species, while the gene coding for caveolin-2 (*CAV2*) was discovered by protein micro-sequencing of purified adipocyte caveolae membrane domains in which the polypeptide revealed a remarkable

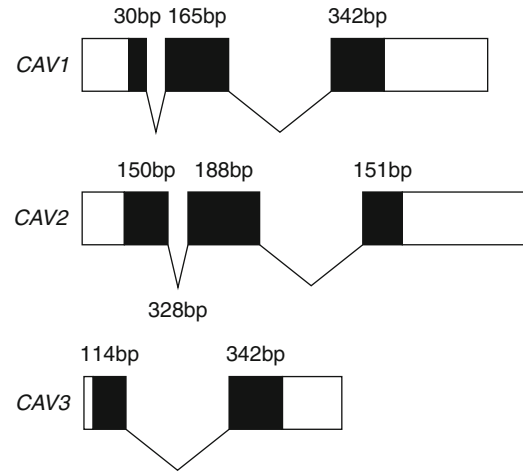

Figure 18–1. Schematic depiction of the caveolin gene family. *Black boxes* indicate the exon coding sequence for each caveolin family member. The *numbers* indicate the number of coding sequence nucleotides in each exon

similarity to caveolin-1, but differing in some key conserved caveolin-1 residues [30].

Finally, using the sequence of *CAV1* as probe, the group of Michael P Lisanti cloned the gene coding for caveolin-3 (*CAV3*), and characterized its expression to be mainly, if not exclusively muscle-specific [31].

The *CAV1* and *CAV2* genes are expressed almost ubiquitously, and in particular, they are co-expressed in most differentiated cells types, especially in adipocytes, endothelial cells, fibroblasts, smooth muscle and type I pneumocytes, but they were initially believed to be absent in mature striated muscle except skeletal muscle satellite cells [32, 33].

However, in the last several years, increasing literature brought forth strong evidence of caveolin-1 expression in both atrial and ventricular cardiomyocytes, although at variable levels and localization [34]. In addition, it appears that caveolin-1 and caveolin-3 interact and form heterooligomeric complexes in atrial cardiomyocytes [35]. Unfortunately, the complexity of the caveolin family took a further step with the discovery of multiple isoforms for both caveolin-1 and -2. Caveolin-1 presents with two isoforms, termed α and β; caveolin-1α consists of residues 1–178, while caveolin-1β, originating from an alternate translation initiation site

occurring at a methionine in position 32, contains residues 32–178, resulting in a protein ~3 kDa smaller in size [32]. Although shorter than caveolin-1α, caveolin-1β represents a functional isoform capable to drive caveolae formation similarly to caveolin-1α in *Drosophila melanogaster* Sf21 cells, which lack endogenous caveolae [36].

The functional significance of these distinct caveolin-1 isoforms remains uncertain, although caveolin-1α appears to be localized primarily to deeply invaginated caveolae and more efficiently drive the formation of caveolae than caveolin-1β [20, 37].

Three isoforms have been identified for caveolin-2, the full-length caveolin-2α and two alternate splice variants, called caveolin-2β and caveolin-2γ, which show distinct subcellular distribution from caveolin-2α, although their functional significance is largely unknown [6].

Caveolin-3

The tissue distribution of CAV3 has been intensely studied in mouse, in which CAV3 expression appears to be restricted to differentiated skeletal and cardiomyocytes, while it is absent in non-proliferating C2C12 skeletal murine myoblasts compared to the proliferating precursor myoblasts, which express both caveolin-1 and -2 [38]. In addition, caveolin-3 levels are intimately associated with muscle development, and experiments performing the differentiation of C2C12 skeletal myoblasts in culture showed CAV3 up-regulation [39], while treatment with a *CAV3*-antisense prevented myotube fusion in vitro [40].

Caveolin-3 has been shown to function similarly to caveolin-1, with which it shares high protein homology. In fact, caveolin-3 is connected to the sarcolemma and modulates the function of the dystrophin glycoprotein complex (DGC), the major protein ensemble linking the contractile apparatus to the plasma membrane in striated muscle and associated with a variety of muscular dystrophies with cardiac involvement and primary heart diseases all presenting with frequent arrhythmias [41–43]. Dystrophin, as well as several members of the DGC including α-sarcoglycan and β-dystroglycan, cofractionate with caveolin-3 in cultured mouse C2C12 myocytes [39]. In addition, Co-IP experiments demonstrated that dystrophin forms a stable complex with caveolin-3 and that dystrophin and caveolin-3 co-localize [44]. Furthermore, since the WW-like domain of caveolin-3 binds the C-terminus of β-dystroglycan, a region containing a PPXY motif it is possible that caveolin-3 competes with dystrophin for the binding to β-dystroglycan; this will inhibit dystrophin binding with subsequent reduced dystrophin presentation to the sarcolemma [44]. In fact, in muscle biopsies from patients suffering from Duchenne Muscular Dystrophy (DMD) with progressive muscle weakness, respiratory failure and the development of DCM associated with arrhythmias, caveolin-3 is shown to be up-regulated along with an increased number and size of caveolae on the plasma membrane [45, 46]. Similarly, caveolin-3 overexpression and increased caveolae number and size occur also in dystrophin-deficient *mdx* mouse [46, 47].

Caveolins and Animal Models

One of the first experimental approaches to study the unknown role of a known protein is to generate an animal model in which the target gene has been ablated. Knockout mice have been generated for all known caveolins, but only the *CAV1* -/- and *CAV3* -/- mice have been particularly interesting in studying caveolin function in the biology of the myocardium.

It is interesting to note that all the caveolin deficient mouse models generated (*CAV1* -/-, *CAV2* -/-, *CAV3* -/-, *CAV1/3* -/- double knockout mice) are viable and fertile despite the tissue distribution and the involvement in such a variety of biological processes observed for caveolins.

The CAV1 -/- Mice

Two independent groups generated *CAV1* null mice. Drab et al., in 2001 showed that upon targeted disruption of *CAV1*, the animals showed absence of caveolae in all tissues usually expressing caveolin-1 and leading to impaired nitric oxide and calcium signaling in the cardiovascular system, causing aberrations in

endothelium-dependent relaxation, contractility, and maintenance of muscular tone [48]. In addition, the lungs of *CAV1* -/- mice exhibited alveolar thickening due to unrestrained endothelial cell proliferation and fibrosis [48].

In the same year, Razani and colleagues generated viable and fertile caveolin-1 null mice demonstrating the same caveolar aberration previously affirmed and showing that absence of caveolin-1 could not prevent normal caveolae to be formed in tissues expressing caveolin-3 such as skeletal and cardiac muscles [49]. In addition, the *CAV1* -/- mouse demonstrated an almost complete loss of caveolin-2 confirming the role of caveolin-1 in hetero-oligomerization with caveolin-2, and the ability of caveolin-1 to recruit caveolin-2 from the Golgi to the plasma membrane [48, 49].

The recent identification of *CAV1* expression in cardiomyocytes supports the observed development of cardiac hypertrophy and cardiomyopathy in *CAV1* -/- animals with reduced lifespan due to sudden cardiac death (SCD) [50–52]. In addition, the cardiac hypertrophy and collagen deposition in caveolin-1-deficient mice are due to the deficient inhibitory effect of cavoelin-1 on the transforming growth factor-β1 (TGFβ1) signaling, a pivotal mediator of cardiac hypertrophy [53].

The CAV3 -/- Mice

The caveolin-3 null mouse demonstrates the absence of muscular caveolae, while expression of caveolin-1 and -2 was normal in non-muscle cells [54, 55]. The animals developed muscular dystrophy phenotype similar to limb girdle muscular dystrophy (LGMD) observed in patients with LGMD-1C. In addition, *CAV3* -/- demonstrates a cardiomyopathy phenotype similar to that described above in *CAV1* -/- animals.

Using cardiac gated MRI and TEE Woodman et al., found that, at 4 mo of age, *CAV3* -/- showed significant cardiac hypertrophy and reduced fractional shortening [56].

Cardiac histological analysis revealed perivascular fibrosis, myocyte hypertrophy, and cellular infiltration, derived from the exclusion of the DGC from lipid rafts and hyper activation of Ras-p42/44 MAP kinase cascade in *CAV3* null cardiac tissue [56]. These changes are consistent with the known role of p42/44 MAP kinase activation in cardiac hypertrophy and the role of caveolin-3 as a negative regulator of the Ras-p42/44 MAP kinase cascade, similar to that of caveolin-1 [56].

Interestingly, the caveolin-3 T63S mutation has been recently identified in patients with HCM and DCM, although the exact pathological mechanism is unknown [57].

The CAV1/3 -/- Mice

The generation of truly caveolae-deficient animals was accomplished by interbreeding Cav-1 and Cav-3 null mice, to produce caveolin-1/3 double knockout mice (Cav-1/3 dKO). Surprisingly, Cav-1/3 dKO mice are viable and fertile, despite a complete absence of morphologically identifiable caveolae in muscle and nonmuscle cells [58]. Additionally, these mice are deficient in the expression of all three caveolin family members, as caveolin-2 is unstable and degraded in the absence of caveolin-1.

The double KO present with a severe cardiomyopathy phenotype, already evident at 2 months of age with more pronounced left ventricular wall thickness and ventricular septum thickness accompanied by dramatic cardiac hypertrophic, interstitial inflammation, perivascular fibrosis, and myocyte necrosis [58]. Thus the combined ablation of both caveolin-1 and -3 profoundly alter cardiac structure and function leading to a remarkable risk of SCD.

Caveolins: The Biochemistry

Caveolins Protein Domains

Regardless of the isoform variability, all three caveolin proteins contain some protein motifs highly conserved throughout evolution such as the eight amino acids FEDVIAEP, which constitutes the “caveolin signature sequence” and is localized proximal to the N-terminus of the protein [6].

Another characteristic protein domain present in caveolins is the “scaffolding domain”, a motif proximal to the hydrophobic transmem-

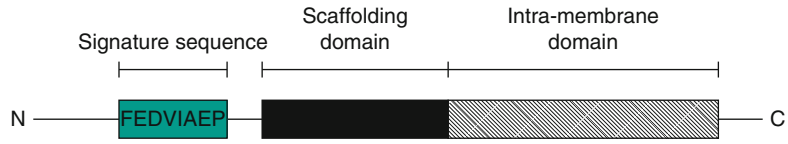

Figure 18–2. Prototypic caveolin structure and critical protein motifs

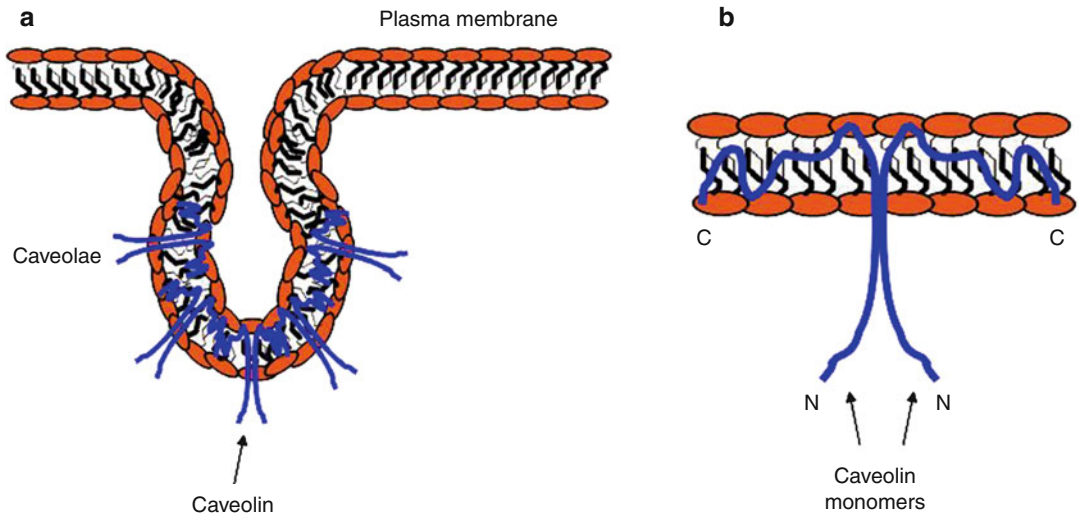

Figure 18–3. Caveolae and caveolin topology. (**a**) Caveolae form plasma membrane invaginations that differ from the clathrincoated endocytic vesicles. (**b**) Caveolins exhibit both N- and C-terminus ends into the cytoplasm, although their central portion is tightly embedded into the plasma membrane, where most of the interactions with ion channels are hypothesized to occur. Caveolin topology dramatically determines the role of these proteins in lipid raft internalization and functional modulation of its binding partners

brane domain, which interacts with different signal transduction molecules (Fig. 18.2) [59, 60]. Although researchers recognize the importance of each protein domain present in caveolins, it appears that proper caveolin function such as lipid raft association, extrusion from the Golgi apparatus and caveolae targeting, greatly if not solely depends on the overall protein folding and tertiary structure [61].

Despite the high amino acids sequence similarity among the different caveolins, human caveolin-1 and-3 share the highest protein homology with ~65 % identity and ~85 % similarity, while caveolin-2 shares only ~38 % identity and ~58 % similarity to human caveolin-1. This remarkable resemblance between caveolin-1 and -3 and their divergence from caveolin-2 may explain why both caveolin-1 and -3, but not caveolin-2 can form caveolae alone or in association with other caveolins in cells lacking caveolae structures such as MDCK and epithelial CaCo-2 cells [62]. In addition, caveolin-1 and -3, but not caveolin-2 undergoes an irreversible post-translational modification inserting a palmitoyl acid molecule on a cysteine residue at their C-terminus [63]. It is believed that palmitoylation of caveolin-1 and -3 stabilizes the caveolin oligomers and increases the association with the membrane through hydrophobic domains [64, 65].

The expression of both caveolin-1 and -3 is also necessary to stabilize caveolin-1/-2 and caveolin-3/-2 heterodimers and to ensure the correct membrane localization of caveolin-2 [66].

Caveolins Protein Topology

Both the N- and the C-termini of the prototypical caveolin are localized to the cytoplasm, while the central hydrophobic domains are "embedded" in the plasma membrane, and no extracellular segments were detected (Fig. 18.3)

[67]. This spatial conformation allows the N-terminal phosphorylation of caveolin-1 by the α subunit of Protein Kinase C (PKCα) and, as previously mentioned, the palmitoylation at the C-terminus of both caveolin-1 and -3 [36, 63]. The ultimate protein structure and topology of caveolins originates in the endoplasmic reticulum (ER), where caveolins are believed to be introduced in the plasma membrane and form a hairpin loop configuration through a transmembrane domain containing 32 hydrophobic amino acids (residues 102–134), which precludes caveolins to completely extend across the plasma membrane [68].

In addition, the scaffolding domain (residues 82–101) proximal to the N-terminus and the segment 135–150, proximal to the C-terminus are tightly connected to the membranes [6]. Caveolin-1 constructs containing residues 1-101 are sufficient for membrane localization, while the segment 1-82 remained in the cytoplasm [69]. It has been established that the (KYWFYR) motif was sufficient to confer membrane localization to a GFP fusion protein [70]. In addition, residues 135–150 include a unique Golgi-targeting sequence, and a construct including only this segment of caveolin shows a prevalent Golgi localization [71, 72].

Caveolin-1 contains an oligomerization domain (residues 61–101), which promotes the homo-oligomerization of up to 16 individual caveolin-1 molecules [59], while a second stage of oligomerization occurs during transport from the *trans*-Golgi to the plasma membrane thus forming a large network of caveolin [73]. In contrast, caveolin-2 is unable to generate large homo-oligomeric complexes and so to develop caveolae by itself [30], while it can form hetero-oligomers with caveolin-1 in the ER to stabilize caveolin-2 against proteasomal degradation and consent to its transport from the Golgi apparatus to the plasma membrane [49, 74–76].

Similarly to caveolin-1, caveolin-3 forms large homo-oligomeric complexes in striated muscle, and co-immunoprecipitation (Co-IP) and membrane co-fractionation studies in rat neonatal cardiomyocytes demonstrated that caveolin-3 associates with caveolin-2 in hetero-oligomers as it occurs for caveolin-1/-2 [77, 78].

Caveolae: The Function

Caveolae and Lipids Regulation

Due to their high lipid content, it is not surprising that caveolae are involved in lipid metabolism, in particular in the regulation of cholesterol.

Studies employing cholesterol-binding agents such as filipin and nystatin resulted in the disruption of caveolae, suggesting the importance of cellular cholesterol balance on caveolar structure [26]. In addition, increase in cholesterol levels up-regulates caveolin-1 expression through specific binding to two steroid regulatory elements (SRE) on the caveolin-1 promoter, while decreased cholesterol levels results in *CAV1* down-regulation [79, 80]. Moreover, oxidation of cholesterol into cholesterone by cholesterol oxidase alters the cellular cholesterol balance and results in caveolin-1 internalization from the plasma membrane to the ER and the Golgi apparatus [81].

Caveolin-1 transports newly synthesized cholesterol from the ER to membrane caveolae, and than to plasma high-density lipoproteins (HDL), while extracellular cholesterol primarily enters cells via clathrin-mediated endocytosis of low density lipoproteins (LDL) [82]. Therefore, caveolae may represent the principal location for cholesterol exchanged between HDL and the cell membrane.

Caveolae and Endocytosis

Caveolae are believed to play a role in endocytosis, oncogenesis and internalization of pathogenic bacteria [83].

Caveolae represent a clathrin-independent mechanism of endocytosis for the turnover of adhesive complexes, and since their discovery, caveolae were believed to play a role in endocytosis owing that they protrude from the plasma-membrane into the cytoplasm. However, there are conflicting evidences involving caveolae in constitutive endocytic trafficking. Caveolin-1 cloned as N- and C-terminus GFP fusion protein and expressed in HeLa, A431, and Madin-Darby canine kidney (MDCK) cells demonstrated that caveolae require both cholesterol and an intact actin cytoskeleton to maintain their integrity

[84]. This may support the hypothesis that caveolae are intimately connected to the actin network. Conversely, Pelkmans and colleagues demonstrated that caveolae play an instrumental role in the internalization of SV40 viral particles, and that this internalization was triggered by molecular signals from the virus, able to activate the caveolae otherwise "dormant" in the stable state previously described [85].

Caveolae and Signal Transduction

Caveolae have been recognized to play an essential role in signal transduction since biochemically purified caveolae contained multiple signaling molecules, including heterotrimeric G proteins, which in the myocardium are involved in sympathetic tone response though β-adrenergic receptors (β-AR) [86, 87]. In addition, caveolae mediate other signaling factors such as H-Ras, Src-family kinases, and endothelial nitric oxide synthase (eNOS) [6].

Recently, it was shown that caveolae integrity and both caveolin-1 and caveolin-3 can modulate atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) production, along with Ras-p42/44 MAP kinase cascade (MEK and ERK) and Akt phosphorylation activity [52, 87]. In addition, caveolae have also been implicated in the modulation of cardiac hypertrophy [88].

The finding of such concentration of signaling molecules in caveolae suggests that caveolins act as scaffolding proteins, through the amino acids 82-101 motif, to concentrate and localize these elements for a rapid and specific cell response.

Caveolae and Ion Channels

Being highly expressed in excitable cells of the nervous system, skeletal muscle and myocardium, there is no surprise to find that a variety of ion channels are localizing to caveolae (Table 18.2). However, it took more than a decade from the first report identifying caveolins in caveolae to obtain evidence that these plasmalemmal vesicles not only cluster and scaffold a plethora of cell membrane proteins including signaling molecules, but also anchor, distribute, transport and possibly modify ion channels on the cell surface.

Table 18–2 Cardiac ion channels localizing to caveolae

Cardiac ion channels
Cardiac sodium channel (Nav1.5) [89]
L-type Ca^{2+} (Cav1.2a) [90, 91]
Voltage-gated Kv1.5 [92]
Pacemaker channel HCN4 [93]
Na/Ca^{2+} exchanger (NCX) [94]
ATP-sensitive K^+ [K_{ATP}] (Kir6.1) [95]
Volume-regulated anion channel (VRAC) [96]
Transient receptor potential canonical 1 (TRPC1) [97]

In 2000, Martens and colleagues identified Shaker-like K^+ channels (Kv2.1) to non-caveolar lipid rafts [98], although the role of these vesicles in Kv2.1 function regulation was elucidated a year later by the same laboratory, showing that cyclodextrin treatment not only led to cholesterol depletion, but also caused a significant shift in steady-state inactivation of the KV2.1 channel [99]. This first evidence that ion channel localize to lipid rafts suggested that other ion channels may be present in caveolae and that caveolins may regulate ion channels function.

In fact, in 2001 Martens and colleagues localized the voltage-gated potassium channel Kv1.5 to caveolae in transfected fibroblasts [92], and later they showed that caveolins are involved in regulating Kv1.5 trafficking to cholesterol-rich membrane microdomains [99]. It is interesting to note that Kv1.5 binds the major protein of the Z-line, α-actinin-2 [100], which is connected to the actin network supporting the concept that caveolae interact with the cytoskeleton (Fig. 18.4).

The Kv1.5 channels are expressed in the intercalated disk of human cardiomyocytes and are associated with connexin 43 and N-cadherin [101]. Cytoskeletal alteration using cytochalasin D, which disrupts the filament actin (F-actin), leads to a massive increase in I_{K+}, while the phenomenon was totally abolished when the cells were by pre-incubated with phalloidin, an F-actin stabilizing agent [100].

Probably the most intriguing discovery concerning the relationship between cardiac ion channels and caveolae was that the *SCN5A*-coded voltage-gated Na^+ channels (Nav1.5) have been reported to localize to 'caveolin-rich membranes' in cardiac myocytes [89]. In addition, the α-subunit of the L-type Ca^{2+} channel (Cav1.2) was

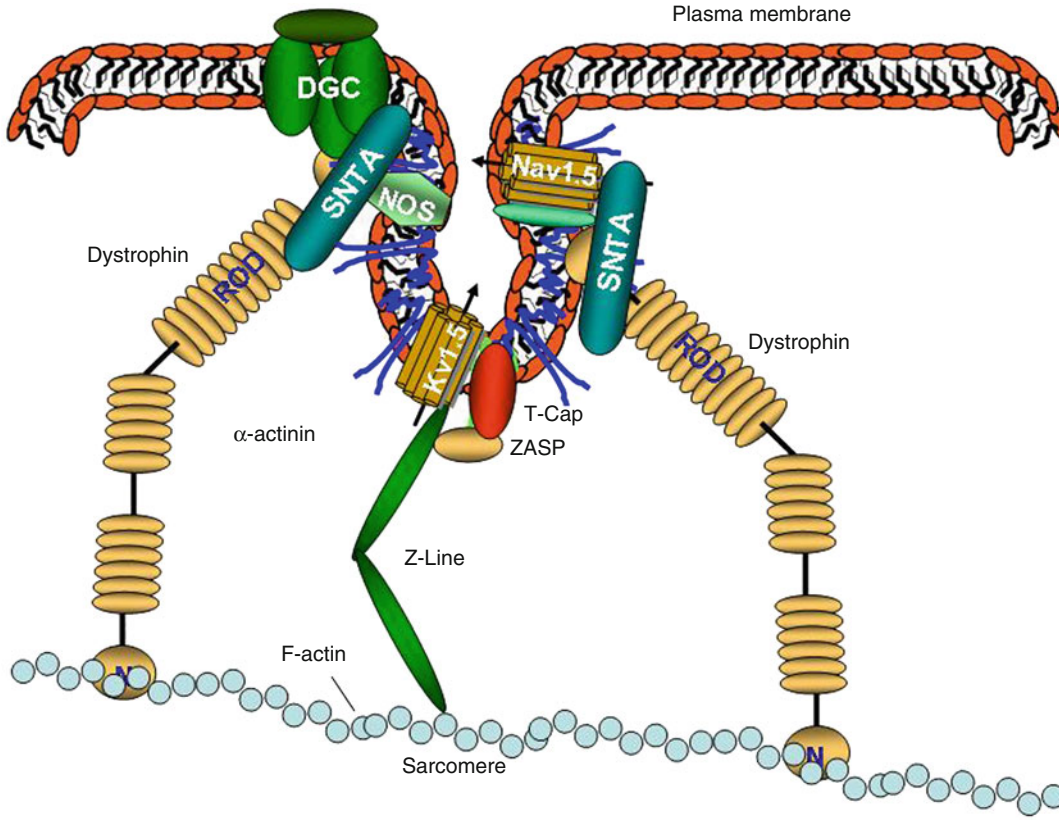

Figure 18–4. Cytoskeleton, caveolae, and caveolins. Caveolae are intimately associated with the cytoskeleton of the cardiomyocytes, and are ultimately linked to the contractile apparatus of the myocardial cells. *DGC* dystrophin glycoprotein complex, *SNTA* α1-syntrophin

originally found in caveolin-enriched membranes in smooth muscle [102].

It is interesting to note that mutations in both Nav1.5 and Cav1.2 have been associated with a primary arrhythmogenic disorder such as the long QT syndrome (LQTS) in human subjects [103–105].

Similarly, skeletal muscle caveolae have been found to contain also the 1,4-dihydropyridine receptor (DHPR or RYR) in the sub-sarcolemmal region of the myofibers [106]. In addition, caveolin expression induces the Cl_2 channel function [97].

It is hypothesized that ion channels, which localize to caveolae probably through the scaffolding domain of caveolin-3, reach these lipid rafts after post-translational modifications such as palmitoylation and myristoylation, or by glycophosphatidylinositol (GPI) membrane anchors, mostly occurring in the Golgi apparatus [107].

It is still unclear whether caveolin, which can independently drive caveolae formation, can organize membrane lipids and stabilize transient membrane rafts, so that channel proteins might perform as a focal point in raft development.

Alternatively, the association between ion channels and caveolae, may not occur through protein/lipid interactions but rather through protein/protein interactions with the direct involvement of caveolins such as caveolin-3, or the direct binding of other caveolin-associated proteins such as the PDZ motif containing protein PSD95 (postsynaptic density protein 95), which has been reported to associate with low-density lipid rafts in mammalian cells [108]. However, the PDZ protein domain may be only one player in the localization of ion channels to raft domains. In fact, Kv2.1 channels do not contain standard PDZ binding sequences [109], and removal of the PDZ binding motif from the

Kv1.5 channel does not prevent its lipid raft association [92].

Caveolae, the Cytoskeleton and Ion Channels

It is interesting to note that many key structural elements, such as the Z-band alternatively spliced PDZ motif protein (ZASP), signaling molecules such as eNOS, and Nav1.5 modulator, such as α_1-syntrophin (SNTA) contain a PDZ domain, and both eNOS and SNTA also localize to caveolae and directly bind caveolin-3 [110–115]. In addition, all the aforementioned proteins localize or are associated with the sarcolemma via direct or indirect interaction with the large protein dystrophin and the dystrophin-associated glycoprotein complex (DGC), thus modulating ion channel regulation [41–43].

Caveolin-3, the Cytoskeleton and Ion Channels

Syntrophins are known to contain two pleckstrin homology (PH) domains, a PDZ domain, and a syntrophin-unique (SU) at its C-terminus. Syntrophins directly binds dystrophin through its PH domain distal to the N-terminus and the highly conserved SU domain [114, 116]. The PH domain proximal to the N-terminus and the PDZ domain interact with other membrane components such as phosphatidyl inositol-4, 5-bisphosphate (PIP_2) [117], neuronal NOS (nNOS) [118], aquaporin-4 [119], stress-activated protein kinase-3 [120], and Nav1.5 [121], thereby linking all these molecules to the dystrophin complex (Fig. 18.4) [122].

In addition, syntrophins bind the C-terminus of Nav1.5 through its PDZ domain, regulating the gating properties of the sodium channel [123].

Remarkably, caveolae not only cluster syntrophins, but caveolin-3 directly binds syntrophin [113] as well as other important focators such as the Na^+/Ca^{2+} exchanger [124], the L-type Ca^{2+} channel [113], eNOS and nNOS, and the DGC (Fig. 18.4) [110–112]. Therefore, the association of caveolin-3 with α_1-syntrophin, through its binding to F-actin, nNOS, and Nav1.5, appears to be involved in regulating structural and electrical functions as well as signal transduction in heart failure [113]. Recently, the p. A257G mutation in the α_1-syntrophin-encoding gene *SNTA1* was identified in LQTS [125]. Electrophysiological analysis in murine cardiomyocytes when Nav1.5 was co-expressed with mutant SNTA1 identified that the mutant SNTA1 had a profound effect on the sodium current causing an increased peak current densities and a shifted onset and peak of the currents toward more negative potentials, as well as a delay of sodium current decay [125]. In addition, coimmunoprecipitation assay demonstrated that human SNTA1 can physically interact with Nav1.5 and that perturbations in SNTA1 might alter Nav1.5 function [125]. Interestingly, Nav1.5 function can also be affected through post-translational modification. In fact, SNTA1 binds nNOS to the nNOS inhibitor plasma membrane Ca-ATPase subtype 4b (PMCA4b), and the p.A390V mutation in SNTA1 identified in LQTS subjects selectively disrupts the association of PMCA4b with the nNOS/SNTA1/Nav1.5 complex leading to the released of nNOS inhibition by PMCA4b and causing increased direct nitrosylation of the sodium channel [126]. The increased nitrosylation of Nav1.5 by p.A390V-SNTA1 causes an increased peak and late sodium current compared with the wild-type SNTA1, while this increase was partially inhibited by NOS blockers. This effect was consistent with the characteristic biophysical dysfunction for sodium-channel-mediated LQTS (LQT3) [126].

Caveolae and Ion Channels Regulation

As discussed in prior paragraphs, due to their physical localization into caveolae and their direct binding to caveolins, it should not be surprising that ion channels might be functionally regulated by lipid rafts such as caveolae, and that genetic mutations or the alteration in epigenetic or post-translational regulations could result in abnormal ion channel function, among the other effects.

The hypothesis that alterations in caveolins could be implicated in abnormal ion channel functions was confirmed by the identification of the p.F97C and p.S141R mutations in caveolin-3 in individuals with LQTS [127] and the p.V14L and p.L79R mutations in sudden infant

death syndrome cases [128]. Mutant caveolin-3 associated with LQTS and SIDS caused a persistent late/tail sodium current resulting in a prolonged depolarizing wave, which is consistent with the prolongation of the QT interval [127, 128].

In addition, caveolin-3 is associated with G protein-coupled receptors (GPCR), which upon stimulation of sympathetic adrenergic receptors triggers G protein-mediated activation of both cAMP-dependent protein kinase A (PKA), and phospholipase C (PLC)-activated protein kinase C (PKC). Both PKA and PKC are known to directly phosphorylate Nav1.5 and modulate its function [129].

Furthermore, Kv channels can be phosphorylated by tyrosine kinase such as c-Src, present particularly in caveolae leading to a reduced I_{K+} [24, 130]. The two proteins interact through the N-terminal proline-rich sequence of the KV1.5 channel and the Src homology region 3 (SH3) of Src [24, 130].

It is also known that both CAV3 bind calmodulin (CaM), which in response to regional increase of Ca2+ concentration binds Nav1.5 increasing its slow-inactivation kinetics [129].

All these evidence point toward an increasingly important role of caveolae, caveolins and caveolin-3 in particular in the regulation of gating, activation/inactivation kinetics, as well as conductance of cardiac ion channels. Therefore, caveolins should be considered as possible genetic diagnostic targets in case of LQTS subjects genotype-negative for the common LQTS genes.

Conclusions and Future Directions

Caveolae are peculiar plasma membrane vesicles with important biological function and roles in various cellular processes. The discovery of their main constituents, the caveolins implicated caveolae and caveolins in a variety of important cellular activity, including vesicular trafficking, cholesterol homeostasis, signal transduction, and ion channel regulation.

Since caveolins act as oligomeric scaffolding elements responsible for the clustering and localization of numerous proteins, it was not surprising to identify caveolin aberration associated with human diseases.

The ultrastructural, genetic, and molecular analysis of caveolae and caveolins both in vitro and *in vivo* provided increasing evidence that these components were instrumental in a range of pathological processes such as muscular dystrophy, cardiac dysfunction and probably arrhythmias.

The generation of caveolin-deficient mice, and in particular the *CAV3* -/-, provides further evidence that, although caveolins are not indispensable for life, caveolin alterations are significant in the pathogenesis of human striated muscle abnormalities such muscular dystrophies, and cardiomyopathies, often associated with arrhythmogenesis. Only recently, investigators are accumulating increasing evidences of an important role of caveolins in arrhythmogenesis.

There is still a need of further investigation the detailed mechanism leading caveolin-3 mutations to cause primary arrhythmogenic syndromes such as LQTS and SIDS. This may help understanding pivotal protein domains for ion channel function regulation and design novel therapeutic approaches to prevent lethal arrhythmic events such as sudden cardiac death.

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Senescence and Arrhythmogenesis

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Abstract

Cardiac arrhythmias are a major cause of morbidity and mortality, particularly in the elderly. With the rapidly changing population demographics, and increasing prevalence of cardiovascular disease, healthcare resource utilization for arrhythmia care is projected to rise exponentially. Aging is associated with electrophysiological alterations due to cardiac structural and functional remodeling, which combined with a blunted response to neurohumoral activation and hemodynamic responsiveness, results in increased propensity to brady and tachy-arrhythmias. These not only affect the quality of life but also contribute to the deterioration in myocardial function leading to heart failure, stroke and death.

Despite progress in the management of cardiovascular disease, arrhythmias in the elderly, continue to be a significant predicament. This is mainly due to limited understanding of the mechanisms and therapeutics directed against the underlying substrate that increases susceptibility of the heart to arrhythmogenesis and paucity of outcome studies in the older-elderly that limits specific practice guidelines in octo- and nona-genarians. Hence, further investigation is warranted to fully define the efficacy of anti-arrhythmic therapeutic modalities in the elderly that have proven to be effective in younger patients. This chapter summarizes the epidemiology, aging-associated cardiac structural and functional changes, basis for arrhythmogenesis, evaluation and management of elderly patients with ventricular tachyarrhythmias that increase predisposition to sudden death.

Keywords

Elderly • Arrhythmia • Sudden cardiac death • Implantable cardioverter defibrillator • Anti-arrhythmic drugs • Ablation • Aging

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Cardiac arrhythmias are a major cause of morbidity and mortality particularly in the elderly [1–4]. Susceptibility to atrial and ventricular arrhythmogenesis is increased in the senescent heart even in the absence of apparent disease and is further exaggerated with underlying comorbidities [5, 6]. Alterations in cardiac energetics, mechanical and electrical properties with aging enhance predisposition to cardiac arrhythmias [2, 7–9]. In the aging heart, there is an overall decline in the number of cardiomyocytes, with enlargement of the residual myocytes and an increase in the elastic and collagenous tissue in the interstitial matrix and conduction system [10]. Adipose tissue accumulates around the sinoatrial node, producing a partial or complete separation of the sinoatrial node from the surrounding musculature [2]. By age 75 years, the number of functional pacemaker cells decrease to less than 10 % of young adults. In addition the expression of ion channels promoting automaticity of the SA node declines [11]. There is calcification of the cardiac skeleton, particularly in areas close to the AV node, His-Purkinje bundle, and the right and left bundle branches, which predispose to conduction abnormalities and both brady- and tachy-arrhythmias [12, 13]. Prolongation of action potential duration with increased refractoriness, slowed conduction and a blunted response to neurohumoral activation also occurs [14, 15]. Together these changes provide the substrate for the age-related increase in the propensity for chronotropic and dromotropic impairment and for the development and maintenance of atrial and ventricular tachyarrhythmias.

Cardiac dysrhythmias in the elderly not only affect the quality of life but also contribute to deterioration in myocardial function increasing susceptibility to heart failure, stroke and sudden death [1, 6, 16–18]. With the projected rise in the elderly population and increasing prevalence of cardiovascular diseases, healthcare resource utilization for arrhythmia care is estimated to increase exponentially [4, 19–21]. Despite progress in the management of cardiovascular disease, arrhythmias in the elderly, continue to be a significant predicament. This is mainly due to limited understanding of the mechanisms underlying aging associated increase in the susceptibility of the heart to arrhythmogenesis and paucity of outcome studies in the very elderly [16, 22]. This chapter summarizes the epidemiology, aging-associated cardiac structural and functional changes, basis for arrhythmogenesis, evaluation and management of elderly patients with ventricular tachyarrhythmias that increase predisposition to sudden death.

Ventricular Arrhythmias and Sudden Cardiac Death

Unexpected death from cardiovascular causes, ranging from 250,000 to 300,000 annually in the United States, continues to be a major cause of death in the elderly [1, 23–25] accounting for 13 % of all natural deaths and 50 % of all deaths from cardiovascular causes [5, 26]. The incidence of sudden cardiac death (SCD) increases with advancing age in individuals with structural heart disease, as well as in those without recognizable risk factors for SCD [5, 27]. In patients with coronary disease, SCD may manifest as the first clinical event in 50 % of the patients [24]. The majority of SCD occurs in the community and results in 90–95 % fatality [27, 28]. Despite advances in the management of cardiovascular disease, the overall incidence of SCD in the general population (0.1–0.2 % per year) has decreased only marginally and is expected to increase with the aging of the population and increased prevalence of chronic heart disease [22, 24, 25, 29]. The overall survival rate despite the advanced first responder system for resuscitation for out of hospital cardiac arrest is dismal at 4.6 % [28]. Therefore, it is imperative to identify those at highest risk of sudden death and develop effective strategies for the primary and secondary prevention of SCD [29].

Substrates for SCD in the Elderly

The substrate for SCD in the elderly varies depending on the underlying heart disease. Defining the effect of advancing age on cardiac structure and function in humans is challenging because of difficulty in isolating the effect of aging from diseases associated with senescence.

The majority of sudden deaths in the elderly occur in the setting of coronary artery disease caused by ventricular arrhythmias, often triggered by acute ischemia [30]. Approximately 80 % of patients who die suddenly from cardiac causes have underlying coronary artery disease and in 50 % sudden death may be the first manifestation of their disease [24, 25, 27, 31]. Active coronary lesions or acute changes in plaque morphology, such as plaque disruption or thrombus may be present in more than 50 % of the victims of sudden death [32–34]. The risk of ventricular arrhythmias increases after a myocardial infarction due to the presence of scar and reduction in left ventricular systolic function. Other substrate for ventricular arrhythmias and sudden death in the elderly include ventricular hypertrophy, nonischemic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, valvulopathy, inflammatory or infiltrative diseases [5, 35–38]. Only a small percentage of SCDs in the elderly occur due to a primary electrical problem resulting from channelopathies which are responsible for sudden death in younger individuals with arrhythmia syndromes, such as the congenital long or short QT syndrome, Brugada syndrome or catecholaminergic polymorphic ventricular tachycardia [39, 40]. Familial clustering of cardiac events, however, do suggest a role of hereditary factors in predisposition to sudden death [41–43], which in the elderly appears to be due to genetic influences that increase the risk of a coronary event [24, 25, 44–46]. Patients with long QT syndrome maintain a high risk for life-threatening cardiac events even in later years, although the risk of aborted cardiac arrest or death conferred by long QT syndrome is attenuated, most likely due to the higher prevalence of comorbidities [47], such as obesity, hypertension, lipid abnormalities and diabetes [48–51] associated with the aging process.

Mechanisms of Sudden Cardiac Death

Despite our understanding of risk factors for arrhythmogenesis, exact mechanisms underlying initiation, propagation, maintenance or prediction of timing for cardiac dysrhythmias causing SCD in the elderly are not fully understood. This is mainly due to the complex interactions between myocardial substrate and the triggers that define the overall risk of arrhythmia susceptibility [52, 53]. Age-related changes in cardiac structure and function occur at macroscopic and microscopic levels in both cellular and extracellular matrix [2, 54]. This results in altered cellular excitability and cell-to-cell coupling creating a proarrhythmic milieu that increases predisposition to arrhythmogenesis due to abnormalities in impulse initiation and/or propagation. Failure of impulse initiation or conduction results in bradyarrhythmias, whereas enhanced impulse generation due to increased automaticity or triggered activity or slowed conduction resulting in re-entry causes tachyarrhythmias.

Bradyarrhythmias, due to reduced “normal automaticity” and delayed conduction, are common in the very elderly, even in the absence of apparent heart disease [55, 56]. The intrinsic heart rate as measured following blockade of the parasympathetic and sympathetic nervous system and heart rate reserve decrease with aging [57]. This is related to aging-associated replacement of pacemaker cells within the sinoatrial node and atrioventricular conduction fibers with collagenous and elastic matrix [58] and impairment of signaling via cardiac G protein–coupled receptors, specifically beta-adrenergic receptors contributing to the diminished cardiac exercise reserve, spontaneous heart rate variability and maximum heart rate achieved during stress resulting in reduction in aerobic work capacity in the elderly [56, 58, 59]. Myocyte hypertrophy and interstitial fibrosis also accompanies aging that alters cellular coupling and exaggerates directional differences in conduction (anisotropy), increasing heterogeneity in conduction and refractoriness promoting formation of zones of functional slowing or conduction block that stabilizes re-entry enhancing susceptibility to arrhythmogenesis [10, 60–62]. In addition aging causes changes in expression, distribution and/or functioning of ion channels that alters action potential waveforms and propagation further increasing vulnerability to dysrhythmias [11, 63]. The action potential duration and repolarization is prolonged in the senescent heart [64–66],

in part due to delay in the inactivation of the calcium current (I_{CaL}) [67–69] and partly due to down regulation of potassium currents, including the transient outward current (I_{to}), Ca^{2+} activated potassium current and ATP-sensitive potassium channel current (I_{KATP}) that along with an increase in sodium-calcium exchanger increases predisposition to Ca^{2+} overload mediated triggered activity and also re-entrant arrhythmias [65, 69–74]. In addition, advanced age is associated with a reduction of expression of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA-2) [75, 76] and post-translational modifications affecting function of SERCA-2, phospholamban and other Ca^{2+} transport proteins, including ryanodine receptor, the sarcoplasmic reticulum Ca^{2+} release channel [77, 78]. The contribution of age-related changes in cardiac microstructure, including mitochondria [21, 79] and other intracellular organelles, cytoskeleton, sarcolemma, intercellular gap junctions, cellular geometry and interstitium on regulation of cardiac excitability or arrhythmogenesis are not well defined and warrant further studies.

Ventricular arrhythmias are common in elderly, present in more than 70 % of persons over the age of 60 years. The incidence, prevalence and complexity of ventricular arrhythmias and their prognostic significance increases with advancing age [48] and presence of heart disease [5, 80–82]. In the absence of heart disease asymptomatic premature ventricular complexes (PVC) observed at rest do not carry adverse prognosis, but when elicited during exercise [83] or post-exercise recovery period [84], are associated with an increased risk of cardiovascular death. In those with structural heart disease, PVCs do indicate an increased mortality risk, especially if ventricular function is reduced [48, 85–91]. The mechanism underlying ventricular arrhythmias varies depending on the underlying substrate. During the acute phase of myocardial infarction or with acute ischemia, ventricular fibrillation may result from functional reentry, whereas in patients with healed myocardial infarction, reentry circuit can form around scarred tissue causing ventricular tachycardia that can then degenerate into fibrillation, even in the absence of active ischemia. In the peri-infarction period, the senescent heart is more vulnerable to arrhythmogenesis, with a higher likelihood of in-hospital cardiac arrest in those 75 years and older compared to younger patients [92]. Although, ventricular tachyarrhythmias occurring within 48 h of the acute coronary syndrome are associated with an increased in-hospital mortality, long-term mortality is however not affected, unless significant ventricular dysfunction persists [93]. The incidence of scar-related re-entrant ventricular arrhythmias, however increases following myocardial infarction, increasing exponentially as the left ventricular ejection fraction falls below 30 % [30, 94].

The exact electrophysiological basis for SCD in the elderly is difficult to determine and results from multiple factors depending on the underlying cardiac substrate with which dynamic transient factors (such as ischemia, hypoxia, catecholamine, pH and electrolyte changes, stretch, or inflammation) interact to precipitate arrhythmias [30]. In addition, an arrhythmia may be initiated by one mechanism, perpetuated by another and then degenerate into a different mechanism. At the time of cardiac arrest, ventricular fibrillation was the most commonly recorded rhythm observed in 75–80 % compared to advanced atrioventricular block or asystole documented in 15–20 % of the cases [95, 96]. An increase in pulseless electrical activity with a proportionate decrease in VF as the presenting arrhythmia in cases of SCD, however, has been reported in more recent studies [97, 98]. The true incidence of bradyarrhythmias causing sudden death in the elderly is not known because by the time the first rhythm is recorded, an arrhythmia beginning as ventricular tachyarrhythmia may degenerate into or appear as asystole.

Evaluation of Elderly Patients at Risk of SCD

Several risk stratification protocols have been developed for the identification of patients at risk for ventricular arrhythmias that may benefit from interventions to reduce the risk of

sudden death. These include non-invasive tests, such as a standard 12 lead electrocardiogram (ECG), exercise test or imaging modalities to determine the severity of left ventricular systolic dysfunction, presence of late potentials on signal-average electrocardiography (SAECG), severity of ventricular arrhythmias by ambulatory cardiac monitoring, detection of repolarization instability by measurement of QT interval, QT dispersion and microvolt T-wave alternans, autonomic balance by heart rate variability or baroreflex sensitivity or invasive tests to determine inducibility of sustained ventricular arrhythmias by programmed electrical stimulation [5].

A standard 12-lead ECG allows identification of underlying structural disease, such as conduction system abnormalities with heart block, bundle-branch block, interventricular conduction delay, ventricular hypertrophy, or prior infarction, as well as primary electrical disorders, such as the long QT syndrome, short QT syndrome, Brugada syndrome or arrhythmogenic right ventricular cardiomyopathy. A prolonged QRS duration >120 ms in patients with a severely depressed ventricular function or a prolonged QTc interval in the elderly predicts a higher risk of SCD [3, 99]. Absence of a slowly conducting zone, the electrophysiologic substrate for re-entrant ventricular arrhythmias that is otherwise detected as late potentials on SAECG may be useful with its high negative predictive value to exclude a wide-complex tachycardia as a cause of unexplained syncope in the elderly patient with coronary artery disease [100, 101]. Exercise-electrocardiogram may also provide useful diagnostic and prognostic information in the evaluation of patients with known or suspected coronary artery disease, cardiomyopathies or with frequent premature ventricular complexes. Appearance of exercise-induced complex ventricular ectopy or ventricular tachycardia in the elderly may predict an increased risk of mortality compared to patients with simple ectopy observed at rest only [83, 84, 102]. T-wave alternans detected as microvolt fluctuation in the amplitude or morphology of the T wave during rest [103], exercise testing or atrial pacing is also a useful tool for identifying high-risk patients after myocardial infarction or with cardiomyopathy and carries a high negative predictive accuracy [104, 105].

Assessment of left ventricular systolic function and other structural and functional information about myocardial dimensions, wall thickness, valvular and congenital heart disorders with imaging technique, such as echocardiogram is an essential part of risk stratification of patients with ventricular arrhythmias at risk of SCD [106]. Cardiac MRI or CT scan is helpful in patients with suspected arrhythmogenic right ventricular cardiomyopathy [107, 108]. Myocardial perfusion SPECT using exercise or pharmacological agents is useful for ischemia detection in those suspected of having ventricular arrhythmias triggered by ischemia [109]. Coronary angiography is useful in the assessment for obstructive coronary artery disease in patients with ventricular arrhythmias or aborted sudden death.

The utility of Electrophysiology (EP) testing with intracardiac recording and electrical stimulation in the elderly varies with the type and severity of heart disease [110–112]. It is useful for arrhythmia assessment and risk stratification for SCD in elderly patients with ischemic heart disease and left ventricular dysfunction or syncope, but plays only a minor role in the evaluation of patients with DCM or inherited arrhythmia syndromes, such as the long or short QT syndrome [110–114]. Its utility in patients with Brugada syndrome or hypertrophic cardiomyopathy is controversial [115–117]. In patients with coronary artery disease, non-sustained ventricular tachycardia, and left ventricular ejection fraction (EF) less than 40 %, inducibility of sustained ventricular tachycardia identifies patients at high risk of ventricular arrhythmias and predicts a worse prognosis [118]. However, in those with severe ventricular dysfunction (EF < 30 %), noninducibility of VT with program electrical stimulation does not exclude those at high risk [119] and is therefore not helpful in risk stratification.

Overall, the use of multiple risk markers in combination may predict future arrhythmogenic events better than use of a single parameter given the complexity and variability of the

underlying substrate predisposing to arrhythmogenesis and SCD [25].

Management of Elderly Patients at Risk of SCD

Antiarrhythmic Drugs

The essential goals of antiarrhythmic therapy in the elderly are acute termination of an ongoing arrhythmia, and/or prevention of arrhythmia recurrence. Although antiarrhythmic agents, except for beta blockers, have not been shown to reduce mortality in randomized trials [120–123], they continue to play an important role for symptom relief by suppression of arrhythmia recurrences in the elderly patients. However, these agents should be used with caution as they can also cause arrhythmia in susceptible individuals [124]. Selection of an effective yet safe medication in the elderly is challenging due to variability in the pathophysiologic substrate, arrhythmia mechanisms, clinical presentation and prognostic implications of the arrhythmia [125]. In addition presence of comorbidities, concomitant drug use and variability in drug disposition and/or responses due to aging-associated physiological changes that alter pharmacokinetics and pharmacodynamics of a drug require careful adjustment of drug regimens and frequent monitoring for efficacy and side effects [126]. The empiric use of antiarrhythmic drugs regardless of the prognostic significance of an arrhythmia or choosing antiarrhythmic by trial and error is not acceptable due to deleterious effects including the risk of proarrhythmia [127, 128], which may be increased in the elderly due to impaired renal clearance and potential for drug interactions [129]. There is no evidence that suppression of asymptomatic non sustained ventricular tachycardia prolongs life and the only indication to treat these arrhythmias are for symptom control due to frequent recurrences of rapid tachycardia compromising hemodynamics. These could be managed with antiarrhythmic therapy, preferably with beta-blockers, sotalol or amiodarone or with catheter ablation. In the presence of structural heart disease or myocardial ischemia, class I antiarrhythmic agents should be avoided as clinical trials, such as the Cardiac Arrhythmia Suppression Trial have demonstrated increased mortality in patients treated with anti-arrhythmic agents compared to placebo [127]. Patients with atrial fibrillation treated with a class I antiarrhythmic agent may become symptomatic with a rapid 1:1 atrioventricular response as the atrial rhythm becomes more organized. If used these agents should be given with drugs that slow AV node conduction. In addition, use of class I antiarrhythmic agents in patients with a pacemaker or ICD is a concern due to the possibility of an increase in pacing threshold or defibrillation energy requirement that requires reprogramming of pacing or ICD systems [130, 131].

Overall, class I or III antiarrhythmic drugs should not be used as primary therapy for prevention of SCD in the elderly. Its use as hybrid treatment with ICD implantation, however, can be considered for symptom control and improvement in quality of life by suppression of arrhythmia recurrences and reducing frequency of ICD discharges [132, 133]. Amiodarone, a complex drug with multiple electrophysiological effects is useful for termination and suppression of ventricular arrhythmias, especially when used with beta-blockers. However, the long-term survival benefit from amiodarone alone was not shown in most of the randomized, placebo-controlled studies, demonstrating no significant benefit over standard of care in high risk patients with heart failure or coronary artery disease [134–137]. Similarly, use of Sotalol, despite its effectiveness in suppressing ventricular arrhythmias has not been shown to improve survival [138]. Use of amiodarone (with a beta blocker) or sotalol is recommended in patients who do not meet the criteria for ICD implantation, or in those who have an ICD with a goal of reducing recurrences of ventricular arrhythmias and frequency of ICD shocks [132, 133].

Drugs that are relatively safe and proven to be effective in improving survival in high risk patients include, beta blockers, angiotensin converting enzyme inhibitors, angiotensin receptor antagonists and statins that do not possess classic antiarrhythmic properties, but should be considered for attenuation of adverse remodeling in high risk patients after myocardial infarction or

with heart failure [139, 140]. The combination of beta blockers and amiodarone appears to be more effective in reducing overall mortality and sudden death than amiodarone alone [141, 142].

Implantable Cardioverter Defibrillator

Patients who had cardiac arrest due to ventricular fibrillation or sustained ventricular tachycardia in the absence of a reversible cause or those with persistent severe left ventricular dysfunction (EF < 35 %) due to non-ischemic or ischemic cardiomyopathy 40 days after the acute myocardial infarction are at increased risk of SCD and should be considered for ICD implantation [5, 143, 144]. Randomized, prospective trials comparing antiarrhythmic drug therapy to ICD have demonstrated the efficacy of ICDs in primary and secondary prevention of sudden death in those at high risk of SCD or resuscitated after cardiac arrest [120–122, 145–147]. However, little information is available from clinical trials specifically focusing on the efficacy of ICD in the elderly. In some studies, the survival benefit of ICD in those 65 years or older appears to be similar to those <65 years of age [148–151] with the benefit of ICD therapy apparently greater in those in whom ICD is implanted for primary prophylaxis of SCD than those for secondary prevention after a life-threatening arrhythmic event or in those with advanced heart failure [152, 153]. In patients 75 years and older, the efficacy of ICD in prolonging survival is reduced compared to younger patients [154]. Because of the limited availability of data, the efficacy of ICD therapy in the older elderly with limited life expectancy is not clear, as only a very small number of elderly above 80 years of age have been included in these trials, that may also suffer from selection bias for the use of more expensive devices in "healthier elderly" with lower risk of non-cardiac or cardiac death [155]. Pooled analysis of the secondary prevention trials, do indicate that the very elderly may derive less benefit from an ICD than younger patients, due to an increased number of non-arrhythmic cardiac and non-cardiac deaths [156]. Similarly, cohort studies reporting an equivalent survival benefit in the elderly and younger patients may not reflect the true benefit of ICD therapy in the overall elderly population as selection bias may be present, with use of more expensive device implantation considered in only "healthier elderly" free of serious comorbidities with a better functional capacity [154, 155, 157–159]. In the absence of symptomatic arrhythmias in those with preserved ventricular function (EF > 40 %) the risk for SCD is relatively low and at this time ICD therapy is not indicated [5]. In addition, in the very elderly patient who has multiple comorbidities with a limited life expectancy from non arrhythmic causes, ICD may not prolong survival and could have adverse impact on quality of life and should be avoided. Patients with ICD with compromised ventricular function who require pacing may have exacerbation of heart failure when paced from the RV apex [160]. The role of resynchronization therapy in the elderly patients has not been systematically assessed in randomized clinical trials [161]. In ICD patients RV pacing should be minimized by selecting low minimum rate, programming long AV interval, selecting an ICD with algorithms utilizing automatic mode selection that favors atrial over ventricular pacing or using ICD with biventricular pacing capabilities [162, 163].

Radiofrequency Ablation

Ablation therapy should be considered in the elderly as adjunctive therapy for recurrent ventricular tachycardia in those with recurrent ICD shocks not manageable by reprogramming of ICD or antiarrhythmic therapy or who do not wish long-term drug therapy [5, 164–167]. Ablation as primary therapy is only indicated in those who are otherwise at low risk for SCD and have symptomatic predominantly monomorphic ventricular arrhythmia that is drug resistant, or in patients who are drug intolerant, or do not wish long-term drug therapy [5, 168]. Ablation of the tachycardia circuit involving the bundle branches in the bundle branch ventricular tachycardia may be curative, but these patients typically have severe ventricular dysfunction with underlying electrophysiological-substrate that increases risk for other arrhythmias and therefore may need ICD. Idiopathic ventricular arrhythmias arising from

the right and less commonly, the left ventricular outflow tract, are usually seen in healthy young individuals, but may present in the elderly. It is associated with a good prognosis [169] and often responds to treatment with beta and calcium channel blockers or class IC antiarrhythmic drugs. Catheter ablation is considered in those that remain symptomatic or do not respond to drug therapy [5] and has been shown to be as effective and safe in the elderly patients as in younger subjects [170, 171].

Other Interventions

Ablation of the tachycardia circuit using surgery to resect or modify the arrhythmia substrate is an alternative therapy that may be suitable for patients in whom catheter ablation is unsuccessful and who are otherwise fit to undergo cardiac surgery. Coronary revascularization with percutaneous coronary intervention or bypass surgery reduces myocardial ischemia and SCD during long-term follow-up [172], but controlled trials evaluating the effect of myocardial revascularization on ventricular arrhythmias in the elderly have not been conducted. If ventricular arrhythmias are triggered by acute ischemia, coronary revascularization may help reduce the frequency and complexity of the arrhythmias. However, sustained monomorphic VT in patients with scarred myocardium from previous infarction is not affected by revascularization [173], neither the risk of recurrent cardiac arrest in patients with markedly reduced ventricular function is eliminated with revascularization even if the original arrhythmia appeared to result from transient ischemia [174].

In summary, cardiac arrhythmias, particularly associated with structural heart disease are common in the elderly and with the changing population demographics, will have a greater impact on healthcare costs. Although clinical practice depends on well designed clinical trials that provide evidence to support management decisions, little information is available in the older elderly (octogenarians and nonagenarians) that limits specific practice guidelines and further investigation is warranted to fully define the efficacy of various therapeutic modalities proven to be effective in younger population.

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20
Comparisons of Substrates Responsible for Atrial Versus Ventricular Fibrillation

Philippe Comtois, Brett Burstein, and Stanley Nattel

Abstract

The fundamental mechanisms underlying fibrillation have long been debated. The classical notion of multiple circuit reentry has been challenged by recent ideas suggesting that in some cases a single high-frequency rotor or even a rapidly-firing focus can underlie fibrillation that can cause wave breakup and fibrillation. There is increasing awareness that arrhythmias do not usually begin in perfectly normal tissue, but require changes in tissue structure or function that constitute the arrhythmic substrate. In this chapter, atrial and ventricular changes (including alterations in ion channel function, intercellular coupling, and fibrosis) playing a role in fibrillation are reviewed. We focus particularly on ischemic substrates, congestive heart failure remodeling, genetic factors, neuroregulatory determinants, and arrhythmic remodeling. Atrial (AF) and ventricular (VF) fibrillation-substrates have many features in common but also a number of important specific differences. Consideration of these mechanistic determinants will lead to improved understanding of the pathophysiology of AF and VF, and ultimately to improve arrhythmia-specific therapeutic approaches.

Keywords

Atrial fibrillation • Ventricular fibrillation • Ischemic disease • Congestive heart failure • Arrhythmic remodeling • Triggered automaticity • Calcium handling • Connexin expression

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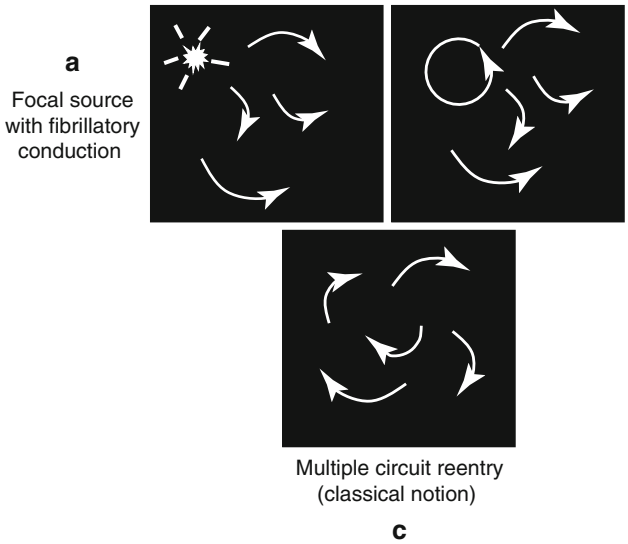

Figure 20–1. Potential mechanisms of fibrillation. Fibrillation may be initiated by a rapidly discharging, spontaneously active, ectopic focus (**a**), a single high frequency rotor (**b**) or by multiple functional reentrant circuits (**c**)

Introduction

The fundamental mechanisms underlying fibrillation have long been debated (Fig. 20.1). The classical notion of multiple circuit reentry (Fig. 20.1c) has been challenged by recent ideas suggesting that in some cases a single high-frequency rotor (Fig. 20.1b) [1] or even a rapidly-firing focus (Fig. 20.1a) [2] can underlie fibrillation. In the latter two cases, although the primary source may be firing regularly, the inability of tissues to follow 1:1 causes wave breakup and fibrillation. Ironically, these recent ideas echo notions first put forward in the early twentieth century [3].

There is increasing awareness that arrhythmias do not usually begin in perfectly normal tissue, but require changes in tissue structure or function that constitute the arrhythmic substrate. The principal mechanisms underlying ectopic complex formation and reentry are illustrated in Fig. 20.2, along with the determinants of their substrates. Abnormal automaticity results from enhanced spontaneous phase 4 depolarization and causes premature beats that can trigger reentry in a vulnerable substrate. Early afterdepolarizations (EADs) are caused by failure of normal repolarization, prolonging the action potential (AP) plateau and allowing L-type Ca^{2+}-channel reactivation to produce abnormal depolarization before repolarization can occur. Delayed afterdepolarizations (DADs) are due to spontaneous Ca^{2+}-leak from sarcoplasmic reticulum (SR) Ca^{2+}-stores. The released Ca^{2+} is exchanged for extracellular Na^+ by the Na^+, Ca^{2+}-exchanger (NCX) in a fashion that generates a depolarizing current and a DAD. EADs and DADs can cause extrasystoles that trigger reentrant VF, but when repetitive can also cause sustained tachyarrhythmias that degenerate to VF. This chapter examines critically the substrates that underlie atrial (AF) and ventricular (VF) fibrillation, to inform the reader about their properties and to evaluate their similarities and differences.

Biophysical Substrate

In biophysical terms, fibrillation represents self-sustaining rapid and irregular electrical activity. Fibrillation can be sustained by either a single rotor, a so-called "mother wave", or by multiple smaller wavelets [4]. Mathematical modeling of AF shows that it can be sustained by either mechanism under different spatial distributions of acetylcholine [5], thus relating the mechanism to the degree of dispersion of refractoriness and regional ion channel expression. Ectopic complexes arising by automatic, DAD or EAD mechanisms contribute to the fibrillation substrate by providing the critical premature activation that initiates reentrant activity.

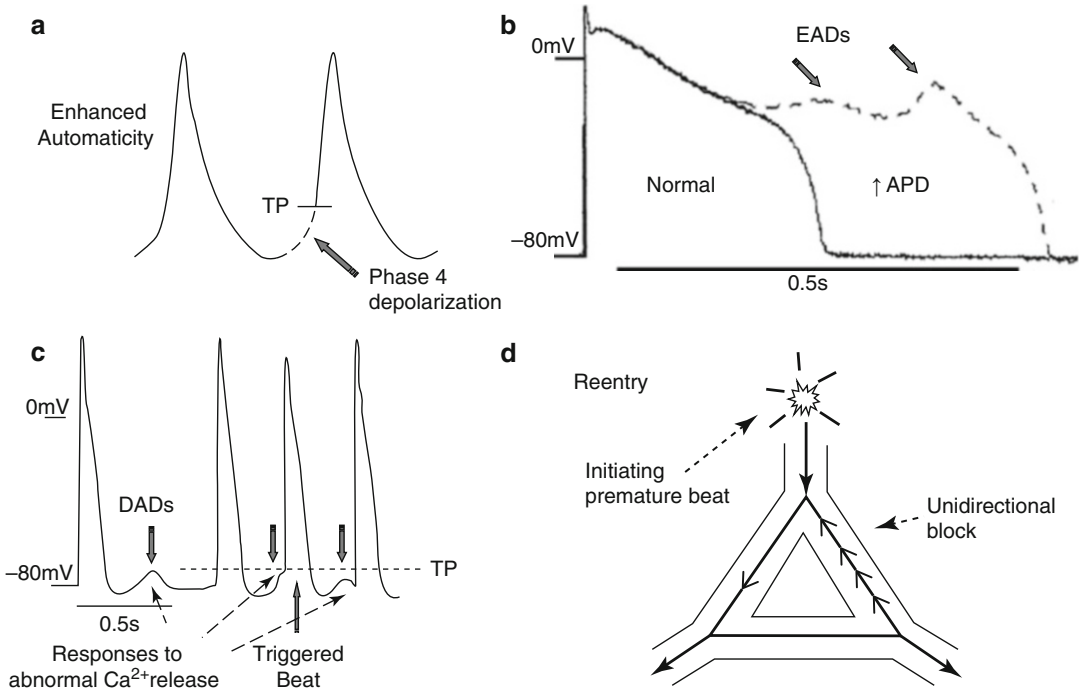

Figure 20–2. Mechanisms of arrhythmia generation. (**a**) Enhanced automaticity: Abnormal impulse formation can result from a region of cardiac tissue prematurely brought to threshold potential (*TP*) by an increased slope of spontaneous phase 4 diastolic depolarization. (**b**) Early afterdepolarizations (*EADs*): Reductions in repolarizing currents and/or increases in depolarizing plateau currents can delay repolarization, prolonging action potential duration (*APD*). This can cause abnormal depolarizations (*EADs*, *arrows*) by reactivation of L-type Ca^{2+}-channels. (**c**) Delayed afterdepolarizations (*DADs*): Under pathological conditions (eg, Ca^{2+} overload, ryanodine receptor dysfunction) Ca^{2+} is released from SR stores in diastole. This Ca^{2+} is exchanged by the Na^+, Ca^{2+}-exchanger for three times as many extracellular Na^+ ions, producing an inward current. The inward current depolarizes the cell, producing delayed afterdepolarizations (*DADs*, *downward arrows*). If DADs are large enough to reach threshold potential (*TP*), they can trigger beats (*upward arrow*). (**d**) Reentry: Reentry may result from a premature initiating beat which fails to conduct in a region that is still refractory (*unidirectional block*), while conducting through an alternative connecting pathway that is no longer refractory. The impulse can then return backwards (retrogradely, *dashed line*) in the previously blocked pathway, and if enough time has elapsed for recovery of excitability, reenter through that pathway. Reentry requires a crucial balance between conduction and refractoriness to be maintained. It is favoured by brief refractory periods and slow conduction. Because variable refractoriness is needed to initiate reentry, spatial variability in refractoriness is an important predisposing condition

Figure 20.3a, b compare the features of the classical leading-circle reentry concept to those of the spiral-wave formalism, which is the presently-accepted biophysical representation of functional reentrant activity in cardiac tissue [6]. The transition between tachycardia and fibrillation proceeds through partial electrical wave break-up and the formation of secondary reentrant spiral waves that can either be sustained (consistent with the "multiple-wavelet hypothesis", Fig. 20.1c) or transient (fibrillatory response to the "mother-wave", Fig. 20.1b). An AP-based atrial model shows that either form of activity can underlie AF, depending on functional properties and tissue dimensions [7]. Figure 20.3c shows how a single primary rotor maintains fibrillation in the model. Figure 20.3d shows how, in a substrate with different properties, multiple unstable rotors can co-exist, each continuing for relatively brief periods of time (up to 1–2 s) but spawning sufficient daughter rotors before extinction to perpetuate AF. For further details and access to on-line movies, the interested reader is referred to the original paper [7]. Recently, it has been shown that generators have the tendency to be anchored to the regions of longer inexcitable period. Thus, the importance of geometry in AF maintenance was seen in situations of moderate anchoring for which rotor-core drifts between regions of longer

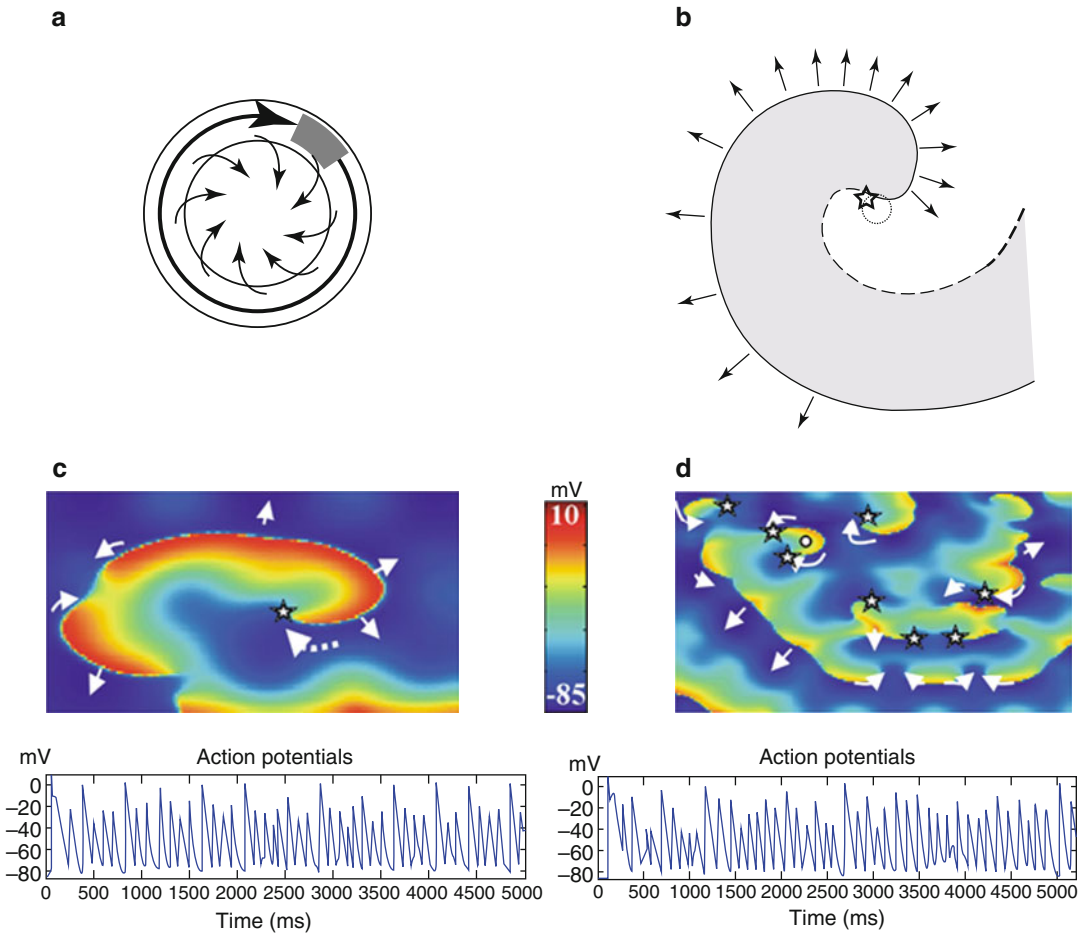

Figure 20–3. (**a**) The leading circle model: Schematic diagram of a leading-circle reentry wave (shown here as a *large black arrow*). Activity establishes itself in the smallest pathway that can support reentry, given by the distance traveled by electrical activity in one refractory period (refractory period × conduction velocity). The length of this pathway is also called the wavelength for reentry. Inside the leading circle, centripetal wavelets (*small arrows*) emanating from the leading-circle wave front constantly maintain the central core in a refractory state. The wave tip and tail are separated by a region (shown in *grey*) that must be excitable, called the "excitable gap". (**b**) Spiral-wave concept: Schematic diagram of a spiral wave with the activation front shown by a *continuous line* and the repolarization front by a *dashed line*. The point at which the *continuous* and *dashed curves* meet (shown with a *star*) has an undefined voltage-state, and is usually called the phase singularity point. There is no unexcitable core in the spiral wave model. (**c**) Example of fibrillation sustained by a single spiral wave. *Top panel*: Snapshot of transmembrane potential (colour coded: *red* fully depolarized tissue, *blue* resting tissue, *yellow* and *green* correspond to the AP plateau) during AF. The phase singularity of the single spiral is indicated by the *star*, and the *dashed arrow* shows the direction of rotation. The *smaller arrows* indicate the directions of electrical propagation. *Bottom panel*: Action potentials over 5 s, showing the non-periodic fibrillatory activity in a cell near the rotor core. (**d**) Example of fibrillation sustained by multiple spiral wave rotors. *Top panel*: Snapshot of transmembrane potential during AF. Generator rotors existing for between 100 ms and 1 s. are indicated by *stars*. In this case, longer-lasting rotors that maintained AF were always multiple and none of them lasted for long periods. However, they have generated daughter waves, some of which avoided annihilation and were able to maintain generator function when the initial generator extinguished. *Bottom panel*: Action potentials over 5 s, showing fibrillatory activity at the position pinpointed by the *white circle* in the *top panel* (For more detailed information and access to illustrative videos, see Zou et al. [7]) (Reproduced with permission of the American Physiological Society Inc.)

inexcitable period exist, favoring generator disappearance at tissue borders [8].

The first stage in the formation of a spiral wave is the occurrence of partial wave-block, equivalent to "unidirectional block" in classical re-entry theory. Wave-block occurs when the electrical wavefront encounters still-refractory tissue, creating a free wave-tip that re-enters through excitable tissue. Sustained spiral waves necessitate a sensitive balance between the time for the wave-tip to re-enter and the refractory period, analogous to the conduction velocity-refractory period requirements for classical reentry. For a more detailed discussion of the relationship between classical reentry theory and the spiral-wave formulation, see Comtois et al. [9].

Two different substrate characteristics play a role in initiating and sustaining fibrillation. Refractoriness (static) heterogeneity can provide the conditions for re-entrant fibrillatory activity. Alternatively, dynamical spatial dispersion related to beat to beat variation in AP duration (APD) may be involved [10]. The APD restitution relation (relation between APD and recovery time) and conduction-velocity restitution relation are crucial determinants of this dynamical instability.

AF Substrate

Primarily non-dynamic mechanisms involving heterogeneity of ERP are thought to initiate breakup within atrial tissue [11]. Variations in neural innervation can also increase ERP heterogeneity. For example, remodeling of atrial electrical characteristics and innervation following left ventricular infarction, causing increased slope of the APD restitution curve, may promote AF via dynamical mechanisms [12]. Atrial shape details, including a range of complicated anatomical factors like pectinate muscles, pulmonary veins and limited inter-atrial connections, are likely important in AF, although the transmural electrical complexity of the ventricles is absent. Only as of recently are computational AF models being developed that consider the anatomical complexity of the atria [13].

The efficacy of Na^+ channel blockade in terminating AF may be a unique feature of the atrial biophysical substrate. I_{Na} blockers terminate AF [14] via mechanisms demonstrated in AP-based computational models [15]. Na^+-channel blockade slows AF and underlying rotors without increasing wavelength. Termination occurs because of increased size and meandering of the primary generator rotor, which becomes more likely to be annihilated against a boundary, as well as a dramatic decrease in the number of daughter wavelets that could provide new generators should the primary rotor extinguish [15, 16]. In contrast, I_{Na} blockers increase VF-related mortality in post-MI patients [17], suggesting a VF-promoting action. In ventricular computational models, qualitatively similar behaviours to those in AF are seen with VF, but spiral-wave stabilization occurs rather than termination [18]. Possible explanations of the failure of VF (in contrast to AF) to terminate with Na^+-channel blockade are [1] spiral-wave stabilization by anchoring to structures like the papillary muscles [19] instead of terminating on boundary conditions, and [2] limited atrial-tissue mass that lacks the 3-dimensional transmural complexities of the ventricle and favours spiral-wave termination at boundaries.

VF Substrate

Wave breakup occurs in VF mainly via a dynamic mechanism, often first preceded by APD alternans, in particular discordant alternans. Although the importance of APD-restitution has been emphasized, recent work points to Ca^{2+}-handling properties as the prime culprit [20, 21]. Tissue thickness and intramural reentry play a key role in VF [22]. Transmural heterogeneity and repolarization gradients [23] favour wave breakup and reentrant wave formation. Because of its tridimensionality, VF is based on scroll waves rotating around filaments, counterparts to spiral waves/core tips in AF. Mathematical modeling shows that transmural changes in ventricular fiber orientation (rotational anisotropy) can induce filament destabilization [24, 25] with filament-twisting leading to wave breakup [26]. Large twisting causes a transition to a turbulent regime characterized by a high density of moving filaments and secondary 3-dimensional spiral sources.

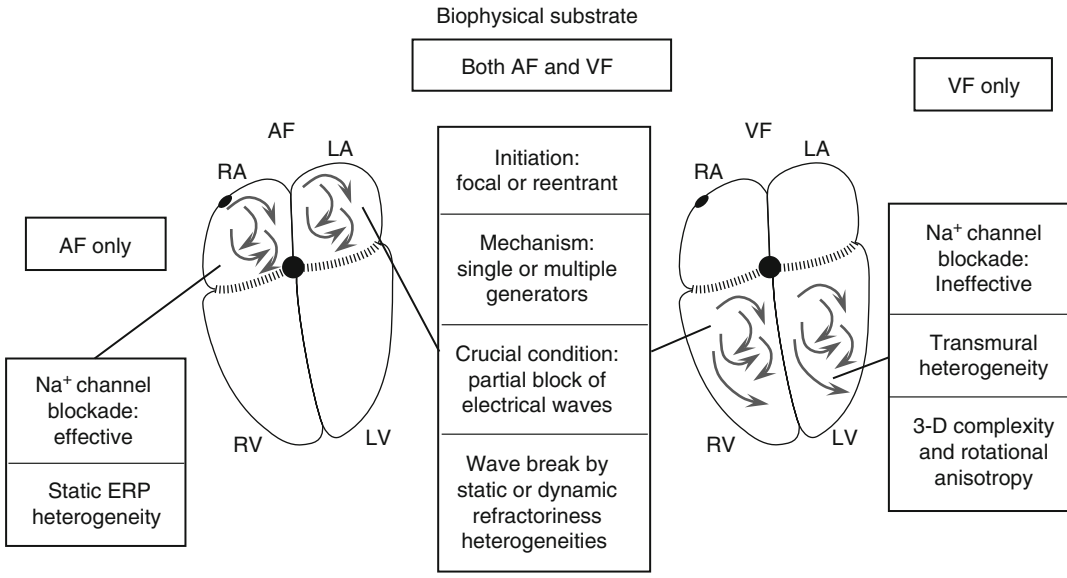

Figure 20–4. A comparison of the biophysical properties of AF and VF substrates. The most important differences relate to the transmural complexities of the ventricle and the differential response to Na^+-channel blockers

Summary of Biophysical Determinants

Similar general biophysical principles apply to AF and VF (Fig. 20.4). Important differences result primarily from discrepant structural characteristics of atria versus ventricles. The much greater thickness and transmural properties of the ventricles add an additional level of complexity to VF that may account for the discrepant responses to Na^+-channel blockers, which terminate AF but increase the likelihood of VF.

Ischemic Substrates

Ventricular tachyarrhythmias are the commonest cause of death, usually sudden, in the early phase of acute myocardial infarction (MI) [27]. Prior MI is a primary risk factor for VF [28]. AF also complicates acute MI quite regularly [29]. Acute ischemia promotes ion-channel dysfunction and tissue electrical dyssynchrony in relation to impairments in cell-to-cell coupling, intracellular acidosis, and accumulating extracellular K^+. Coronary artery disease (CAD) is one of the conditions most commonly associated with AF [30]. The association could be due to secondary factors like congestive heart failure (CHF) caused by CAD, but could also be due to ischemia-induced AF.

AF Substrate

Atrial ischemia in itself promotes AF by impairing conduction, thus stabilizing atrial reentry that underlies AF and allowing AF to be much more readily sustained [31]. In contrast to ventricular MI [32], the effects of healed atrial MI have just been recently studied. Over time, tissue remodeling in chronic atrial ischemia creates substrates for both spontaneous ectopy, favouring reentry formation, and sustained reentry [33]. Border zone (BZ) tissue that separates ischemic from normal tissue, showed increased fibrous tissue content with augmentation in conduction heterogeneity. Studies of AF in small-animal MI models have described changes that more likely reflect the effects of post-MI hypertrophy and/or CHF than ischemia per se. Atrial fibrosis provides a substrate for AF in MI rats with CHF [34]. Chronic ventricular MI with no atrial involvement causes heterogeneous alteration of atrial electrical restitution related to atrial sympathetic hyperinnervation, a form of neural remodeling that provides a substrate for increased AF vulnerability [12].

VF Substrate

The presence of a zone of slow propagation and propagation block is an important common feature shared by the AF and VF substrates caused by acute myocardial ischemia/infarction. With subacute and healed ventricular infarction, a complex set of electrophysiological changes occur that create a substrate for ventricular tachycardia (VT) and VF [35]. Surviving cardiomyocytes in the MI border have reduced AP amplitude and phase 0 upstroke velocity (dV/dt_{max}) [36] and increased phase 4 depolarization slope, in addition to prolonged APD [37]. Most K^+-currents are downregulated in post-MI border-zone cells [38]. These conditions are associated with enhanced automaticity and EADs [39].

Ca^{2+}-handling is altered in border-zone and Purkinje cells post-MI, with I_{CaL} diminished in several large animal models [40, 41], showing slower recovery [40] and hyperpolarizing shifts in inactivation voltage-dependence [41]. Cardiac contraction is based on Ca^{2+} released into the cytoplasm from SR Ca^{2+} stores in response to Ca^{2+} entry through L-type Ca^{2+} channels during the AP. The SR has specialized channels, called Ca^{2+}-release channels or ryanodine receptors (RyRs, co-called because they were first isolated based on high affinity to the blocker ryanodine) that are responsible for releasing Ca^{2+} with the appropriate stimulus and then remaining closed until the next AP. Altered RyR function is importantly altered post-MI [42, 43], whereas NCX function appears unaltered [44]. These MI-induced disturbances in SR Ca^{2+}-handling favour the occurrence of arrhythmogenic DADs.

Abnormalities of activation cause slow and often discontinuous conduction within and around the MI region [45, 46]. Marked changes in border-zone I_{Na}, including reduced current density, accelerated inactivation and slowed reactivation [47], impair conduction and excitability, promoting unidirectional block and reentry. Electrical conduction is also hindered by altered cell-to-cell coupling caused by gap junction dysfunction [46]. Gap junctions are smaller and less numerous in the peri-MI region [48]. Decreased side-to-side connections favour conduction block perpendicular to fiber orientation, producing a substrate for anisotropic reentry that underlies inducible VT/VF [49,50]. Connexin proteins form the cell-to-cell connections in gap junctions that maintain low-resistance intercellular coupling. Altered expression of the principal ventricular connexin, connexin43, is a primary factor in post-MI gap-junction dysfunction and the substrate for VT/VF [49].

Summary of the Role of Myocardial Ischemia

Figure 20.5 summarizes the mechanisms demonstrated to provide substrates for AF and VF in relation to myocardial ischemia and infarction. A favourable AF and VF substrate is present at various phases of the acute ventricular ischemic process: immediately after acute MI, during the subacute phase and during the chronic healed phase. Important contributors include abnormalities in impulse propagation, heterogeneity in electrical properties, impairments in myocardial repolarization and disturbances in Ca^{2+}-handling.

CHF

In the CHF population, sudden cardiac death, largely due to VF, is responsible for up to 50 % of CHF-associated mortality [51]. CHF is also one of the most common clinical causes of AF [52].

AF Substrate

Sinoatrial (SA) node function is abnormal in CHF [53, 54]. Bradycardia enhances refractoriness heterogeneity and thereby facilitates reentry [55], and can also promote the emergence of ectopic foci [56]. Atrial APD is unchanged or increased in CHF [57]. The only study available of atrial ionic-current changes in CHF showed decreased transient-outward current (I_{to}) and slow delayed-rectifier current (I_{Ks}), along with increased NCX current [57]. NCX carries a depolarizing diastolic current because it exchanges 1 intracellular Ca^{2+} ion (total charge +2) for 3 extracellular Na^+ ions (total charge +3), carrying net inward current,

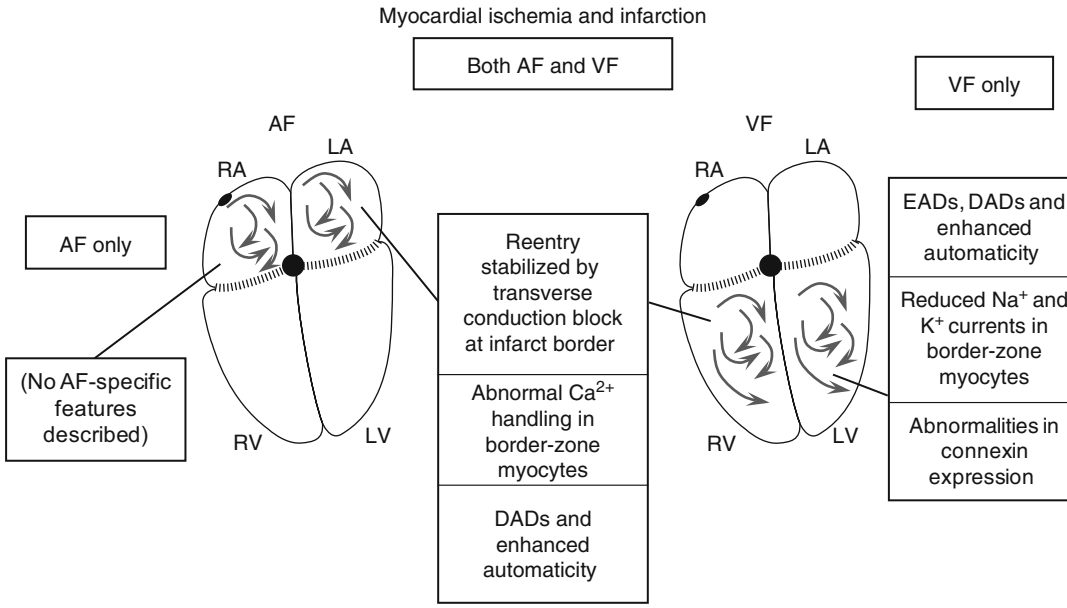

Figure 20–5. Promotion of AF and VF by myocardial ischemia and infarction. Acute ischemia seems to affect the atria and ventricles similarly. There is nothing known about longer-term effects of previous ischemia/infarction on the atria. Vastly more work has been done on the ventricles

when Ca^{2+} is extruded. This depolarizing current generates DADs, which can reach threshold and result in triggered activity, particularly in a setting of Ca^{2+}-overload. There is evidence for DAD-induced focal atrial tachyarrhythmias in experimental CHF [58, 59]. These tachyarrhythmias may be very rapid and mimic AF. In addition, mapping studies have provided evidence for rapidly-discharging focal sources with fibrillatory conduction during AF in CHF dogs [60], as in Fig. 20.1a, suggesting that DAD-induced sustained triggered activity may be able to maintain AF.

Structural remodeling and fibrosis appear to be a significant factor in the CHF-induced AF substrate [61]. Localized regions of conduction slowing occur and manifest as increased conduction heterogeneity that favours AF [62, 63]. No significant overall atrial connexins Cx40 and Cx43 changes in mRNA and protein expression levels has been found but Cx43 dephosphorylation and redistribution toward transverse cell-boundaries has been documented [63]. In some cases, this has been shown to result in single macro-reentry circuits underlying AF [64], consistent with the mechanism shown in Fig. 20.1b. Recently, it has been proposed that heterogeneous spatial distribution of fibrosis at the posterior left atrial endocardium may govern AF dynamics driven by micro-reentry circuits [65].

VF Substrate

As in the atria, ventricular K^+-currents, including both I_{to} and I_{Ks}, are down-regulated by CHF [66,67]. Additionally, many studies show reduced I_{K1} [66]. One study showed reduced I_{Kr} [68], but most found I_{Kr} to be unchanged [66, 67]. Down-regulation of K^+-currents increases APD (APD prolongation is a consistent feature in CHF [69]) and can lead to EAD-related tachyarrhythmias.

Significant Ca^{2+}-handling changes occur in CHF ventricles. NCX expression and activity are both enhanced [70,71]. CHF also causes SR Ca^{2+}-leak due to abnormal RyR function [72]. Hyperphosphorylated RyRs, whether by protein-kinase A or Ca^{2+}-calmodulin dependent protein-kinase II (CaMKII), are prone to abnormal spontaneous diastolic Ca^{2+}-release [72]. CaMKII-induced RyR-hyperphosphorylation produces

important SR diastolic Ca^{2+}-leaks despite the decreased SR Ca load in CHF [72]. Ca^{2+} leaked from hyperphosphorylated RyRs is exchanged for extracellular Na^+ by the NCX, producing DADs. These DADs can cause extrasystoles that may initiate reentry in a vulnerable VF substrate or directly lead to triggered-activity tachyarrhythmias that degenerate to VF [73]. In addition, spontaneous SR Ca^{2+} release promotes abnormal automaticity in latent pacemaker cells [74]. Ca^{2+}-release induced DADs are enhanced by two other factors [70]: (1) increased NCX activity, which increases the amount of current generated for any level of Ca^{2+}-release, and (2) downregulation of I_{K1}, which increases membrane resistance, resulting in a larger depolarization for a given inward current.

Extensive ventricular structural remodeling occurs with end-stage CHF in humans [75] and experimental models [49]. Areas of interstitial fibrosis cause ventricular conduction slowing and promote reentry. However, CHF-related fibrosis is much more extensive in atria than ventricles [76], suggesting a more important role in AF than VF. Connexin43 expression is downregulated in human and experimental CHF [77–80]. Moreover, gap-junctional distribution and regulation are altered, with connexins redistributed to the cardiomyocyte lateral borders and dephosphorylated [78]. In animal models, along with tissue fibrosis, the changes in connexin43 expression and phosphorylation lead to conduction slowing [77, 78] and APD heterogeneity [80] in the failing heart, predisposing to reentry. In humans, HF resulted in the heterogeneous prolongation of APD, which significantly reduced the transmural APD gradients [81] along with heterogeneous remodeling of excitation-contraction coupling and calcium handling [82].

Summary of the Role of CHF

CHF creates a substrate for both AF and VF. Table 20.1 compares the underlying changes in the two tissues and Fig. 20.6 summarizes the known contributors to AF and VF substrates in CHF. Many similar alterations occur in each, and form substrates for EAD, DAD and reentry based arrhythmic activity. The main differences appear to be more prominent and functionally important fibrosis at the atrial level, and a clearer role for connexin alterations and EADs at the ventricular level.

Table 20–1. Substrate: congestive heart failure

Feature	AF	VF
Potassium currents		
Ito	↓	↓
I_{K1}	↔	↓
I_{Kr}	↔	↔ (↓)
I_{Ks}	↓	↓
Sodium currents		
I_{Na}	?	↓
Calcium handling		
I_{CaL}	↓	↓, ↔
NCX	↑	↑
SERCA	?	↓
CaRC	?	DS, Ph ↑
Structural changes		
Fibrosis	↑↑↑	↑
Connexins	Ph↓, lateralization	↓, lateralization
APD	↔ (↑)	↑
CV	↓	↓
EADs	?	+
DADs	+	+
Reentry	+	+

increased, ↓ decreased, ↔ unaffected, + present, *Ph* phosphorylation, *DS* dyssynchronous release, () effect reported less frequently than the primary effect shown, *?* unknown

Genetic Factors

Recent advances in genetics have pinpointed a wide range of primary fibrillation-inducing mutations [83], which provide novel insights into molecular mechanisms. Two important fibrillation-promoting disorders of repolarization are the short QT syndromes (SQTSs) and long QT syndromes (LQTSs). SQTS causes early-onset paroxysmal AF [84] and VF [85]. The prolonged APD in LQTS patients often leads to the EAD-associated polymorphic ventricular tachyarrhythmia Torsades de Pointes, TdP, which can degenerate to VF [86]. Arrhythmic mutations can also affect conduction properties, like the loss-of-function mutations in cardiac Na^+-channels that cause the arrhythmic Brugada Syndrome [87] and probably also AF [88]. Inherited arrhythmia predilections can also affect Ca^{2+}-handling and connexin function.

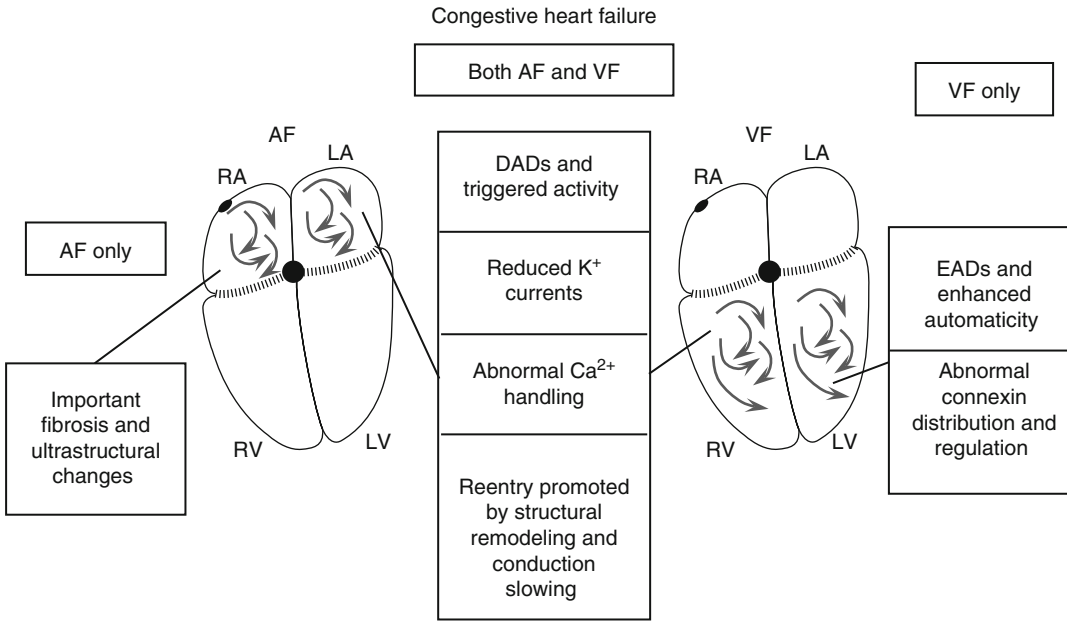

Figure 20–6. CHF-related changes underlying AF and VF substrates. Many similar changes occur with CHF in both atria and ventricles. The roles of EADs and connexin changes are more clearly established in the ventricles

AF Substrate

While SQTS most strikingly causes VF-related sudden death, SQTS clearly also predisposes to AF [84, 85, 89, 90]. The AF substrate is presumably based on accelerated repolarization, which makes the atria highly vulnerable to reentry, as seen with many acquired arrhythmia paradigms [91]. Atrial and ventricular ERPs are very short and both AF and VF are readily inducible in SQTS [84]. A *KCNQ1* mutation causes familial AF by increasing I_{Ks} and giving it a time-independent behaviour [92]. A mutation in *KCNE2*, a β-subunit of uncertain function, has been described in familial AF kindred [93]. Mutated subunits produce gain-of-function in a time-independent I_{Ks} component upon co-expression with *KCNQ1*. It is not clear why these two gain-of-function I_{Ks} mutations do not cause SQTS and ventricular tachyarrhythmias. Loss-of-function mutations in genes encoding the cardiac L-type calcium channel (*CACNA1C* and *CACNB2b*) have also been associated with a familial sudden cardiac death syndrome and increase AF sensitivity [94]. There is some evidence suggesting that LQTS can lead to AF [95], but this remains controversial. Recently, other paradigms of AF apparently associated with delayed repolarization have been described [96, 97]. Prolonged atrial APs could lead to AF either by EAD-mediated mechanisms in susceptible patients or by favouring wave-front breakup at critical rates [96].

Slow conduction favours reentry by leaving more time for recovery of excitability in potential reentrant pathways (Fig. 20.2). Conduction can be slowed by altering the Na^+-current that provides the energy for electrical conduction or by affecting cell-to-cell coupling. Several mutations in *SCN5A*, which encodes the cardiac Na^+-channel, lead to a variety of phenotypes including AF [88]. A single-nucleotide polymorphism (SNP) in the connexin40 promoter that reduces Cx40 transcription is associated with AF vulnerability [98,99]. Gollob et al. have recently reported the intriguing observation that several patients with idiopathic, early-onset AF have somatic mutations in Cx40 [100].

VF Substrate

SQTS produces transmurally-heterogeneous APD abbreviation by preferentially abbreviating

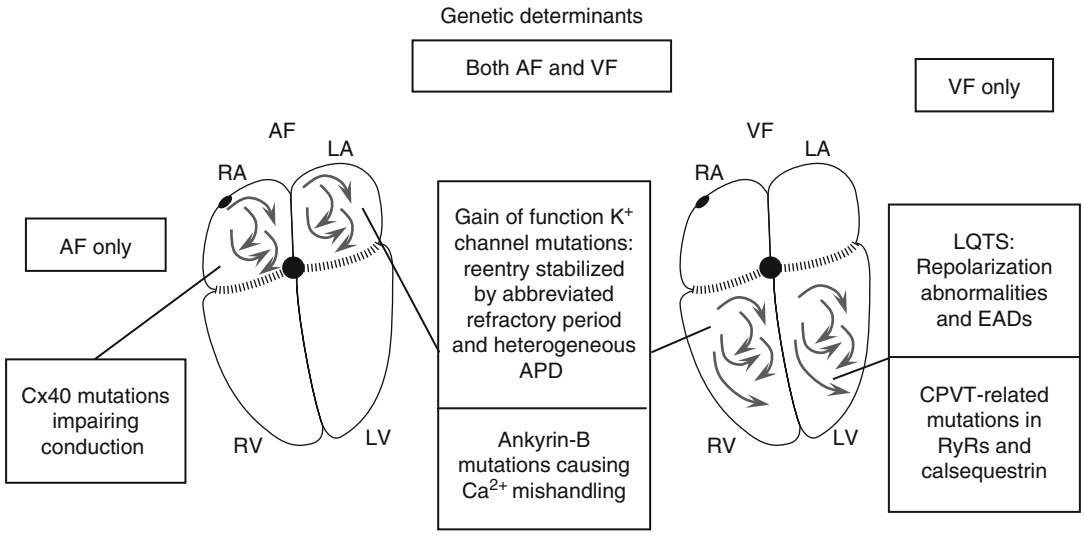

Figure 20–7. Genetically determined AF and VF substrates. The role of connexin40 abnormalities in AF is clear, as is the role of SQTS, LQTS and CPVT mutations in VF. In addition, gain-of-function K^+-channel mutations have been described that appear to cause AF but not VF, for presently unclear reasons. Ankyrin-B mutations cause both AF and VF. Recently, repolarization-delaying genetic alterations have been described as associated with AF, but the precise mechanisms remain unclear

repolarization in M-cells, promoting reentry [101]. A variety of loss-of-function K^+ channel mutations, or gain-of-function Na^+ and Ca^{2+} channel mutations can lead to impaired repolarization, LQTS, TdP and VF precipitation [102].

The Brugada Syndrome is characterized by ST segment elevation in right precordial leads, right bundle branch block, and susceptibility to VF [87]. Loss-of-function *SCN5A* genes are causal in ~25 % of Brugada syndrome patients [103]. Loss of *SCN5A* function can promote reentry by slowing conduction. However, a major component of the pathophysiology in the Brugada Syndrome appears to be loss of the action potential plateau in the epicardium, where large I_{to} can cause very early repolarization in the absence of countervailing I_{Na}, with current spread from normal AP plateaus in the endocardium causing "phase 2 reentry" (for detailed review, see Antzelevitch et al. [104]).

A variety of genetic syndromes promote VF by impairing Ca^{2+}-handling. Catecholaminergic polymorphic ventricular tachycardia (CPVT) mutations affect Ca^{2+}-handling and buffering. Missense mutations in the *CASQ2* gene, encoding the principal SR Ca^{2+}-buffer calsequestrin, are associated with autosomal-recessive CPVT [105]. Mutations in the gene encoding RyRs are the most common cause of CPVT [106]. Alterations in these CPVT-related gene products cause abnormal diastolic SR Ca^{2+} release, producing DADs. Catecholamine-induced β-adrenergic receptor stimulation promotes VT/VF in CPVT patients by increasing Ca^{2+}-entry through L-type Ca^{2+}-channels, increasing Ca^{2+}-loading and enhancing spontaneous diastolic Ca^{2+}-release and DADs. Ankyrin-B mutations disrupt the cellular localization of a variety of proteins, including Na^+/K^+-ATPase and NCX, and ankyrin-B deficiency produces defects in Ca^{2+} handling and afterdepolarizations that may be responsible for fibrillation-induced cardiac death, as well as AF [107, 108].

Summary of the Role of Genetic Determinants

There is considerable overlap in genetic AF and VF determinants (Fig. 20.7), but the unique role of connexin40 in the atrium is reflected in the lack of ventricular arrhythmias with connexin40 gene abnormalities. Repolarization abnormalities predispose much more clearly to VF than AF, presumably because of the much longer AP durations in Purkinje and M cells

than the atrium, predisposing to EADs and repolarization heterogeneity. CPVT-related mutations also seem to be much more likely to lead to VF than AF.

Neuroregulatory Factors

The autonomic nervous system (ANS) has long been recognized to play a potentially important role in AF and VF [109, 110]. The spatial heterogeneity of ANS actions increases refractoriness dispersion [111], favouring fibrillation. This is particularly important in vagal AF, a well-recognized clinical entity [112]. Alterations in neurohormonal function can also play a critical modulating role for fibrillation substrates. For example, adrenergic stimulation is a well-recognized precipitator of ventricular tachyarrhythmias in LQTS and CPVT. β-Adrenergic stimulation is also an important contributor to ischemic VF, since β-adrenoceptor blockers are among the only drugs known to prevent VF post-MI in patients with coronary artery disease [113].

AF Substrate

Vagal stimulation produces high vulnerability to sustained AF [114]. Spatially-heterogeneous refractoriness abbreviation appears particularly important in the pathogenesis of vagal AF [115]. The local (intrinsic) cardiac nervous system is affected by pathology associated with AF [116]. Atrial arrhythmias can be induced by stimulating mediastinal nerve branches of the thoracic vagosympathetic complex [117], or mediastinal nerves associated with pulmonary veins [118, 119].

Significant nerve sprouting and spatially-heterogeneous sympathetic hyperinnervation occur in a canine model of sustained AF produced by prolonged right atrial pacing [120]. Heterogeneous sympathetic denervation in a canine model also facilitates AF [121]. Sympathetic hyperinnervation may also increase AF vulnerability in a canine post-MI model [12]. Cryoablation of the left and right stellate ganglia and the cardiac branch of the left vagal nerve only delayed but did not prevent sustained atrial fibrillation [122]. Thus autonomic nerve activity seems not to be the only factor that determines atrial fibrillation maintenance.

VF Substrate

In contrast to AF, vagal influences do not appear to play a major role in VF substrates. Ventricular nerve sprouting is related to ventricular tachyarrhythmia-associated sudden death [12, 123], apparently by effects on repolarization [124, 125]. Mice with MIs show sympathetic nerve sprouting with up-regulation of nerve growth factor peri-MI and, to a lesser extent, in remote areas [126]. The ability of β-adrenergic stimulation to increase I_{CaL} accounts for its well-recognized role as a promoting effects on EAD-related arrhythmias associated with the LQTS and DAD-related arrhythmias in CPVT. The contribution of β-adrenergic stimulation to the VF substrate in acute MI and CHF may be related to the promotion of EADs and DADs, but also to other factors such as an enhancement in metabolic needs, stimulation of hypertrophic/remodeling pathways and increased ischemia.

Summary of the Role of Neuroregulatory Determinants

Figure 20.8 summarizes the contribution of neural regulation to AF and VF substrates. Vagal factors play a prominent role in the AF substrate. Adrenergic stimulation contributes particularly to EAD and DAD mechanisms, and potentially plays a role in reentrant substrates by enhancing refractoriness heterogeneity.

Arrhythmic Remodeling

Accumulating evidence indicates that cardiac rhythm disturbances may cause persistent changes in cardiac electrophysiology. The clearest example is AF itself, which by virtue of the rapid atrial rate causes atrial changes that promote AF occurrence ("AF begets AF") [127, 128], a process sometimes called "atrial tachycardia-remodeling" (ATR). AF-induced remodeling has most clearly been demonstrated in man by the reversal of remodeling changes in atrial

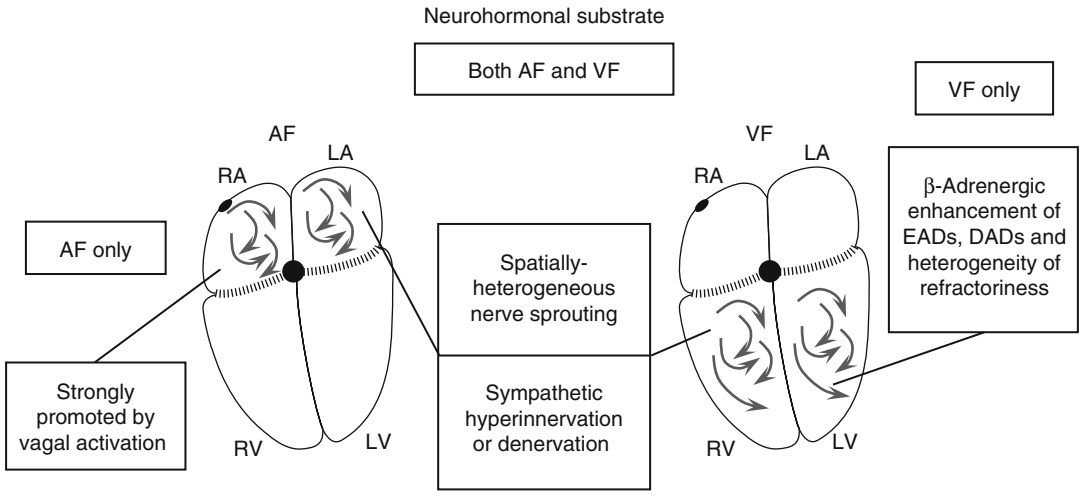

Figure 20–8. Neuroregulatory contributions to AF and VF substrates. Vagal effects are particularly important in AF and much less so in VF. Sympathetic enhancement of EAD- and DAD-related arrhythmia syndromes are well-established in the ventricles but have not been shown in the atria

refractoriness and conduction after AF cardioversion [129]. Remodeling is believed to underlie the tendency of paroxysmal AF to become permanent, of longer-lasting AF to be refractory to drug therapy, and of AF recurrence to be most likely within the first couple of days post-cardioversion [127]. Ventricular arrhythmic remodeling is most clearly manifest by electrical changes caused by abnormal activation sequences, as occurs with ectopic beats, so-called "T-wave memory" [130]. The implications of T-wave memory for VF are poorly defined.

AF Substrate

A consistent feature of ATR is a reduction in APD and consequently ERP [127, 128, 131]. ATR-induced refractoriness-shortening is maximal within 2 days, whereas AF-promotion occurs over several weeks [127, 128]. APD-abbreviation results from reduced I_{CaL} [132], along with increased I_{K1} [133] and acetylcholine-regulated K^+-current [134]. I_{Kr} and I_{Ks} are unchanged but I_{to} is reduced [130]. Rapid atrial cardiomyocyte activation causes Ca^{2+} loading, activating the calcium-dependent calmodulin-calcineurin-NFAT system causing downregulation of I_{CaL}, restoring Ca^{2+} to normal leading to APD reduction [135]. There is evidence for ATR-induced I_{Na} reductions in dogs [136], which could contribute to conduction-slowing and AF-promotion, but these have not been seen in atrial cardiomyocytes from AF patients [137].

ATR alters Ca^{2+}-handling, reducing cellular Ca^{2+}-transients [138]. There is evidence for defective RyR-function related to hyperphosphorylation in ATR/AF [139], as well as for spontaneous, potentially DAD-promoting, SR Ca^{2+}-release [140].

A variety of changes in connexin expression and distribution have been described in ATR and AF. The most consistent findings are connexin lateralization [141, 142] and increased hemichannel subunit heterogeneity [141] with spatially-variable loss of connexin40 [143]. The connexin alterations may promote AF by causing spatially-heterogeneous conduction disturbances.

VF Substrate

Sustained ventricular tachyarrhythmias [144] and even frequent ventricular ectopy [145] can produce ventricular cardiomyopathy and CHF; however, most associated electrical-remodeling changes are likely due to associated CHF. Electrical storm is characterized by a clustering of intractable VT or VF episodes [146], suggesting a positive-feedback system caused by ventricular tachyarrhythmias. The pathophysiology

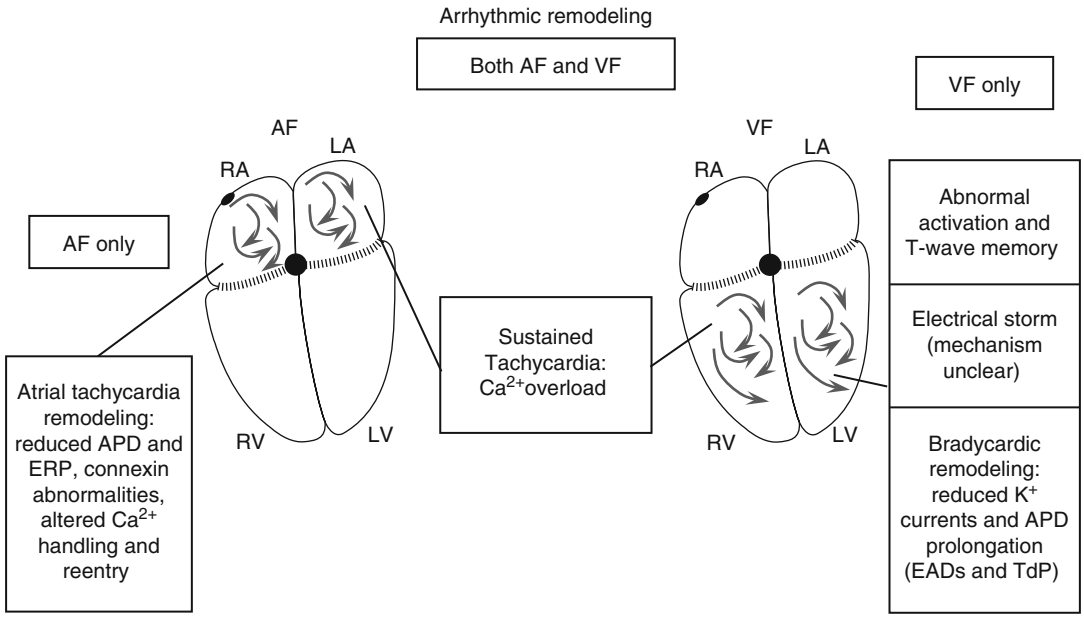

Figure 20–9. Contribution of arrhythmic remodeling to fibrillation substrates. Arrhythmia itself can cause electrical and structural changes that promote the initiation and maintenance of fibrillation. The classical example of such arrhythmic remodeling is atrial tachycardia remodeling (ATR, or so called 'AF begets AF'). ATR results in alterations of ionic currents and Ca^{2+}-handling that lead to refractoriness shortening, conduction disturbances and triggered activity. The purely rate-dependent mechanisms underlying ventricular tachycardia-induced remodeling cannot be dissociated from the major changes cause by the accompanying CHF, and so are unknown. The mechanisms underlying refractory VT/VF episodes of electrical storm have yet to be defined. Bradycardic states produce a substrate for VF by causing K^+-current downregulation, repolarization delays, and a potential for TdP

of electrical storm is poorly understood. Ca^{2+}-overload likely plays a pivotal role by reducing myofilament responsiveness and promoting post-defibrillation reinitiation of VF [147–149]. Electrical storm has also been shown to induce Ca^{2+}/calmodulin-dependent protein kinase II activation and phospholamban dephosphorylation [150].

Bradycardic states are also associated with ventricular electrical remodeling, predisposing to lethal ventricular tachyarrhythmias [151, 152]. Sustained bradycardia decreases I_{Kr} and I_{Ks} [67, 153, 154], causing QT interval prolongation and prominent repolarization delays leading to spontaneous TdP. I_{Kr} alterations in the face of simultaneous I_{Ks} down-regulation (reduced repolarization reserve) appear to be particularly important in the resulting long-QT phenotype, which may explain the association of AV block and clinical TdP syndromes [155]. Calcium handling abnormalities is also implicated in bradycardia remodelling with enhanced intracellular $[Ca^{2+}]$ and activation of the Ca^{2+}-calmodulin-CaMKII system [156].

Summary of the Role of Arrhythmic Remodeling

Arrhythmic remodeling contributions to fibrillation substrates are shown in Fig. 20.9. ATR promotes AF by abbreviating refractoriness and possibly by causing abnormal SR Ca^{2+}-handling and connexin remodeling. Bradycardic remodeling produces a substrate for VF due to repolarization abnormalities caused by K^+-current downregulation, and electrical storm may be an extreme form of ventricular tachyarrhythmia-induced VF promotion.

Conclusions

AF and VF substrates have many features in common but also a number of important specific differences. Consideration of these mechanistic features will lead to improved understanding of the pathophysiology of AF and VF, and possibly to newer, arrhythmia-specific therapeutic approaches.

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21
Single Nucleotide Polymorphisms in Health and Cardiac Disease

Eric Schulze-Bahr

Abstract

Recent advances in knowledge of the genome structure of ion channel genes and their physiologic role in myocardial repolarization have shown that genetic alterations of these key molecular components are associated with slight in-vitro effects and changes in fine tune of normal repolarization. It is expected that next-generation sequencing technologies (e.g., targeted resequencing of ion channel genes) will booster knowledge for individual arrhythmia predisposition and will enable researchers to lower the costs of complex genotyping and to implement these data into a personalized, genomic-oriented medicine. In this chapter, the role of natural genomic variation according to two main hypotheses, i.e. the 'common variant-common disease' hypothesis and the 'rare variants in common disease' hypothesis will be discussed upon current knowledge.

Keywords

Human genome • SNP • Complex heart diseases • Arrhythmias • QT interval • Repolarisation • Genetic predisposition

Prevalence of many common and genetically complex human diseases such as asthma, cardiovascular disease, and diabetes has risen greatly over the past two decades in developed countries and significantly contribute to the society's health burden. In addition, the genetic causes of monogenic diseases have been increasingly identified. In general, these conditions are declared as 'rare diseases' (RD) and are defined by the EU as rare when they affect less than one persons in 2,000. The fact that there are more than 8,000 such diseases means that the overall number of patients is considerable despite the low prevalence of individual clinical cases. It is estimated that more than 80 % of rare diseases are genetic in origin, yet in most patients the aetiology of their disease remains undetected. However, large scale investigations (e.g., exome sequencing) has been made to better understand their pathogenesis, and to develop preventive strategies, diagnostic tools, and treatment. Together with deciphering the human genome and its natural complexity of variance [1–3], considerable effort has been made to detect genetic loci contributing to quantitative phenotypes and complex arrhythmogenic diseases.

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Since the introduction of high-throughput DNA sequencing technologies, the costs of re-sequencing (i.e., non-repetitive portions) human genome felt down, and it has been reasoned that in a few years from now, costs will drop down to less than 1,000 US$. Therefore, the question is no longer whether, but when deep sequencing approaches will become routine in the diagnosis of genetic disorders and SNP detection. Since the first description of next-generation sequencing (NGS) systems 14–15 and the development of methods for genome partitioning [4, 5], numerous groups have successfully combined these methods to identify the molecular causes of monogenic disorders. In consequence, whole exome sequencing has been established as a potent and affordable strategy to identify disease-causing mutations in the 1 % of the human genome that codes for protein [6]. Thus, it is likely that whole genome approach will replace whole-exome sequence analysis in order to identify disease-causing mutations; by the way, also neutral variability in the human genome (e.g., see http://www.1000genomes.org/) will be investigated and further available to facilitate knowledge of disease modifying variants in non-coding sequences. This may further allow systematic elucidation of monogenic disorders as a clue to understand the pathogenesis of complex diseases [7].

Genetic association and linkage studies thereby comprise the two dominant strategies: association studies aim to find disease-predisposing alleles (from single nucleotide polymorphisms (SNPs) or microsatellite markers) at the population level, whereas linkage studies focus on familial segregation. A novel approach is a family-based association study design [8, 9]. Arrhythmia predisposition, e.g. acquired QT prolongation or torsade de pointes during treatment with cardiac and non-cardiac drugs, is still a major challenge for physicians. Recent advances in knowledge on the genomic and physiologic regulation of myocardial repolarization suggest that common alterations of cardiac (ion channel) genes might be associated with slight electrophysiologic changes and an increased susceptibility for ventricular arrhythmia. The extent, to which common genetic factors play a role, is under current investigations and remains to be determined. To date, no prospective data are available that link presence of certain SNP genotypes with a favorable or worse arrhythmia outcome.

Human Genome and Single Nucleotide Polymorphisms (SNPs): A Revival in Genomic Medicine

The annotated draft sequence of approximately three billion base pairs (bp) of the human genome has been completed earlier than expected [1, 3]. This was a major scientific and technologic development for researchers with an interest in the molecular bases of rare and common disorders, since awareness of the genomic diversity and molecular differences are expected to help in the understanding of role of a genetic contribution between individuals and disease [2]. The variations at the nucleotide level are implemented to determine the physiological differences and individual phenotypic variance, including major biological functions at the cellular and body level. **Single nucleotide polymorphisms (SNPs)** were the first type of genetic markers that were used to make chromosomal genetic maps [10]. However, due to their lower degree of heterozygosity and genetic informativeness when compared with polymorphic length (repeat) markers, SNPs became temporarily less attractive, until the completion of the human genome was done. In general, SNPs are single nucleotide base substitutions at a certain gene or genomic position and represent the major part of interindividual variability that accounts for only 0.1 % of genome sequences between individuals in health and disease. These small differences in the genetic code can be linked with unique personal features (e.g., eye color, tallness, …) and alterations of regular physiologic function, varied response to environmental conditions and predisposition for certain diseases. Of the approximate 10^6 million SNPs in the human genome, only a fraction is directly associated with functional significance and related to complex traits so far. Thus, the complexity of the entire human genome map is undermined by distinct effects of SNPs that

Table 21–1 Types and sequence location of DNA variation [11]

Polymorphism type	Sequence location	Predicted protein and potential functional effects	Occurrence in genome	Potential disease impact
Nonsense	Coding	Prematurely truncated, most likely loss of protein function	Very low	High
Missense, non-synonymous	Coding, non-conserved	Altered amino acid chain, mostly similar protein properties	Low	Low (to high)
Missense, non-synonymous	Coding, conserved	Altered amino acid chain, mostly different protein properties	Low	Medium to high
Rearrangements (insertion/deletion)	Coding	Altered amino acid chain, mostly different protein properties	Low	High
Sense, synonymous	Coding	Unchanged amino acid chain, rarely an effect on exon splicing	Medium	Low (to medium)
Promotor and regulatory sequences	Non-coding, promotor/UTR	Unchanged amino acid chain, but may affect gene expression	Low to medium	Low to high, depending on site
Intronic nucleotide exchange (<40 bp)	Non-coding, splice/lariat sites	Altered amino acid chain, failed recognition of exonic structure	Low	Low to high, depending on site
Intronic nucleotide exchange (>40 bp)	Non-coding, between introns	Unchanged amino acid chain, rarely abnormal splicing or mRNA instability, site for gene rearrangements	Medium	Very low
Intergenic nucleotide exchange	Non-coding, between genes	Unchanged amino acid chain, may effect gene expression, site for gross rearrangements	High	Very low

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Abbreviations: *UTR* untranslated region (5′ or 3′ region of a gene), *bp* base pairs

depend on the nucleotide subtype, their genomic location and effect on the protein structure/function, their abundance (allele frequency) and contribution to subchromosomal compartments of SNPs in linkage disequilibrium (haplotypes). SNPs differ from their location within the genomic sequence (coding vs. non-coding areas), from the type of nucleotide exchange and the consequence for the amino acid sequence, and from the frequency (relative occurrence) in the human genome (Table 21.1). Polymorphisms with the potentially highest phenotypic disease impact are rare within the genome [11]. A well-recognized example is variation in the Factor V gene (e.g., the Leiden variant) and its association with deep vein thrombosis. An understanding of the genetic diversity and of its contribution to variations in normal and abnormal physiology will have a potentially powerful effect on cardiovascular and genomic medicine.

Genetic association studies (or: case-control studies) are an analysis of statistically significant relationships between SNP alleles and phenotypic differences. The power of a genetic association study is a direct function of the number and quality of the SNPs used to screen a population for phenotypic variability. SNPs and haplotypes can vary in their prevalence among different populations. Thus, a SNP associated with a particular phenotype or quantitative trait in one population may not have the same frequency or effect in another population, e.g., when the population is of different ethnicity, age or gender. Large datasets of chromosomal SNPs have been published since 2000 [1, 13–17], along with improved methods to screen immense numbers of SNP candidates. More than three million variants have been reported and are catalogued in public databases (e.g., http://www.ncbi.nlm.nih.gov/projects/SNP/). Newer techniques allow high-throughput genotyping to study simultaneously large numbers of SNP loci (currently: >4.0 M markers per sample/chip; e.g., HumanOmni5-Quad, Illumina Inc.) and are based on matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF; e.g., Sequenom MassARRAY), pyrosequencing, or hybridisation.

A huge and as yet unsolved problem is the identification of clinically relevant mutations in

a plethora of functionally neutral single nucleotide polymorphisms. Common SNPs can be filtered out through comparison with genomes and exomes from healthy individuals (e.g., http://www.1000genomes.org/) [18] or dbSNP (see http://www.ncbi.nlm.nih.gov/projects/SNP/, despite an increasing contamination with clinically relevant mutations), but this approach is not possible for the many rare SNPs in the human genome. Indeed, comprehensive NGS-based reanalyses have recently found that 12 % of the previously reported mutations are not disease-causing itself [19]. In principle, large-scale whole-genome sequencing may reduce the number of novel variants from 3.4 million to a mere 150.000 per genome. Therefore, sequencing 100,000 individuals and comparing the results with their complete medical records (e.g., see http://www.personalgenomes.org/), would identify the vast majority of changes that do not give rise to disease. A comparable project is the NHLBI GO Exome Sequencing Project that focuses to discover novel genes and mechanisms contributing to heart, lung and blood disorders by pioneering the application of next-generation sequencing of the protein coding regions of the human genome across diverse, richly-phenotyped populations. These datasets and findings – obtained from potentially affected individuals – are also shared with the scientific community (Exome Variant Server; http://evs.gs.washington.edu/EVS/) and now showed that rare variants (e.g., less than 1 in 1,000 alleles) can be commonly identified in many cardiovascular genes. The presence of these databases, in contrary, enhances the need for certified and proven mutation databases for genes, such as such as the Human Gene Mutation Database (HGMD, see http://www.hgmd.org/), or potentially the Human Variome Project (http://www.humanvariomeproject.org/) or the Human Genome Organization (http://www.hugo-international.org/). Without theses, the clear clinical significance of many genetic variants and the role of the relevant genes in disease may remain uncertain for a long time. Similarly to SNPs and recently being more recognized [2], also many genomic imbalances were recurrently detected and were found in both, patients and healthy individuals (see central databases like Decipher, http://decipher.sanger.ac.uk/ or the Database of Genomic Structural Variations, http://www.ncbi.nlm.nih.gov/dbvar).

The clinical use of SNPs is still far away from being established, at least in arrhythmia prediction. This might be related to some inherent limitations with SNP studies [20, 21]. The two major issues are *statistical power* and *replication* of genetic findings in another, independent population set of same origin to avoid population stratification. In association studies, the prevalence of genetic marker alleles in unrelated subjects with a certain phenotype and (unaffected) controls will be compared and aim to correlate differences in disease frequencies between groups (or in trait levels for continuously varying characters) with differences in allele frequencies at an SNP. Thus, the frequencies of the two variant forms (alleles) of an SNP are of primary interest for identification of genes affecting disease. The traditional 'case-control' approach assumes that any noted difference in allele frequencies is related to the outcome measured and that there are no unobserved confounding effects. Unfortunately, allele frequencies are known to vary widely within and between populations, irrespective of disease status. For an appropriate study, an adequate sample size of the groups and a relatively high frequency of the minor SNP allele (to facilitate detection of allele frequency differences between the investigated populations) are needed. Usually, haplotype tagging SNPs (tagSNPs) were selected on chip-based arrays to systematically analyze nearly every genes approach. Typical criteria for tagSNP selection are a pairwise-only tagging with $r^2 > 0.8$ and a minor allele frequency (MAF) >0.1. Studies with small sample sizes may commit **type II errors**, i.e., not declaring a statistically significant result when there may be a difference. These underpowered studies can be misleading because genes may be undetected, and reporting of the odds ratio and 95 %-confidence interval are recommended [22]. The term β is defined as the chance of making a type II error. Values for β are typically 10–20 %, meaning a power $(1 - \beta)$ between 80 and 90 %. In contrast, a sample size that is much larger than required may declare small differences to be statistically significant and thus commit **type I errors** (i.e., declaring a statistically significant difference when it may

not be present). The term α refers to the chance of making a type I error; usually, a level of 0.05 or less is chosen. Due to the increasing, but also inconsistent number of GWAS publications, proposed guidelines have been developed which should facilitate the quality of association studies [23, 24], including strategies to ascertain *heritability and exact phenotyping of a trait*, to perform *population stratification of cases and controls* (ethnicity, age and gender distribution), *to select physiologically and genetically meaningful markers*, to address the *probability of association*, and to *replicate* initial results in independent studies [25, 26]. A p-value $<5\times10^{8}$ is considered as statistically significant for GWAS results. This quite stringent significance threshold, that is frequently used when studying samples of European ancestry, accounts for about 1,000,000 independent common variant tests in the human genome. To date, only a few of the several thousand published association studies strictly meet the criteria to ascertain a ('true') genetic association. For arrhythmogenic disorders, first studies exist [27–30], but the majority of data is still unreplicated by independent approaches. Differences in study outcome may be related to population stratification, study design, still inappropriate marker selection, and lack of statistical power [11]. Discovery of meaningful SNP markers [31], e.g., indicating an elevated risk of SCD, is still far from being established. Common weaknesses of many association studies include study design failing to adequately identify true positives while eliminating false positives, poorly defined phenotypes and sampling from heterogeneous patient populations, inappropriately matched controls, small sample sizes relative to the magnitude of the genetic effects, failure to account for multiple testing, population and sample stratification, failure to replicate marginal findings and overemphasizing interpretation of study results. In the past, the optimum study design for association studies has been discussed because, often, studies were prone to population stratification and biased or spurious results. Thus, replication of the findings from genetic association studies in other populations became a cornerstone for the data quality, and, so far, only a few studies merit these criteria. In this line, a shift from case-control and cohort studies towards family-based association designs has been noted. These study designs have fewer problems with population stratification, but have greater genotyping and sampling requirements, and data can be difficult or impossible to gather.

Analysis of SNPs in Cardiac Arrhythmogenesis: Towards a Dissection of Common ECG Traits

Phenotypic variation in arrhythmia development is well known from families with inherited, arrhythmogenic disorders that have demonstrated an important phenotypic spectrum of the same mutation in affected family members [32, 33]. Recent reports have highlighted the importance of a family history of sudden death as a risk for **ventricular fibrillation** (VF) in patients experiencing acute myocardial infarction (AMI), pointing to the possibility of a genetic predisposition. Familial aggregation demonstrated an increased risk of SCD among patients with a parental history of cardiac arrest [34, 35], but a clearly defined genetic basis is not known to date [36]. Sudden death was found to share the same profile of risk factors for coronary artery disease and, thus, was not specifically predictable in the general population. These observations are also recognized from in patients with more polygenic disorders, such as myocardial infarction, for which not every patient develops ventricular fibrillation during acute ischemia [37, 38]. In a case-control study in patients with a first ST-elevation myocardial infarction (STEMI) and similar infarct sizes and locations, it was recently shown that (cumulative) ST-segment elevation was significantly higher among cases and that familial sudden death occurred more frequently among cases than controls [37]. Two population-based studies of the late 1990s demonstrated an increased risk of SCD in first-degree relatives of SCD victims and provided some evidence that genetic components may be involved in SCD of unknown (probably atherosclerotic) origin [35, 39]. A family history of MI/SCD was associated

with SCD (RR = 1.57), after adjustment for other common risk factors and person–years at risk among (first degree) relatives [39]. After differentiating between family history of MI or of SCD, the positive family history of early-onset SCD finally was associated with a 2.7-fold increase in risk of SCD. In victims of VF in the setting of their first, acute MI it has been also reported that SCD of degree relative is a strong risk factor for ventricular fibrillation (OR = 2.72) [37].

Thus, arrhythmia development may have a common and modifiable substrate in both, rare inherited (monogenic) and common (polygenic) forms of various arrhythmias and a positive family history can be noted in both. In addition, multiple factors – such as age, gender, and environmental condition – play an important role in the modulation of the phenotype. Structural and electrical remodeling during acute ischemia, altered hemodynamic loads, or changes in neurohormonal signaling are recognized key features that alter ion channel gene expression. Down-regulation of major repolarizing potassium currents, I_{to}, I_{Kr}, I_{Ks}, and I_{K1}, has been described in several models of heart failure and resembles a condition of "acquired QT prolongation" and reduced, but reversible repolarisation reserve [40]. Cellular abnormalities through disturbances in the electrical cell-cell coupling and a local reduction of conduction velocity facilitate re-entrant ventricular arrhythmias. These cellular abnormalities can be found in the structurally diseased heart. The extent of genetically controlled variation is not clear to date, but it is of potential interest and under recent investigations. Of note, some studies already focused on associations of SNPs in ion channel genes and a relation with myocardial infarction. Since KATP channels are involved in membrane regulation during metabolic stress, studies focused to identify variants in the *KCNJ11* gene associated with SCD after myocardial infarction [41]. These channels are composed of four pore-forming Kir6.2 (KCNJ11) subunits and four sulfonylurea receptor subunits (SUR2A); sarcolemmal KATP channels regulate membrane potential and action potential duration, whereas the mitochondrial KATP channels are involved in ischemic preconditioning. So far, two non-synonymous polymorphisms (R371H, P266T) in two highly conserved pore regions are known that showed altered modulation by intracellular ATP and protons and differences in channel density [42] and, thus, are potential candidates for genetically determined electrophysiologic differences under ischemic conditions. Interestingly, mutations in the *KCNJ8* gene have been associated with idiopathic ventricular fibrillation [43–45]. Phase 2 re-entry is a key mechanism for ventricular fibrillation complicating acute myocardial infarction as well as arrhythmias associated with Brugada syndrome. In this line, a heterozygous SCN5A gene mutation (G400A, located in *cis* with the H558R polymorphism) was reported in a patient who developed an arrhythmic electrical storm during acute myocardial infarction and suggested a hidden genetic predisposition to the severity of arrhythmias that in the setting of acute myocardial ischemia [46]. Another study indicated significant changes (up to 63 % down-regulation) of sodium channel transcription in dependence of the SNP composition within the potential SCN5A promoter region when investigated by transient transfection of promoter-reporter constructs in CHO cells or in neonatal cardiomyocytes [47]. These results may further support a concept of interindividual variability in transcription of this cardiac ion channel gene and arrhythmogenesis.

Population-based studies for SCD are ongoing to highlight potential causes among patients with a positive parental history of cardiac arrest [34, 35], but a clearly defined genetic basis is not known to date [36]. In contrast to patients with cardiac dysfunction, in patients without intraventricular conduction defects or a normal cardiac function, QTc prolongation is a non-negligible risk factor for sudden cardiac death independent of age, history of myocardial infarction, heart rate, and drug use. This has been shown in the Rotterdam Study, a prospective population-based cohort study, in which 125 patients died of sudden cardiac death (mean follow-up 6.7 years) and a prolonged QTc interval had a threefold increased risk [36, 48, 49]. So far, first GWAS have been directed to evaluate the role of common genetic variation in modulation of SCD or VF risk [38, 50, 51]. Since the causes and confounding factors for SCD are

diverse, it is not unexpected to note that in two GWAS studies reported [38, 50], did not confirm and replicate each other, i.e., the SNP at chromosome 2q24.2 (rs4665058) at the *BAZ2B* gene locus was not seen in the Dutch case-control set. Similarly, the *CXADR* gene signal was not detected in the study involving European ancestors; this may be due to several factors, including not only insufficient statistical power and random chance, but also differences in study design (population stratification) and phenotype definition. Future studies, entailing expression quantitative trait locus (eQTL) analysis in cardiac tissue as well as the genome-wide identification by ChiP-Seq of regulatory regions occupied by transcriptional enhancers and transcription factors [52] will shed additional light on the pathophysiology.

Recently, a quantitative influence of ion channel gene variation on the myocellular repolarization has been described in twins [29] and in the general population [27, 28, 30]. Of note, the heritability reflecting the degree of variance in ECG indices between individuals is for the QTc interval in the range of 25–50 % and for the PR interval between 34 and 40 %, at least depending on the population set studied, see [53]. Genomic studies are currently on the way to narrow candidate these gene regions and to identify these variants (SNPs or haplotype constellations) in coding and non-coding sequences. An example has been shown very recently [54]; in the study by Amin and co-workers sequence variance of the 3′-UTR at the LQT-1 (*KCNQ1*) locus was investigated by microRNA binding sites that may influence *KCNQ1* expression of mutant or wild-type allele. As a novel concept, three single nucleotide polymorphisms (rs2519184, rs8234, and rs10798) were associated in an allele-specific manner with QTc and symptom occurrence and, intriguingly, with concordant, but altered gene expression upon luciferase reporter assays. These data raised the idea that clinical disease expression may be a function of the ratio between normal and mutant allele expression and other factors [54].

It has long been surmised that **drug-induced torsade de pointes** is an acquired condition that may occur in the context of a mutation encoding for a cardiac ion channel gene responsible for repolarization. This was enhanced due to the recognition of LQTS gene mutation carriers with normal or nearly normal ECGs (incomplete or non-penetrance) [55, 56]. First reports on patients with drug-induced QT interval prolongation and LQTS ion channel gene mutations and were reported nearly a decade ago [57–59] and are listed in Table 21.2. In at least 15–20 % of patients LQTS genes mutations can be found [60], even some reports are indicative for a higher ratio [73, 74]. Altogether, these factors further diminish 'repolarization reserve' [75] to a critical extent and allow the generation of afterdepolarizations and triggered activity preceding torsade de pointes. The observation that in the majority of patients with idiosyncratic drug reactions and TdP development a LQTS gene mutation cannot be found, is possibly related to undetected mutations in these, predisposing variants or other target genes responsible for cardiac repolarization [11].

Heritability and a **quantitative influence on the QT interval** has been described particularly in twins [29] as well as in the general population [27, 28, 30, 76]. In consequence, a 'common variant – common phenotype' hypothesis has been proposed that implies the influence of frequent (ion channel and other) genetic variance (e.g., SNPs) to modulate the QT interval in terms of a quantitative trait. The expectation is that multiple SNPs, whether alone or in combination, have protective or deleterious effects on the QT interval [27]. A series of genome-wide or candidate gene SNP studies have been conducted and tested for the propensity of single SNPs to modulate repolarization or the arrhythmia phenotype. In Fig. 21.1, those with a significant p-value ($<10^{-8}$) are shown. Some of them are known in close location of cardiac ion channel and LQT genes, others not. Meanwhile, also in cardiac ion channels common protein variants (non-synonymous SNPs) have been identified and were seen in a setting with QT drug prolonging drugs (Table 21.2). Yang et al. screened the coding regions of the three major LQT genes (LQT1-3) in 92 patients with drug-induced LQTS and controls [59]. The allele frequencies of three, common, non-synonymous polymorphisms (SCN5A-H558R, SCN5A-R34C, HERG-K897T), however, did not significantly differ between the

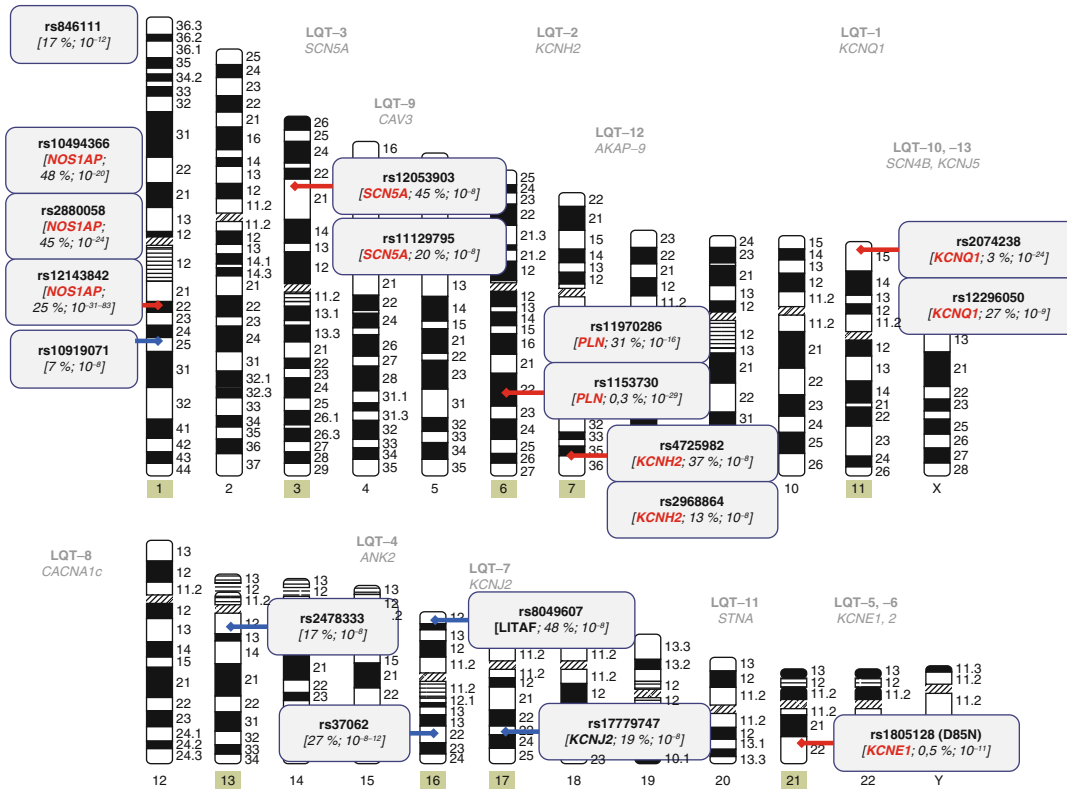

Figure 21–1. Human chromosomes (ideograms) and SNPs (rs identifier; gene symbol in *red*; minor allele frequency [%]; p-value, only $<10^{-8}$) with a reported effect on QT interval duration

two groups. Similar findings were reported by others [77]. In the African population, a particular SNP in the cardiac sodium channel gene *SCN5A* (LQT-3) gene was reported to predispose to prolong the QT interval, which appears to be ethnic-related (SCN5A_S1102Y) [78]. This SNP that was primarily found in West African and Caribbean (10.1 % frequency for Y1102 allele) increases the risk of cardiac arrhythmias in the presence of drugs such as amiodarone. SCN5A_Y1102 itself does not cause LQTS, but induces a small and potentially inherent and chronic risk of acquired arrhythmia in the setting of additional risk factors, such as medications, hypokalemia, or structural heart disease. Additional, longitudinal studies are still required to confirm the predictive utility of the Y1102 allele. Meanwhile, an association of SCN5A_Y1102 with sudden cardiac death and sudden infant death in blacks [79–81] has been described; in-vitro data indicate susceptibility for repolarization delay and arrhythmia during acidotic/ischemic environmental conditions. Recently, another propensity for ventricular arrhythmias in black patients with heart failure and reduced ejection fraction was proposed [82]. Moreover, gene-gene interaction intragenic variance may affect function of wild-type sodium (SCN5A) channels and modulate the cardiac arrhythmia phenotype. This particularly refers to naturally occurring splice variants of LQTS genes [83–85] upon quantitative mRNA analysis from the cardiac tissue and to the observation that these common variants had a different electrophysiologic behavior than full-length clones. Of interest, baseline differences in isoform-mediated sodium currents were profoundly modulated when a common SCN5A polymorphism (SCN5A_H558R) was present and exhibited functional differences. In addition to SCN5A_Y1102, a series of other, non-synonymous SNPs have been noticed in the sodium channel [86, 87]

Table 21–2 Ion channel gene variants identified in patients with drug-induced QT prolongation or TdP occurrence

Gene	Current	Amino acid alteration	Drug/setting	Functional assay	Minor allele	Reference
KCNE2	I_{Kr}	T8E	Trimethoprim (TMX)/ sulfa-methoxazole (SMX); quinidine; amiodarone	E8-MiRP1 weakly reduced I_{Kr} current peak density; (CHO cells); SMX and TMX had almost no effect on wild-type channels, but SMX was reported to inhibit more than 50 % of A8-MiRP1 at −40 mV. Mutant channels were 4× more sensitive to SMX than wild-type	1.6 %	[60–62]
	I_{Kr}	Q9E	Clarithromycin, low K^+		Rare in Caucasians, but not in Afro-Americans	[62, 63]
	I_{Kr}	M54T	Procainamide	T54-MiRP1 significantly reduced I_{Kr} current peak density (CHO cells). No influence on drug-related channel inhibition was seen	Rare	[61]
	I_{Kr}	M57T	Oxatomide	T57-MiRP1 significantly reduced I_{Kr} current peak density (CHO cells). No influence on drug-related channel inhibition was seen	Rare	[61]
	I_{Kr}	A116V	Quinidine	V116-MiRP1 significantly reduced I_{Kr} current peak density (CHO cells). No influence on drug-related channel inhibition was seen	Rare	[61]
KCNH2	I_{Kr}	M124T	Probucol	Co-expression of wild-type HERG and T124-HERG resulted in markedly smaller amplitudes of I_{Kr} (Xenopus oocytes). Probucol decreased the amplitude of the HERG tail current, decelerated the rate of channel activation, accelerated the rate of channel deactivation, and shifted the reversal potential to a more positive value	Rare	[64]
	I_{Kr}	R328C	Not reported		Rare	[65]
	I_{Kr}	P347S	Cisapride/clarithromycin		Rare	[60, 66]
	I_{Kr}	R486H	Quinidine		Rare[b]	[57]
	I_{Kr}	A561P	Clobutinol	P561-HERG led to an intracellular trafficking defect; when co-expressed with wild-type HERG, voltage-dependence was shifted towards more negative potentials (3–3.5-mV). Clobutinol further blocked heteromeric channels	Rare[b]	[67, 68]
	I_{Kr}	R784W	Amiodarone	W784-HERG mediated a reduced I_{Kr} current (by ~75 %) and a positive shift of voltage dependence of activation	Rare	[59]
KCNQ1	I_{Ks}	R243H	Halofantrine; hydrochinine		Rare[a, b]	[57]
	I_{Ks}	Y315C	Cisapride	In-vitro expression of mutant KvLQT1 protein showed a severe loss of current with a dominant negative effect on the WT-KvLQT1 channel	Rare[b]	[58]
	I_{Ks}	R555C	Terfenadine		Rare	[69, 70]
	I_{Ks}	R583C	Dofetilide	C583C-KvLQT1 mediated I_{Ks} was reduced by ~50 % compared with wild-type, and the voltage dependence of activation was shifted positively by 19.6 mV (CHO cells)	Rare	[59]
SCN5A	I_{Na}	G615E	Quinidine	E615-SCN5A indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	[59]
	I_{Na}	L618F	Quinidine	E615-SCN5A indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	[59]
	I_{Na}	S1103Y	Amiodarone	Y1103-SCN5A mediated an increased I_{Na} channel activation (HEK 293)	7–10 % (Afro-Americans or West Africans/ Caribbeans only)	[59]
	I_{Na}	F1250L	Sotalol	E615-SCN5A indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	[59]
	I_{Na}	P1825L	Cisapride	The C-terminal mutant P1825-SCN5A mediated I_{Na} current with slow decay and prominent TTX-insensitive, non-inactivating component (gain-of-function), a reduced peak density (loss of function), shifted voltage dependence of activation (more positive potentials) and of inactivation (more negative potentials) (tsA-201 cells)		[71]
				P1825-SCN5A channels showed impairment of intracellular trafficking (CHO cells) and failed to generate QT prolongation. Exposure with cisapride rescued cell surface expression of P1825-SCN5A and exaggerating the LQT3 phenotype		[72]

[a]Reported from recessive forms of LQTS

[b]Heterozygous mutation carriers may have a normal QT interval; for SCN5A, amino acid residues are numerated according to position on the long splice variant

and other LQTS genes [61, 63], but a role as a modulating component of repolarization is undetermined. Very recently, a set of 176 drug-induced LQTS patients as genotyped for a total of 1,424 single nucleotide polymorphisms in 18 candidate genes (among them 1,386 SNPs tagging common haplotype blocks) were compared to controls [88]. After all, the *KCNE1* gene polymorphism D85N (rs1805128) was present in 8.6 % of cases, 2.9 % of drug-exposed controls, and 1.8 % of population controls (odds ratio of 9.0) and suggestive for a susceptibility allele that may be associated with the rare adverse drug reaction torsade de pointes.

In the first study of the general (healthy) population, the influence of 174 (out of around 270 possible) common LQTS gene variants (LQT-1, -2, -5, -6) on the QT interval were investigated in a total of 3,966 unrelated individuals from the general population [27]. Using a two-step design and a population-based linear regression formula to calculate gender- and age-specific QT values (named QTc_RAS), four SNPs (one in intron 1 of LQT-1 gene, one 5′ of LQT-5, KCNH2_K897T, and another one in the same KCNH2 haploblock, respectively) were detected with a slightly lower QTc_RAS value (for each of the four SNPs < 2.0 ms). Genetic association data were not reported for the more commonly used QT formulas. In another publication [28], confirmed linkage was found between a 5′ SNP (rs10494366) in NOS1AP gene with QT interval with an average genetic effect for QTc_RAS of ~4 ms. NOS1AP (CAPON) is a regulator of neuronal nitric oxide synthase, as a new target that potentially is involved in modulating cardiac repolarization and the minor allele of the NOS1AP genetic variant was reported to explain around 1.5 % of QT interval variation [28]. In a subsequent study, replication of this association was addressed in the Old Order Amish, a genetically isolated population, where a heritability of the QT interval was 0.50–0.09 [89]. Two of the four NOS1AP SNPs were significantly associated with variation in adjusted QT interval and explained a fraction of 0.9 % of QT interval variability, with an average genetic effect on adjusted QT of 6.1 ms [89]. Subsequently, NOS1AP SNPs were proposed to influence the clinical course in congenital LQTS and sudden cardiac death in blacks [90, 91], probably through the modulation of L-type channel activity [92]. Meanwhile, increasing numbers of publications using GWAS approaches in large population sets [93–95] are indicating the presence of small-sized SNP effects (1–10 ms) on the QT interval (see Fig. 21.1 and [53]). The majority of these SNPs are located in intronic regions and further studies are needed to evaluate the mechanisms for repolarisation modulation.

Future Directions

Recent advances in knowledge of the genome structure of ion channel genes and their physiologic role in myocardial repolarization have shown that genetic alterations of these key molecular components are associated with slight in-vitro effects and changes in fine tune of normal repolarization. The extent to which minor genetic factors altogether are associated with susceptibility to arrhythmias remains to be determined, but first evidence is present. Following the concept of 'repolarization reserve' [40], it is likely that torsade de pointes occurrence, arrhythmia occurrence during acute myocardial infarction or drug response (as well as side effects) are also dependent on an individual genetic background. Genetics of arrhythmogenesis switches from gene identification and single pathway understanding to genomic medicine by integrating complex gene and environmental information. Future research will

- identify all relevant genes and their genomic structure for repolarization,
- determine the extent of the variability of the QT interval and of the response to action potential-prolongation that is genetically controlled,
- investigate the role of functionally relevant SNPs and haplotype constellations in LQTS and other gene loci for their quantitative contribution to repolarization,
- integrate identified genetic factors with other known factors for cardiac risk, according to their relative importance, in a network algorithm for arrhythmogenesis.

These data should be available within the next few years and advances, along with additional technological improvements in DNA analysis and data management. It is expected that next-generation sequencing technologies (e.g., targeted re-sequencing of ion channel genes) will booster knowledge for individual arrhythmia predisposition and will enable researchers to lower the costs of complex genotyping and to implement these data into a personalized, genomic-oriented medicine. Apart from the 'common variant-common disease' hypothesis, the increasing evidence for a role of 'rare variants in common disease' will be elucidated [96–99].

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Electrophysiological Remodeling in Heart Failure

Fadi G. Akar and Gordon F. Tomaselli

Abstract

Ion channel remodeling in heart failure modulates key cellular electrophysiological properties, predisposing to arrhythmias and sudden death. Heart failure induced ion channel dysfunction prolongs the action potential, increases spatio-temporal gradients of repolarization, promotes arrhythmogenic triggers and results in conduction abnormalities. Understanding fundamental ionic mechanisms of normal and abnormal electrogenesis is a key requirement for the development of effective and safe therapies. Elucidating the underlying molecular mechanisms and functional consequences of ion channel remodeling at the cellular and organ levels presents a unique opportunity for the development of novel pharmacological, device, gene, and cell based approaches for the treatment of arrhythmias in heart failure.

Keywords

Heart failure • Cardiac resynchronization therapy • Arrhythmias • Ion channels • Conduction • Repolarization

Introduction

Heart failure (HF) claims over 200,000 lives annually in the US alone [1]. Approximately 50 % of these deaths are sudden and unexpected, and presumably the consequence of lethal ventricular arrhythmias [2]. Our limited understanding of fundamental arrhythmia mechanisms at the ionic and molecular levels has hampered the development of effective pharmacological treatments for these patients [3]. In fact, the proarrhythmic tendency of ion channel targeting agents has resulted in the premature termination of the Cardiac Arrhythmia Suppression Trial (CAST) [4]. These proarrhythmic effects are due to the heterogeneity of ion channel expression and function [5, 6], the nonspecific nature of most pharmacological

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agents that target ion channels, the cross-talk between individual ion channels, and the complex remodeling that occurs dynamically in the context of left ventricular dysfunction. Indeed, elucidation of arrhythmia mechanisms in HF at the basic ionic level is expected to improve existing pharmacological therapies and facilitate the design of novel approaches, including cell- and gene-transfer strategies designed to target ion channels and transporters. In this chapter, we focus on key HF-induced ion channel remodeling at the cellular level and its pro-arrhythmic manifestations at the tissue level, with the intent of identifying molecular targets for anti-arrhythmic therapy in HF.

Ionic Basis of the Ventricular Action Potential

A unique signature of any excitable tissue is its action potential (AP) profile, which reflects a delicate balance in the activity of several depolarizing and repolarizing ion channels, transporters and exchangers. Myocytes are characterized by a long AP, which is initiated by the influx of sodium ions (Na^+) when voltage-gated Na^+ channels (encoded by $Na_v1.5$) open. The transient opening of these channels gives rise to a rapid phase of depolarization (phase 0) which is terminated by the inactivation of the fast inward Na^+ current (I_{Na}) within a few milliseconds. The AP upstroke is followed by a brief interval of early repolarization (phase 1), caused by the activation of the voltage-gated transient outward potassium (K^+) current (I_{to}). Influx of calcium ions via L-type Ca^{2+} channels carries a small depolarizing current ($I_{Ca\text{-}L}$) which stimulates the release of calcium from the sarcoplasmic reticulum (SR) though ryanodine receptors (RyR2). This regenerative process known as calcium-induced calcium-release results in tropomyosin translocation and myofilament contraction. Cytosolic Ca^{2+} levels are restored to diastolic levels by the coordinated activities of the Na^+–Ca^{2+} exchanger (NCX) and the SR calcium ATPase (SERCA2a). A delicate balance between inward and outward currents results in the plateau phase of the AP. Finally, AP repolarization is sculpted by the orchestrated activities of the delayed rectifier K^+ currents (I_{Kr} and I_{Ks}), the inward rectifier K^+ current (I_{K1}) along with a gradual decrease in net depolarizing currents. The membrane potential of fast response myocytes is effectively locked at rest (phase 4) by the background I_{K1} until the next wave of depolarization activates I_{Na} resulting in a new propagating AP.

Since most ion channels are time- and voltage-dependent, even a subtle change in one ionic transport mechanism can modulate the activity of many other channels and transporters, often profoundly altering the AP duration and profile. As in the long QT syndrome, HF results in a major prolongation of the cellular AP, which translates clinically into QT interval prolongation on the surface electrocardiogram (ECG). Numerous studies using cellular electrophysiology, molecular biology, protein chemistry, high-throughout gene expression profiling and proteomics have considerably advanced our understanding of ion channel remodeling in HF [5, 7]. Complementary studies in multicellular tissue preparations are bridging major gaps in our knowledge of the functional consequences of ion channel remodeling and dysfunction in generating arrhythmias [8–10]. In this Chapter, we summarize some of the key developments in this rapidly evolving field of investigation, with a view towards identifying appropriate ion channel targets for antiarrhythmic therapy in HF.

Potassium Channel Remodeling in Heart Failure

Transient Outward K^+ Current

I_{to} inscribes the notch of the AP during early repolarization and sets the membrane potential at which activator calcium is initiated. As such I_{to} indirectly controls excitation–contraction coupling and AP duration by modulating the take-off potential at which subsequent plateau currents are activated [11]. I_{to} varies regionally (right ventricle > left ventricle) and transmurally (epicardium > midmyocardium > endocardium). Functional down-regulation of I_{to} is consistently observed in most models of HF [7]. The effect of HF-induced I_{to} down-regulation on

AP duration varies across species. Since I_{to} is the major repolarizing current in rodents (with the exception of guinea pigs), its reduction is directly responsible for AP prolongation in these species [12]. On the other hand, in humans and large animal models reduction of I_{to} influences the early part of the AP (phase 1), reducing Ca^{2+} entry through $I_{Ca\text{-}L}$ and shortening the AP duration [13]. The discrepancy in how I_{to} modulates AP duration highlights the importance of exercising caution when applying knowledge gained from rodent studies of repolarizing currents to humans before carefully investigating large animal models.

Kv4.3 (Shal-related subfamily, member 3) encodes the pore-forming alpha subunit of cardiac I_{to} in large mammals [14]. An additional component of I_{to} (encoded by *Kv1.4*) with slower inactivation kinetics also exists preferentially in the endocardial layer of rodents [15]. The role of *Kv1.4* channels in large animals and humans remains unclear. Despite abundant expression of *Kv1.4* in the myocardium, we recently argued against its functional role in the formation of native I_{to} in human and canine ventricular myocytes [16]. Our findings highlight important differences in the molecular composition of I_{to} and other repolarizing currents across species. Such species differences may profoundly impact the response to pharmacological agents targeting ion channel components. I_{to} down-regulation in HF is transcriptionally regulated as *Kv4* mRNA levels are reduced in both humans [14] and dogs with HF [6]. Interestingly, gene transfer of *Kv4.3* shortened AP duration and limited the hypertrophic response in rat hearts that underwent transaortic constriction [17]. The effects of *Kv4.3* over-expression in large animal models whose terminal repolarization is sculpted by delayed rectifier K^+ currents, however, remain unknown.

Other accessory subunits participate in the formation of native I_{to}, including Kv-channel interacting proteins (KChIPs) [18] and the CD26-related dipeptidyl aminopeptidase-like protein 6 (DPPX) [19]. Interestingly, both of these accessory subunits likely contribute to the formation of the transmural I_{to} gradient [20, 21]. Recently, another beta subunit (MiRP-1; potassium voltage-gated channel subfamily E member 2) originally known to modulate I_{Kr} has also been shown to physically associate with, and functionally regulate Kv4- and Kv1-encoded channels in mammalian expression systems [22]. Elucidation of the macromolecular complex that forms native I_{to} is expected to offer new opportunities for regulating this current, which is strongly remodeled by HF, and whose dysregulation has been linked to various arrhythmic syndromes [23, 24]. The strong influence of KChIP2 and MiRP-1 on multiple ion channels suggests that macromolecular complexes involving the same accessory subunits could indeed regulate diverse electrophysiological properties [18, 22]. As such, selective targeting of these proteins is expected to produce complex physiological outcomes that are difficult to predict, especially in the setting of HF, in which these subunits are differentially remodeled.

Inward Rectifying K^+ Current

I_{K1}, encoded by the *Kir2.x* family of genes, maintains the resting membrane potential and contributes to terminal repolarization. Reduced I_{K1} density in HF promotes AP prolongation and enhanced susceptibility to spontaneous membrane depolarizations [25, 26]. Indeed, the genetic suppression of I_{K1} converts fast-response myocytes to cells with automatic activity (i.e. biological pacemakers) [27]. Disease-induced downregulation of I_{K1} could, therefore, enhance automaticity in the failing heart. Interventricular differences in I_{K1} density (left ventricle > right ventricle) underlie the localization of high-frequency sources of activation during ventricular fibrillation [28]. Moreover, pharmacological suppression of I_{K1} reduces the dominant frequency of the so-called 'mother rotor' underlying arrhythmias in structurally normal hearts [28]. Conversely, overexpression of I_{K1} leads to the acceleration and maintenance of fibrillatory rotors [29]. While I_{K1} down-regulation promotes triggers and enhanced automaticity, I_{K1} overexpression can shorten the AP and the cardiac wavelength, thereby stabilizing reentrant activity. This delicate balance supports the notion that ion channels are regulated to operate within a narrow physiological range, such that excessive modulation (reduction or enhancement) of ion channel activity or expression can have equally

deleterious effects. This property adds another challenge to pharmacological and gene transfer approaches targeting ion channels, whose expression is differentially altered across myocardial layers and regions of the failing heart.

The underlying basis for I_{K1} downregulation in HF remains unknown, as no consistent changes in the steady-state levels of *Kir2.1* (inward rectifier potassium channel 2) mRNA [14] or protein [6] have been found. Surprisingly, *Kir2.1* mRNA and protein levels are up-regulated in terminally failing human hearts [30]. Clearly, further investigation into the molecular identity and regulation of I_{K1} by HF is warranted.

Delayed Rectifier K^+ Currents

The delayed rectifier K^+ currents (I_{Kr} and I_{Ks}) play a prominent role in the control of terminal cardiac repolarization in large animals and humans. In fact, a relatively small reduction of either component leads to significant AP prolongation [31]. The reduction in the outward current, over the plateau range of voltages in various models of HF, predisposes to the development of arrhythmogenic early afterdepolarizations (EADs) [8, 32]. Indeed, K^+ current downregulation in HF underlies key phenotypic similarities between HF and the long QT syndrome, including AP and QT-interval prolongation, reduced repolarization reserve and arrhythmia propensity [33, 34].

Despite knowledge of the major alpha and beta subunits that form I_{Kr} and I_{Ks}, the molecular mechanisms underlying I_{Kr} and I_{Ks} downregulation in HF remain controversial. We measured the expression of *HERG* (encoding I_{Kr}) and *KvLQT1* (encoding I_{Ks}) in normal and failing canine ventricles. Surprisingly, we found a paradoxical increase in *HERG* expression with no change in *KvLQT1* protein levels [6]. Other groups reported decreased *HERG* expression in human HF [35]. These discrepant findings highlight the fact that ion channel function is dependent on complex factors that are well beyond the expression levels of the alpha and beta subunits that form these channels. For example, changes in protein assembly, folding, trafficking, membrane insertion, internalization, and degradation can significantly modulate channel behavior.

Evidence is emerging for the existence of macromolecular complexes that include diverse proteins in the proper assembly, trafficking and function of cardiac ion channels, such as I_{Kr} and I_{Ks}. Interestingly, a strong physical and functional interaction between *KvLQT1* and *HERG* was recently demonstrated [36]. Specifically, *KvLQT1* was shown to modulate both the distribution and biophysical properties of *HERG*. Indeed, this elegant demonstration of an interaction between two K^+ channel alphasubunits underscores the complexity of selectively targeting individual ion channels without affecting others [36].

Gene transfer of various alpha and beta subunits that encode I_{Kr} and I_{Ks} has been used to modulate AP duration in small animal models [26, 37]. Whether or not these strategies can reverse remodel the failing heart, normalize AP duration, reduce repolarization gradients, and prevent arrhythmias remain to be seen. Indeed, the complex regulation of ion channel function by post-translational modifications may strongly limit the ability of gene transfer of individual alpha and beta subunits to completely and safely restore ion channel function in the failing heart.

ATP-Sensitive K^+ Current

Sarcolemmal (Sarc) K_{ATP} channels link membrane excitability to metabolism [38]. They are regulated by intracellular nucleotides, membrane phospholipids, protein kinases and phosphatases [38]. The ATP-sensitive K^+ current ($I_{K\text{-}ATP}$) is the principal mediator of AP shortening under conditions of increased metabolic demand and/or energy deficit. Due to their abundance in the plasma membrane, the opening of sarcK_{ATP} channels causes rapid AP shortening, loss of intracellular K^+, and reduction in myocyte excitability. Interestingly, AP shortening in ischemia is exaggerated in cells from hypertrophied compared to normal ventricles [39]. We described a novel mechanism by which oscillations in the mitochondrial membrane potential ($\Delta(\text{delta})\Psi(\text{psi})_m$) under conditions of oxidative stress, can produce oscillations in the AP duration via cyclical activation of $I_{K\text{-}ATP}$, setting the stage for conduction block (which was termed 'metabolic sink') and arrhythmias [40, 41]. While hypertrophied and

failing hearts are associated with oxidative stress and $\Delta(\text{delta})\Psi(\text{psi})_m$ instability [42, 43], the exact role of sarcK_{ATP} channels in HF-mediated arrhythmias remains unclear.

Pacemaker Current

The hyperpolarization-activated pacemaker or 'funny' current (I_f) is a nonselective cation current originally described in automatic tissues such as the sinoatrial node [44]. Although I_f has been observed in human ventricular myocytes [45, 46], it appears to activate at negative voltages outside the physiological range of membrane potential. Nonetheless, increased I_f density in hypertrophy suggests its possible role in either promoting disease progression or arrhythmic triggers [47]. The *HCN* (hyperpolarization-activated mammalian cation channels) family of genes encoding I_f has been cloned [48, 49]. In a canine model of HF, *HCN* expression is significantly decreased in the sinoatrial node and increased in the right atrium, likely contributing to sinus node dysfunction and atrial ectopy [50].

Adenoviral-mediated gene transfer of an engineered *HCN* construct, which exhibits a shift in its inactivation kinetics to more depolarized potentials, unleashes a biological pacemaking source in guinea pig ventricles and pig atria [51]. Increased I_f in the setting of reduced I_{K1} is likely to predispose the failing heart to enhanced automaticity. A strategy for suppressing ventricular I_f could, therefore, be appealing in HF. Indeed, Ivabradine, a potent I_f inhibitor that suppresses the spontaneous depolarization rate in sinoatrial nodal cells, improved cardiac structure and function in HF [52]. The **S**ystolic **H**eart Failure Treatment with the $\boldsymbol{I_f}$ Inhibitor **T**rial (SHIFT) has shown significant efficacy in reversing left ventricular remodeling in a large cohort of patients with HF [53].

Calcium Channel Remodeling in Heart Failure

Excitation–contraction coupling refers to the fundamental principle by which a myocyte's ionic (excitation) properties tightly coordinate its mechanical function. Defective Ca^{2+} handling in HF has a profound electrophysiological impact because the intracellular calcium transient and the AP are intricately linked by a variety of Ca^{2+}-mediated channels and transporters.

L-Type and T-Type Ca^{2+} Channels

Ca^{2+} entry through $I_{Ca\text{-}L}$ triggers SR Ca^{2+} release. The density of $I_{Ca\text{-}L}$, dictated in part by the stage of HF [54, 55], is increased in mild-to-moderate hypertrophy and decreased in more advanced stages of hypertrophy and failure [56, 57]. Interestingly, myocytes from failing hearts also exhibit attenuated augmentation of $I_{Ca\text{-}L}$ in response to beta-adrenergic stimulation [58]. Finally, slowing of $I_{Ca\text{-}L}$ inactivation in HF alters Ca^{2+} handling and prolongs the AP [59]. The molecular mechanisms underlying these changes are unknown, but could involve a dephosphorylation defect that alters open channel probability [60]. Sipido et al. demonstrated that the negative force–frequency relationship in dilated cardiomyopathy is due to impaired recovery of $I_{Ca\text{-}L}$ inactivation at fast stimulation frequencies. These findings provided a potential ionic target for improving myocardial contractile reserve [61].

The pore-forming subunit of $I_{Ca\text{-}L}$ is encoded by *CACNA1C* (calcium channel, voltage-dependent, L type, alpha 1C subunit) and is highly regulated by a variety of accessory subunits that affect channel trafficking, current density and kinetics. *In vitro* and *in vivo* knockdown of the L-type Ca^{2+} channel accessory beta subunit, using a short hairpin RNA template sequence, reduced $I_{Ca\text{-}L}$ and attenuated the hypertrophic response, without compromising systolic performance [62].

Another class of sarcolemmal calcium channels is the low-voltage-activated transient Ca^{2+} channel ($I_{Ca\text{-}T}$), encoded by the *Cav3.x* family of genes [63]. Since these channels activate at hyperpolarized potentials, they might contribute to enhanced automaticity [64], especially in the failing heart, in which $I_{Ca\text{-}T}$ density is considerably enhanced [65]. Interestingly, $I_{Ca\text{-}T}$ was observed in myocytes from endocardial, but not epicardial, layers of the normal canine ventricle [66]. As such, $I_{Ca\text{-}T}$ could also contribute to the electrical and contractile heterogeneity seen

across the transmural wall. Whether or not this intrinsic heterogeneity is increased or decreased in HF remains unknown.

Since abnormal calcium cycling is a central feature of mechano-electrical dysfunction of the failing heart, pharmacological and genetic modulation of individual Ca^{2+} channel subunits could be useful strategies for restoring defective excitation-contraction coupling and electrophysiological properties. However, these strategies have the strong potential to exacerbate intracellular calcium overload and associated dysfunction (increased calcium entry) or result in atrioventricular conduction delays and block (decreased calcium entry).

Sarcoplasmic Reticulum (SR) Ca^{2+} Pump

The amplitude and rate of decay of the intracellular calcium transient are blunted in cells and tissues from failing hearts [67]. These changes are caused by defective sequestration of Ca^{2+} by the SR due, in large part, to reduced SERCA2a expression and function [68]. Since compromised SR Ca^{2+} re-uptake causes abnormal contractile and electrical function in HF, increasing the expression and activity of SERCA2a could be clinically beneficial. Pharmacological stimulation of the pump enhances mechanical function [69]. Furthermore, numerous studies using adenoviral-mediated gene transfer approaches to increase myocardial SERCA2a expression, have demonstrated the efficacy of restoring impaired intracellular Ca^{2+} handling and normalizing contractile dysfunction [70]. The safety of this approach has been confirmed by experiments that demonstrated improved contractility at no metabolic cost (i.e. cost of oxygen) in normal [71] and failing hearts [72]. Targeted gene-transfer techniques to increase the expression levels of SERCA2a using adeno-associated vectors have also been developed [72], and a First-in-man multicenter trial (CUPID, **C**alcium **U**pregulation by **P**ercutaneous Administration of Gene Therapy **I**n Cardiac **D**isease) has been completed [73, 74]. Whether or not enhancing SERCA2a expression and/or function in the failing heart will protect against or exacerbate arrhythmias remains to be fully determined, although no adverse electrical outcomes were reported in the Phase 1 and Phase 2a CUPID trials [73, 74]. Finally, SERCA2a over-expression using gene transfer has been recently shown to suppress arrhythmogenic repolarization alternans in structurally normal hearts [75].

Phospholamban

Phospholamban (PLB), a key regulator of SERCA2a, blunts the rate of SR Ca^{2+} re-uptake [76]. The inhibitory influence of PLB on SERCA2a is reduced when the protein is phosphorylated [77]. Therefore, restoration of intracellular calcium cycling in HF could be achieved by targeting PLB. To that end, PLB silencing using an anti-sense strategy successfully enhanced SERCA2a activity and improved contractile function [78]. In contrast, PLB gene ablation in a transgenic mouse model failed to improve global cardiac function, possibly because of compromised metabolic properties in that animal model [79]. Pharmacological manipulation aimed at dissociating PLB from SERCA2a has also been successfully tested in the laboratory [80]. Potential differences in the electrophysiological consequences of enhancing SR calcium uptake by targeting SERCA2a versus PLB requires direct investigation. Indeed, it is expected that these complementary approaches may be associated with unique benefits and potential pitfalls depending on the disease etiology and the sympatho-adrenergic state, as these calcium regulatory proteins are modulated by local kinase and phosphatase signaling mechanisms.

Na^+/Ca^+ Exchanger

In its 'forward' mode of operation, NCX extrudes intracellular Ca^{2+} via an electrogenic exchange for extracellular Na^+ (one Ca^{2+} for three Na^+ ions), thereby generating a net inward current [81]. Indeed, forward-mode NCX function compensates for defective SR Ca^{2+} uptake at the expense of depleting the releasable pool of Ca^{2+} with repetitive stimulation. In contrast, reverse-mode exchange (Na^+ out and Ca^{2+} in) could provide inotropic support to the failing ventricle while shortening AP duration [82].

NCX expression and function are increased in HF [83, 84], contributing to AP prolongation and

repolarization instability. Importantly, partial inhibition of NCX enhances SR Ca^{2+} load by shifting the balance of Ca^{2+} flux away from transsarcolemmal efflux [85]. As such, NCX blockade could represent an effective therapeutic strategy for improving contractility in HF [85]. Unfortunately, the efficacy of abrogating arrhythmias by targeting NCX will have to await the development of more selective pharmacological agents with improved lipophilicity and bioavailability properties.

Ryanodine Receptors

RyR2 channels are downregulated in HF, both at the mRNA [86] and protein [87] levels. Hyperphosphorylation of RyR2 causes FKBP12.6 (FK506-binding protein 1B) dissociation resulting in diastolic Ca^{2+} leak and the generation of Ca^{2+} waves underlying triggered activity [88]. Whether protein kinase A [88], CaMKII [89], or both mediate RyR2 hyperphosporylation and diastolic calcium leak in HF has been the subject of intense debate. Identification of the specific molecular sites of RyR2 phosphorylation might lead to pharmacological strategies designed to suppress diastolic Ca^{2+} leak and associated arrhythmias. Marks and colleagues identified a class of small molecules that enhance the binding affinity of FKBP12.6 for RyR2 [90]. While these compounds are effective in suppressing catecholaminergic polymorphic ventricular tachycardia in mouse models [91, 92], their utility in preventing HF-related arrhythmias remains to be tested in clinically relevant large animal models of acquired structural heart diseases.

Calmodulin and Calcium/Calmodulin-Dependent Protein Kinase II

Calmodulin kinase II (CaMKII), the main target of Calmodulin binding, is a multifunctional protein capable of phosphorylating several Ca^{2+}-handling proteins, including RyR2, PLB, and SERCA2a. In human HF, CaMKII activity is markedly increased [93, 94], possibly as a compensatory mechanism for altered Ca^{2+} homeostasis. Since phosphatase activity is also enhanced in human HF, the net effect with respect to the phosphorylation state of any given target protein is locally controlled within the subcellular milieu [95]. Over-expression of CaMKII has been shown to increase SR Ca^{2+} leak through RYR2, without altering myocyte contractility [96]. Anderson and colleagues demonstrated that CaMKII inhibition could represent an effective strategy for improving myocardial function and preventing atrial and ventricular arrhythmias in various animal models [97].

Sodium Channel Remodeling in Heart Failure

In light of the important interplay between Na^+ and Ca^{2+} in the cardiac cell, changes in intracellular Na^+ levels are expected to alter both mechanical and electrophysiological properties [98]. Moreover, as Na^+ channels are critical to normal impulse propagation in the heart, perturbations in I_{Na} could lead to conduction slowing, block, and arrhythmias. In what follows, we summarize some of the major changes in Na^+ transport mechanisms that occur in HF.

Na^+ Channels

Normal impulse formation and conduction depend on the fast inward I_{Na}. An increase in the late component of this current can markedly prolong AP duration and promote polymorphic ventricular tachycardia [99]. Changes in I_{Na} density and kinetics could, therefore, promote arrhythmias, either by disrupting conduction or prolonging repolarization. In a canine model of myocardial infarction, major downregulation of I_{Na}, acceleration of its inactivation kinetics and slowing of its recovery from inactivation were observed in myocytes isolated from the infarct border zone [100]. In another canine model of repeated microembolization-induced HF, a considerable increase in the late component of I_{Na} was demonstrated [101]. As such, changes in I_{Na} are likely to depend on the specific disease etiology, and could have profound implications for arrhythmogenesis given the relative abundance and importance of this current to myocyte excitability.

Over-expression of $Na_V1.5$ (also known as *SCN5A*), which forms the alpha subunit of I_{Na} exerted an antifibrillatory effect by enhancing excitability in monolayers of neonatal rat ventricular myocytes [102]. In addition to *Nav1.5*, I_{Na} activity is modulated by a variety of auxiliary beta subunits, kinases, phosphatases and cytoskeletal proteins. HF-induced disruption of the macromolecular complex that regulates I_{Na} results in marked changes in current density and kinetics, which alter conduction (by affecting the amplitude of I_{Na}) and repolarization (by affecting the late component of I_{Na}). Finally, pharmacological inhibition of the late I_{Na} with Ranolazine has shown significant promise in improving mechano-electrical function and suppressing atrial and ventricular arrhythmias [103–106]. Indeed, a prospective, proof-of-concept study (RAnolazine for the Treatment of Diastolic Heart Failure, RALI-DHF) was recently initiated to determine the efficiacy of late I_{Na} blockade in improving diastolic function in HF patients with preserved ejection fraction [107].

Na^+/K^+ ATPase

Intracellular Na^+ is increased in ventricular hypertrophy and HF by approximately two to three fold [108]. This causes a secondary rise in Ca^{2+} influx via reverse-mode NCX [108], which in turn increases SR Ca^{2+} load and enhances contractility. This seemingly beneficial, positive inotropic effect comes at a price — enhanced susceptibility to spontaneous SR Ca^{2+} release through RyR2, activation of the transient inward current and the development of delayed afterdepolarizations, especially in the setting of beta-adrenergic stimulation [81]. Hence, tight regulation of intracellular Na^+ is required for normal electrogenesis.

The Na^+/K^+ ATPase exchanges extracellular K^+ for intracellular Na^+, with a stoichiometry of 2:3, thereby generating a net outward repolarizing current. Reduced Na^+/K^+ ATPase activity in some models of HF contributes to intracellular Na^+ and Ca^{2+} overload, AP prolongation and arrhythmias. Molecular mechanisms underlying altered Na^+/K^+ ATPase activity may include changes in the expression of its three alpha isoforms. In addition, major changes in the expression and phosphorylation of Phospholemman, a key regulatory subunit of the Na^+/K^+ ATPase pump have been identified in a rabbit model and in human HF samples. Indeed, recent advances in our understanding of the regulation and physiological properties of Phospholemman under normal and stress conditions are likely to yield new opportunities for controlling the Na^+/K^+ ATPase pump, intarcellular Na^+ homeostasis and electromechanical function in HF.

Na^+–H^+ Exchanger

The Na^+–H^+ exchanger (NHE) regulates intracellular pH via proton extrusion, driven by the transmembrane Na^+ gradient. NHE inhibition is cardioprotective against myocardial ischemia–reperfusion injury [109]. Prevention of intracellular Na^+ accumulation and excessive Ca^{2+} influx via reverse-mode NCX, have been proposed as the mechanism of cardioprotection by NHE inhibition. Since HF is associated with elevated intracellular Na^+ levels, NHE could represent a promising target for antiarrhythmic therapy. The **GUARD** During **I**schemia **A**gainst **N**ecrosis (GUARDIAN) trial was designed to determine the efficacy of Cariporide, a selective NHE inhibitor, in reducing mortality. Although GUARDIAN failed to demonstrate an overall clinical benefit in patients at risk of myocardial necrosis [110], it is clear that NHE inhibition preserves mitochondrial function and therefore the metabolic status of the myocyte. As such, NHE inhibition is likely to play a critical role in preventing cellular apoptosis and related dysfunction under conditions of oxidative stress, such ischemia-reperfusion injury. The role of NHE inhibition in modulating the electrophysiological substrate and preventing arrhythmias in HF requires direct exploration. Given the central role of mitochondria in regulating excitability and arrhythmias, it is conceivable that NHE inhibition may play a prominent role in restoring normal electrogenesis to the failing heart.

In what follows, we illustrate how ion channel remodeling at the cellular level alters the electrophysiological substrate at the organ level.

Particularly, we focus on mechanisms by which transmural and intra-ventricular repolarization gradients predispose the failing heart to lethal arrhythmias.

Electrophysiological Remodeling at the Tissue Level

Abnormal AP prolongation at the cellular level readily translates to the level of the intact organ resulting in a long and variable QT interval on the surface ECG [2, 111, 112]. Key changes in the early and late phases of AP repolarization have been documented in numerous studies using the patch clamp technique in isolated cardiomyocytes from various small and large animal models of HF. At the opposite end of the spectrum, studies in humans and animal models showed delayed global repolarization and enhanced temporal repolarization instability using clinical non-invasive metrics, such as the QT-interval variability index and T wave alternans on the surface ECG. Rosenbaum, Berger, and others have developed sophisticated algorithms that detect various ECG metrics of global cardiac repolarization to identify patients at high risk of sudden cardiac death (SCD) [113–122]. Of key importance are recent findings of a multi-center clinical trial (**A**lternans **B**efore **C**ardioverter **D**efibrillator, ABCD), in which non-invasive T wave alternans testing was shown to significantly enhance the predictability of impending SCD in patients with HF when combined with standard electrophysiological testing [116].

These cellular and clinical studies highlight the importance of repolarization changes occurring at the tissue level for arrhythmia genesis in HF. Until relatively recently, efforts to investigate the mechanistic link between repolarization changes and reentrant arrhythmias were hampered by technical difficulties in assessing spatio-temporal repolarization gradients across the heart. With the advent of optical imaging techniques using voltage sensitive dyes, a high-resolution measurement of cardiac repolarization at a cellular level within the intact syncytium has become possible [8, 9, 87, 123, 124]. Importantly, a quantitative relationship between altered spatio-temporal repolarization gradients and the incidence of arrhythmias in various animal models of HF have recently emerged [8, 125]. In what follows, we focus on the role of spatial heterogeneities of repolarization on the incidence of reentrant arrhythmias in clinically relevant large animal models of HF that are prone to arrhythmias. Specifically, we discuss changes in transmural and intra-ventricular repolarization gradients as mechanisms for ventricular arrhythmias in non-ischemic dilated cardiomyopathy and dyssynchronous heart failure, respectively.

Transmural Repolarization Heterogeneity in Dilated Cardiomyopathy

Antzelevitch and colleagues [126, 127] advanced the notion that heterogeneities of cellular repolarization in different cell types (epicardial, mid-myocardial, and endocardial) represent a unifying mechanism underlying a host of arrhythmias in congenital and/or acquired cardiac diseases, such as the long QT, short QT [128], Brugada [129], Andersen-Tawil [130], and Timothy syndromes [131, 132]. Of particular importance was the role of mid-myocardial (M) cells in the establishment of transmural repolarization heterogeneity under conditions of prolonged QT interval in various *ex vivo* models of the long QT syndrome [127, 133, 134]. Because QT interval prolongation represents an electrophysiological hallmark of the failing heart, we hypothesized that both disease states (long QT syndrome and HF) may share important phenotypic properties at the multi-cellular tissue level that predispose them to arrhythmias via similar mechanisms [8]. This hypothesis was directly investigated by determining the functional expression of repolarization gradients across myocardial layers and their potential role in the mechanism(s) of arrhythmias in a model of non-ischemic dilated cardiomyopathy produced by chronic rapid pacing in the dog [8]. As expected, HF was associated with a marked AP prolongation across all myocardial layers, consistent with findings in isolated myocytes and

whole animals. Interestingly, AP prolongation was heterogeneous across the left ventricular (LV) wall, affecting mid-myocardial and endocardial muscle layers more selectively; thereby, increasing the effective transmural repolarization gradient by ~2-fold [8]. In support of transmural dispersion of repolarization as a unifying mechanism for arrhythmias associated with various disease etiologies, Yan et al. elegantly demonstrated that LV hypertrophy in a rabbit model of renovascular hypertension was also associated with significant enhancement of transmural dispersion of repolarization because of selective prolongation of subendocardial APs [135]. Transmural repolarization heterogeneity in HF produced an arrhythmic substrate, as premature stimuli introduced at a critical window during AP repolarization resulted in intramural decremental conduction and block. Importantly, conduction block which was localized at the interface of the mid-myocardial layer was followed by the initiation of ventricular tachycardia.

Mechanisms underlying increased transmural repolarization heterogeneity in HF remain unresolved. These changes, however, likely involve multiple factors, including heterogeneous remodeling of cell-to-cell coupling, ionic currents/exchangers, and calcium handling proteins. Li et al. [32] investigated the ionic basis of transmural AP remodeling in HF by measuring the density of key repolarizing K^+ currents, including I_{to}, I_{K1}, I_{Kr} and I_{Ks}. In general, K^+ current changes were uniform in epicardial, mid-myocardial, and endocardial myocytes of failing hearts, indicating that transmural repolarization heterogeneity observed at the tissue level could not be explained by cell-type specific remodeling of repolarizing K^+ currents. In a subsequent study, we measured the expression levels of key alpha and beta subunits encoding these K^+ currents in the three principal myocardial layers of normal and failing hearts [136]. In support of the findings of Li et al. [32] we also did not find a K^+ channel molecular basis (neither at the mRNA or protein levels) for the enhanced transmural repolarization heterogeneity observed in the failing heart.

Poelzing and Rosenbaum [137] attributed the location of the maximum transmural repolarization gradient to increased electrical resistivity at that location. Furthermore, they converted transmural repolarization gradients measured across normal preparations into gradients that mimicked those in HF simply by perfusing normal preparations with the gap junction inhibitor, Carbenoxolone [137]. These findings highlight the potential importance of gap junction uncoupling in the mechanism of increased transmural dispersion of repolarization in HF. We and others have investigated the molecular basis for gap junction uncoupling in HF and have found major changes in the expression, distribution, and phosphorylation state of the main ventricular gap junction protein, Cx43 that develop with varying time-courses during disease progression [9, 125, 138, 139]. Specifically, end-stage HF was associated with over-all Cx43 downregulation, dephosphorylation, and lateralization. In addition, we recently reported the loss of interaction between Cx43 and the cytoskeletal protein, ZO-1 as a potentially critical event underlying severe conduction slowing and therefore gap junction uncoupling at late stages of remodeling in a model of pressure overload hypertrophy [125]. Interestingly, hyperphosphorylation of Cx43 also occurred at earlier stages of remodeling that were associated with a milder form of conduction slowing [125]. As such disrupted phosphorylation (either increased or decreased) at critical residues within the carboxyl domain of Cx43 may lead to loss of gap junction function via distinct mechanisms. The individual contribution of these complex molecular changes to the establishment of transmural repolarization heterogeneity across the failing heart requires direct investigation.

Intra-ventricular Repolarization Heterogeneity in Dyssynchronous Heart Failure

Left ventricular (LV) dyssynchrony caused by delayed activation of the lateral free wall results in heterogeneous mechanical stress across the ventricle [140]. We hypothesized that regional differences in local mechanical function across the dyssynchronously contracting failing ventricle produce important changes in electrophysiological, metabolic, and molecular properties, which ultimately worsen outcome in

these patients. We further hypothesized that re-establishing LV synchrony using bi-ventricular pacing in cardiac resynchronization therapy (CRT) could potentially reverse-remodel the electrophysiological substrate. Indeed, the late activating lateral LV endocardium of dyssynchronously failing hearts is distinguished by selective remodeling of calcium handling, gap junction, and stress related molecules [141]. These regionally heterogeneous molecular changes were linked to key electrophysiological abnormalities. Specifically, Aiba et al. [142] found that myocytes isolated from the lateral LV of dyssynchonrously failing hearts were characterized by excessive AP prolongation and enhanced propensity for early after depolarization mediated triggered beats. The ion channel basis of these changes was also investigated. Specifically, dyssynchronous mechanical contraction of the failing heart reduced K^+ current densities (I_{to}, I_K, and I_{K1}) in both anterior and lateral regions. In contrast, $I_{Ca\text{-}L}$ was differentially remodeled across the dyssynchronously failing heart, potentially underpinning differences in repolarization properties and susceptibility to early afterdepolarizations. In particular, a significantly greater reduction in $I_{Ca\text{-}L}$ amplitude and decay rate in myocytes isolated from the high-stress lateral compared to the low-stress anterior LV wall was described. Interestingly, CRT partially restored abnormal repolarization of lateral LV myocytes, reducing the regional disparity in electrophysiological function across the failing ventricle.

In a subsequent study, the effect of mechanical dyssynchrony on changes in regional gene expression was also investigated [143]. Electromechanical dyssynchrony resulted in complex cardiac transcriptome remodeling, causing major gene expression changes in the anterior wall. Once again, CRT corrected the region specific alterations in gene expression, highlighting the importance of mechanical synchrony/dyssynchrony on global and regional gene expression remodeling, which is likely to impact diverse cellular processes. As such, the electrophysiological and molecular changes induced by CRT may indeed suppress ventricular arrhythmias and potentially promote mechanical and metabolic function.

Finally, the influence of mechanical dyssynchrony per se on key electrophysiological properties was investigated independently of LV dysfunction [144]. Normal hearts were subjected to chronic left bundle branch block, inducing mechanical dyssynchrony with preserved LV function. We found that mechanical dyssynchrony in non-failing canine hearts was sufficient to cause regional changes in conduction and repolarization properties [144]. In addition, the distribution of the main ventricular gap junction protein, Cx43 was significantly more lateralized in the late-activating lateral LV, which was associated with slower conduction and faster repolarization [144]. Taken together, these findings suggest an important mechanosensitive component to chronic remodeling associated with dyssynchronous LV contraction which causes regional changes in protein expression and electrophysiological properties that enhance intra-ventricular repolarization gradients and potentially predispose to reentrant arrhythmias. Understanding mechanisms by which local mechanical stresses and strains regulate electrophysiological properties offers the potential for designing novel device-based strategies for the control of arrhythmias in heart failure.

Conclusions

Complex remodeling of a host of ion channels and Ca^{2+}-cycling proteins modulates key cellular electrophysiological properties, predisposing to arrhythmias and sudden death. HF-induced ion channel dysfunction prolongs the AP, increases spatiotemporal gradients of repolarization, promotes arrhythmogenic triggers and results in conduction abnormalities. Understanding fundamental ionic mechanisms of normal and abnormal electrogenesis is a key requirement for the development of modern therapies. Elucidating the role of individual current components and their underlying molecular identities presents a unique opportunity for the development of novel pharmacological, device, gene, and cell based approaches for the treatment of arrhythmias in HF.

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23
Ventricular Electrical Remodeling in Compensated Cardiac Hypertrophy

Vincent J.A. Bourgonje, Toon A.B. van Veen, and Marc A. Vos

Abstract

Ventricular hypertrophy is an adaptation of the heart that develops in response to congenital or acquired pathologies to reduce wall stress and to maintain cardiac output. These adaptations can be successful (compensated) or inadequate, leading to deterioration of cardiac function, resulting in heart failure. A model of compensated hypertrophy is the dog with chronic AV-block (CAVB), in which cardiac remodeling occurs after AV-block, without deterioration towards heart failure.

Electrically, the most notable change is a lengthening of the QT-interval on the ECG. This is due to a lengthening of the action potential, which in turn depends on down regulation of repolarizing potassium currents. This long-QT phenotype leads to increased propensity for Torsade de Pointes (TdP) arrhythmias.

Contractile remodeling is observed in an increased systolic calcium concentration, while diastolic levels are not elevated, leading to improved contraction and increased dP/dT in the left ventricle. The increased calcium transient is linked to an increased loading of the sarcoplasmic reticulum.

Structurally, remodeling is characterized by hypertrophy of both ventricles. However, this is not accompanied by increased fibrosis or decreased intercellular coupling or conduction.

Intracellular signaling in the CAVB dog is reminiscent of exercise-induced hypertrophy. Calcineurin is not activated, while the CaMKII signaling cascade is interrupted at the HDAC4 level. Akt however, likewise in physiological hypertrophy, is activated.

The precise mechanism of arrhythmogenesis in this model is unclear, but could be due to an L-type calcium window current or an increased sodium late current, as inhibition of these currents is anti-arrhythmic. Reentry is probably not involved, as conduction is not slowed. Also, the presence of early afterdepolarizations in isolated cardiomyocytes suggests triggered activity as a leading mechanism instead.

Concluding, the compensated hypertrophy in the CAVB dog is accompanied by electrical remodeling, leading to a severely increased sensitivity for arrhythmias.

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Introduction

Ventricular hypertrophy is an adaptation of the heart that develops in response to either congenital or acquired pathologies in order to reduce wall stress [1] and to maintain cardiac output. Initially, the term remodeling was reserved for structural changes (hypertrophy, dilatation). More recently, it has been recognized that adaptive mechanisms are present on different levels (structural, contractile, and electrical) [2]. After an insult, these adaptations can be successful (compensated) or inadequate leading to deterioration of cardiac function, resulting in heart failure (Fig. 23.1a). Structurally, the compensated heart has regained the balance between wall thickness and internal cavity dimension [3], whereas the failing heart becomes quite dilated in time [4] (Fig. 23.1b). On the cellular level, there is an increase in the duration of the cellular action potential [5] (electrical remodeling, Fig. 23.1c) that is accompanied by an increased intracellular calcium transient [6], or by a severely depressed one [7, 8] (contractile remodeling). In case of the latter, the intracellular diastolic calcium levels ($[Ca^{2+}]_i$) are often elevated too [7] (Fig. 23.1c). Also the signal transduction pathways involved in the remodeling process may differ [9, 10]. It is known that exercise leads to a physiological hypertrophy in which the pAkt pathway is dominant [11, 12], whereas heart failure or pathological hypertrophy is ruled, among others, by the calcineurin [13, 14] and possibly the

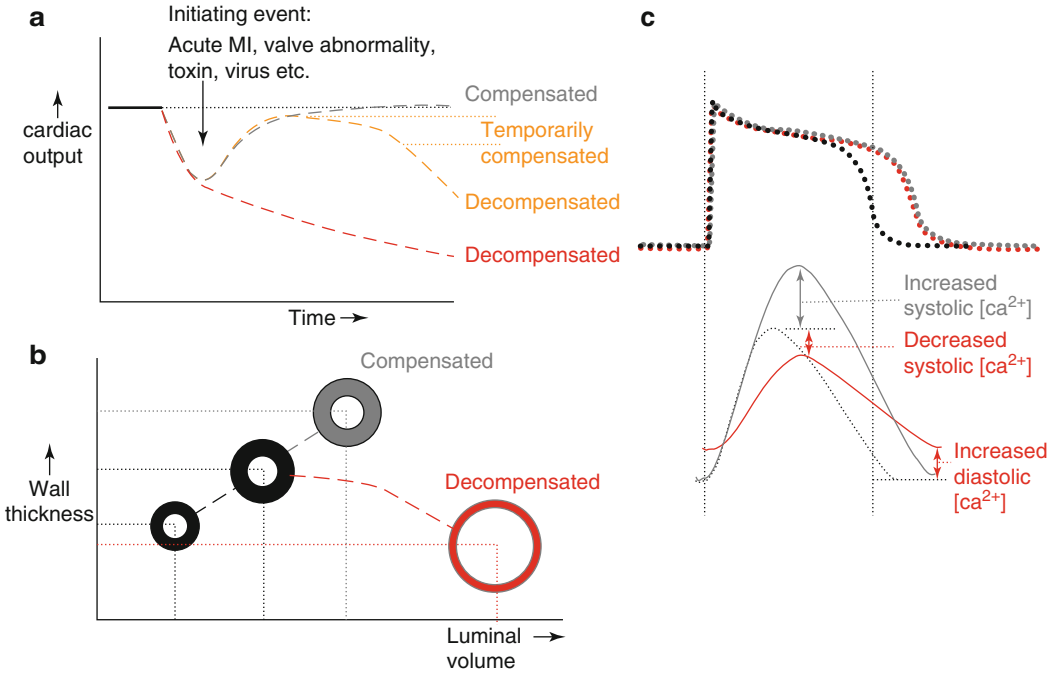

Figure 23–1. Differences between decompensated and compensated hypertrophy. (**a**) Evolvement of cardiac output over time in compensated (*grey line*) and decompensated (*yellow* and *red lines*) hypertrophy after an initiating event. (**b**) In compensated hypertrophy wall thickness and luminal volume increase (*grey circle*), while in decompensated hypertrophy dilatation occurs (*red circle*). (**c**) *Lower part*: the differences in intracellular calcium levels at baseline (*black*) and in compensated (*grey*) and decompensated (*red*) hearts. In the *upper part* the accompanying cardiac action potentials are shown for reference

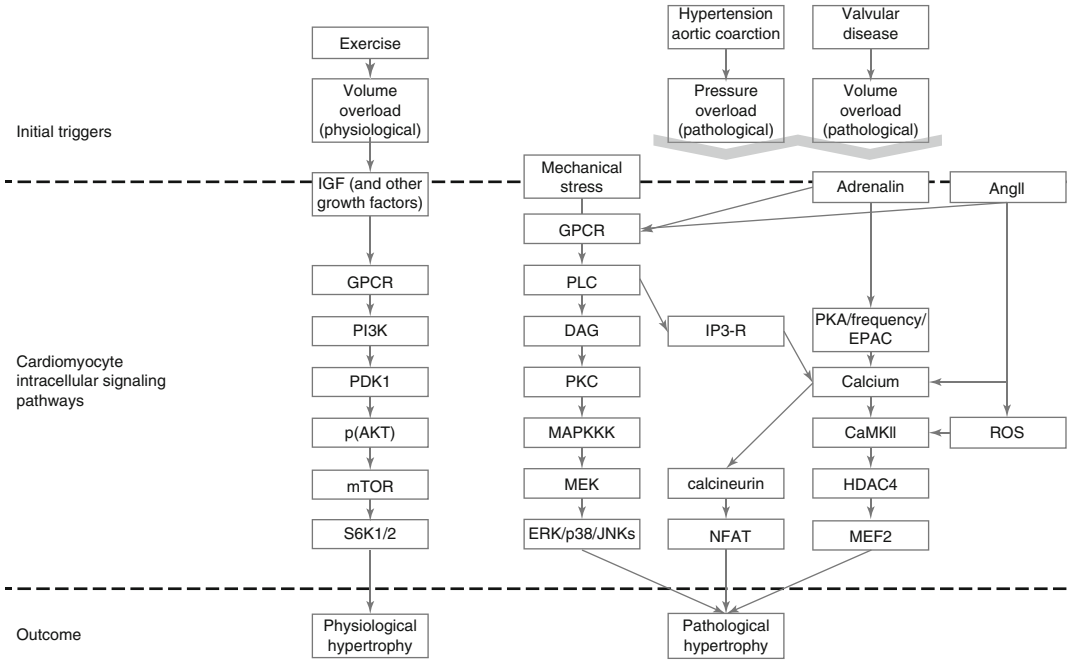

Figure 23–2. The intracellular signal transduction pathways involved in cardiac hypertrophy leading to either a more physiological or pathological phenotype

Ca^{2+}-calmodulin-kinase (CaMKII) pathways [15] (Fig. 23.2).

Ventricular remodeling is known to increase the propensity for ventricular arrhythmias and may lead to sudden cardiac death. Despite a clear association between left ventricular mechanical dysfunction, hypertrophy and ventricular arrhythmias [16], the majority of sudden cardiac death occurs at earlier stages of the disease process, even in circumstances in which mechanical dysfunction or ventricular hypertrophy, are absent [16– 18]. The mechanisms by which contractile and electrical remodeling predispose to arrhythmias remain unclear, but the changes in $[Ca^{2+}]_i$ handling and repolarization as seen in the compensated form (Fig. 23.1c) may contribute. Therefore, in this chapter, we will concentrate on a canine model of complete atrio-ventricular block (CAVB) in which the beneficial adaptations leading to compensated biventricular hypertrophy are accompanied by a detrimental one, being an increased susceptibility for repolarization related ventricular arrhythmias. We will discuss the specific remodeling changes that are related to this phenotype.

Ventricular Remodeling in the CAVB Dog

Creation of chronic CAVB by radiofrequency ablation results in numerous adaptations initiated to overcome, acutely and in the long run (weeks), the abrupt decrease in cardiac output (Fig. 23.3a, black line) due to the bradycardia (Fig. 23.3a, red line). The CAVB dog [19] is a model of compensated biventricular hypertrophy with a long QT phenotype [3, 6, 19, 20]. The primary compensatory parameter is illustrated in the recovery of cardiac output after the initial decline. Initially, this is reached by increasing cardiac contractility as can be seen in left ventricular (LV) pressure development over time (LV dP/dt, Fig. 23.3a, green line). However, as time progresses, a decline is seen, until it reaches a stable state at >10 weeks that accounts for 120 % of baseline values. Nonetheless, also on this longer time scale cardiac output is maintained, because biventricular hypertrophy develops (increased heart weight to body weight, Fig. 23.3a, yellow line) creating a new equilibrium between wall thickness and LV volume,

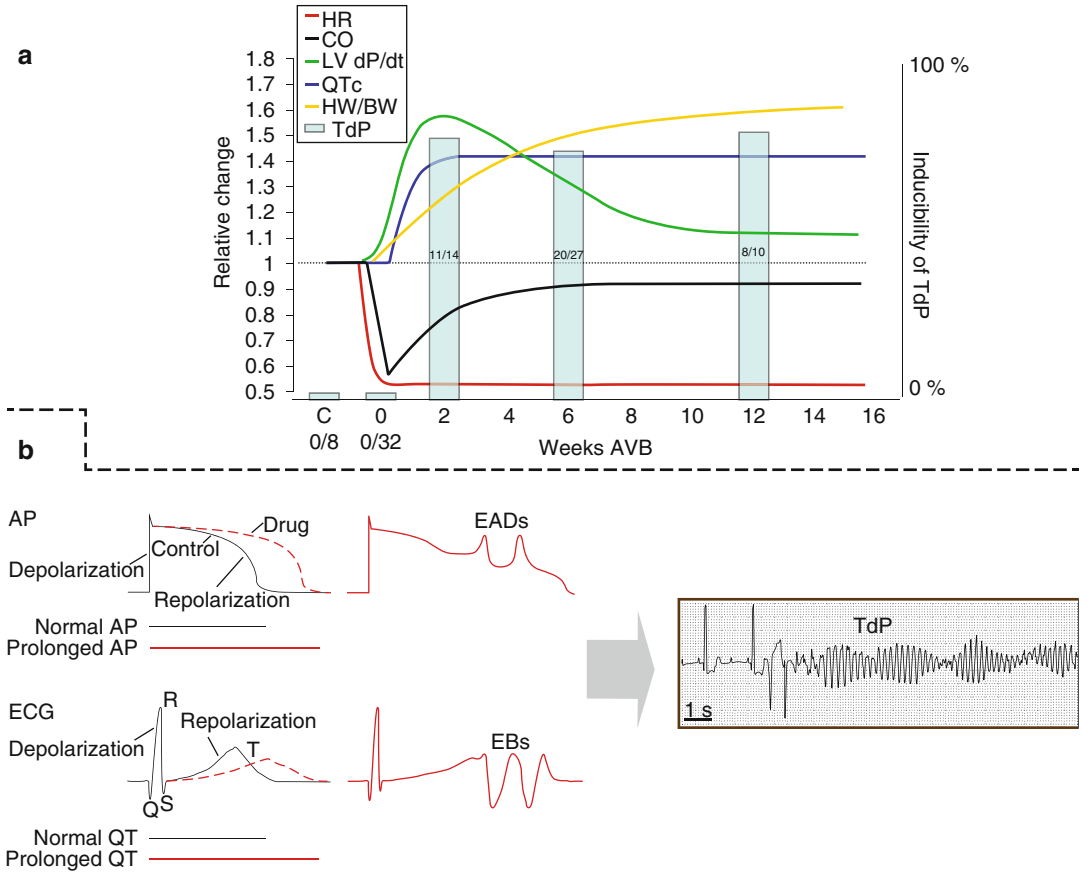

Figure 23–3. Progress of remodeling in the CAVB dog. (**a**) Schematic figure summarizing the development of cardiac remodeling in the CAVB dog over time. Scale is relative. The *red line* shows development of heart rate. The *black line* is an approximation of cardiac output. The *green line* shows contractile remodeling as left ventricular pressure changes. The *yellow line* represents structural remodeling, via cardiac hypertrophy as heart weight to body weight. The blue line depicts QT interval lengthening on the ECG, as a parameter for electrical remodeling. Finally, the bars represent fraction of dogs sensitive to drug-induced TdPs (see *right* Y-axis) at 2, 6, or 12 weeks of chronic AV-block. (**b**) Summary of the mechanism of drug-induced repolarization-dependent arrhythmias. From lengthening of the QT interval/APD, to early afterdepolarizations (*EADs*) and extra beats (*EBs*), to Torsade de Pointes arrhythmias (*TdP*)

which prevents deterioration into dilated cardiomyopathy (for reference: see Fig. 23.1) [3, 21].

Aside from the structural and mechanical changes, electrical remodeling is also present. Grossly, this is identified in a lengthening of the cellular action potential duration, that in vivo is reflected in prolongation of the QT-time (Fig. 23.1, blue line) [3, 7, 17, 19–21]. This is pro-arrhythmogenic, as further drug-induced lengthening leads to early afterdepolarizations (EADs), extra beats, and ultimately life threatening Torsade de Pointes arrhythmias (TdP) (Fig. 23.3b) [17, 19, 22–24].

In the following paragraphs, we will describe in depth the electrical, contractile, and structural remodeling. Moreover, the possible mechanisms responsible for the enhanced arrhythmogeneity (arrhythmogenic outcome) and possible responsible intracellular signaling pathways will be discussed. Special focus will be on the existing intricate connections between ventricular remodeling and their effect on arrhythmogeneity.

Electrical Remodeling

As mentioned above, the most striking electrical remodeling is seen in a lengthening of the action potential duration, which can be observed via lengthening of the QT interval on the ECG or,

more regionally, via an increase in the duration of the catheter-based monophasic action potentials (MAPD, Table 23.1) [17, 19, 22, 24] or the activation recovery interval (ARI) of a needle measurement [25]. The prolongation of the MAPD is larger in the LV versus the right ventricle (RV), indicating that interventricular dispersion assessed as LV MAPD – RV MAPD is increasing too (Table 23.1) [17, 19, 24, 25]. More recently, spatial dispersion was further assessed using 66 needles with 4 electrodes, demonstrating increases in transseptal, interventricular, LV transmural as LV apex to base dispersion [25]. Also temporal dispersion, quantified as short term variability of beat-to-beat repolarization was increased after CAVB [24]. So not only is the repolarization prolonged, but it is also non-uniform in space and time.

Lengthening of the QT-duration develops in the first 2 weeks after AV-block and then stabilizes (Fig. 23.3a, blue line) [23]. Cellular experiments performed at 3–9 weeks of CAVB in isolated cardiomyocytes confirm this increase in repolarization times [26, 27]. Since potassium currents are largely responsible for proper repolarization [28], the most logical assumption would be to expect a decrease in these currents in the CAVB dog. Indeed, both the slow component of the delayed rectifier current Ik_s, as to a lesser extend the rapid component (Ik_r), are downregulated in the LV and in the RV (Table 23.2) [29]. Other repolarizing currents as the inward rectifying potassium current (Ik_1) and the transient outward current (I_{to}) are not changed functionally in either of the two ventricles [29].

Also the inward currents are attenuated when compared to their functioning before AV-block. The peak sodium current (I_{Na}) is down in the LV (Table 23.2) [25], whereas I_{Na} late, which occurs in the plateau of the action potential, is also reduced [30]. The current through the L-type calcium current ($I_{Ca\text{-}L}$) does not change after AV-block [6], but a more frequent occurrence of the window current is observed [31]. Sipido et al. [6] observed an increased current via the sodium calcium exchanger (NCX), in both modes, responsible for a more active sarcolemmal exchange of calcium and natrium ions through this channel. Also this increase may be pro-arrhythmic (see further). In line with this observation, $[Na^+]_i$ seems to be increased, while activity of the Na^+/K^+ exchanger remained similar [32]. The reduction in both peak and late sodium current are not in line with an increase in $[Na^+]_i$ or $[Ca^{2+}]_i$ nor with an increase in cellular APD. Recently we studied the relevance of the sodium-proton exchanger (Na^+/H^+) (van Borren et al., 2009, unpublished data). The activity of this pump seems elevated thereby possibly explaining the increased $[Na^+]_i$. Taken together, repolarization reserve in the CAVB model has been diminished.

For a complete summary of the observed changes in ion currents in the CAVB dog, see Tables 23.1 and 23.2. On mRNA and protein level, appropriate changes of the (alpha)α-subunits of ion channel proteins have been observed [26, 33]. A reference for the role of ion currents in a normal action potential can be found in Fig. 23.4a.

Table 23–1. Characterization of electrical remodeling in the CAVB dog: summary of studies which report APD lengthening and variability, via monophasic action potential duration (MAPD) or QT interval lengthening on the ECG in AV-block dogs

Reference	QT	MAPD	Spatial dispersion	Temporal dispersion
Vos et al. [19]	↑	↑	↑	
Schoenmakers et al. [17]	↑	↑	↑	
Boulaksil et al. [25]	↑	↑(ARI)	↑(ARI)	
Thomsen et al. [24]	↑	↑	↑	↑
Dunnink et al. [22]	↑	↑	↑	↑

Table 23–2. Characterization of electrical remodeling in the CAVB dog: summary of the ion current changes observed in the CAVB dog

Reference	Etiology	INa	ICaL	Ito	Ik_r	Ik_s	Ik1	NCX	Na/K pump
Sipido et al. [6]	CAVB		= =			=		↑ ↑	
Volders et al. [29]	CAVB			=. =	=	↓ ↓ ↓	=. =		
Boulaksil et al. [25]	CAVB	↓ (peak) =							
Antoons et al. [30]	CAVB	↓ Late							
Antoons et al. [31]	CAVB		Window current						
Stengl et al. [26]	CAVB					↓			
Verdonck et al. [32]									=

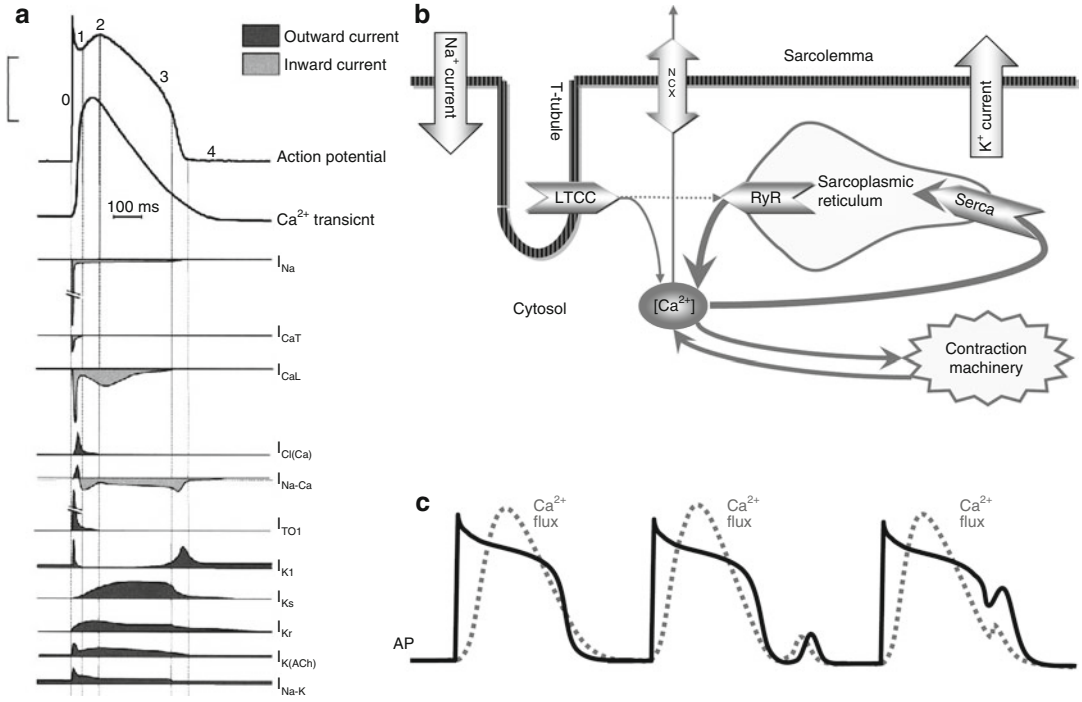

Figure 23–4. Ion currents and calcium handling in the cardiomyocyte. (**a**) Schematic illustration of the depolarizing and repolarizing currents that shape the action potential in the normal mammalian ventricle. Time course of each of the currents is shown together with the course of the Ca^{2+} transient. (**b**) Depiction of intracellular calcium movement during an action potential. Upon depolarization the L-type calcium channel opens (*LTCC*) and a relatively small amount of calcium enters the cardiomyocyte. The ryanodine receptor (*RyR*) reacts to this calcium and opens as well, leading to release of calcium out of the sarcoplasmatic reticulum. The myofilaments start contracting in response to the increased calcium concentration. At the end of the action potential the intracellular calcium concentration is reduced to baseline by the sarcoplasmic reticulum calcium-ATPase (*SERCA*) pump, which pumps calcium back in to the sarcoplasmic reticulum, and via the sodium/calcium exchanger (*NCX*), which removes a smaller amount of calcium in exchange for sodium ions (three sodium ions for one calcium ion). (**c**) Behaviour of the intracellular calcium flux during a normal action potential, and a delayed and early afterdepolarization. The *dotted line* depicts the intracellular calcium concentration. Note that a delayed afterdepolarization occurs after the end of the action potential, while a early afterdepolarization occurs during the repolarization phase of an action potential

Contractile Remodeling

The ion current responsible for coupling the electrical impulse to contraction is I_{CaL} via the L-type calcium channel (LTCC) (Fig. 23.4b) [34], which is voltage-sensitive and activates upon depolarization. The ryanodine receptor (RyR), located at the sarcoplasmic reticulum (SR), opens in accordance with the influx of calcium through LTCC, and even more calcium will be released in the cytoplasm; a process called 'calcium-induced calcium release' [34]. The SR functions as an intracellular calcium store. Cytosolic calcium binds to the myofilaments and myocyte shortening/contraction occurs. Thereafter $[Ca^{2+}]_i$ returns to baseline values with the assistance of the NCX, transporting calcium out of the cell, while the (larger) remainder of cytoplasmic Ca^{2+} is pumped back into the SR via the sarcoplasmic reticulum calcium ATPase (SERCA). This is summarized in Fig. 23.4b and in the left part of Fig. 23.4c. Also, for a more detailed review of excitation-contraction coupling, see Bers [34].

Contractile strength can be varied under regulation of adrenergic stimuli, like epinephrine. The ß(beta)-adrenergic pathway modifies contraction by phosphorylation of a number of key proteins: (1) LTCC, (2) RyR release, and (3) SR reuptake of calcium by phosphorylation of phospholamban, the ancillary inhibitor protein of SERCA [34]. Also, CamKII has a positive, facilitating function in increasing contraction

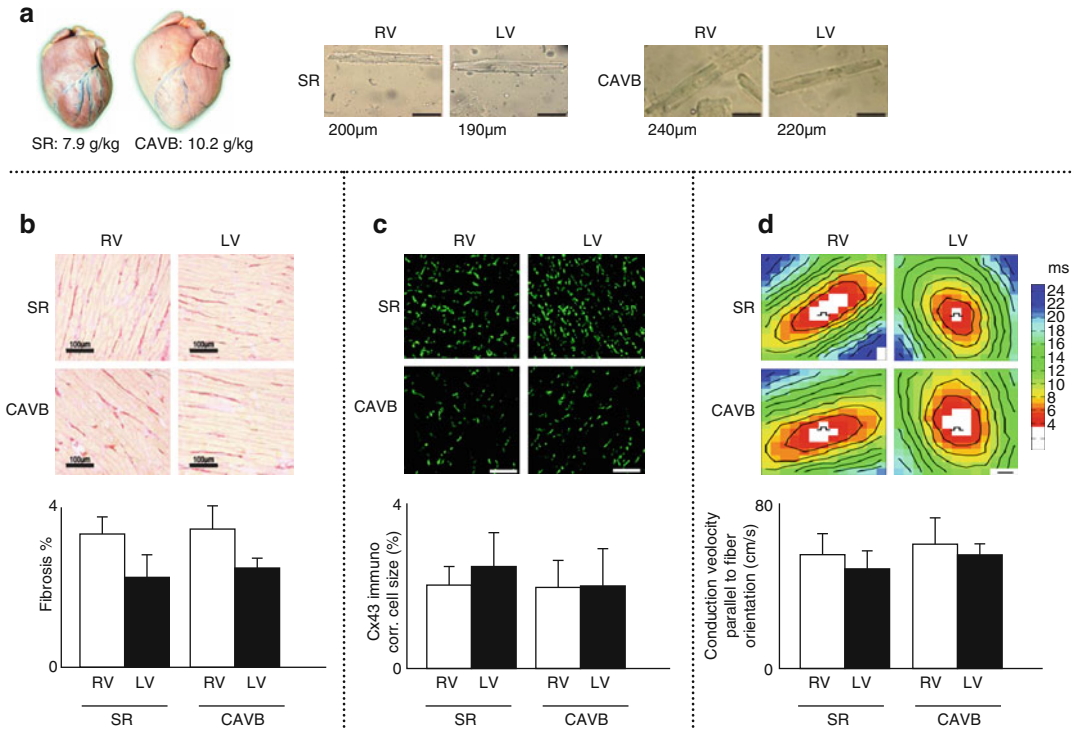

Figure 23–5. Structural remodeling in the CAVB dog and its consequences for conduction. (**a**) Cardiac hypertrophy in the CAVB dog heart and at the cellular level. (**b**) Comparison of ventricular fibros in sinus (*SR*) rhythm and chronic AV-block (*CAVB*) dogs as assessed by Sirius red staining. (**c**) Comparison of Connexin43 (Cx43) expression level and distribution in SR and CAVB dogs as assessed by immunohistochemistry. (**d**) Measurement of ventricular impulse propagation, both in SR and CAVB dogs via epicardial mapping. *Red* depicts early activation, *blue* late. The pacing site (indicated with the pacing symbol) was from the center of the epicardial placed electrode grid

by phosphorylation of the same set of proteins, although at a different amino-acid residue [35].

In the CAVB dog, the $[Ca^{2+}]_i$ transient is longer and in amplitude increased (Fig. 23.1c), while diastolic calcium levels are unaltered, thereby enhancing the systolic calcium fluxes as compared to non-hypertrophied, non-remodeled cardiomyocytes [6]. These increased $[Ca^{2+}]_i$ levels result in more contractile power at the slow heart rhythm (bradycardia) in this animal model. A negative force frequency relationship occurs (in both ventricles) [6, 20], which is in contrast to normal physiological behavior [7, 36]. Also, potentiation of contraction, as achieved by extra-stimuli, is increased in the CAVB dog [20]. As mentioned before, both the Na^+/Ca^{2+} and possibly Na^+/H^+ exchange, and SERCA have increased functional activity in order to handle this larger sarcolemmal and SR calcium movements. There is a close relation between electrical and contractile remodeling (see section "Arrhythmogenic Outcome").

The stronger contractility can be measured in vivo using LV or RV dP/dt+, which is clearly increased at 2 weeks of CAVB (Fig. 23.3a, green line) [20]. Neurohumoral activation as seen in temporarily increased levels of (nor) epinephrine, angiotensin II and aldosteron in the blood plasma of these dogs is in agreement. Both contractility and neurohormonal levels are transiently increased, since after 4–6 weeks of CAVB, all measured neurohumoral plasma concentrations are back to baseline [19, 26].

Structural Remodeling

The most obvious structural change is of course the biventricular hypertrophy. This can be seen on the whole heart level as an increase in the heart to body weight (Fig. 23.5a), as well as on LV and RV weight determinations [19], as on the cellular level, where the cardiomyocytes are

both lengthened and broadened (Fig. 23.5a) [3, 25, 27]. In this animal model, hypertrophy is more pronounced in the RV than in the LV as is reflected in the averaged increase in the length of the individual cardiomyocytes of Fig. 23.5a.

Profound hypertrophy, especially of the pathological kind, can be accompanied by extensive fibrosis, decoupling of the cardiomyocytes and slowing of the conduction velocity [37, 38]. In the CAVB dog, neither is present: interstitial fibrosis, quantified using Sirius red staining, does not increase (Fig. 23.5b), nor is there a decrease in connexin43, a gap junction protein that constitutes channels responsible for ventricular electrical coupling (Fig. 23.5c) [37]. Finally, the capillary-fiber ratio of the myocytes remains similar [19]. As a consequence, CAVB does not affect electrical conduction over the myocardium (Fig. 23.5d) [25].

Arrhythmogenic Outcome

The enhanced susceptibility of this animal model for drug induced Torsade de Pointes arrhythmias (Fig. 23.3b) indicates that the beneficial adaptations resulting in compensated biventricular hypertrophy have deleterious effects on electrophysiology and ventricular repolarization. Especially, repolarization reserve is severely diminished in these animals.

Generally, there are roughly two ways in which arrhythmias can develop: reentry or triggered-activity [39]. Reentry based arrhythmias are dependent on conduction slowing and unidirectional block of conduction, circumstances which promote self-sustaining electrical wavefronts. This mechanism of arrhythmogeneity can probably be excluded in case of the CAVB dog, as the necessities for these kind of arrhythmias are not present, which is most clearly shown by its retained conduction velocity (see Fig. 23.5d), and inability to demonstrate contribution of reentry in the initiation and perpetuation of TdPs [25].

Triggered activity either resulting from delayed (DADs, Fig. 23.4b, middle panel) or early afterdepolarizations (EADs, Fig. 23.4b, right panel) are likely involved in the initiation of ectopic beats and ventricular arrhythmias [40]. In this model, their occurrence, alone or in combination, has been demonstrated in numerous conditions, both in vivo [19, 20], as in isolated cells [27, 41]. There is a close relation with the excitation-contraction activity, as can be seen in Fig. 23.4b: spontaneous release of Ca^{2+} from the (overloaded) SR through RyR can (re) trigger depolarization of the action potential, either after (delayed) or within (early) the AP. The sequence is now reversed to mechanical-electrical coupling, as calcium release induces a change in membrane potential, instead of the opposite. In case of DADs, the NCX is most likely responsible for the transient inward current (I_{ti}) [42]. For EADs, window currents carried by the LTCC have been mentioned to underlie these events [31], probably assisted in a conditional phase induced by the NCX. Especially in conditions when intracellular Ca^{2+}-handling is combined with a decreased repolarization reserve, these interactions may cause this arrhythmogenic mechanism. The drug-induced TdPs as observed, are both initiated and perpetuated by EADs, and triggered ectopic beats, which accumulate to self terminating polymorphic ventricular tachyarrhythmias (Fig. 23.3b, right panel), as we have recently shown in this model [25].

Controlling the $[Ca^{2+}]_i$ is anti-arrhythmic and block of the LTCC, by verapamil or flunarizine, is clearly effective in preventing and suppressing these arrhythmias in vivo and on the cellular level [43].

This arrhythmogenic outcome is stable over time (Fig. 23.3, the bars) in the CAVB dog: 60–80 % of the dogs show reproducible TdPs, at 2, 6, or 12 weeks after AV-block, whereas no TdP can be induced in dogs without cardiac remodeling, like dogs still in sinus rhythm or directly after AV-block. When evaluating the different remodeling processes (structural, contractile, and electrical), it is clear that electrical remodeling is the only one that remains stable in the weeks after CAVB (Fig. 23.3a). Structural remodeling develops more slowly, whereas contractile adaptations after being profoundly increased transiently, are returning to less increased values in time [23].

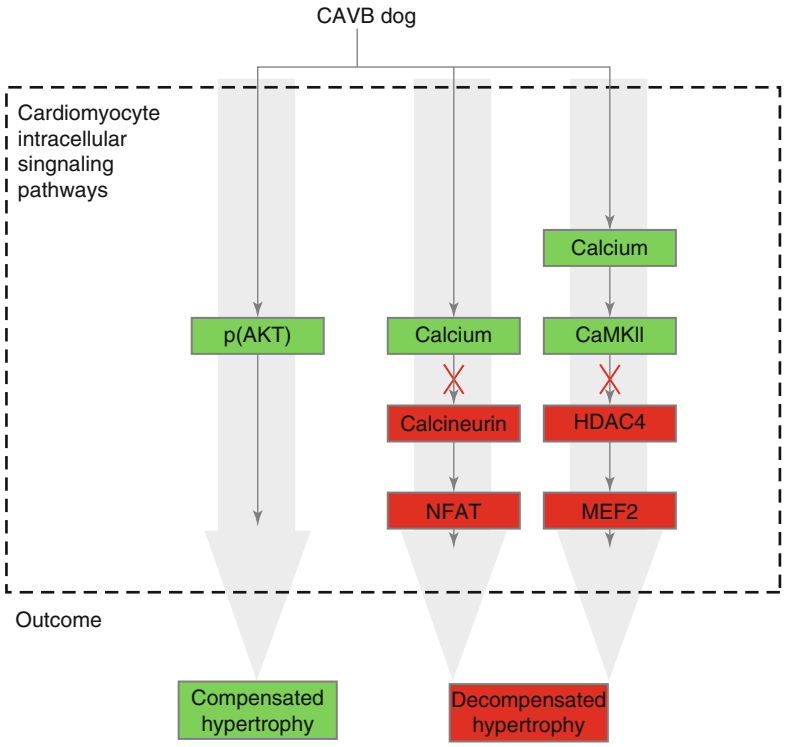

Figure 23–6. Hypertrophic signaling in the CAVB dog. Pathways involved in decompensated hypertrophy were not activated while the phospho-Akt pathway involved in the compensated phenotype was

Intracellular Signaling

As has been summarized in Fig. 23.2, hypertrophic remodeling is accompanied by activation of a number of signaling pathways in the cardiomyocyte. Of these, some, like calcineurin [13, 14] and CaMKII [15] (in the heart, CaMKII almost always refers to the CaMKIIδ variant, as this is the most expressed isoform in the heart) [44], are related to heart failure, while others, like Akt [11, 12], are more closely linked to physiological hypertrophy. In the CAVB model, mechanotransduction seems to play an prominent role [3]. Besides bradycardia, volume overload and altered ventricular activation are important in generating this different phenotype [45].

As the CAVB dog has a compensated hypertrophy, one would expect to indentify signaling reminiscent of physiological hypertrophy. This is indeed the case, as Akt was shown to be activated [3]. In contrast, calcineurin, another signaling molecule involved in pathological remodeling, appeared not to be involved in cardiac remodeling of the CAVB dog, which was assessed via calcineurin inhibition through chronic cyclosporin A treatment [46]. Cyclosporin A did not affect electrical, contractile, or structural remodeling, thereby suggesting no role of calcineurin.

CaMKII activation in the dog was more paradoxical. We have recently established that CaMKII total levels were not changed, but autophosphorylation levels were increased. This is indicative for increased CaMKII activity in the CAVB dog. However, the CaMKII-dependent pathway that leads to MEF2-dependent changes in gene expression was not activated, as HDAC4, the link between MEF2 and CaMKII [47], was not phosphorylated. On the other hand, CaMKII also phosphorylates numerous targets involved in intracellular calcium handling, like RyR, LTCC, and phospholamban (the inhibitor of SERCA) [48]. This implies a regulatory role of CamKII in $[Ca^{2+}]_i$ handling, but no involvement in alterations of the gene expression profile associated with maladaptive remodeling. A summary of the involved signaling pathways in the CAVB dog, as we have observed, can be seen in Fig. 23.6.

Conclusion

The CAVB dog has a heart with profound adaptations on the structural, electrical, and contractile levels. This remodeling is compensatory, as the cardiac output is retained in the long-term due to stable biventricular hypertrophy and increased contractility. However, the action potential is heterogeneously lengthened, both in space and time, which is pro-arrhythmic and maladaptive, as the remodeled heart appears much more sensitive to EADs, extra beats, and TdP arrhythmias.

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24
Physiological and Other Biological Pacemakers

Richard B. Robinson, Peter R. Brink, Ira S. Cohen, and Michael R. Rosen

Abstract

Physiological pacemaking in the heart is the province of the sinus node, in which transmembrane ionic currents and Ca^{2+} homeostasis mechanisms interact to generate the pacemaker potential. A key transmembrane component for initiating pacemaker function is I_f, an inward current carried by sodium through a family of channels that are hyperpolarization-activated and cyclic nucleotide-gated (HCN channels). In many settings where physiological pacemaking fails, therapy involves electronic pacing. Because of shortcomings in this otherwise excellent technology, there has been a search for biological alternatives in which either gene or cell therapy is used to decrease outward current or increase inward current to provide pacemaker function. The various technologies used are summarized in the following pages as are directions for optimizing biological pacemaker function and for using them in tandem with electronic units.

Keywords

Hyperpolarization-activated • Cyclic nucleotide-gated channels • Pacemaker current • Electronic pacemaking • I_f current • Autonomic regulation

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Introduction

Pacemaker therapy is an established part of medical practice, such that few of us remember what the world was like before the electronic pacemaker era. As recently as the early 1960s many patients experiencing Adams-Stokes seizures resulting from high degree heart block were given sublingual catecholamines as therapy. The diagnosis was effectively a death sentence and the therapy was both inconvenient and not very efficacious: every 2 h the patient had to take his/her drug; although it maintained a faster heart rate than that of the baseline idioventricular rhythm it was often arrhythmogenic [1].

During their infancy implantable electronic pacemakers were clumsy, hockey-puck-sized

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devices delivering impulses at a fixed rate. While certainly better than preexisting therapy, there were problems: limited battery life, infection and lead dislodgement and parasystolic competition from the patient's own idioventricular rhythm [2]. The subsequent 50 years have seen dramatic advances, through demand pacing, AV sequential pacing and through the application of pacing for treating a variety of arrhythmias as well as heart failure. All these advances have been made while modifying the power packs such that their mass is a fraction of that of the original implantable devices [2].

Why attempt to improve upon this situation? Because as good as they are, electronic pacemakers have limitations. These include poor responsiveness to the demands of exercise and emotion, the requirements of monitoring and maintenance (including at times battery and/or electrode replacement), suboptimal flexibility for pediatric treatment, limited flexibility with regard to electrode placement site, infection and occasional interference from other devices [2–4]. This is not to say continued advances have not occurred: rate responsive pacing and leadless electronic pacing are two examples of where the field is attempting to go. But because of the shortcomings of electronic therapy, a truly disruptive technology is being explored: biological pacing. In this chapter we will first consider the physiological pacemaker of the heart, and then will review the field of biological pacemakers.

Physiological Pacemakers

The physiological cardiac pacemaker is the sinus node, which generates a rate and rhythm having great stability under normal circumstances. In addition, the heart incorporates biological redundancy with a complex backup system in place. Hence, in addition to the sinus node, there are pacemaker cells in the atrium, atrioventricular junction and Purkinje system, each of which is capable of driving the heart (at progressively lower rates as one proceeds distally from sinus node through Purkinje system) in the event that higher pacemaker centers fail [5].

The property central to sinoatrial impulse initiation is automaticity, the gradual depolarization of cells during phase 4 of the transmembrane potential until threshold potential is reached and an action potential initiated [6]. While there continues to be controversy as to the relative contributions of different mechanisms to sinoatrial impulse initiation [7], many in the field now agree that sinus node pacemaking relies on some combination of transmembrane ionic currents and Ca^{2+} homeostasis mechanisms. In this respect, the transmembrane current most associated with initiation of phase 4 depolarization is an inward current carried by sodium and potassium, and referred to as I_f (the f stands for "funny," given the observation that this inward current depends on hyperpolarization for its initiation and increases in magnitude with increasingly hyperpolarizing voltage steps) [8]. These characteristics result in a time-dependent inward current that increases during diastole. The current is carried by the HCN (hyperpolarization activated, cyclic nucleotide gated) [6, 9] family of channel subunits, illustrated schematically in Fig. 24.1a. There are four isoforms of the HCN channel, designated HCN1-4; isoforms four and one are preponderant in sinus node, and two in myocardium and Purkinje system.

In addition to impulse initiation, the other key attribute of the sinus node is its autonomic responsiveness, which is dependent on modulation of intracellular cAMP levels by sympathetic and parasympathetic neurohumors. Critical to this modulation is the structure of the HCN channel, which has six transmembrane spanning domains, and incorporates a cyclic-AMP binding site near its carboxy-terminus [6, 9]. β-adrenergic catecholamines via a G-protein-transduced pathway operate through adenylyl cyclase to break down ATP to cyclic AMP, which then can interact with its HCN channel site to increase the rate of phase 4 depolarization and thus pacemaker rate (Fig. 24.1b) [10]. The increase in rate results from an increase in activation kinetics and a positive shift along the voltage axis of the current. In contrast, acetylcholine utilizes its G-protein-transduced pathway to brake the increase in rate by decreasing cAMP levels and reducing the rate of phase 4 depolarization. At higher concentrations, acetylcholine also increases the potassium current $I_{K,Ach}$ to accelerate repolarization, hyperpolarize

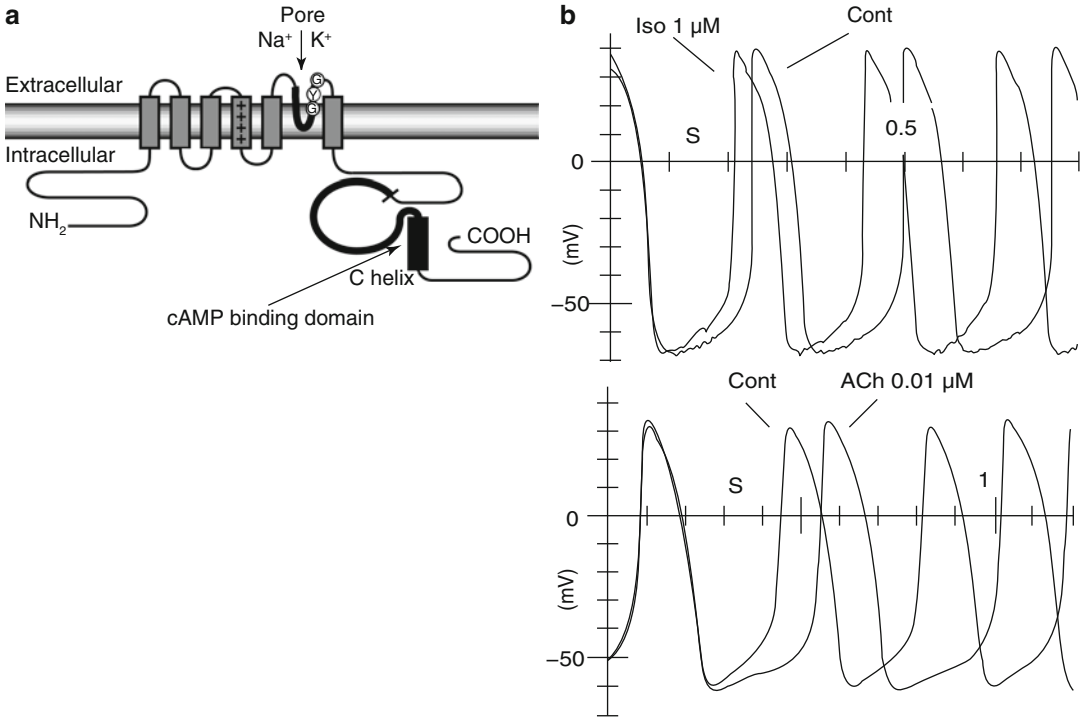

Figure 24–1. *Panel* **a**: Representation of an HCN channel subunit, four of which together form a functional channel generating I_f current. Shown are the six membrane spanning segments, the pore loop and the cAMP binding domain (Modified from Robinson and Siegelbaum [9] with permission). *Panel* **b**: Spontaneous action potential recordings from single rabbit sinus node cells showing the effect of the β-adrenergic agonist isoproterenol (Iso, *top*) and the muscarinic agonist acetylcholine (ACh, *bottom*) on spontaneous rate and slope of phase 4 depolarization (Modified from Bucchi et al. [10]. With permission from Wolters Kluwer Health)

the membrane potential and suppress phase 4 [11]. This further slows the rate of impulse initiation.

Although the information just reviewed highlights the importance of I_f to sinus node pacemaker function, the other components contributing to nodal impulse initiation are such that administering highly selective I_f –blocking drugs such as ivabradine only decreases heart rate by 10–30 % [12].

The other contributors of inward current during phase 4 include the Na/Ca exchanger and the inward Ca currents, $I_{Ca,L}$ and $I_{Ca,T}$ [6]. These contributors also are responsive to autonomic regulation via cAMP, in this case via PKA dependent phosphorylation either of these proteins or of other proteins involved in regulation of Ca^{2+} homeostasis that in turn modulate their function [13, 14]. Recent evidence suggests that there is an additional cAMP dependent interaction between I_f and these Ca^{2+} dependent currents and exchanger. This interaction arises from the fact that, unlike ventricular myocardium, sinus node expresses Ca^{2+}-activated adenylyl cyclase isoforms [15, 16]. The presence of such isoforms provides a mechanism by which Ca^{2+} dynamics can influence – via cAMP levels – not only Na/Ca exchanger and Ca^{2+} currents, but also I_f [17].

The sinus node action potential itself is largely Ca-dependent (in contrast to the faster-rising Na-dependent action potentials of working myocardium). However, in some species and at some ages – particularly in settings where basal heart rate is high – fast inward Na current also is present [18, 19]. In these cases, the Na channel isoform is not the "cardiac" Nav1.5, but other isoforms (e.g. Nav1.1) that exhibit a less negative inactivation relation, thereby allowing the channels to operate at the membrane potential typical of the sinus node.

Finally, repolarizing current is provided via potassium channels, generating an outward

current [6]. Hence, a balance between inward and outward currents determines sinus rate, and any intervention that increases inward or decreases outward current during diastole will increase pacemaker rate.

Biological Pacemakers

Three situations in which one might want to use biological materials as opposed to an electronic pacemaker are (1) repair or creation of a sinoatrial node in sick sinus syndrome with intact AV nodal function, (2) creation of a demand ventricular pacemaker in individuals with high degree or complete heart block and atrial fibrillation; (3) creation of an atrioventricular bridge in individuals with normal sinus node function and AV nodal disease. Note that the latter is not a biological pacemaker, but a biological alternative to electronic atrioventricular sequential pacing that could be combined with a biological pacemaker, if needed. This presentation will focus on biological pacemakers rather than on atrioventricular bridges.

In creating a biological pacemaker we recognize that any intervention that increases inward current or decreases outward current during diastole in an excitable cell might induce pacemaker function. We have characterized what we believe to be the optimal characteristics of a biological pacemaker as follows [3]: It should create a stable physiologic rhythm for the life of the patient, require no battery or electrode, and no replacement, compete effectively in direct comparison with electronic pacemakers, confer no risk of inflammation/infection or neoplasia, adapt to changes in physical activity and/or emotion with appropriate and rapid changes in heart rate, propagate through an optimal pathway of activation to maximize efficiency of contraction and cardiac output, and have limited and preferably no arrhythmic potential. We have deliberately set a high bar here because electronic pacemakers provide a standard for excellence as palliative therapy. Indeed, to exceed the success of electronic pacemakers, the biological pacemaker should represent cure, not palliation.

Biological pacing has incorporated two general approaches, that of gene therapy and that of cell therapy. Initial studies focused on gene therapy and generated transient successes, in keeping with the episomal delivery of the gene. While gene therapy remains an important field for investigation, cell therapies have also been explored. In this presentation we will discuss gene and cell therapies sequentially.

Gene Therapy

Initial Attempts at Biological Pacemaking

Initial attempts at biological pacing focused on sympathomimetic modulation of existing pacemaker current. Edelberg et al. [20] reasoned that since β-adrenergic catecholamines can increase sinus rate, overexpressing β-adrenergic receptors should enhance heart rate. Hence, they incorporated the gene encoding the β_2-adrenergic receptor into a plasmid and injected this into porcine atrium in situ. Although the transfection was transient, they demonstrated both a faster atrial rate and an enhanced atrial rate response to catecholamine infusion in the injected animals. Concerns about this approach were: first, therapy would rely on the ability of the existing sinoatrial node (or other endogenous pacemaker) to respond to catecholamines. If the node were in any way damaged or diseased the response might be unpredictable. Second, catecholamines can be arrhythmogenic. However, as detailed below, a recent variant on this approach involves manipulating adenylyl cyclase to influence cAMP levels.

Miake et al. provided the first proof of concept experiments that alteration of ion channel function could create a biological pacemaker [21]. They reasoned that if repolarizing current were reduced, this would permit inward currents to depolarize the membrane during electrical diastole, resulting in a functioning biological pacemaker. To this end, they replaced three amino acid residues in the pore of the Kir2.1 gene that regulates the ion current, I_{K1} and created a dominant negative construct. This was presumed to form multimeric, nonfunctional channels with endogenously expressed wild-type Kir2.1 and/or Kir2.2. The construct and green fluorescent protein (GFP) were packaged in a replication-deficient adenoviral vector which was injected into the left ventricular cavity of guinea pigs. After 3–4 days I_{K1} was about 80 % reduced in isolated ventricular myocytes,

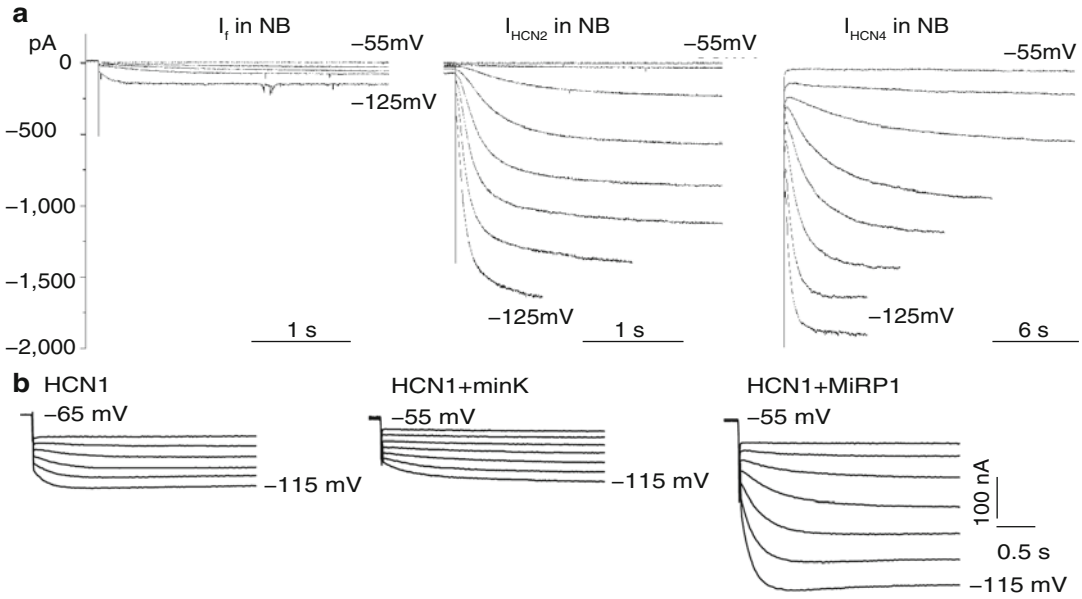

Figure 24–2. *Panel* **a**: Representative tracings from newborn (NB) rat ventricular myocytes transfected with HCN genes. *Left*, native I_f in newborn myocyte; *center*, transfection with HCN2 gene; *right*, transfection with HCN4 gene. Note the markedly larger currents in center and *right panels* as compared to the *left*. *Panel* **b**: Effect of MiRP1 transfection to increase current in xenopus oocytes transfected with HCN2. *Left* represents HCN2, alone; *center*, HCN2 plus the β subunit minK; *right* HCN2 plus the β subunit MiRP1. Note the markedly larger current n the *right panel* (Modified from Qu et al. [24] and Yu et al. [26], with permission)

and ventricular ectopic rhythms were seen on ECG. Recently this approach has been combined with HCN over-expression [22]. The major concern about reducing outward repolarizing currents was that it might excessively prolong repolarization, as was borne out in later experiments [23].

Using HCN to Create Biological Pacemakers

Proof of concept that increasing I_f in myocytes would lead to increases in pacemaker rate was provided by our group [24–26]. We elected to overexpress the HCN channel as the biological pacemaker because it not only carries the primary pacemaker current but because I_f activates on hyperpolarization and turns off at depolarized potentials. Therefore it has no effect to prolong action potential duration. Further, it directly binds cAMP [9], providing inherent autonomic responsiveness. After showing in rat ventricular myocytes in culture that I_f could be overexpressed by administering the HCN2 gene using a plasmid as the vector [24], we asked whether the channel expressed was autonomically regulated. Here, we infected ventricular myocytes with an adenoviral construct incorporating HCN2 and found cAMP regulation of the current was preserved, similar to that for wild-type I_f [25]. We also found that a β subunit, MiRP1, administered via a plasmid, increased pacemaker current density and kinetics of exogenously expressed HCN2 in xenopus oocytes [26] and in myocytes [27]. The final in vitro experiment involved HCN2 overexpression in neonatal myocytes in culture using an adenoviral vector. Spontaneous rate increased, suggesting a similar approach might result in biological pacemaking in situ [24, 25]. These results are demonstrated in Fig. 24.2.

Our initial attempt at proof of concept in vivo involved injecting HCN2 into left atrial myocardium [28]. We used an adenoviral vector incorporating HCN2, with a second adenovirus expressing green fluorescent protein (to facilitate visualization of transfected cells). In terminal experiments we used vagal stimulation to induce sinoatrial slowing and/or AV nodal block, and studied the subsequent escape rhythms. The approach was successful and we pace-mapped the ectopic rhythms to the site of

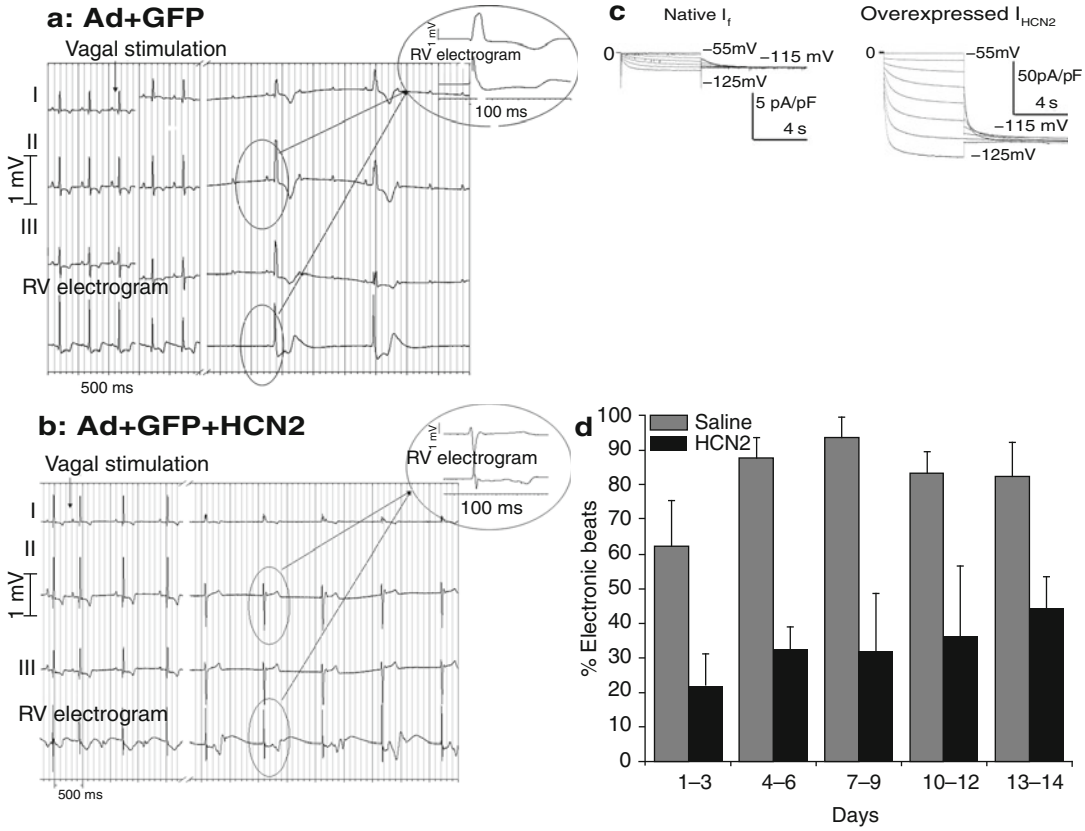

Figure 24–3. *Panels* **a** and **b** are respectively Leads I, II and III and a right ventricular electrogram (*top* to *bottom*) of the ECGs of two different dogs. That in (a) was injected with an adenoviral vector carrying green fluorescent protein (GFP), alone; that in (b) received the same vector incorporating GFP plus the HCN2 gene. Animals were anesthetized 6 days after injection and the vagi isolated and stimulated (stimulation marked by the *arrows*). Interval between traces is 22 s in (a) and 5 s in (b). The animal that received GFP developed an idioventricular rhythm having a slower rate than that receiving GFP+HCN2. Insets show the temporal relationship between the lead II QRS complex and the electrogram. *Panel* **c**: I_f in a Purkinje myocyte isolated from the LBB of a control animal (*left*) and one receiving HCN2 (*right*). The current is markedly greater in the latter (note the vertical gains of 5 pA/pF on the *left* and 50 pA/pF on the *right*). *Panel* **d**: Percent of electronic beats as a function of days after injection into the LBB of saline or an HCN2 adenovirus (Modified from Plotnikov et al. [29] and Bucchi et al. [30], with permission)

injection of the construct. We then isolated and studied cells from that region, which showed I_f at least 100-fold greater than native current [28].

Although this approach successfully demonstrated functional escape rhythms, we believed it important to test the concept in the ventricle. Hence, under fluoroscopic control, we injected the same construct into the left bundle branch system of intact dogs [29]. We again used vagal stimulation to slow sinus rate or induce atrioventricular block and found that HCN2-injected dogs developed escape rhythms whose rates were about 25 % faster than the idioventricular rates of control or sham injected (GFP without HCN2) animals (Fig. 24.3a, b) [29]. We then isolated left bundle branch fibers from the injection site and studied them with microelectrode techniques. They showed a significantly faster automatic rate than those generated at the same sites by tissues from control or sham-injected animals [29]. Current recordings from cells disaggregated from the same sites showed approximately 100-fold overexpression of I_f in HCN2 injected animals (Fig. 24.3c). Finally, immunohistochemistry demonstrated increased fluorescence with an anti-HCN2 antibody.

In a subsequent study [30] we demonstrated that an HCN2 biological pacemaker implanted in the left bundle branch could function smoothly in tandem with an electronic pacemaker set at VVI 45 bpm. The biological pacemaker significantly reduced the incidence of electronic beats (Fig. 24.3d) while the electronic pacemaker's memory function provided a record of pacemaker functionality. Finally, a recent study confirms that an HCN2 based biological pacemaker responds to natural physiological stimuli, in this case using arousal to induce an increase in rate [31].

Other experiments focused on fine-tuning pacemaker properties using mutant or chimeric constructs. For example, we have used the E324A mutant channel, incorporating a single point mutation at position 324 of the parent mHCN2 and found that this elicits a sequence of complex changes: first, there is less channel and current expression with the mutant than the parent HCN2; however there is a positive shift of both activation and cyclic AMP responsiveness: the result is expressed as a baseline rate not very different from that with HCN2 alone, but there is a greater rate response to catecholamines [30]. We also employed a chimeric HCN1/HCN2 channel which combined the transmembrane region of HCN1 with the cytoplasmic N- and C-termini of HCN2. The rationale was that the HCN1 component would provide faster gating kinetics while the HCN2 C-terminus, with that cAMP binding domain, would provide a robust cAMP response. This prediction proved correct, but the resultant biological pacemaker generated excessive rates, resulting in periods of ventricular tachycardia [32]. The HCN basis for the tachycardia was confirmed by the efficacy of ivabradine to terminate it.

Another group has employed an HCN1 mutation that deletes a portion of the S3–S4 linker, shifting activation positively [33]. The resultant pacemaker, implanted in atrial tissue, exhibited a good range of rates in a porcine model of SAN dysfunction and a demonstrable catecholamine response. This occurred despite the poor inherent catecholamine response of the HCN1 channel subunit, suggesting contributions from endogenous elements.

While interventions such as these provide means to optimize pacemaker constructs, no single gene construct based on HCN has yet achieved optimal results in terms of the basal or autonomically stimulated rate attained. This suggests that a combination gene approach may be necessary, and several initial attempts along that line have been pursued.

In considering combination gene approaches, Nature has given us at least two clues. The first derives from the observation that the sinus node expresses Ca^{2+}-activated adenylyl cyclase (AC) isoforms [15, 16], which provides a possible intrinsic feedback mechanism between cytosolic Ca^{2+} changes and activation of transmembrane currents, in particular HCN based currents. We have pursued this by over-expressing AC1 (an isoform activated by Ca^{2+} in the submicromolar range [34]) in the canine left bundle branch in combination with HCN2. AC1+HCN2 expression resulted in a significantly greater escape rate and greater maximal rate following adrenergic agonists than HCN2 alone [35]. While we have not yet compared this to a pacemaker based on a different AC isoform, another group has studied the effect of AC6 over-expression (without HCN co-expression) [36]. This isoform, which is inhibited by Ca^{2+} in the micromolar range, was able to generate a biological pacemaker, but only in the setting of catecholamine stimulation.

A second idea for combination gene therapy derives from the observation that in animals with intrinsic high heart rate a Na channel, and in particular an isoform that is not inactivated at the typical membrane potential of the sinus node, contributes to diastolic depolarization [19, 37]. We explored this concept by overexpressing the Nav1.4 Na channel isoform along with HCN2 [38]. At low membrane potentials at which more than 50 % of cardiac Na channels are inactivated, cells expressing Nav1.4 generate action potentials having a high maximum rate of action potential upstroke [39, 40]. We administered combined SkM1/HCN2 to the left bundle branch of dogs in chronic heart block. Impulse initiation was improved over HCN2 or SkM1 alone, yielding basal rates of 70–100 bpm and maximal rates of 150–170 bpm [38]. While the mechanism underlying this combination approach requires further exploration, the result was a biological pacemaker operating in the normal physiological range and possessing a robust response to adrenergic stimuli.

Why Seek Alternatives to Gene Therapy?

An obvious advantage of the HCN-based approach to gene therapy is that it not only overexpresses members of the primary pacemaker gene family, but it does not induce excess prolongation of repolarization and it retains the function of autonomic responsiveness. However, there are problems with the viral vector approach: replication-deficient adenoviruses or naked plasmids represent episomal strategies and the construct administered is not incorporated into the cell's genome. Such expression of biological pacemaking is transitory. Moreover, the viral vector induces inflammation and there is also the risk of infection. Finally, some viruses that might be expected to result in genomic incorporation of a pacemaker construct carry an already-demonstrated risk of neoplasia [3, 4]. Concerns such as these have led us and others to examine cell therapy approaches to biological pacemaking. These approaches have largely (albeit not exclusively) depended on xenografting human stem cells into animal hearts.

Cell Therapies

Human Embryonic Stem Cells

Research on embryonic stem cells has been proceeding for over 25 years, having been initiated by biologists interested in maturation during early stages of development [41, 42]. Research on human embryonic stem cells is far more recent. The major contribution to date in human embryonic stem cell research on biological pacemaking has been provided by the Gepstein group [43–45]. They have used embryonic stem cells to create a cardiogenic line that has been implanted into the hearts of immunosuppressed pigs in complete heart block. Their implantation has resulted in stable idioventricular rhythms that have persisted for months, and whose initiating current appears consistent with I_f.

Yet, questions remain regarding the long-term consistency of function as well as whether the current providing pacemaker function is I_f or a family of currents. A potential limitation of this approach is that the cells must be driven down a cardiac lineage that results in spontaneously active cardiac-like cells (as opposed to, e.g., ventricular like cells), and in fact the resulting cardiac-like population is quite heterogeneous [46]. Even with a pure population of pacemaking cells, the magnitude and kinetics of the diastolic current generated will be determined by the endogenous channels expressed in the cell. In contrast, expression of exogenous genes makes it possible to consider channel mutagenesis and/or combination therapy to optimize pacemaker function, as described above. This is one of the considerations that led us to explore the use of engineered adult mesenchymal stem cells, as discussed in the next section.

In addition, a major concern with embryonic stem cells is the need for immunosuppression: most physicians and patients faced with a decision to pace biologically and immunosuppress, versus to pace electronically and not immunosuppress would elect the latter approach. An alternative approach that does not require immunosuppression (but does not address the issue of optimization of pacemaker function) is creation of autologous biological pacemakers using induced pluripotent stem cells (IPSCs) [47, 48]. Retroviral administration of transcription factors to adult cells reprograms them, resulting in pluripotent IPSCs that can then be driven down a cardiac lineage in a manner analogous to ESCs. As with ESCs, concerns remain regarding possible tumorgenicity and control of the terminally differentiated state. Some of these concerns for IPSCs may be alleviated by more recent approaches, including ones that result in the direct reprogramming of fibroblasts to automatic, differentiated cardiomyocytes without involving a pluripotent intermediate or the use of viruses [48, 49]. However, concerns remain about abnormal phenotype in these cells, as reflected in changes at the chromosomal and subchromosomal levels [50, 51].

Adult Human Mesenchymal Stem Cells

Because of the limitations of gene therapy and concerns about embryonic stem cells we turned to adult mesenchymal stem cells (hMSCs) to provide pacemaker function in the intact heart. hMSCs are multipotent stem cells that are expected to differentiate along mesenchymally-derived lineages. Our strategy was to use them as platforms to carry HCN or other genes of interest to regions of the heart. We realized the

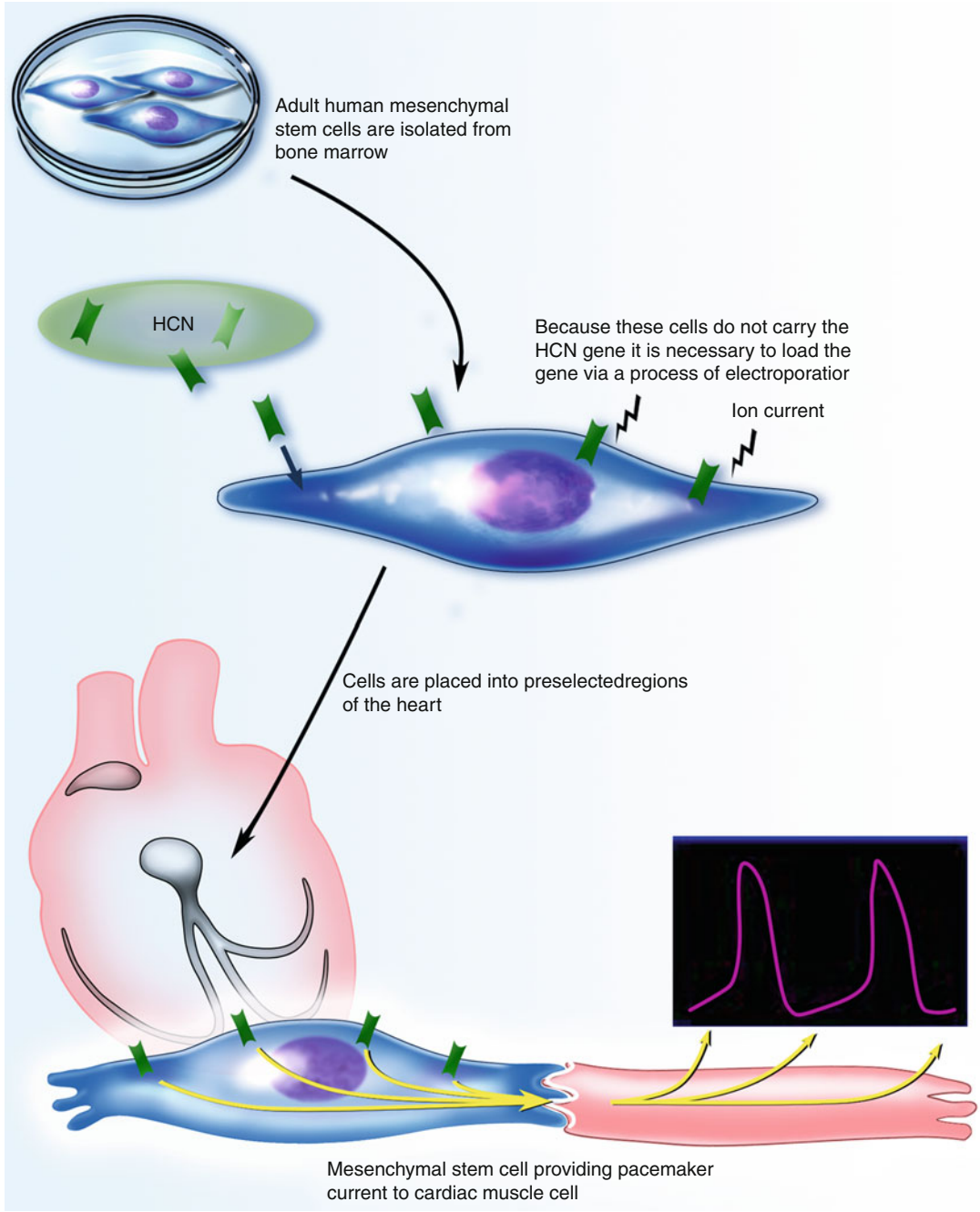

Figure 24–4. Experimental plan for hMSC-based pacemaker. See text for discussion (Modified from Dougherty [53], with permission from Columbia University)

key property needed for them to be effective platforms for gene delivery would be gap junctional coupling with adjacent myocytes. Without such coupling, the pacemaker signal could not be transmitted. Another complexity of cell implantation is the possibility of rejection by the host animal. We were encouraged here by reports suggesting hMSCs might be immunoprivileged [52]. For this reason, we studied them without the use of immunosuppression.

Figure 24.4 summarizes the strategy for working with hMSCs to create biological pacemakers

[3, 4]. Preliminary gene chip analysis showed no message for HCN2 or HCN4, some message for KCNQ1 (the gene encoding the α-subunit for I_{Ks}), and abundant message for the gap junctional protein, connexin43 (Cx43). Heubach et al. reported a similar result [54] and performed biophysical studies indicating that hMSCs express a Ca-activated K current, a clofilium sensitive outward current and, occasionally, L-type Ca current.

Our initial experiments in vitro tested the coupling of hMSCs to one another, to other cell lines and to cardiac myocytes [55]. Effective gap junctional coupling was demonstrated physiologically via intercellular dye transfer and by passage of current between cells in a pair. The current traces were asymmetrical, reflecting the presence of Cx43 in myocytes and Cx43 and Cx40 in hMSCs (Fig. 24.5a, b) and the formation of heterotypic channels.

The critical importance of having functional gap junctions in the cell pairs can be appreciated from the following: In the normal sinus node, cells incorporate a family of ion channel genes that include the pacemaker gene HCN4 [6, 9]. I_f activates on hyperpolarization, initiates diastolic depolarization and action potentials that propagate to the rest of the atrium via low resistance gap junctions. While hMSCs can serve as a platform for HCN genes if appropriately loaded with them, they haven't the complement of channels required for initiating action potentials. Neither have they the means to hyperpolarize such that overexpressed HCN channels will open. We hypothesized that if the hMSCs coupled to ventricular myocytes, then hyperpolarization of the latter would open the HCN channels in the hMSCs, and the HCN current generated by the stem cells would initiate an action potential in the myocyte [3]. The action potential then can propagate to other cells in the myocardium. Hence, we proposed to use two independent cell types, each generating an essential component (the hMSC providing pacemaker current and the myocyte, the ion channels needed to initiate an action potential). We hypothesized the two cell types might then operate as a single functional unit as suggested in Fig. 24.4. We validated this approach by studying cell pairs in culture [56], consisting of an adult myocyte coupled to a HeLa cell. HeLa cells lack endogenous connexins, permitting full control of cell-cell communication. Alone, the ventricular cell is quiescent but excitable (Fig. 24.5c). When the HeLa cell is co-transfected with HCN2 and Cx43 and coupled to the myocyte, the myocyte exhibits automaticity (Fig. 24.5d).

The other advantage of the hMSC was that it permitted us to build a pacemaker without using viral vectors. Rather, we used electroporation, which provides a transfection efficacy approximating 50 % [57]. Having loaded hMSCs with HCN2, we demonstrated that the hMSC now generated I_f-like current (Fig. 24.6) that responded to cesium and to autonomic modulation similarly to native I_f.

In subsequent experiments we overlaid cultures of rat ventricular myocytes on an island of HCN2 and GFP-loaded hMSCs. These manifested a significantly faster beating rate than control cultures. These tissue culture experiments were predictive of subsequent intact animal studies [57]. Here, we loaded approximately one million hMSCs with HCN2+GFP or GFP alone and injected them trans-epicardially into the left ventricular free wall. Vagal stimulation resulted in expression of an idioventricular rhythm at approximately 60 bpm that was pace-mapped to the injection site. The rate generated was significantly faster than the rates of hearts that received hMSCs with GFP [57]. A subsequent study [58] explored the effect of cell number as well as persistence of expression. Animals were studied for up to 6 weeks and pacemaker function persisted through this time period. While maximum rates of the pace-mapped beats, measured at the 6 week time point, increased with the number of cells administered (Fig. 24.7a), other data suggested an effective threshold level. Specifically, there was a clear distinction, in terms of percent of beats that pace-mapped to the injection site, when >700,000 cells (about 50 % loaded with HCN2) were injected compared to a lesser number (Fig. 24.7b). When the injected sites were excised at 6 weeks for histological analysis (Fig. 24.7c), the transfected hMSCs could be detected using a GFP antibody. There was no evidence of either humoral (canine IgG staining) or cellular CD3+ T lymphocyte staining) rejection, nor was there evidence of apoptosis

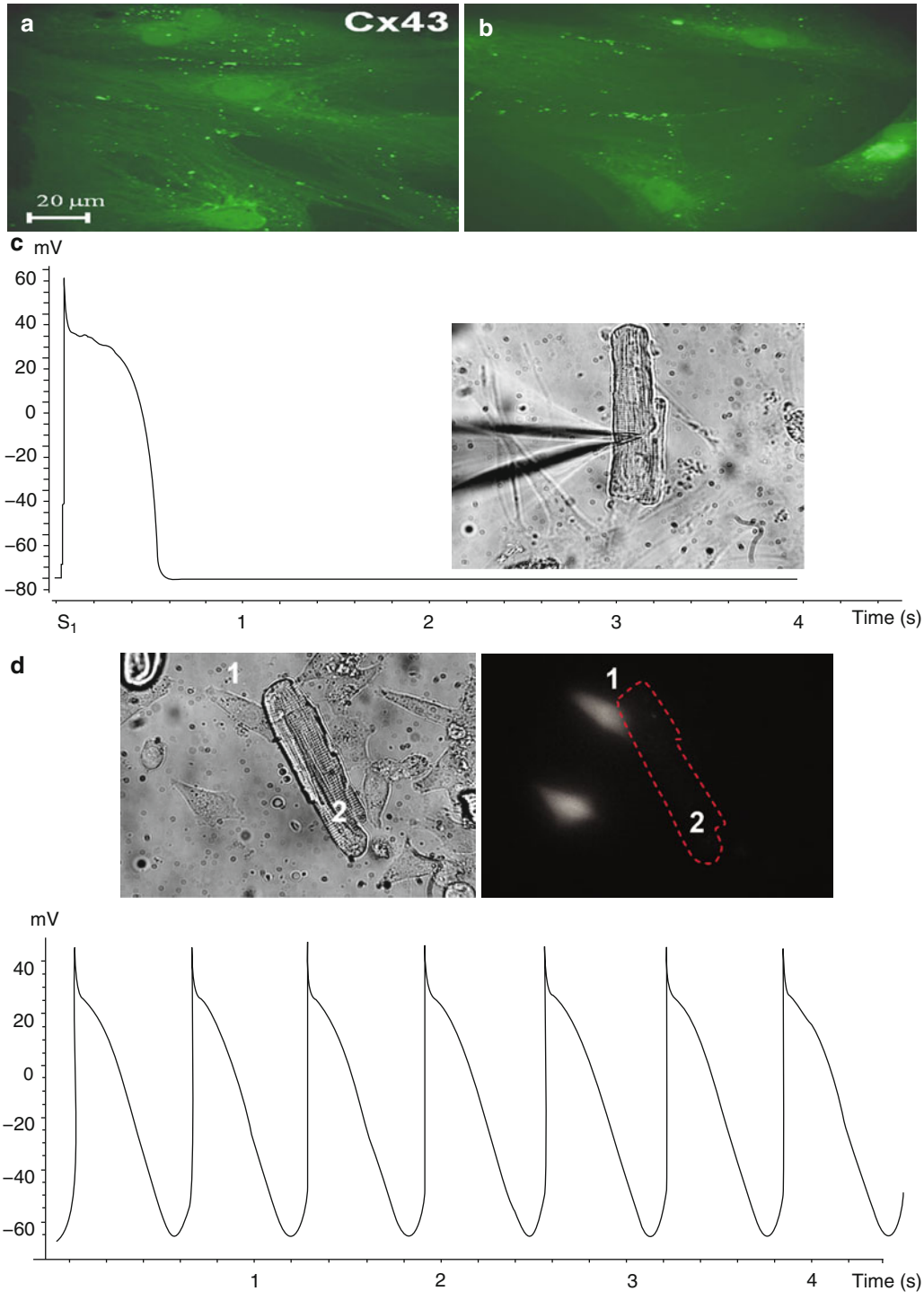

Figure 24–5. *Panels* **a**, **b**: Immunohistochemical stains for connexin 43 (a) and connexin 40 (b) in hMSCs. Note the punctate staining that is positive for Cx43 and Cx40. There is no staining for Cx45 (not shown). *Panels* **c**, **d**: An isolated canine ventricle myocyte (c) is excitable but not spontaneously active, while another myocyte coupled to a HeLa cell expressing Cx43 and HCN2 exhibits spontaneous action potentials. *Upper* photograph shows the isolated myocyte with a patch electrode (*top*); *lower left* photograph shows a myocyte (*labeled 2*) coupled to a HeLa cell (*labeled 1*); *lower right* photograph is a fluorescent image of the latter field, where the HeLa cells are visible due to expression of GFP (Modified from Valiunas et al. [55, 56], with permission)

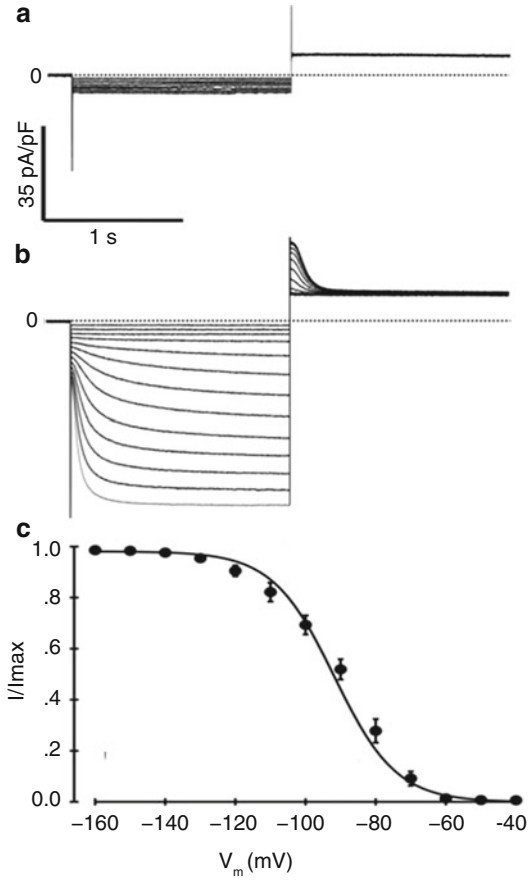

Figure 24–6. I_f recorded from a native hMSC (*panel* **a**) and I_{HCN2} from an hMSC in which HCN2 has been overexpressed via electroporation (*panel* **b**). Note the essential absence of I_f in the former. *Panel* **c**: Current voltage relationship for I_{HCN2} (note tail currents in *inset*), which is seen to activate in a physiologically relevant voltage range (Reprinted from Potapova et al. [57], with permission)

(cleaved/activated caspase-3 staining). Abundant gap junctions were also demonstrated immunohistochemically among hMSCs and between hMSCs and muscle cells [57].

What Remains to Be Learned?

There is much that remains in the learning curve regarding biological pacemaking. For approaches based on genetic engineering (whether direct gene delivery or cell based delivery, e.g. within an hMSC), the optimal gene(s) remains to be determined. At present, a combination approach such as HCN2 and Nav1.4 has yielded the most promising outcome, but questions remain. Further, the optimal construct will not necessarily be the same in all locations/substrates within the heart. An atrial based implantation may require a different solution than a bundle branch or ventricular wall implantation, and direct expression of gene(s) within a myocyte at any location may require a different construct than when placed within the environment of a stem or other packaging cell. For cell based approaches that depend on creating an automatic cell without addition of exogenous currents, it remains to be determined if ESCs or IPSCs will be more successful, the best way to direct these toward a lineage of an automatic cardiac cell and constrain them at that stage of differentiation and, most importantly, whether such an endogenous cell will generate sufficient depolarizing current at all locations to function adequately.

In addition, although experiments to date have been promising there is much to learn about viral approaches and about stem cells. For the viruses, inflammation, infection and the potential for neoplasia remain concerns. Also essential is to understand whether genomic incorporation of the gene construct will be possible, thereby conferring life-long function.

There is a different range of concerns for stem cells. First, will they evolve into other cell lineages? While we would not want to see cartilage or hematopoietic cells in the heart, their evolution into other cell types might not be problematic in the case of genetically engineered cells as long as gap junctional continuity and HCN function persisted. Will they migrate elsewhere in the body? This might create problems regardless of site and may necessitate the incorporation of a "death gene" within the construct. Initial results are encouraging in that they indicate little to no inflammation and no rejection over a 6 week period post implantation [58]. But long-term studies relating to both effectiveness of the pacemaker and its potential toxicity are still needed, as is a direct comparison with electronic pacemakers.

Finally, delivery systems for biological pacemakers are a challenge currently being addressed

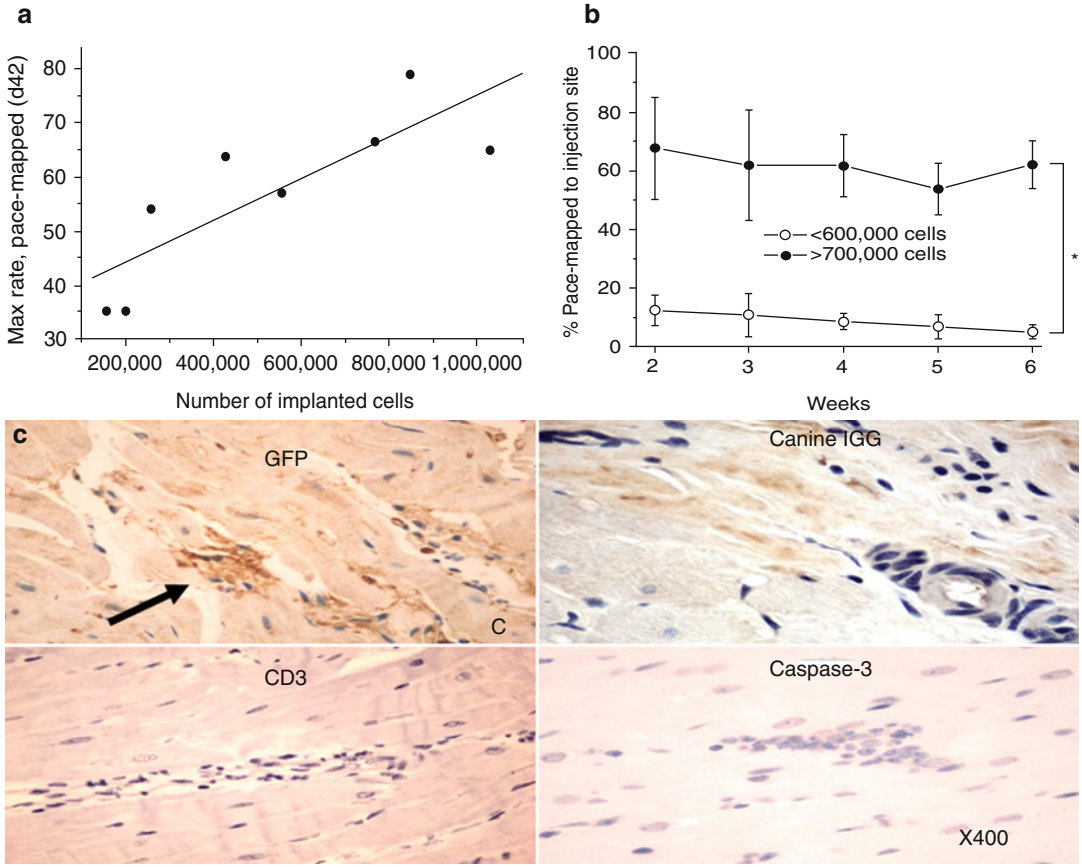

Figure 24–7. Expression of hMSC-based biological pacemaker function in canine heart. *Panel* **a**: Relation between number of injected stem cells and maximum rate of beats pace-mapped to injection site. *Panel* **b**: Persistence of efficacy during 6 week study period and effect of number of injected cells, as determined by measuring % of beats pace-mapped to injection site. *Panel* **c**: Immunohistochemical study 6 weeks after implantation showing persistence of GFP positive cells (*upper left*; *arrow*), lack of humoral (*upper right*) or cellular (*lower left*) rejection and lack of apoptosis (*lower right*) (Modified from Plotnikov et al. [58], with permission)

via needle/electrode combinations operating through a catheter or hollow-lumen steerable catheters or combinations of both. The goal here is to tailor placement to the optimal site in any patient while minimizing trauma.

Conclusion

In closing there are many approaches in what is an active and competitive field. Will we have biological pacemakers? Highly likely. But which one will be the ultimate construct and how it will be administered and with what success are all matters still to be sorted out.

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25
Cardiac Memory: From Electrical Curiosity to Clinical Diagnostic and Research Tool

Alexei Shvilkin

Abstract

The term "Cardiac memory" (CM) refers to persistent changes in myocardial repolarization upon resumption of normal ventricular conduction sequence after a period of abnormal ventricular activation (such as ventricular arrhythmias, pacing, or transient conduction abnormalities) manifesting by the typical pattern of the T wave changes on the ECG. In CM T wave direction follows the direction of the preceding aberrant QRS complex.

CM is believed to reflect adaptation of the heart to the new activation sequence and is triggered by changes in myocardial strain causing local release of angiotensin II. CM involves alterations in ion channel trafficking (short-term CM), transcription and expression of multiple ion channels including Ito, IKr, ICa-L, and gap junction redistribution (long-term CM).

In the clinical setting T wave inversions due to CM are often confused with myocardial ischemia.

Recent studies demonstrated that CM can be observed not only during normal ventricular activation but continuous aberrant conduction and its magnitude depends on the aberrant QRST morphology (particularly QRS/T vector magnitude ratio) and the degree of the left ventricular dyssynchrony.

Clinical applications of CM reviewed including differential diagnosis of T wave inversions, left bundle branch block age determination and potential use in pacemaker-related clinical research.

Keywords

Memory • Cardiac repolarization • T wave • Electrocardiography • Vectorcardiography • Pacing • Remodeling • Action potentials • Ion channels • Connexins • Gene transcription

Phenomenology of Cardiac Memory

The term "Cardiac memory" refers to the persistent changes in myocardial repolarization (manifested by typical T wave changes on the ECG) upon resumption of a normal ventricular activation sequence after a period of abnormal ventricular activation (such as ventricular

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arrhythmias, pacing, or transient conduction abnormalities). This term is closely related to the somewhat broader term "electrical remodeling" sometimes used to describe not only electrocardiographic phenomena but also local electrophysiological changes in the myocardium following a period of abnormal ventricular activation [1, 2]. Transient T wave inversions after single ventricular premature beats were remarked on by Paul Dudley White in 1915 [3], and a series of case reports describing T wave inversions after paroxysmal tachycardias were published in the 1940s [4–6]. Among the explanations of these findings were "anoxia, exhaustion" of the cardiac muscle as well as changes in ventricular filling due to a longer diastole. Later, T wave inversions were described following intermittent WPW syndrome [7–9], transient ventricular pacing [10, 11], left bundle branch block [12–15], and QRS widening due to sodium channel blocker toxicity [16].

In 1982 Mauricio Rosenbaum and associates published a cornerstone paper [17] in which they introduced the term "Cardiac memory" (CM) and presented a unified hypothesis of how abnormal ventricular activation leads to the development of T wave abnormalities by "electrotonic modulation." Based on a known inverse relationship between ventricular activation sequence and the local duration of repolarization [18] they concluded that the depolarization sequence "teaches" the cardiac cells to repolarize in a particular order and "the longer the teaching lasts, the better the cells will learn the lesson" [17].

Later observations by Costard-Jackle et al. [19] in a Langendorff-perfused isolated rabbit heart confirmed gradual development of an inverse relationship between activation time and duration of repolarization upon initiation of ventricular pacing suggesting that repolarization changes related to CM are indeed part of adaptation to the new activation sequence.

Using ventricular pacing and rate-related left bundle branch block (LBBB) as a means to alter the normal myocardial activation sequence in humans Rosenbaum et al. [17] summarized the major features of cardiac memory:

1. Spatial direction of the T wave follows the direction of the preceding abnormal QRS complex (hence the term itself);
2. Observed T wave changes manifest *accumulation* (increase in the magnitude of T wave change with increasing duration of preceding conduction abnormality), and
3. Property of *memory* (repeated interventions of the same magnitude/duration result in more rapid and intense accumulation of T wave changes).

The authors concluded that abnormal ventricular activation induces two opposite changes in repolarization:

1. Immediately apparent "secondary" T wave changes discordant to the main QRS direction, and
2. More gradual T wave changes induced by "electrotonic interaction" that develop in order to tailor repolarization to the new activation pattern. These changes were thought to be obscured by the more pronounced secondary T wave changes until the normal activation sequence resumes; however their presence can be inferred by the changes in the ventricular gradient even during abnormal activation.

The more rapid accumulation of T wave changes with repeat interventions suggested an analogy to the process of memory development in the central nervous system [20–22] which became the framework in which subsequent animal studies was carried out.

Following this analogy, cardiac memory was postulated to comprise two overlapping phenomena: short-term CM based on a selective modification of existing proteins and long-term CM involving new protein synthesis, changes in the cell coupling, etc. [23–25].

Experimental Models of Cardiac Memory

Based on the continuum of CM expression from the short-lived T wave changes after minutes of pacing to stable long-lasting T wave inversions following days and weeks after prolonged pacing the animal models were used to study both "short-term" and "long-term" CM. An acute open chest canine model was employed to study short-term memory while a chronically instrumented closed chest canine model with surgically implanted epicardial pacing electrodes

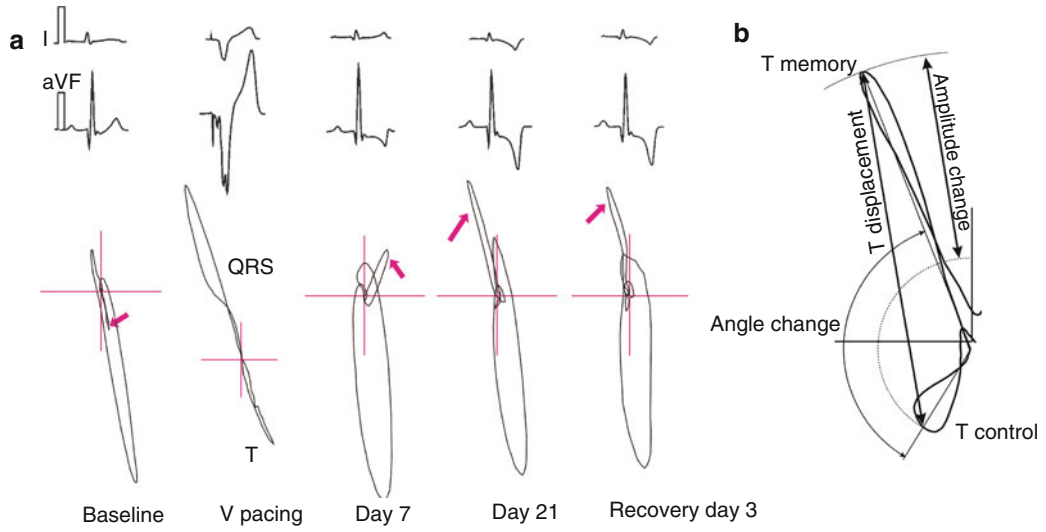

Figure 25–1. (**a**) Electrocardiographic (*top*) and vectorcardiographic (*bottom*) representation of a long-term CM development in a chronic canine model. Leads I, aVF, and frontal plane VCG shown at baseline, during ventricular pacing, 1 h after ventricular pacing for 7, 21 days, and 3 days after cessation of ventricular pacing. Note the increase in T vector amplitude and rotation towards the direction of the paced QRS complex (*arrows*). Cross-hair represents calibration at 1 mV (From Yu et al. [31]. Reprinted with permission from Wolters Kluwer Health). (**b**) Quantitative assessment of CM. The control and memory T vector loops superimposed. CM can be measured by the change in T vector direction, amplitude, and 3-dimensional distance (T peak displacement, TPD measured in mV) between T vector peaks ("T control" represents the baseline T vector loop, "T memory" represents T vector loop after CM induction). TPD is the preferred method of CM measurement (From Plotnikov et al. [27]. Reprinted with permission from Oxford University Press)

was used for studies of long-term cardiac memory [24–26]. Further development of the long-term CM model included application of additional externalized epicardial pacing electrodes (atrium, different areas of the ventricles) allowing performance of electrophysiological studies including pacing from multiple epicardial sites [27].

Due to rapid resolution of the T wave changes of short term CM, these experiments were limited initially to observations of pharmacological inhibition of T wave changes by ion channel blocking drugs and receptor antagonists [24,27,28] although some biochemical [29] and electrophysiological [30] studies were performed with longer (2 h) duration of pacing.

The chronic canine model permitted recordings in the conscious non-sedated state, provided the opportunity for multiple epicardial electrogram recordings and programmed stimulation, and was the source of myocardial tissue for histological, isolated cell and ion channel analysis [25, 27, 31]. Further refinements of the chronic CM model included dual chamber pacing [1, 32–34] eliminating confounding effect of the increased heart rate necessary with single chamber pacing and a transvenously placed ventricular pacing electrode [35] which eliminated the effects of pericardiotomy on the electrocardiogram and sped post-operative recovery.

In the initial experiments CM was induced by epicardial ventricular pacing from the posterolateral left ventricular base as the area activated the latest during sinus rhythm in both dogs [36] and humans [37, 38]. At the time this was presumed to achieve the most drastic reversal of the ventricular activation sequence (although later data suggested that right ventricular apical pacing produces the most abnormal left ventricular contraction pattern triggering CM) [39]. In this canine model short-term CM can be reliably induced by 20–60 min of ventricular pacing at a rate 15–20 % faster than sinus [24, 26, 27]. T wave changes due to the long-term CM approach plateau after 3 weeks of pacing (Fig. 25.1a) [25].

A vectorcardiographic-based method of CM measurement facilitated quantitative assessment of T wave changes independent of the individual

ECG lead characteristics. It involves measuring a 3-dimensional distance (in mV) between the T vector peaks at baseline and after the induction of CM [27], Fig. 25.1b. The proposed measurement (T peak distance, TPD) has become used in CM quantification in both animal and human studies [33, 40–42].

Although prolonged abnormal ventricular activation eventually results in structural changes in the left ventricle regardless of its mechanism [43–45], the ventricular pacing necessary for the development of the long term CM was shown to produce no changes in myocardial blood flow, cell size, and no histological evidence of hypertrophy, ischemia, or necrosis [1, 25, 31]. Therefore, CM can be considered an early, purely electrical stage of the long-term ventricular remodeling that can eventually progress to asymmetric ventricular hypertrophy, dilatation, and heart failure [39, 44, 45].

Molecular Mechanisms of CM

The first and most extensively studied ion channel implicated in the development of short-term CM was I_{to}. In an acute open-chest canine model Del Balzo et al. used 4-aminopyridine (4-AP), which blocks I_{to} blocker to abolish the T wave changes induced by 20-min ventricular pacing [26]. Based on the preferential expression of I_{to} in canine epicardium [46] the mechanism of short-term CM was proposed to include a change in transmural voltage repolarization gradient. Further support for this hypothesis was found by studying action potentials in isolated endocardial and epicardial slabs while simulating induction of CM by pacing in the direction perpendicular to the fiber direction. This intervention abolished phase 1 notch, prolonged the action potential of the epicardial cells, and reduced the transmural repolarization gradient. Pretreatment of the slabs with 4-AP reduced the phase 1 notch in the epicardial tissue and no further changes in action potential shape and duration were observed during pacing [47].

In long-term CM experiments prolongation of the epicardial action potential duration [25], loss of the phase 1 notch, decrease in I_{to} density, and decreased mRNA levels for Kv4.3, the gene controlling the pore-forming unit of the channel carrying canine I_{to} [31] and in the protein of its accessory subunit, KChIP2 [29] were observed in the left ventricular epicardium, indicating a role for I_{to} in the evolution of long-term CM as well (Fig. 25.2).

While the direction of changes in I_{to} density and distribution is similar in short- and long-term CM, their mechanisms appear to be different. In short-term CM the decrease in I_{to} is believed to result from internalization of the AT1 angiotensin receptor/Kv4.3/KchIP2 complex (discussed further below) [48]. In long-term CM it is achieved by the reduction in the transcription factor CREB (cAMP response element binding protein). Critical to this observation is a cyclic AMP response element near the promoter region of KChIP2 and Kv4.3. A reduction in CREB would lead to decreased synthesis of the channel components. Both the short- and long-term processes appear to be initiated by angiotensin II synthesis and release and may involve the actions of reactive oxygen species [29].

Several additional ion channels are implicated in short- and long-term CM including I_{Kr} [32], $I_{Ca,L}$ [24], possibly by the mechanism of CREB affecting transcription of some of them [29]. The reversal of the normal epicardial > endocardial gradient for the I_{Kr} density and the loss of the gradient for ERG mRNA and protein were documented in long-term CM around the ventricular pacing site also contributing to the local epicardial APD prolongation [32]. Reduction in epicardial connexin43 expression close to the pacing site (but not at remote ventricular areas) with gap junction redistribution was also observed along with the decrease in the conduction velocity around the pacing site [49] and an increase in the activation time during normal conduction [33] in long-term CM. A schematic of the short- and long-term CM development is presented in Fig. 25.3.

It is important to point out that the majority of the biochemical studies in CM involved tissue samples obtained from the areas 1–2 cm away from the left ventricular pacing site and although a gradient change in the transcription pathways across the ventricle was documented [34] the regional differences (especially in relation to the

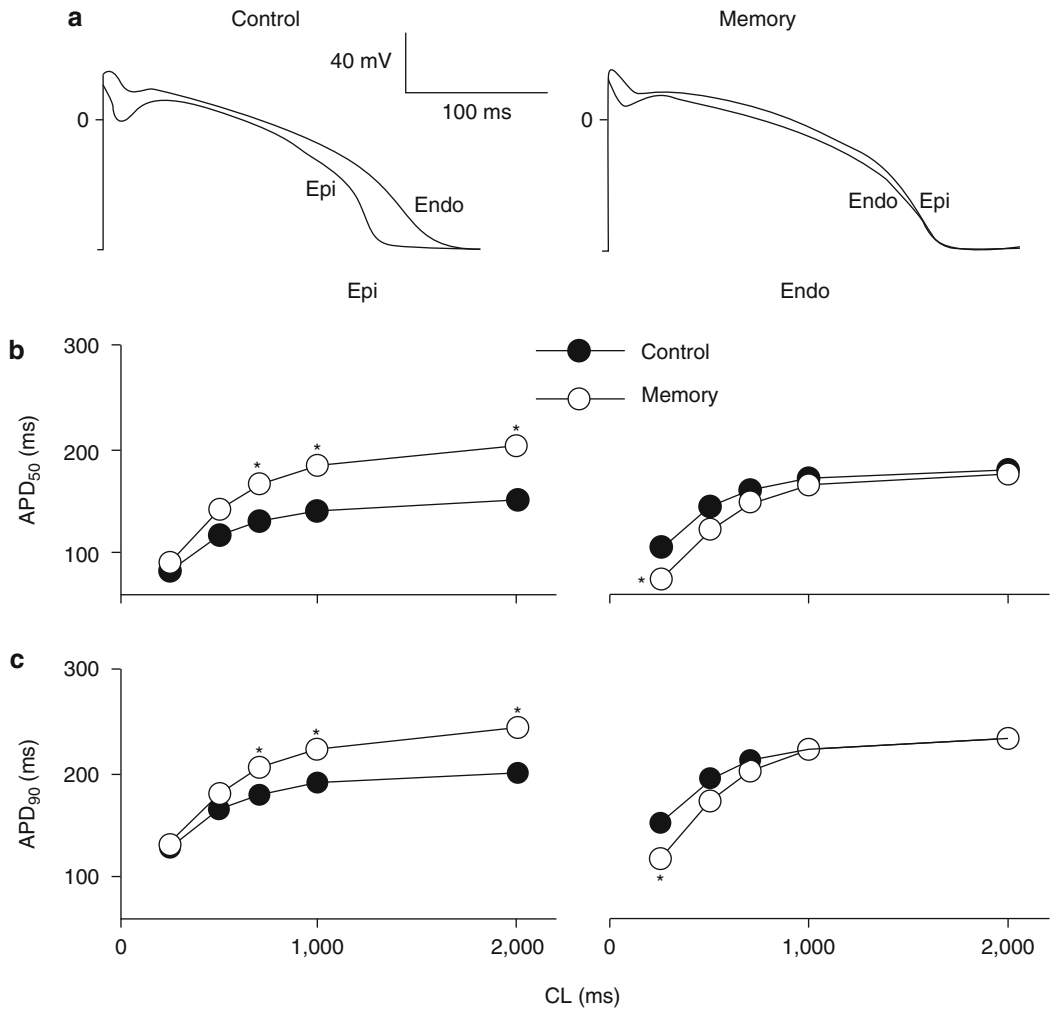

Figure 25–2. CM-induced changes in the action potential shape and duration. (**a**) Representative experiments showing superimposed epicardial and endocardial AP in a control dog and a dog with CM at CL = 1,000 ms. (**b**, **c**) Effects of CM on APD50 (b) and APD 90 (c) in epicardium and endocardium. *P < 0.05 vs respective control a the same CL (From Yu et al. (31) Reprinted with permission from Elsevier).

local mechanical function) have not been fully explored.

The processes of ventricular remodeling operate in the different directions in early- and late-contracting areas. The late results of this differential remodeling of the ventricle are quite opposite: thinning and atrophy of the early-contracting areas and hypertrophy of the late-contracting regions [44, 45]. Therefore it is plausible to predict that different mechanisms of remodeling operate in different areas of the ventricle at earlier stages as well which requires further investigation.

CM Triggers: Mechanical Forces Versus Electrical Activation Sequence

Along with the signal transduction and molecular control of CM its triggering factors have been extensively studied. It was clear that changes on a cellular and molecular level cannot be directly explained by the change in electrical activation sequence itself (a single cell cannot possess the knowledge of the timing of the activation front); therefore other mechanisms have to be involved. From the early experiments the alteration in the

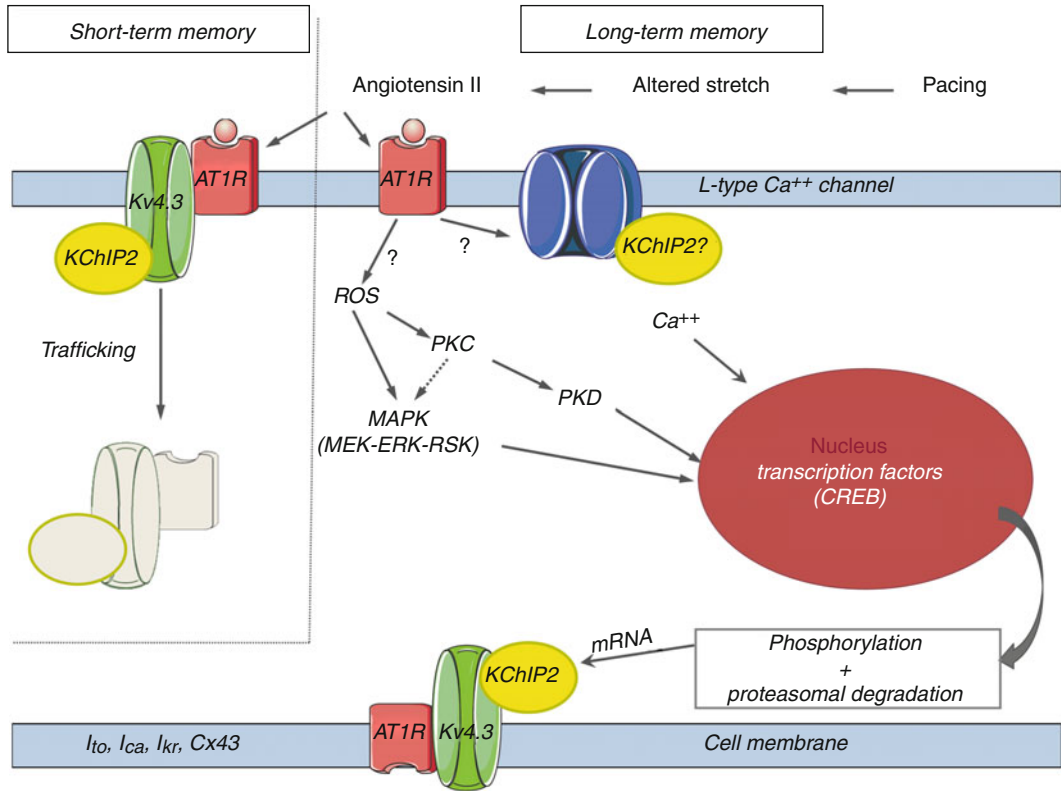

Figure 25–3. Schematic of pathways already identified and under study in short-term and long-term cardiac memory. Aberrant activation alters stretch resulting in increased cardiac angiotensin II synthesis/release. Short-term memory results from angiotensin II-induced trafficking of an AT1 receptor/Kv4.3/KChIP2 macromolecular complex. The result is reduction of Ito. Long-term memory is initiated by angiotensin II binding to its receptor leading to reduction of the transcription factor CREB. Production of reactive oxygen species (ROS) leading to MAPkinase and protein kinase C/D-determined pathways are possilbe intermediary steps. CREB is phosphorylated and then proteasomally degraded, resulting in reduced KChIP2 transcription and expression and decreased Ito. Other ion currents altered in memory include ICa, L and IKr. Connexin43 is also involved. Ca^{2+} is an important cofactor throughout (From Ozgen and Rosen [23]. Reprinted with permission from Elsevier)

local stress-strain relationship and geometry of ventricular contraction triggering activation of the renin-angiotensin system was suspected as the CM trigger and confirmed experimentally. Pretreatment of animals with saralasin, (AT1 and AT2 receptor antagonist), captopril (angiotensin converting enzyme inhibitor), and chymostatin (inhibitor of tissue chymase, which converts angiotensin I to angiotensin II) abolished the development of short-term CM suggesting a role for local angiotensin II production in its initiation [50].

Additional proof of local angiotensin II release as the CM trigger came from the demonstration that AT1 receptors co-localize with the α (alpha) (Kv4.3) and accessory (KChIP2) subunits of the I_{to} channel. Exposure to angiotensin II caused internalization of the AT1R–Kv4.3-KChIP2 macromolecular complex [48] reducing I_{to} current in HEK cells in which the channel components of the complex were overexpressed. The results were validated in co-immunoprecipitation experiments in cardiac myocytes, and in experiments using FRET. Another mechanism by which altered strain triggers CM is the stretch activated receptors (SAR) [2, 40]. In a canine model SAR blockade with streptomycin dramatically attenuated CM [40].

Sosunov et al. [51] in an isolated perfused rabbit heart obtained direct evidence that it is indeed mechanical factors and not the electrical activation sequence itself that serves as the primary

CM trigger. The excitation-contraction uncoupler blebbistatin completely abolished CM after ventricular pacing, while local myocardial stretch without ventricular pacing (and therefore unchanged activation) produced T wave changes similar to CM. This observation suggests potential common mechanisms of T wave changes observed in CM and various forms of structural heart disease associated with mechanical dyssynchrony without significant QRS prolongation such as myocardial infarction, hypertrophy, certain cardiomyopathies, etc.

Electrophysiological Effects of Cardiac Memory

While the studies of molecular mechanisms associated with CM in a large part have reached consensus, the electrophysiological effects of CM at the level of the whole heart are less clear.

There is an ongoing debate regarding to what degree transmural vs regional repolarization gradients are responsible for the T wave changes in CM. In perfused myocardial wedge preparations Jeyaraj et al. found APD prolongation in late- (and, to lesser extent early-activated regions) suggesting that regional repolarization gradient is responsible for long-term CM, while small transmural repolarization gradient remained unchanged [1]. In contrast, Spragg et al. using ablation-induced LBBB demonstrated shortening of action potential duration, effective refractory periods, and conduction velocity in the wedge preparations from the late-activated areas with increased tissue strain [2]. APD shortening in the late-activated areas was also consistently observed in the isolated rabbit heart [19, 51]. In the intact in-situ canine heart Coronel et al. showed the development of the transmural repolarization gradient localized at the area close to the pacing site with no evidence for significant regional gradient during long-term CM [33]. In-situ study of the short-term CM by the same group showed baseline apical-basal repolarization gradient that did not change significantly although repolarization time shortened uniformly in the setting of short-term CM [30].

Using non-invasive ECG imaging (ECGI) in patients with WPW syndrome before and after ablation of an accessory pathway Ghosh et al. detected the presence of a large (85–95 ms) regional repolarization gradient between the accessory pathway ventricular insertion site and the rest of the myocardium (translating into apical-basal gradient given the annular location of the accessory pathways). This gradient was caused by a significantly prolonged activation-recovery time in the area of the bypass tract insertion that gradually resolved within 1 month consistent with the time course of the long-term CM [52].

The challenges observed in extrapolating the results obtained in isolated myocytes, tissue slabs, and wedge preparations to the effects of CM in the heart in situ mirror those in explaining the genesis of the normal T wave [53, 54] (see [55] for potential explanations and discussion).

The relevance of these findings to human CM (the major contributors to which are right ventricular pacing and LBBB) is unclear as well. The vast majority of animal CM models followed the original idea of complete reversal of left ventricular activation by stimulating the latest activated site during normal conduction (i.e. posterolateral LV base) [36, 37]. However the degree of mechanical dyssynchrony which (rather than the electrical conduction delay) is the primary CM trigger does not correspond to the degree of activation reversal. Instead, endocardial right ventricular apical (rather than the epicardial left ventricular basal) pacing was found to create the most dyssynchronous contraction [39, 43]. This is one of the possible reasons why CM following right ventricular pacing in humans achieves the steady state more rapidly (1 week) [56, 57] than left ventricular pacing.

Human Studies of Cardiac Memory

Time Course of CM Development

Unlike the majority of animal experiments most human data on CM were obtained using right ventricular apical endocardial pacing. Compared to the long-term animal models, CM in humans evolves faster, achieving steady state within a

week of right ventricular pacing at physiologic rates when assessed semi-quantitatively [56, 57]. However, after 1 week of ventricular pacing T vector during normal conduction still has not been fully aligned with a paced QRS [58] and T vector loop morphology continued to evolve [57] suggesting that further subtle changes in T vector likely to occur beyond this period. In a single patient with experimentally induced rate-related LBBB M. Rosenbaum observed apparent plateau in ECG changes as shortly as after 24 h of abnormal conduction [17] although in cases of rate-dependent block pre-existing memory cannot be ruled out.

Effects on Myocardial Repolarization

Several studies documented the opposite effects of CM in humans on repolarization during normal and aberrant conduction. While during normal conduction (sinus rhythm or atrial pacing with narrow QRS) QT interval prolongs in the setting of long-term CM by 4–5 %, QT interval during ventricular pacing shortens with the increased duration of pacing by approximately 9 % [57–59]. This conforms to the concept that CM is an adaptive reaction to the new activation sequence [19, 51]. The fact that in the setting of CM changes in ventricular activation sequence (switching from sinus rhythm to ventricular pacing and back) produce instantaneous contrasting changes in the duration of repolarization (shortening with ventricular pacing and prolongation in sinus rhythm) indicate that CM involves a complex change in both transmural and regional repolarization gradients. Changes in T vector loop morphology that could be consistent with pro-arrhythmogenic effect during normal activation were observed [57].

It is unclear whether these changes result in untoward clinical consequences. An isolated case report of pro-arrhythmic effect of CM after ablation of an accessory pathway [60] was confounded by an iatrogenic right bundle branch block and therefore is questionable. Although long-term CM affects the action of antiarrhythmic drugs (for example exaggerating QT-prolonging effects of I_{Kr} blockers) in animals [27], the clinical significance of it is unclear.

Effects on Contractile Function

Little is known regarding contractile function of the heart and CM in humans. Alessandrini et al. [59] demonstrated that long-term CM is associated with transient diastolic dysfunction (prolongation of the isovolumic ventricular relaxation time and decrease in the left ventricular filling rate, dD/dt/D). The causality of the relation to CM was proven by correlation of the magnitude of diastolic dysfunction to the change in QT interval and T wave amplitude. These changes in myocardial function resolved within 1 h after cessation of ventricular pacing.

Longer-lasting myocardial systolic dysfunction (a decrease in the LV ejection fraction lasting over 24 h) was observed after 1 week of ventricular pacing [61].

Little is known regarding CM in structural heart disease besides a small observation series in hypertrophic obstructive cardiomyopathy [62]. Patients with compromised baseline ventricular function are more likely to develop pacing-induced ventricular dysfunction and heart failure [63, 64]. How exactly the phenomenon of CM relates to the pacing-induced heart failure in patients with compromised systolic function is unknown.

Clinical Implications of CM

Differential Diagnosis Between CM and Ischemia

Since the time CM was first recognized its similarity to ischemic T wave inversions (TWI) made it a significant confounder in the diagnosis of myocardial ischemia. This diagnostic dilemma becomes even more important because the pattern of the precordial TWI due to CM can closely resemble the pattern seen in proximal left anterior descending artery (LAD) disease (so called Wellens' syndrome) which portends poor prognosis unless urgent revascularization is performed (Fig. 25.4) [65, 66]. These changes are believed to be due to a transient LAD occlusion that resolves spontaneously and at the time of presentation these patients are often asymptomatic, further complicating the diagnosis

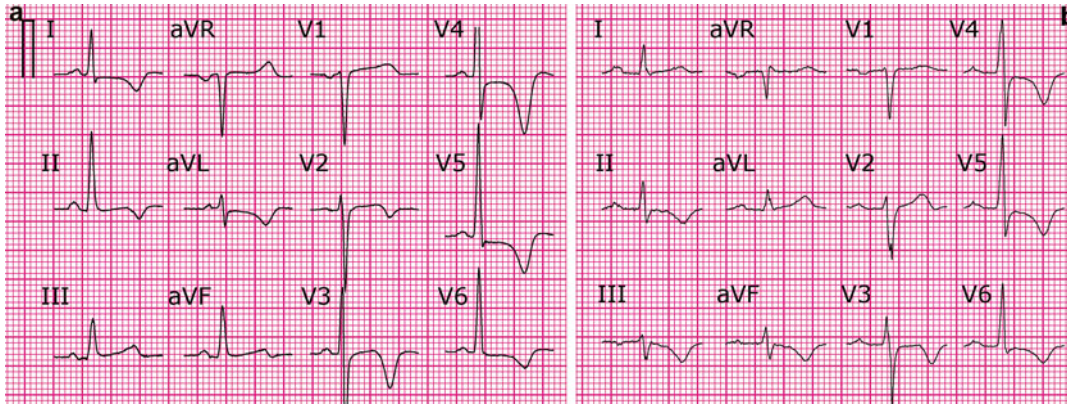

Figure 25–4. (**a**) Precordial T wave inversions (TWI) in a patient with transient proximal LAD occlusion (Wellens' syndrome). TWI in leads I, aVL is present. (**b**) Precordial T wave inversions due to CM. Note positive T waves in leads I, aVL and TWI V_{4-5} > TWI III

[66]. As the result, asymptomatic precordial TWI is a common referral reason for cardiac catheterization.

In developing diagnostic criteria to distinguish between TWI due to ischemia and CM due to intermittent ventricular pacing we used the following premises:

1. The direction of the ischemic T waves points away from the area of ischemia. Since in most cases the ischemic region involves the left ventricle, the ischemic TWI should have a rightward axis in the frontal plane. Repolarization abnormalities due to anterolateral ischemia in particular are characterized by TWI in leads I and aVL.
2. The vast majority of pacemaker leads are implanted in or close to the right ventricular apex producing left superior QRS axis during ventricular pacing with QRS positive in leads I and aVL. Pacing from alternative right ventricular sites (such as septal inflow tract and high interventricular septum close to the His bundle) usually also produce positive QRS complex in these leads during pacing. Based on the principles of CM this should result in positive T waves in these leads upon resumption of normal conduction.
3. Maximal repolarization changes due to CM occur in two non-contiguous areas (the earliest and latest activation sites) which is likely to produce a unique direction of T wave vector that cannot be replicated by repolarization changes in any single territory.

Based on these premises we compared in a retrospective study the direction of the TWI caused by ischemia and CM [67]. As predicted, the frontal plane CM-induced TWI direction was quite different from ischemia in the LAD and left circumflex artery (LCX) distributions. When translated to the 12-lead ECG terms, the presence of positive/isoelectric T wave in lead I and positive T wave in lead aVL while typical for CM was never seen in LAD/LCX ischemia. It was observed in some cases of right coronary artery (RCA) ischemia; however those cases differ from CM-induced TWI in other vector planes. When translated to the 12-lead ECG terms this pattern was characterized by TWI that was deeper in the inferior leads (II, III, aVF) compared to the deepest TWI in the precordial leads whereas the reverse was true for CM-induced TWI. Overall, the combination of the two criteria:

1. Positive/isoelectric T lead I and positive T aVL and
2. Precordial TWI > TWI in lead III was 92 % sensitive and 100 % specific for CM in discriminating it from ischemia.

Although not confirmed in larger prospective trials, these criteria have been used successfully in clinical practice by us as well as others [68, 69]. Since the axis during right ventricular pacing and left bundle branch block (LBBB) is similar with positive QRS complex in leads I and aVL, the criteria described can also be used in the cases of TWI due to intermittent LBBB [69].

One of the limitations in using this diagnostic tool is the concern that T wave changes due to CM can counter-balance or obscure ischemic TWI when CM and ischemia occur together in the same patient. Although CM has been studied in populations of patients with structural heart disease such as hypertrophic obstructive cardiomyopathy [62], no published data are available in patients with T wave abnormalities due to coronary artery disease. Our preliminary results in this population indicate that CM does not "override" ischemic TWI eliminating the possibility of missing myocardial ischemia. However, when TWI in leads I and aVL are present at baseline as the result of the structural heart disease, T waves stay inverted despite the development of CM (T vector never fully aligns with the paced QRS vector) which can result in over-diagnosis of ischemia.

CM During Continuous Abnormal Ventricular Activation

Since the introduction of the original concept of cardiac memory in 1982 and until recently, two important issues remained unresolved.

First, it was universally accepted that the return to the normal ventricular activation sequence is pre-requisite to observe it. Although it was clear that CM develops gradually during the period of abnormal activation secondary T wave changes were thought to completely obscure it.

The most common causes of CM in clinical practice – LBBB and atrioventricular block requiring ventricular pacing are rarely reversible. This significantly limited clinical application of CM as the majority of the patients developing CM never return to normal conduction.

Second, although obvious individual variations in the degree of CM, were observed [70], it was unclear what factors determine this variability. The only factor associated with CM magnitude was the amount of ventricular pacing [57].

Using further refinement of the quantitative methods of CM assessment developed previously in the animal studies [27], we sought to resolve these issues.

In our clinical practice we observed that following pacemaker implantation the amplitude of T waves during ventricular pacing decreases in time. We hypothesized that this change in T wave amplitude is related to the development of CM. Using vectorcardiographic software [71] we compared 3 dimensional T vector loop displacement after 1 week of ventricular pacing (DDD with short atrioventricular delay) in humans in AAI mode (conventional measure of CM) to that in DDD mode with continuous ventricular pacing [58].

We found that not only the 3-dimensional direction of the T wave shift was identical when measured in AAI and DDD mode (Fig. 25.5), but the magnitude of the shift in the two pacing modes correlated closely (Fig. 25.6). In contrast to AAI mode (conventional CM) which demonstrated typical rotation of the T wave vector towards the paced QRS direction, CM in DDD mode (continuous ventricular pacing) was characterized by a progressive decrease in discordant T vector magnitude without change in direction. The strong correlation between T vector changes in both pacing modes suggested that they reflect the same physiological phenomenon. These findings have important implications.

First, quantitative measurement of CM is of critical importance in elucidation of its mechanisms. Various methods of CM quantification in clinical practice have been used, including TPD, changes in T-wave amplitude, T-wave area [72], or T-vector direction [56, 57]. CM alters T vector during normal ventricular activation in a complex manner, including variable changes in amplitude, direction, and morphology [56, 57]. Some of these changes are non-uniform and time dependent. For example, T-vector magnitude often decreases after the first 24 h of pacing [57] before it starts increasing. T-vector direction change depends on its initial orientation, and in cases with abnormal baseline T wave axis can be minimal or nonexistent. Changes in the shape of the T vector loop include increased roundness and distortion of the loop [57], which can interfere with TPD measurements. Therefore, it is unlikely that a single measurement during normal ventricular activation can reliably reflect

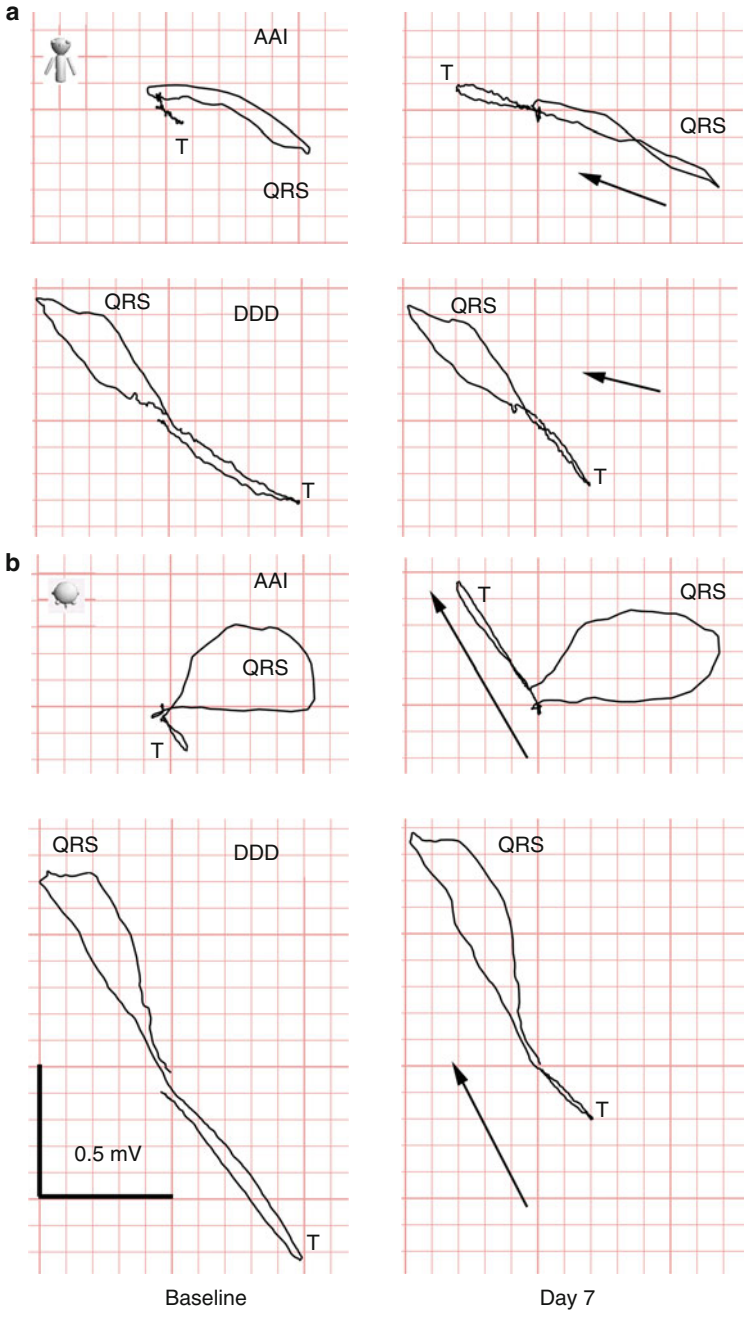

Figure 25–5. Vectorcardiogram of the same patient during AAI and DDD pacing in frontal (**a**) and transverse (**b**) projections before (baseline) and after induction of cardiac memory (Day 7). On Day 7 during AAI pacing, the T vector assumes the direction of the paced QRS complex while increasing in magnitude. At the same time in DDD mode, the T-vector magnitude decreases without significant change in direction. *Black arrows* indicate the direction and magnitude of the projection of T-peak displacement (TPD) as the result of cardiac memory and are very similar in AAI and DDD modes. Sagittal plane (not shown) showed similar changes (From Shvilkin et al. [58]. Reprinted with permission from Elsevier)

these complex changes and adequately quantify CM. We believe that CM measurement during DDD pacing offers a superior alternative. T-vector change during DDD pacing consists of a uniform time-dependent decrease in vector magnitude with no significant change in direction eliminating variability attributable to the differences in baseline T-wave axis and T-vector angle change.

Second, the ability to detect CM during continuously abnormal ventricular activation expands the application of this concept to a wide

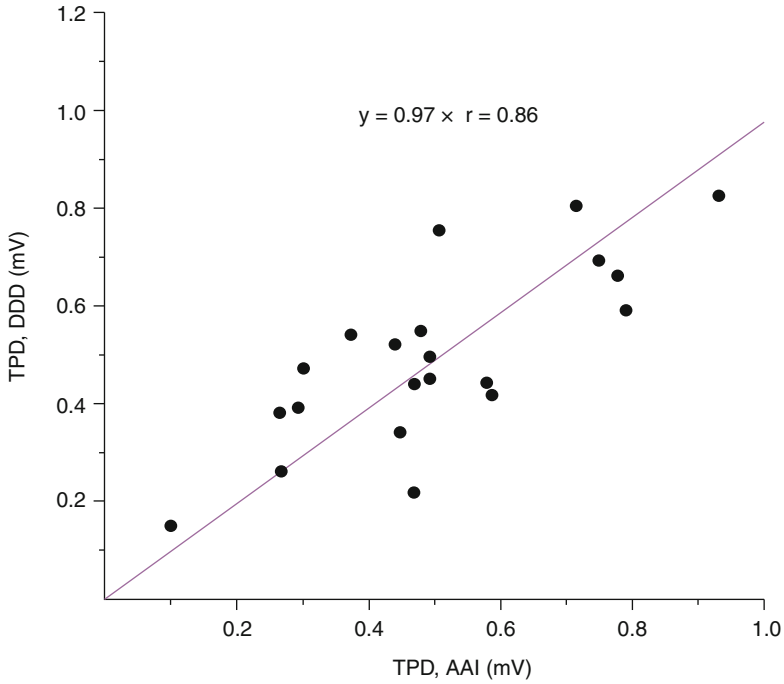

Figure 25–6. CM magnitude (T peak displacement, *TPD*) measured during normal (AAI pacing) and aberrant (DDD pacing) ventricular activation. CM magnitude measured in the two pacing modes correlates closely (From Shvilkin et al. [58]. Reprinted with permission from Elsevier)

variety of clinical situations when no return to normal conduction is possible (pacemaker dependency, persistent LBBB).

Our observations of CM during continuous abnormal activation recently have been confirmed and expanded by description of T wave changes compatible with CM during [73] and after biventricular pacing [73, 74].

CM in LBBB Age Determination

One of the obvious clinical applications of CM during continuous abnormal ventricular activation (progressive decrease in the amplitude of T vector without directional changes with increased duration of pacing) is to determine the duration of the conduction abnormality. We applied this concept to determine the age of the LBBB. This determination is essential in the setting of the suspected acute coronary syndrome. Current American College of Cardiology/American Heart Association guidelines for the management of patients with ST elevation myocardial infarction (MI) consider new or presumed new LBBB associated with symptoms suggestive of ischemia a class I indication for reperfusion therapy [75] based on increased mortality in this patient population [76, 77]. The premise of this recommendation is the acute onset of LBBB (within minutes or hours) in a setting of a proximal coronary artery thrombosis.

Recently, the validity of these recommendations has been challenged in several studies that demonstrated a low prevalence of MI and mortality in LBBB patients presenting to the emergency room with chest pain [78, 79]. One of the possible reasons for this discrepancy is the inclusion of patients with "presumed new LBBB," which in fact is old and not associated with the acute ischemic event, but prior ECGs are lacking. Therefore, the ability to determine whether LBBB is new or old using a single ECG tracing would facilitate the triage of patients with chest pain and LBBB and potentially help to avoid unnecessary cardiac interventions, reduce complications, and the overall cost of care.

We compared QRS/T vector ratios between the new onset (<24 h duration) and old (>24 h duration) LBBB. Similar to the right ventricular pacing, T vector amplitude decreased with the increase in the LBBB duration (Fig. 25.7). In addition, QRS vector amplitude in the old

LBBB group was significantly larger than in the new LBBB group. These two opposite trends resulted in wide separation of QRS/T vector amplitude ratios between the groups (Fig. 25.8) allowing reliable LBBB age diagnosis [41]. When adapted to the 12-lead ECG terms suitable for manual analysis the ratio of the deepest S wave to the tallest T wave in the precordial leads <2.5 had 100 % sensitivity and 89 % specificity in diagnosing new onset LBBB. The validity of these findings was preserved in a small subgroup of patients with acute coronary syndrome. If confirmed in larger studies these criteria can significantly simplify diagnostic decisions in this difficult category of patients.

Individual Variability in CM: Quantitative Extension of the Original Concept

In addressing the issue of individual variability in CM magnitude we studied multiple clinical, electrocardiographic, and vectorcardiographic factors using ventricular pacing in DDD mode for 1 week. This duration of pacing was shown to produce maximal steady-state pacing-induced T wave changes in humans [56]. Despite comparable amount of ventricular pacing we found wide (up to eightfold) variation in the CM magnitude among patients. The strongest correlation in univariate analysis was found between the magnitude of CM and QRS/T vector amplitude ratio in DDD mode at the beginning of ventricular pacing explaining over 80 % of variance in CM magnitude (Fig. 25.9). This parameter remained the only one significant variable in multivariate analysis. Patients with smaller QRS/T ratio (taller T waves) in ventricular paced beats demonstrated more pronounced CM.

This finding provides a quantitative extension of the original concept of CM: not only the direction of the paced QRS determines the direction of the memory T vector but the paced QRS/T configuration (namely QRS/T vector amplitude ratio) to a large extent determines its magnitude.

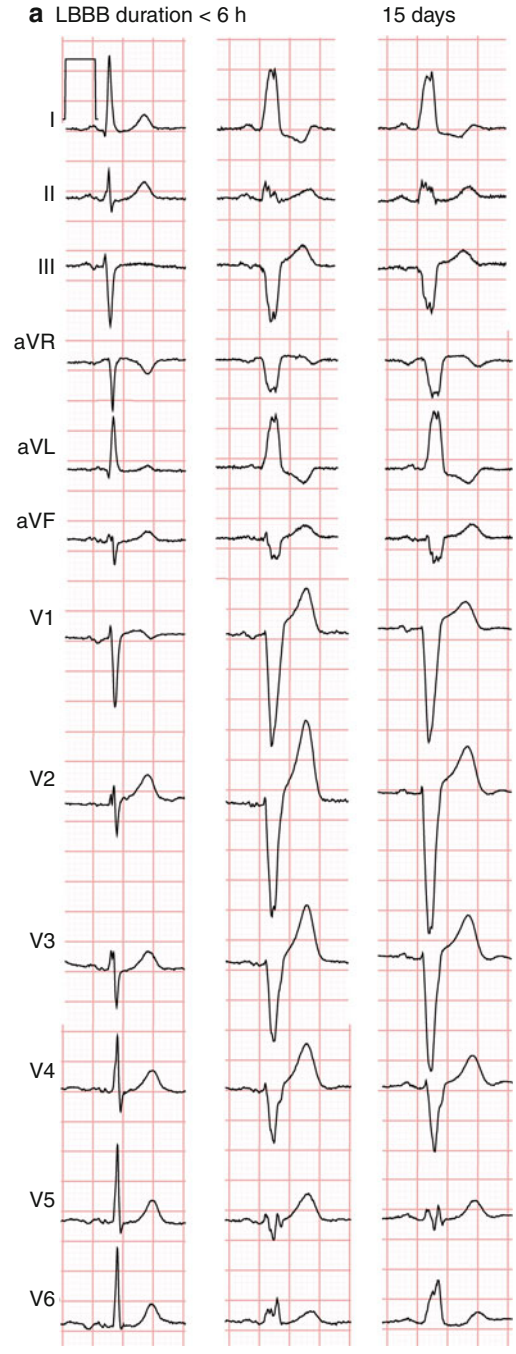

Figure 25–7. Electrocardiogram (*Panel* **a**) and vectorcardiogram (*Panel* **b**) of the new (<6 h) and old (15 days) LBBB of the same patient. (*Panel* **a**) ECG during sinus rhythm (*left*), new (*middle*) and old (*right*) LBBB. Note the decrease in the T wave amplitude (best seen in the precordial leads) with the increase in LBBB duration. (*Panel* **b**) Orthogonal VCG projections (transverse, frontal, left sagittal planes) of a new (*Left*) and old (*Right*) LBBB. VCG measurements demonstrate peak QRS and T vector magnitudes, azimuth (ϕ, PHI), and elevation (θ THETA). Note the decrease in the T vector magnitude without change in direction in the old LBBB (From Shvilkin et al. [41]. Reprinted with permission from Elsevier)

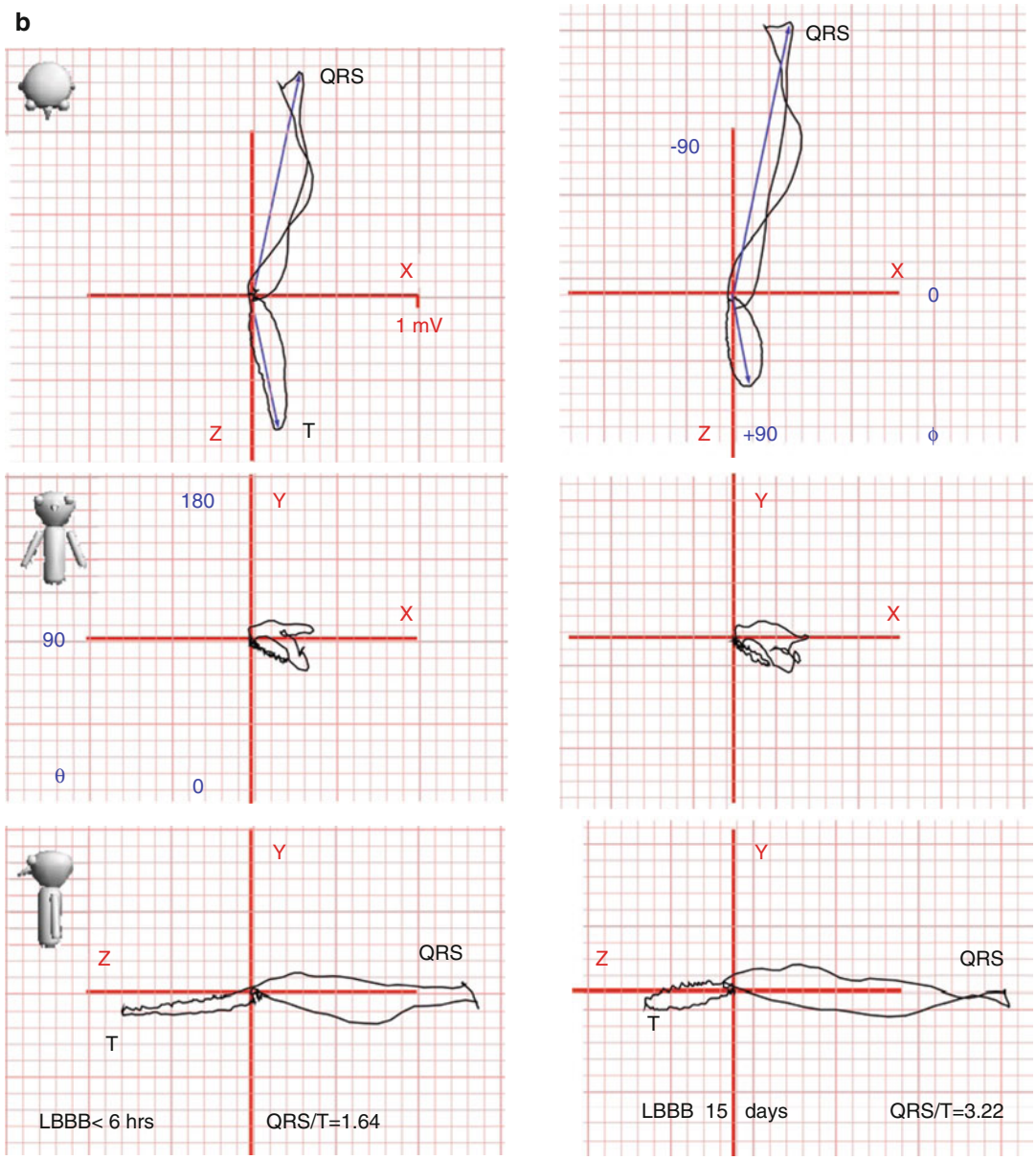

Figure 25–7. (continued)

Cardiac Memory: Parallels Between Electrical and Mechanical Functions of the Heart

Although the alteration of the mechanical function of the heart was convincingly proven to be the primary trigger for CM development, the relationship between the two is complex. Both global (developed ventricular pressure) and local (mechanical dyssynchrony) function affect CM magnitude [51]. The contribution of these two factors can be to some extent counter-balancing: the increase in mechanical dyssynchrony results in the decrease of global ventricular contractility and developed pressure. Therefore, the final CM magnitude will likely reflect the interplay between local and global contractility changes.

Based on the strong correlation between paced QRS/T vector amplitude ratio and CM

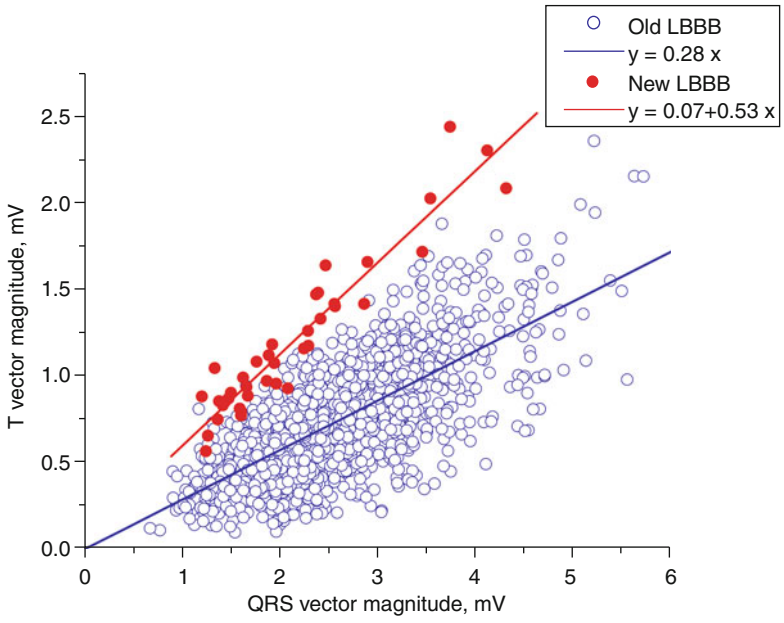

Figure 25–8. Relationship between QRS and T vector ratio in the new (*solid circles*, n = 39) and old (*open circles*, n = 1,760) LBBB. Note very similar QRS/T ratio in the new LBBB and much more variable QRS/T ratio in the old LBBB (From Shvilkin et al. [41]. Reprinted with permission from Elsevier)

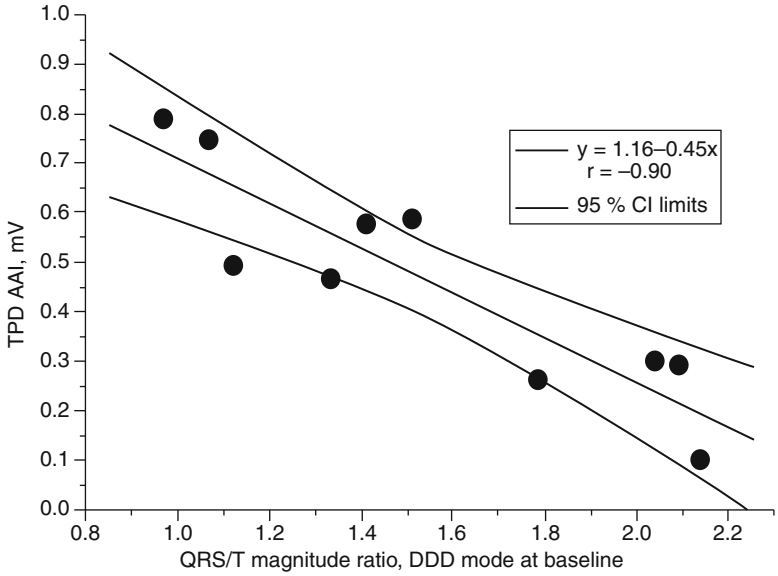

Figure 25–9. Correlation between QRS/T ratio and CM magnitude (TPD measurement during AAI pacing with narrow QRS are presented) (From Shvilkin et al. [58]. Reprinted with permission from Elsevier)

magnitude, the former can be viewed as a surrogate of the CM triggering stimulus intensity.

When studying QRS/T vector amplitude ratios we were intrigued by the fact that in patients with the new onset LBBB this ratio was remarkably similar (approximately 1.65). This indicated that the identical change in ventricular activation sequence (proceeding from the point of the right bundle branch insertion into the right ventricular myocardium) generates a very similar trigger for CM. At the same time, right ventricular pacing while activating the ventricle close to the point of the right bundle branch insertion produces wide variation of QRS/T ratios. This paradox has a few potential explanations. First, dual chamber pacing in patients with preserved AV conduction often results in a fusion of the ventricular activation from the pacing electrode and intrinsic conduction. This fusion can be very subtle and sometimes only observed using

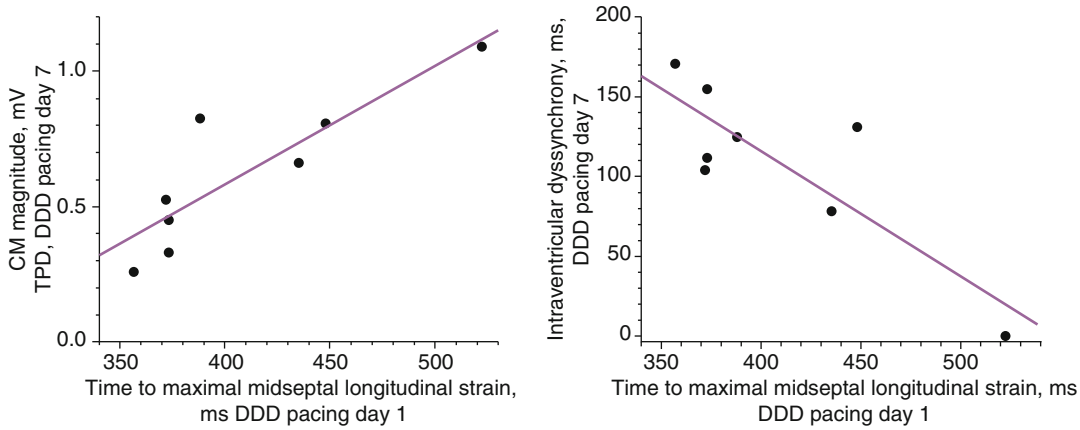

Figure 25–10. Relationship between CM and regional wall motion. *Left*: CM magnitude (measured as TPD in DDD mode after 7 days of DDD pacing) correlates with the delay in septal contraction. *Right*: The delay in septal contraction (time to the maximal strain, X axis) is inversely related to the degree of the interventricular dyssynchrony

quantitative vectorcardiographic techniques. In a few patients we were not able to achieve full ventricular capture despite using the shortest pacemaker AV delay settings. The ventricular fusion can also be produced by the retrograde invasion of the pacemaker activation front into the terminal branches of the conduction system. Finally, variation in the ventricular pacing electrode position can be large enough to produce significantly different patterns of activation.

Based on these assumptions we hypothesized that the variability in the magnitude of CM and paced QRS/T vector ratio will be reflected in the differences of the contractile disturbance produced by ventricular pacing. Our preliminary observations demonstrated strong correlation between CM magnitude after 1 week of the right ventricular pacing and geometry of the left ventricular contraction. In particular, CM magnitude correlated positively with the time to the maximal septal strain which in turn was inversely related to the left ventricular dyssynchrony (measured as time difference between the maximal strain at the earliest and latest sites), Fig. 25.10. A possible interpretation of these findings is that it is an abnormal early septal contraction that contributes to the pacing-induced dyssynchrony associated with a negative impact on the stroke volume and reflected by the small CM magnitude. When the septal activation and contraction are delayed (more synchronous with the rest of the delayed left ventricle), the amount of dyssynchrony decreases and the stroke volume increases which is reflected by the more pronounced CM.

If these findings are confirmed it will make possible to estimate the amount of dyssynchrony generated by abnormal activation based on the QRST morphology of a single aberrant beat. This can have profound clinical implications in choosing the site of ventricular electrode placement (for the right and left ventricular pacing), predicting long-term effects of ventricular pacing, etc.

Conclusion

Cardiac memory is an intriguing phenomenon that over the years provided researchers with great insights into the physiology of the heart. CM greatly enriched our understanding of the function of the heart from the molecular level to the bedside. From a clinical standpoint especially exciting are the advances in the electrocardiography of wide QRS rhythms and their potential relationship to the contractile function of the heart.

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Part II
Heritable Arrhythmogenic Channelopathies, Primary Electrical Diseases, and Sudden Cardiac Death

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Introduction to Part Two: Celebrating the Challenge of Cardiac Arrhythmias

Jeffrey A. Towbin

The field of cardiac arrhythmias has matured dramatically over the past 15 years, initially spurred on by the studies performed on the genetics of arrhythmia disorders. Once the genetic basis of disorders such as the long QT syndrome (LQTS) and Brugada syndrome (BrS) was discovered to occur due to disruption in ion channel encoding genes, basic electrophysiologists joined the fray, opening up a new area of study with significant clinical relevance. This book is a result of such bedside-to-bench collaboration.

Today, the genetic foundation of arrhythmia disorders is known to be based on disruption of a "final common pathway," now called ion channelopathies. In addition to LQTS and BrS, catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT syndrome (SQTS), atrial fibrillation (AF), and Wolf–Parkinson–White (WPW) syndrome have, at least in part, been defined genetically. Overlap disorders including arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), dilated cardiomyopathy (DCM), Andersen–Tawil syndrome, and Timothy syndrome are also defined, with ion channel disruption central to the clinical features or secondarily dysfunctional.

These new genetic and biophysical discoveries have opened up several new sciences as well. Drug discovery now requires analysis of potential effects on channel function. Pharmacogenomics is also the key to new approaches to medical therapy of "old" disorders. The concepts of gene-targeted therapies have grown out of this work. The pioneering work by Arthur Moss, Peter Schwartz, G. Michael Vincent, and others on the use of mexilitine and other similar therapies in LQT3, a gain-of- function persistent sodium channel leak treated with a sodium channel blocking agent, as well as the use of potassium supplementation in other forms of treatments to be devised have caused a reawakening to older therapies during studies of outcomes in patients treated with classic modalities such as β(beta) blockers and have resulted in newer approaches such as internal cardioverter defibrillator (ICD) implantation. In addition, fee-for-service genetic testing has finally become available for clinical use.

In the chapters that follow, an excellent group of assembled authors outline the current state of knowledge in the area of arrhythmias, as well as novel new concepts that are likely to define the future of diagnosis and treatment of these high-risk disorders. This has been an exciting field and, as demonstrated in this book by Gussak and Antzelevitch et al., the best is yet to come.

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Congenital Long QT Syndrome

David J. Tester, Peter J. Schwartz, and Michael J. Ackerman

Abstract

With an incidence as high as 1 in 2,000–2,500 live births, long QT syndrome (LQTS) is often characterized clinically by prolongation of the heart rate corrected QT interval (QTc) on a 12-lead surface electrocardiogram (ECG) and is associated with syncope, seizures, and sudden cardiac death due to ventricular arrhythmias usually following a precipitating event such as exertion, extreme emotion, or auditory stimulation. The phenotypic expression of LQTS varies profoundly from asymptomatic longevity to premature sudden cardiac death despite medical therapy. Therefore the clinical/genetic diagnostic evaluation and risk-stratification are highly important issues in the clinical management of LQTS. This chapter will review the historical background, epidemiology and prevalence, molecular genetics, and clinical presentations of LQTS, explore unique genotype – phenotype relationships that help define the various forms of the disorder, and provide a detailed outline for the diagnostic evaluation and clinical management of LQTS patients including current treatment strategies and recommendations.

Keywords

Long QT syndrome • Ion channels • QT interval • Genetics • Genetic testing • Electrocardiogram • Sudden death

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Introduction

Once considered an extremely rare yet lethal arrhythmogenic peculiarity, congenital long QT syndrome (LQTS) is understood today as a primary cardiac arrhythmia syndrome/electrical cardiomyopathy ("Cardiac Channelopathy") that is both far more common and overall much less lethal than previously recognized. Clinically, LQTS is often characterized by prolongation of the heart rate corrected QT interval (QTc) on a 12-lead surface electrocardiogram (ECG) and is associated with syncope, seizures, and sudden cardiac death due to ventricular arrhythmias (*torsades des pointes*,

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TdP) usually following a precipitating event such as exertion, extreme emotion, or auditory stimulation. The molecular breakthroughs of the 1990s led in large measure by the research/clinical laboratories of Mark Keating and Michael Vincent in conjunction with international LQTS registries containing meticulously phenotyped patients directed by Arthur Moss and Peter Schwartz revealed the fundamental molecular underpinnings of LQTS – namely, defective cardiac channels [1].

Maturing from the decade (1995–2004) of research based genetic testing, LQTS genetic testing has been a routine, commercially available molecular diagnostic test in the United States since 2004. Nevertheless, the cornerstone of the clinical evaluation for LQTS remains a time honored careful personal/family history evaluation and meticulous inspection of the ECG to detect either overt prolongation of the QT interval or suspicious T wave abnormalities. Once a patient exhibits the clinical and/or genetic substrate for LQTS, it is critical to delineate whether or not he/she possesses a substrate that resembles a "ticking time bomb" waiting for the necessary trigger to detonate, or perhaps a "dud" destined for asymptomatic longevity.

In this chapter, we will first present some case vignettes followed by a review of the historical background, epidemiology and prevalence, molecular genetics, and clinical presentations of LQTS. Next, we will explore unique genotype – phenotype relationships that help define the various forms of LQTS. We then will offer a detailed outline for the diagnostic evaluation and clinical management of LQTS including current treatment strategies involving current and potential pharmacotherapies, device therapy with implantable cardioverter defibrillators (ICD), and surgery involving the left cardiac sympathetic denervation (LCSD) procedure. Lifestyle recommendations, prevention, and competitive sport issues will be reviewed. Finally, we will conclude with clinical pearls derived from a dissection of the three case vignettes.

Case Vignettes

Case #1 – Do I or Don't I Have Long QT Syndrome? That Is the Question

JPA is a 15-year-old male referred for a second opinion and possible ICD implantation following the diagnosis of LQT1. At a preparticipation sports physical, an innocent murmur was heard, an ECG was obtained and a QTc of 440 ms was noted. The patient fainted once in the setting of having his blood drawn. His family history was completely negative. No ECGs were performed on the parents. Holter monitoring revealed a QTc maximum of 501 ms occurring at 2:30 AM. An epinephrine QT stress test using the Mayo continuous infusion protocol demonstrated a 100 ms increase in the QTc during low dose epinephrine. Genetic testing revealed a rare amino acid substitution in the *KCNQ1*-encoded Kv7.1/I_{Ks} potassium channel localizing to the C-terminus of the channel. The patient was diagnosed with LQT1, placed on once-a-day atenolol beta-blocker therapy, restricted from all competitive sports, and a recommendation for sudden death primary prevention with an ICD was rendered.

Case #2 – Oh Great, an Infant with LQT3, Now What Do I Do?

JMA is a healthy asymptomatic newborn female born to a mother with genetically proven LQT3. The family history is significant for a nocturnal sudden death involving a maternal aunt at age 40. The initial newborn ECG shows a QTc of 420 ms. Would you perform any additional tests or dismiss as normal?

Case #3 – Doctor Please Do Something, My Defibrillator Keeps Shocking Me

LLA is a 32-year-old female with previously symptomatic LQT2, QTc of 550 ms, and a positive family history of multiple

premature sudden deaths. Despite adequate beta blocker therapy with nadolol, she continues to receive frequent ventricular fibrillation-terminating ICD therapies. What therapeutic strategy would you consider next?

These three cases highlight several aspects in the clinical evaluation, risk stratification, and management of LQTS and the approach to each case will be summarized at the chapter's conclusion.

Historical Background

Congenital LQTS was first described by Drs. Anton Jervell and Fred Lange-Nielsen in 1957 in a Norwegian family in which four of six children had prolonged QT interval, congenital sensorineural hearing loss and recurrent syncope during exercise or emotion; three of them died suddenly at ages 4, 5 and 9 years [2]. The QT prolongation on the electrocardiogram (ECG) was obvious and the inheritance pattern appeared autosomal recessive. A similar clinical syndrome of sudden death during exercise and emotion, but with normal hearing and autosomal dominant inheritance was first described separately by Drs. Romano [3] and Ward [4] in the early 1960s after observing families with similar presentations including prolongation of the QT interval, syncope and sudden death. These two inherited forms of LQTS with different modes of inheritance have become known respectively as the Jervell and Lange-Nielsen syndrome (JLNS) and the Romano-Ward syndrome (RWS).

Epidemiology and Prevalence

The prevalence of congenital LQTS in the USA has been speculated to be approximately 1 in 5,000 persons [5], causing hundreds to thousands of sudden deaths in children, adolescents and young adults each year [6]. However, the latest data from Italy presented in 2005 suggests the incidence may be as high as 1 in 2,000–2,500 live births [7]. In addition, LQTS is one of the most common causes of autopsy negative sudden unexplained death [8].

RWS is the most common inherited form of LQTS accounting for over 99 % of cases and is transmitted as an autosomal dominant trait, whereby, each child of an affected parent has a 50 % chance of inheriting the abnormal allele and males and females are equally affected. In contrast to RWS, JLNS is associated with congenital deafness and is extremely rare. Males and females are also equally affected. Initially understood exclusively as an autosomal recessive disorder, JLNS is now appreciated to be a syndrome where the auditory phenotype (deafness) is autosomal recessive, but the cardiac phenotype is autosomal dominant [9]. Thus, technically, both parents of a child with JLNS actually have RWS, albeit curiously with a generally mild/negligible cardiac phenotype.

Molecular Genetics

With respect to its pathogenic mechanisms, hundreds of mutations in 13 distinct LQTS susceptibility genes have been identified so far and principally involve either loss-of-function potassium channel mutations or gain-of-function sodium channel mutations (Fig. 27.1). The vast majority of all genotyped LQTS and 75 % of all clinically robust LQTS is a pure "channelopathy" stemming from mutations involving cardiac channel alpha subunits: *KCNQ1*-encoded Kv7.1/I_{Ks} potassium channel (LQT1, 30–35 %), the *KCNH2*-encoded Kv11.1/I_{Kr} potassium channel (LQT2, 25–30 %), or the *SCN5A*-encoded Nav1.5/I_{Na} sodium channel (LQT3, 5–10 %).

Rare subtypes stem from perturbations in key cardiac channel interacting proteins [Kv7.1, Kv11.1, or Nav1.5 beta subunits (LQT5, LQT6, LQT10, LQT11, LQT12) or structural membrane scaffolding proteins such as ankyrin B (LQT4) or

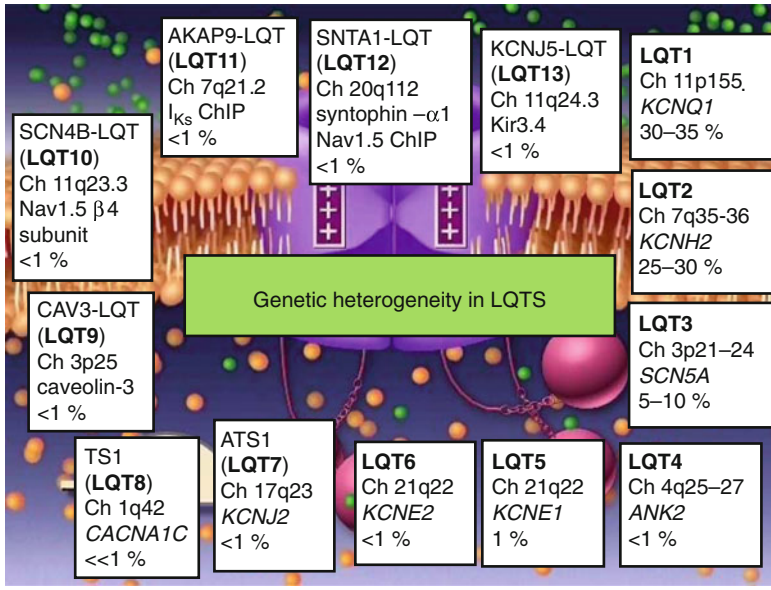

Figure 27–1. Compendium of LQTS-susceptibility genes

caveolin 3 (LQT9)]. The latest edition, LQT13, identified in 2010 in a single large Chinese pedigree with concomitant prolonged QTc and persistent atrial fibrillation, stems from mutations in the *KCNJ5*-encoded Kir3.4 potassium channel [10].

Complex, multi-system syndromes that include abnormal repolarization have been included as variants of LQTS. These syndromes include Andersen Tawil syndrome (ATS1, also annotated as LQT7) due in part to mutations in the *KCNJ2*-encoded Kir2.1/I_{K1} potassium channel and Timothy syndrome (TS1, also annotated as LQT8) due to mutations in the L-type calcium channel (Cav1.2) alpha subunit. This chapter will focus mostly on the clinically pure forms of LQTS, particularly the three most common subtypes, LQT1-3.

Clinical Presentations

The clinical manifestation of LQTS ranges from a lifelong asymptomatic course in some cases to premature sudden cardiac death (SCD) during infancy in others. As a simple rule of thumb, approximately 50 % of patients with genetic evidence of LQTS will never have a symptom attributable to LQTS and a significant proportion (ranging from 10 to 40 % depending on genotype) of those with genetic LQTS do not show overt QT prolongation [5, 11, 12]. Patients failing to display QT prolongation at rest are said to have **Concealed LQTS** or **Normal QT Interval LQTS.** While risk of a LQTS-precipitated event generally increases with increasing QTc, it is nevertheless possible to experience SCD as a sentinel event despite having Concealed LQTS (i.e. normal resting QTc). This is fortunately quite uncommon. The most common presenting symptoms are recurrent syncope, seizures, and sudden cardiac death (Fig. 27.2). These events are due to the hallmark feature of LQTS, namely polymorphic ventricular tachyarrhythmias called *Torsade des Pointes* (TdP) which are most often self terminating. Rarely, TdP continue to degenerate, yielding ventricular fibrillation and sudden death. Overall, less than 5 % of patients with LQTS will present with a sentinel event of sudden death or aborted cardiac arrest. Conversely, nearly half of LQTS sudden death victims experienced a prior warning episode of syncope.

Syncope is the most frequent symptom occurring commonly between age 5 and 15 years. Among symptomatic probands, 50 % experience their first cardiac event by 12 years of age (particularly boys), and by 40 years of age the proportion increases to almost 90 %

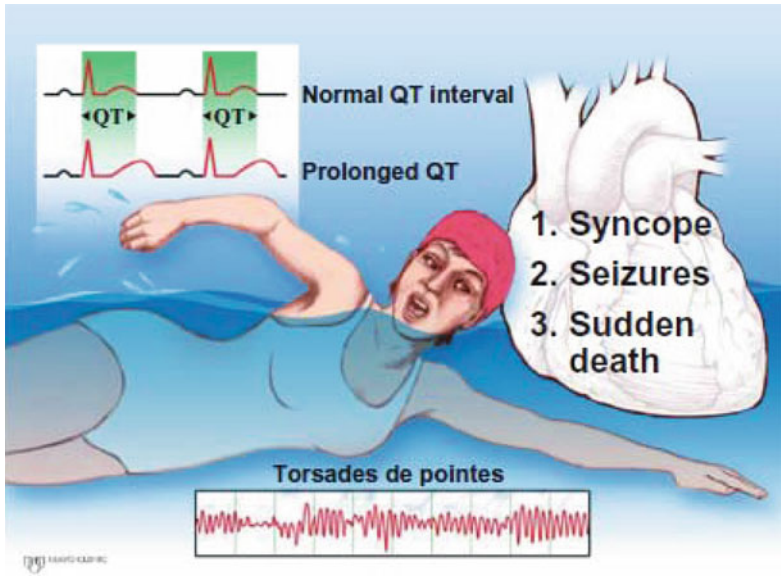

Figure 27–2. Summary of the key clinical features of LQTS

[13]. In general, approximately 50 % of patients present with activity – or emotion-related symptoms – primarily syncope, seizures, or palpitations. The majority of cardiac events are related to physical activity or emotional stress. To be certain, a "fight-flight-fright"-triggered faint must be considered potentially ominous until proven otherwise and a meticulous LQTS evaluation must be conducted. In fact, data from the LQTS Registry indicates that recent syncope is the most significant harbinger of future sudden death, greater than genotype and degree of QT prolongation [14]. Thus, it is critically important to properly distinguish an ordinary vasovagal faint from a possibly torsadogenic one.

In general and without consideration of the underlying LQTS-causing genotype, the risk of the first cardiac event in males is typically higher in childhood and decreases after puberty, perhaps due in part to regression of QTc duration [15, 16]. On the other hand, during adolescence and adulthood, females appear more vulnerable to LQTS-related cardiac events. In addition, females are at significant risk for cardiac events during the postpartum period. In fact, Rashba and colleagues reported nearly 10 % of female probands experienced their first cardiac events during the postpartum period [17].

Genotype-Phenotype Specific Correlations

Some of the phenotypic heterogeneity in LQTS is now understood because of the underlying genetic heterogeneity particular with respect to gene-specific triggers for cardiac events [18]. Figure 27.3 summarizes some of the genotype-specific features observed in LQT1, LQT2 and LQT3.

LQT1 Phenotype

Patients with the most common genetic subtype (LQT1) predominantly have exertional-triggered symptoms [18]. Swimming is a relatively gene-specific arrhythmogenic trigger associated almost exclusively with LQT1 [20, 21]. With few exceptions to date, all LQTS patients, with either a personal history or an extended family history of a near drowning, have a defective *KCNQ1* gene facilitating strategic genotyping [21]. The common triggers other than swimming include running, startle, anger and fright. LQT1 mutations cause defective I_{Ks} channel which is responsive to adrenergic stimulation, so the usual shortening of QT in response to increased heart rate is impaired and QTc

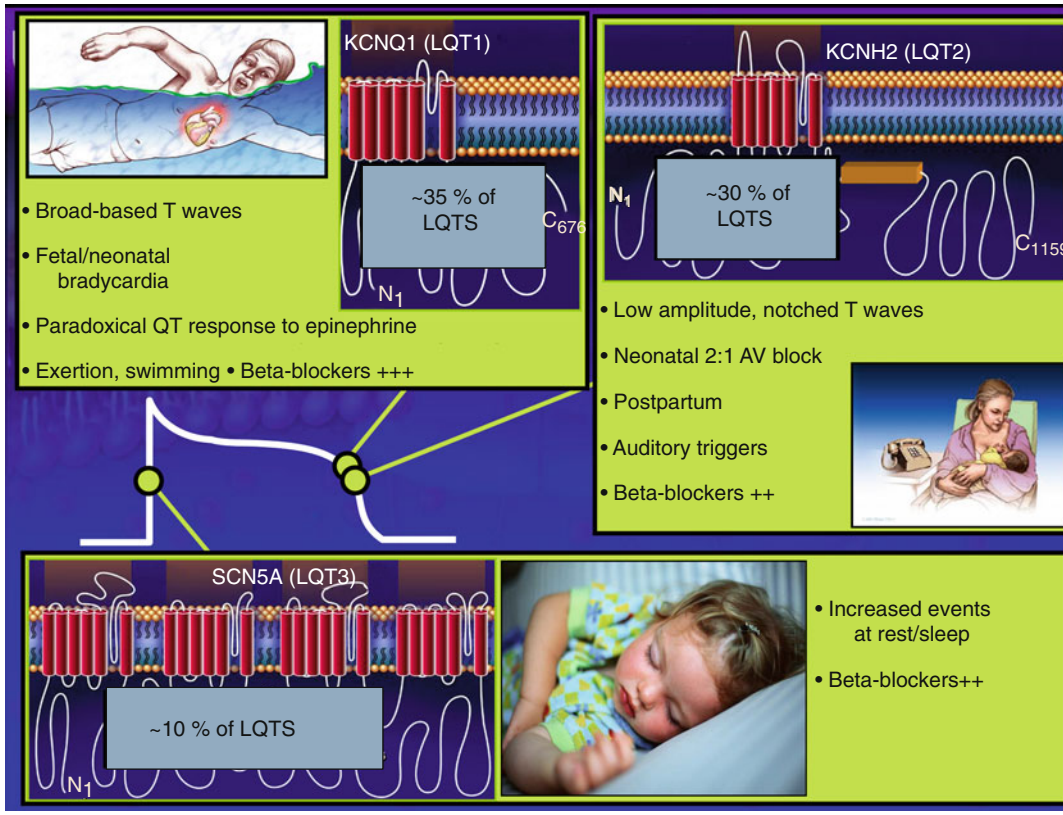

Figure 27–3. Summary of key genotype-phenotype correlations (Modified from Tester and Ackerman [19] with permission from Elsevier)

progressively lengthens during exercise and early recovery. The common ECG finding in LQT1 is prolonged T wave duration or a broad based T wave pattern [22, 23].

LQT2 Phenotype

Auditory stimuli, such as a telephone ringing or an alarm clock sounding, is a common trigger in LQTS and often indicates the presence of a *KCNH2* (LQT2) defect [24]. Additionally, there is a relatively gene-specific molecular basis underlying cardiac events during the postpartum period in LQTS [25]. Postpartum cardiac events were found more common in LQT2 (16 %) than in LQT1 (<1 %) [25, 26]. Fifteen percent of LQT2-associated cardiac events occur during rest or sleep. The finding of bifid T waves in the inferior and lateral leads is characteristic of LQT2 [27].

LQT3 Phenotype

Sleep/rest-triggered events seem most common in patients harboring a defective sodium channel Nav1.5 encoded by *SCN5A* [18, 28]. In LQT3, *SCN5A* mutations impair inactivation of I_{Na}, causing repetitive re-openings throughout the action potential and persistent inward current, resulting in prolongation of the action potential duration and QT interval [29].

The underlying genotype has a profound influence on the clinical course [30]. LQT1 and LQT2 families comprise approximately 70 % of LQTS and have a much higher risk of cardiac events than patients with LQT3. However, the "lethality" of a given cardiac event appears to be greatest in LQT3. Fortunately, *SCN5A*-based LQTS (LQT3) is approximately ten-fold less common than the potassium channel LQTS subtypes.

Table 27.1 Schwartz/Moss score for LQTS diagnostic criteria

Variable	Points
ECG findings	
QTc ms[a]	
≥480	3
460–479	2
450–459 (in males)	1
QTc[a] fourth minute of recovery from exercise stress test ≥480 ms	1
Torsades de pointes[b]	2
T-wave alternans (macroscopic)	1
Notched T wave in three leads	1
Low heart rate for age[b]	0.5
Clinical history	
Syncope[c]	
With stress	2
Without stress	1
Congenital deafness	0.5
Family history[d]	
Family members with definite LQTS[e]	1
Unexplained sudden cardiac death < age 30 years among immediate family members	0.5

[a]QTc calculated using Bazett's formula (QTc = QT/square root RR)
[b]Mutually exclusive
[c] Resting heart rate below the second percentile for age
[d]The same family member cannot be counted in both
[e]Definite LQTS is defined by and LQTS score ≥3.5

Diagnostic Evaluation

Schwartz et al. proposed the first diagnostic criteria for LQTS in 1985, that included the following major criteria: QTc > 440 ms, stress induced syncope, family members with LQTS, and minor criteria: congenital deafness, episodes of T-wave alternans, low heart rate (in children) and abnormal ventricular repolarization [31]. However, the more recent understanding of the "overlap zone" between LQTS and health has rendered this cut-off value of 440 ms a major limitation of the original "Schwartz criteria."

A modified "Schwartz score" containing new criteria and a point system based upon a range of QTc values and the clinical/family history was formulated in 1993 and in 2011, the diagnostic utility of the QTc at the 3rd/4th minute of recovery from exercise stress testing was added (Table 27.1) [32, 33]. The "Schwartz score", recently modified for what concerns the points necessary for a "high clinical probability of LQTS" ranges from 0 to 9 and contains three diagnostic probabilities: ≤1 point, low probability of LQTS; 2 or 3 points, intermediate probability of LQTS; and ≥3.5 points, high probability of LQTS [34]. The Schwartz criteria provide a useful guide for contemplating a clinical diagnosis of LQTS with the positive predictive value of a modified "Schwartz score" ≥3.5 approaching 100 %.

The Schwartz criteria should be used to reasonably select the candidates for genetic testing; in turn, the results should be used to identify by cascade screening the "silent" mutation positive family members. By definition, these criteria **CAN NOT** identify the patients or family members with **Concealed LQTS**. This critical point stems from the observation that a significant proportion of LQTS is concealed and that the penetrance, defined as the ratio between patients with a manifest clinical phenotype (QT prolongation, symptoms, etc.) and the total number of family members who are positive for the mutation identified in the LQTS proband may be as low as 25 %. Although the clinical risk of the patient with Concealed LQTS is much lower than the patient with manifest LQTS, the risk of an untoward cardiac event is not zero and hence, it is essential to correctly identify all family members. **Thus, in the evaluation of first degree relatives of a definitely affected LQTS proband, it is no longer acceptable to exclude LQTS among individuals based upon an equivocal or even normal QTc or a low Schwartz score.** Instead, genotyping, for the estimated 75 % of families who possess an LQTS-causing mutation, is the only definitive diagnostic test for the rest of the family.

Symptomatic patients, who have an ECG with a so-called borderline QTc (440–460 ms) or normal QTc, will have a borderline or questionable (score of 2 or 3) LQTS diagnosis based on clinical criteria. In this situation, serial ECGs should be performed since the QTc value in LQTS patients may vary from time to time. Furthermore, a careful inquiry about family history of sudden death as well as screening ECGs from other family members may be informative. In addition, stress testing using exercise protocols or the epinephrine QT stress test may be helpful in exposing LQTS, particularly LQT1 [33, 35], and a simple brisk "stand-up" test may be useful in unmasking LQT1 and LQT2 [36].

The 12-Lead Electrocardiogram

The 12-lead ECG remains the cornerstone in the LQTS clinical diagnosis and the principal screening tool. The hallmark ECG feature of LQTS includes prolongation of the rate-corrected QT interval (QTc) as measured by Bazett's formula (QTc = QT interval/square root of RR interval) [37]. The QT interval is defined as the time interval between the onset of QRS and the end of the T wave. This value is corrected for the heart rate by dividing it by the square root of the preceding RR interval (Bazett's QTc formula). Note, with a heart rate of 60 beats per minute (RR interval = 1 s), the QTc equals the QT interval. More rapid heart rates cause the calculated QTc to increase relative to the measured QT interval.

Lead II is generally the accepted lead for QTc calculation because the inscription of the T wave is usually discrete [38]. Alternatively, the lateral precordial leads V5 and V6 are sometimes quite informative [38, 39]. When sinus arrhythmia is present, an average QTc from the entire lead II strip (at least three consecutive determinations) must be determined [28, 40]. **Simply taking the longest observed QTc will result in too many false positive classifications.**

It is vital that the ECG be visualized directly and the QTc manually calculated by a physician with expertise in LQTS. **The computer calculated QTc is NOT acceptable in the evaluation of LQTS. Independent manual calculation is mandatory.** Unfortunately, Viskin and colleagues have exposed that humans are not much better than the computer, in fact, humans are worse, when it comes to accurately calculating the QT interval. Compared to "QT aficionados" who made significant QTc miscalculations <5 % of the time, heart rhythm specialists and general cardiologists often failed to correctly calculate the QTc (>60 and >75 % of the time respectively) [41].

Even when the QTc has been calculated accurately, the abundance of **Concealed LQTS** and misconceptions about the normal distribution of the QTc pose a serious diagnostic challenge (Fig. 27.4). Before the molecular revelations in LQTS, a QTc of ≥440 ms (males) and ≥460 ms (females) was considered prolonged [42]. Subsequently, Vincent et al. [28] examined the QTc distribution in three families with LQT1 and found that no genotype positive individuals had a QTc ≤ 410 ms and no unaffected (genotype negative) persons had a QTc ≥ 470 ms in men or ≥480 ms in women, and that a significant number of patients were classified erroneously using the 440 ms cut-off. From a screening standpoint, if a QTc of 460 ms is used as a cutoff point, the positive predictive value is 92 % and the negative predictive value is 94 % [5]. **Again for screening purposes, we advocate designating QTc values ≥470 ms during infancy or ≥500 ms in adolescents/adults as "prolonged" to maximize both the positive and negative predictive values as a screening test.**

Figure 27.4 depicts the distribution of QTc values among healthy postpubertal adult men and women as well as the QTc distribution seen for all genotype positive patients evaluated in Mayo Clinic's LQTS clinic. Despite our "indoor track record" of a genotyped LQT1 family member with a QTc of 365 ms, the interpretation of a QTc < 400 ms is statistically easy – Normal, no LQTS – with virtually 100 % negative predictive value. On the other end, a QTc ≥ 500 ms almost always indicates repolarization pathology due to either **acquired** or **congenital** LQTS. More importantly, the overlap zone (400–480 ms) is where great consternation occurs. Here, the physician must understand the normal QTc distribution in healthy subjects versus the 1 in 2,500 LQTS-affected persons. **For example, an asymptomatic patient with a negative family history and a screening QTc of 450 ms has >500:1 odds favoring normalcy rather than happening to catch someone with Concealed LQTS.**

Complicating matters further is the observation that LQTS individuals with a "normal" ECG (i.e. "low-penetrant" LQTS or "concealed" LQTS) can indeed, albeit uncommonly, have cardiac events including sudden death. Approximately 10 % of genotype positive index cases presented for LQTS genetic testing with a QTc ≤ 440 ms [43]. These observations have transformed this extensive "overlap zone" into the "nightmare zone." **Thus, although the risk for sudden death in a patient with a QTc < 440 ms remains exceedingly rare, the presence of a normal QTc in a genotype positive LQTS patient must NOT be interpreted as a risk free marker.** As will be discussed later in

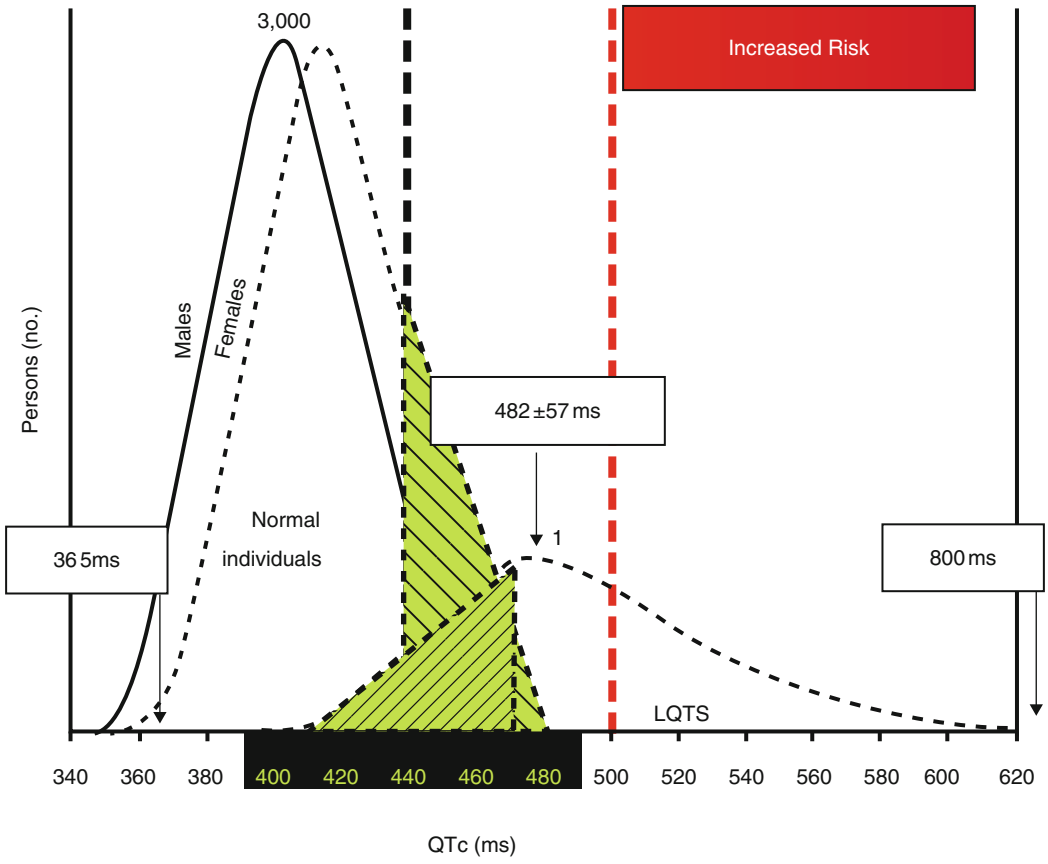

Figure 27–4. QTc distribution in health and LQTS

the treatment section, such genotype positive/phenotype negative patients must be advised of simple, potentially life saving preventative measures including avoidance of medications that prolong the QT interval. Understanding this QTc overlap zone is foundational to the LQTS evaluation and constitutes one of the major misunderstandings in the clinical evaluation of LQTS.

T-U Wave Abnormalities and T Wave Alternans

Besides manually calculating the QTc and understanding the QTc overlap zone, sleuth-like inspection of the morphology of the T waves should also be conducted in a LQTS evaluation (Fig. 27.5). Unusual T waves – wide-based slowly generated, notched, bifids, biphasic, low amplitude humps and bumps on the down-slope limb, indistinct termination due to U waves, sinusoidal oscillation, or simply a delayed inscription of normally appearing T waves may lead to the diagnosis of LQTS despite a normal or borderline QTc [22]. These T wave abnormalities may be evident particularly in the lateral precordial leads. However, caution must be exercised with concluding LQT-suspicious T wave peculiarities based upon changes in the precordial leads of V2 and V3. In addition, the T wave morphology may be somewhat gene-specific providing another piece of evidence permitting strategic genotyping [22, 44]. Patients with LQT3 tend to have a late-appearing T wave clearly distinct from the low-amplitude, moderately delayed T wave observed in LQT2. Both of these T-wave profiles are different from the broad-based, prolonged T-wave pattern seen in LQT1 [22, 23]. However, the classic LQT3-like T wave pattern can be seen often in patients with LQT1.

In 1994, Malfatto and colleagues provided the first evidence that morphological analysis of T

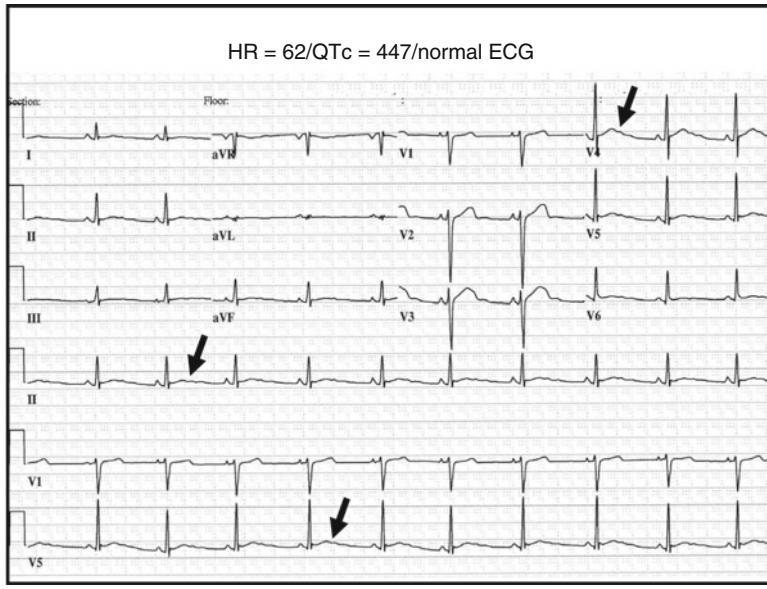

Figure 27–5. Example of T-wave sleuthing – 12-lead ECG in proband with LQT2, borderline QT prolongation, and abnormal T wave morphology

wave abnormalities, namely notched or biphasic T waves, may contribute to the diagnosis and risk stratification of LQTS. Herein, notched or biphasic T waves were present in 62 % of LQTS patients compared to only 15 % of control subjects ($p < 0.001$). Moreover, 81 % of symptomatic patients compared to 19 % of asymptomatic patients had notched or biphasic T waves ($p < 0.001$). This same distribution was also observed within LQTS families, in which symptomatic members had more pronounced T wave abnormalities then their asymptomatic family members. In symptomatic patients, the occurrence of T wave abnormalities was independent of the length of repolarization (QTc) [45].

Next, Lupoglazoff and colleagues classified a notched T wave as a grade 1 (G1) notch when it occurred at or below the apex whatever the amplitude, and as grade 2 (G2) when the protuberance occurred above the apex [46]. G2 notches appear more specific and often indicate LQT2. However, baseline ECG showing G2 notches is not found commonly. Recently, Khositseth et al. [47] reported G2 notching elicited during low-dose epinephrine may unmask some patients with concealed LQT2. The mandate for careful inspection of T wave morphology is two-fold: (1) unmask a patient with concealed LQTS – i.e. a normal resting QTc with a peculiar T wave and (2) provide a possible starting point for the mutational analysis based upon the suggested ECG pattern.

Besides QTc and T wave morphology, macrovoltage and microvoltage T wave alternans (TWA) and QT dispersion (QTd) may be informative. TWA is characterized by beat-to-beat alternation of the morphology, amplitude, and/or polarity of the T wave and is a marker of major electrical instability and regional heterogeneity of repolarization and is likely to be associated with an increased risk of cardiac events [48, 49]. QTd is defined as the difference between the maximal and minimal QT intervals in the 12 standard leads and may reflect spatial repolarization [50]. It has been described as an arrhythmic marker for LQTS. There is evidence that QT dispersion is increased in LQTS patients compared to normal controls [51, 52]. Moenning and colleagues have confirmed the finding that a significant and independent difference in QT dispersion between mutation carriers and unaffected family members exists [53]. However, a cut-off value of QT dispersion to distinguish LQTS from health is not available.

Holter Monitoring

In patients with a non-diagnostic QTc, Holter monitoring may aid in the evaluation of LQTS. Again, caution must be exercised with interpreting

Holters in a patient with an equivocal history and a borderline QTc. Presently, the normal distribution of 24-h maximal QTc values is poorly understood and concerns have been raised regarding the filter settings in Holter recordings and hence, the precision/accuracy of Holter QT intervals. **Critically, a Holter-recorded maximum QTc exceeding 500 ms does not equal LQTS.** Instead, the value of repeated Holter recordings lies in capturing the appearance of T-wave patterns (such as T wave notching) that might suggest LQTS in patients with borderline QT prolongation and an uncertain clinical diagnosis [46].

Exercise Testing

Exercise testing may enhance the diagnostic accuracy of the LQTS evaluation as inadequate shortening of QTc with increasing heart rate has been observed [54]. Swan et al. [55] studied 19 LQTS patients and 19 healthy controls undergoing exercise testing. During the recovery phase of exercise, the QT interval lengthened abnormally and the inhomogeneity of repolarization increased in LQTS. LQT2 patients had a lesser degree of QT interval shortening than LQT3 patients in response to increasing heart rate [56]. Moreover, LQT1 patients displayed a diminished chronotropic response and exaggerated prolongation of the QT interval after exercise. In contrast, the QT interval shortens more in LQT2 than in LQT1 patients when heart rate increases and the sinus nodal rate response is normal [57]. The majority of these studies have been conducted in LQTS patients having a diagnostic QTc at rest. Provocative testing is most needed, however, in exposing the patient with concealed LQTS. Here, exercise testing is capable of unmasking patients with concealed LQT1 by detecting inadequate QT adaptation during heart rate increase and especially during the recovery phase [58].

Besides the Ackerman study that demonstrated the ability of the recovery phase QTc, at 2–4 min recovery, to unmask LQT1 whether or not there was QT prolongation at rest and even in the setting of beta blocker therapy [58], Sy and colleagues also proposed a simple 3-step algorithm that incorporates resting ECG, 4-min recovery QTc, and 1-min recovery QTc for the identification of LQTS in asymptomatic relatives and the discrimination between LQT1 and LQT2 [35]. In a derivation cohort of 69 asymptomatic first-degree relatives of LQTS probands (28 with LQT1, 20 with LQT2, and 21 mutation negative), the combination of resting and 4-min recovery QTc (QTc ≥445 ms) in a screening algorithm yielded a sensitivity of 0.94 and specificity of 0.90 for detecting individuals with LQTS mutations. When applied to a validation cohort of 152 LQTS relatives (58 LQT1, 61 LQT2, and 33 mutation negative), the sensitivity was 0.92 and specificity was 0.82. In terms of predicting genotype, the abnormal prolongation of 1-min recovery QTc ≥ 460 ms correctly differentiated LQT1 subtype in 86 of 115 patients (overall accuracy) in their validation cohort, with a sensitivity of 0.73 and specificity of 0.76. This 3-step algorithm was also applied to an independent cohort of 45 possible LQTS probands that were confirmed subsequently to have disease-causing mutations in *KCNQ1* or *KCNH2*. In this third cohort, among patients with a normal or borderline resting QTc, a 4-min recovery QTc ≥ 445 ms correctly identified 25 of 28 patients as having LQTS. The combined diagnostic algorithm had an overall sensitivity of 0.93 for identifying mutation-positive probands [35].

These recent studies provide the general cardiologist with an easily employable tool to be used whenever a LQTS case is suspected [35, 58]. While there is some value in using this algorithm for the identification of asymptomatic relatives of genotyped LQTS probands, the real promise is the potential for confirming the clinical suspicion of LQTS in an ungenotyped borderline diagnosed LQTS patient, where initial genetic testing remains a lengthy process yet there is an urgency to determine if the patient is truly affected so that appropriate prophylactic therapy may be initiated [33, 58]. In fact, given the clinical value delineated in these two studies [35, 59], the evaluation of the recovery phase of exercise has been added to the latest Schwartz score LQTS diagnostic criteria [33].

It is important to note that exercise-induced ventricular ectopy is uncommon in LQTS [59]. In fact, induction of premature ventricular contractions in bigeminy or as couplets and of course,

bi-directional ventricular tachycardia, should prompt suspicion for a mimicker of LQTS, namely catecholaminergic polymorphic ventricular tachycardia.

Macrovoltage TWA at rest or during exercise/epinephrine QT stress testing is abnormal and prognosticates increased risk. However, exercise-induced macrovoltage TWA is extremely rare and we have seen epinephrine-induced macrovoltage TWA in only 1 of the past 600 epinephrine QT stress tests [60]. On the other hand, after conducting nearly 100 μV T wave studies using either the epinephrine QT stress test or treadmill stress testing, we cannot assign any clinical utility to this test. In fact, we have discontinued microvoltage T wave testing in both of our Long QT Syndrome Clinics.

Epinephrine QT Stress Test

The epinephrine QT stress test can also unmask concealed LQTS, particularly LQT1. Whether using the Shimizu protocol or the Ackerman/Mayo protocol, the chief finding that points to LQT1 involves a paradoxical lengthening of the QTc (Shimizu protocol) or the absolute QT interval (Ackerman/Mayo protocol) during infusion of epinephrine [60, 61]. When present, a tentative diagnosis of LQT1 is rendered (75 % positive predictive value) and beta blocker therapy is initiated while waiting for confirmation by genetic testing [62]. Given a 96 % negative predictive value, this test is also performed in patients hosting a novel LQT1-associated mutation to provide an *in vivo* physiological challenge of the patient's I_{Ks} pathway. Such testing provides independent conformation for the pathogenicity of the newly discovered mutation. Importantly, beta blockers significantly confound the interpretation of the epinephrine QT stress test and therefore, unlike the treadmill stress test, it should not be performed in a patient already taking beta blockers. The epinephrine QT stress test is as safe as exercise testing in terms of inducing a dangerous arrhythmia. In over 10 years and over 600 epinephrine QT stress tests conducted at Mayo Clinic, epinephrine-induced torsades has occurred only once. The reader is directed to the Provocative (Drug) Testing in Inherited Arrhythmias chapter by Shimizu and Ackerman which details catecholamine stress testing in the evaluation heritable arrhythmia syndromes with particular focus on the epinephrine QT stress test.

Echocardiographic Analysis by Tissue Doppler Imaging

Echocardiographic analysis by tissue Doppler imaging (TDI) may be useful tool in risk stratification in LQTS. In the early 1990s, Schwartz and colleagues illustrated for the first time, a relationship between motion abnormalities of the left ventricle (LV) assessed by echocardiography and symptomology (syncope or cardiac arrest) in patients with LQTS [63–65]. Using an M-mode technique, these investigators demonstrated the presence of a very slow contraction phase before rapid relaxation that manifested as either a plateau or as a double-peaked contraction in over half of the studied patients with LQTS. These contraction abnormalities, in particular the double-peaked morphology, were associated strongly with a clinical history of syncope or cardiac arrest [63]. Subsequently, calcium channel blockade by verapamil was illustrated to normalize even the most severe abnormality patterns, including those with a double-peak morphology [64]. The presence of a slow contraction phase before rapid relaxation in LQTS patients was confirmed in 1998 by Nakayama [66]. In 2003, Savoye and colleagues utilized TDI to assess wall motion and myocardial velocities in ten patients with 'mild' LQTS and 14 control subjects and observed similar results as Savoye and colleagues [67]. In 2009, Haugaa and colleagues investigated whether prolonged and dispersed myocardial contraction duration assessed by TDI may serve as a risk marker for cardiac events in patients with LQTS [68]. Here, 73 patients with genetically confirmed LQTS (9 with JLNS, 33 symptomatic subjects with single mutations, and 31 asymptomatic subjects with single mutations) and 20 healthy control subjects were studied. Myocardial contraction was significantly prolonged in each LQTS patient group compared to the controls. Furthermore, contraction duration and dispersion of contraction was significantly longer in symptomatic

patients (0.46 ± 0.06 s; 0.048 ± 0.018 s) compared to asymptomatic LQTS patients (0.40 ± 0.06 s, P = 0.001; 0.031 ± 0.019 s, P = 0.001) [68]. In fact, while a QTc ≥ 0.46 s showed a sensitivity of 70 % (95 % CI 67–88) and specificity of 50 % (95 %, CI 40–61) in identifying symptomatic individuals, contraction duration with an optimal cut off value of 0.44 s had a sensitivity of 79 % (95 % CI 68–87) and specificity of 74 % (95 % CI 62–83). Thus, assessment of myocardial duration may add an important clinical parameter for risk stratification in LQTS mutation carriers, in particular for those subjects with a normal or mildly prolonged QTc [68].

Molecular Genetic Testing

The discipline of cardiac channelopathies was birthed publicly on March 10, 1995, with tandem publications by the Keating laboratory in *Cell* that revealed LQTS to be a disease of the cardiac channels [69, 70]. These foundational discoveries permitted research-based genetic testing for LQTS over the ensuing decade. In May 2004, akin to the evolution from research testing to clinical testing for its sister channelopathy, cystic fibrosis, LQTS genetic testing completed its maturation into a clinical diagnostic genetic test involving a comprehensive open reading frame analysis of the translated exons for the genes associated with LQT1, LQT2, LQT3, LQT5, and LQT6. In the United States, Transgenomic (www.familion.com), GeneDx (www.genedx.com), and Correlagen Diagnostics (www.correlagen.com) currently represent three CLIA-approved clinical laboratories that offer fee-based clinical genetic testing for LQTS, with LQTS gene testing panels that now include LQT1–LQT13.

In 2011, two consensus documents, the Heart Rhythm Society (HRS)/European Heart Rhythm Association (EHRA) Expert Consensus Statement [71] and the Canadian Cardiovascular Society (CCS)/Canadian Heart Rhythm Society (CHRS) joint position paper [72] were published on the use of genetic testing in the clinical evaluation of cardiac channelopathies and cardiomyopathies. Accordingly, "comprehensive or LQT1-3 targeted genetic testing **is recommended** for any patient in whom a cardiologist has established a strong clinical index of suspicion for LQTS based on examination of the patient's clinical history, family history, and expressed electrocardiographic phenotype." Genetic testing is also **recommended** for any asymptomatic patient with QT prolongation (QTc > 480 ms, prepubertal; QTc > 500 ms, adult) in the absence of other clinical conditions that might prolong the QT interval (i.e. electrolyte abnormalities, hypertrophy, bundle branch block, etc.). Mutation-specific genetic testing **is recommended** for family members following the identification of a LQTS-causing mutation in the index case. For any asymptomatic patient with otherwise idiopathic "borderline" QTc values of >460 ms (pubertal) or >480 ms (adult), LQTS genetic testing **may be considered** [71].

When there is no doubt about the clinical diagnosis, the genetic test will be positive about 75 % of the time [43, 73, 74]. Identification of the specific LQTS-associated mutation then assists with genotype-guided therapy and provides the gold standard diagnostic marker for the unambiguous genetic classification of all relatives. An estimated 5–10 % of LQTS probands have spontaneous germline mutations (sporadic) and 5–10 % of patients who are genotype positive have >1 mutation [73–77]. Like patients with JLNS (autosomal recessive LQTS plus deafness), this subset of patients with multiple mutations tend to have a more severe phenotype [76]. These observations underscore the critical importance for comprehensive genetic testing rather than a "find-and-stop" approach.

To be sure, a negative genetic test MUST NOT result in removal of the diagnosis in a patient with a high probability/definite "Schwartz score". Remember, 20–25 % of patients with clinically definite LQTS will have a negative genetic test. On the other hand, there appears to be an abundance of patients for whom the diagnosis of LQTS may have been assigned prematurely based upon rather soft criteria. In this setting, a negative genetic test result which objectively and unemotionally "**RULES OUT**" at least 75 % of LQTS may be a helpful piece of data for a specialist to help the patient/family move away from the diagnosis. Among patients referred for LQTS genetic testing despite an intermediate probability "Schwartz score", 44 % had a positive genetic test enabling their status to be elevated from diagnostic ambiguity (borderline LQTS) to

diagnostic certainty [43]. Finally, the physician must be aware that the LQTS genetic test has an estimated 5 % false positive rate [78–80]. That is, approximately 5 % of healthy individuals are positive for a rare genetic variant in these LQTS-susceptibility genes. These rare variants tend to localize to the N- and C-termini of the LQT1- and LQT2-associated potassium channels and to the N-terminus, C-terminus, and cytoplasmic inter-domain linkers of the *SCN5A*-encoded sodium channel (LQT3) [80, 81].

Given the severe consequences surrounding the misdiagnosis and mismanagement of patients with these potentially lethal cardiac disorders, the clinical evaluation and management of a patient and family suspected of having LQTS should be performed under the supervision of a pediatric or adult cardiologist with experience and expertise in heritable cardiac channelopathies [82]. Because of the issues associated with incomplete penetrance and variable expressivity, the genetic test result must be interpreted cautiously and incorporated into the overall diagnostic evaluation for this disorder. Counter to current widespread thought, genetic testing in general should **not** be considered binary but rather probabilistic in nature. In fact, even when a genetic variant has been published previously as a putative pathogenic mutation, assignment of a specific genetic variant as a true disease-causing mutation still requires some vigilant scrutiny.

Establishing the "background genetic noise rate" for the three most common LQTS genes has enabled a case: control mutational analysis of the properties and localization of case-associated mutations compared to the compendium of presumably innocuous variants [83]. Algorithms based on mutation location, species conservation, and the biophysical nature of the amino acid substitution may assist in distinguishing pathogenic mutations from otherwise rare variants of uncertain significance (VUS) and perhaps allow for the assignment of estimated predictive values for the probability of pathogenicity of each novel mutation identified within a specific gene [80]. The probabilistic rather than binary nature of genetic testing for LQTS is depicted in Fig. 27.6 showing that rare "radical" mutations (i.e. insertion/deletion, frame-shift, nonsense, or splice-site mutations which represent ~20 % of the LQTS spectrum of mutations) are high probability LQTS-associated mutations whereas the probability of pathogenicity for the most common mutation type, missense mutations (i.e. single amino acid substitutions), is strongly location dependent. For example, missense mutations localizing to the transmembrane spanning/pore domains of the LQT1- and LQT2-associated potassium channels are high probability disease mutations whereas a similarly rare missense mutation that localizes to the domain I-II linker of the Nav1.5 sodium channel is indeterminate, a variant of uncertain significance (VUS). Without co-segregation or functional data, such a mutation has a point estimate for probability of pathogenicity of <50 % [80].

Thus, it is critical that the physician carefully synthesize together all components of the diagnostic evaluation. This meticulous, comprehensive approach is vital to ensure that the wisest recommendations are rendered.

Management

When LQTS was estimated to affect 1:20,000 persons, the disease was viewed as a "death sentence" with an untreated annual mortality of 5–10 %. Akin to the "tip of the iceberg" phenomenon, LQTS has become much more common (1 in 2,000) and much less severe (overall annual mortality ~1 %) over the last half century since its original description in 1957.

Risk Stratification

The issue of risk stratification in LQTS is clinically important. The phenotypic expression of LQTS varies profoundly from asymptomatic longevity to premature sudden cardiac death despite medical therapy. The great challenge is to try to discern which of these divergent outcomes is most likely in each of our patients. Occurrence of a LQTS-related cardiac event like syncope before 5 years of age suggests a serious LQTS phenotype and syncope occurring in the first year of life is associated with an extremely poor prognosis [84].

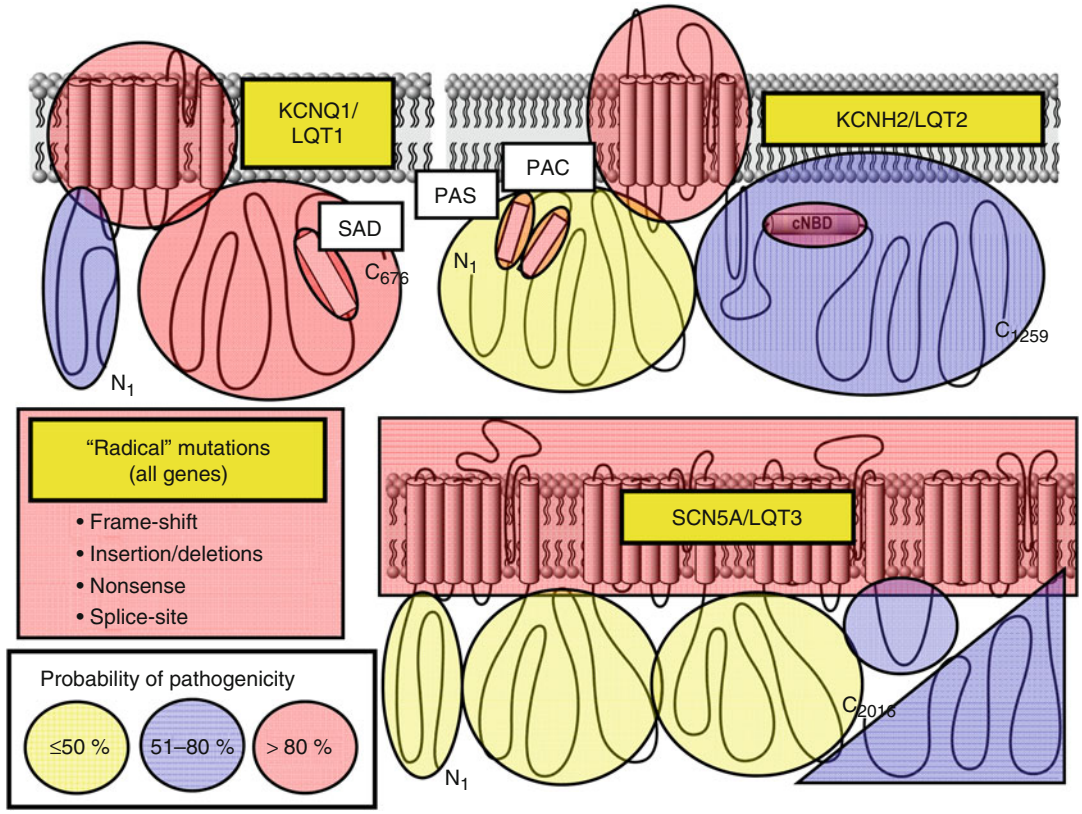

Figure 27–6. Probabilistic nature of LQTS genetic testing. Depicted are the three major ion channels causative for LQTS with areas of probability of pathogenicity shown for mutations localizing to these respective areas. While "radical" mutations have a >90 % probability of being a true pathogenic mutation, the level of probability for missense mutations vary depending on their location for each channel protein. Missense mutations residing in red shaded areas have a high probability (>80 %) of being pathogenic, those in blue are possibly (51–80 %) pathogenic, and those in yellow shaded areas truly represent variants of uncertain significance (VUS, ≤50 % probability) clinically [80] (Adapted from Tester and Ackerman [19] with permission from Elsevier)

Overall among LQTS patients, the risk of cardiac events is higher in males until puberty and higher in females during adulthood [16]. Events tend to occur earlier in males than females, and males who are still asymptomatic at age 20 years may be considered at lower risk (lowest in LQT1) for manifesting cardiac events [16, 85]. Even if event free for the first 20 years of life, females continue to have discernible risk for cardiac events in adulthood and may be at increased vulnerability to an arrhythmic event during the postpartum period [17].

Among the known LQTS genotypes, patients with LQT1 are "frequent fainters" while patients with LQT3 have the highest lethality rate per cardiac event [18, 30]. The frequency of cardiac events including syncope, aborted cardiac arrest and sudden death was highest in LQT1 (60 % of patients), then LQT2 (40 %) and lowest in LQT3 (18 %) [30]. Since the rate of death was the same in each of these three genotypes, the percentage of lethal events was highest in LQT3 patients. JLNS is associated with very early clinical manifestations and a poorer prognosis than autosomal dominant Romano-Ward LQTS [9, 86]. Patients with JLNS due to compound homozygous/heterozygous mutations involving *KCNQ1* (JLN1) have a significantly greater risk than patients with JLN2 due to mutations involving *KCNE1* [86]. Patients with the very rare form of LQTS with syndactyly, now known as type 1 Timothy syndrome secondary to mutations in

the L-type calcium channel, have a very poor prognosis [87, 88].

Intra-genotype risk stratification and in rare instances, mutation-specific risk stratification, has also been realized for the two most common subtypes of LQTS based upon mutation type, mutation location, and cellular function [89–94]. In general, LQT1 patients with KCNQ1 (Kv7.1) missense mutations localizing to the transmembrane-spanning domains clinically have a two-fold greater risk of a LQT1-triggered cardiac event than LQT1 patients with C-terminal region localizing mutations. Trumping location, patients with mutations resulting in a greater degree of Kv7.1 loss-of-function at the cellular *in vitro* level (i.e. dominant negative) have a two-fold greater clinical risk than those mutations that damage the biology of the Kv7.1 channel less severely (haploinsufficiency).

Besides the observations that transmembrane localizing and dominant negative functioning mutations represent higher risk LQT1 mutations than C-terminal localizing and haploinsufficient ones, Crotti and colleagues illustrated the potential for mutation-specific risk stratification by providing a phenotypic clinical severity data from a large group of individuals (n = 78) from 21 families and 8 countries (different parts of the world and ethnic background) and a South African founder population that hosted a specific LQT1 mutation (A341V-KCNQ1) compared to a group of 205 patients with non-A341V LQT1[95]. Despite the *in vitro* mild dominant negative functional effect of this Kv7.1 transmembrane region mutation, the LQT1 A341V population had a significantly greater percentage of symptomatic patients, manifesting with their first cardiac event at an earlier age, higher incidence of life-threatening arrhythmias, more prolonged mean QTc, lower frequency of asymptomatic ("silent") mutation positive subjects, and twice the proportion of patients with a QTc ≥ 500 ms compared to the non-A341V group [95].

Akin to molecular risk stratification in LQT1, patients with LQT2 secondary to KCNH2 (Kv11.1) pore region mutations have a longer QTc, a more severe clinical manifestation of the disorder, and experience significantly more arrhythmia-related cardiac events occurring at a younger age than those LQT2 patients with non-pore mutations in Kv11.1 [89]. Similarly, in a Japanese cohort of LQT2 patients, those with pore mutations had a longer QTc, and while not significant among probands, non-probands with pore mutations experienced their first cardiac event at an earlier age than those with a non-pore mutation [93]. In addition, LQT2 patients with mutations involving the transmembrane pore region have the greatest risk for cardiac events, those with frame-shift/nonsense mutations in any region have an intermediate risk, and those with missense mutations in the C-terminus have the lowest risk for cardiac events [94]. Adding to the traditional clinical risk factors, molecular location and cellular function are independent risk factors used in the evaluation of patients with LQTS [92].

Now, in 2012, a recent discovery has elucidated a potent genetic determinant that underlies penetrance and expressivity in LQT1 [96]. Here, a specific set of *KCNQ1* single nucleotide polymorphisms (SNP, rs2519184, rs8234, and rs10798) residing in the 3′untranslated region (UTR) of the gene modify LQT1 disease severity in an allele-specific manner, by suppressing the allele on which they reside through microRNA-mediated repression of transcription and translation [96]. When these SNPs reside on the same allele that contains the LQT1 mutation, the expressivity is attenuated (less mutant protein is made by the cardiomyocyte, thus proportionally more normal protein). However, when they reside on the normal allele (allele not containing the mutation), the disease phenotype is more severe (less normal protein is made by the cardiomyocyte, thus proportionally more mutant protein) [96]. If validated, this observation may cause a paradigm shift in our understanding of autosomal dominant genetic diseases like LQTS with the discovery that one of the single greatest determinants of disease severity depends on what was inherited from the **normal** parent.

A history of recurrent cardiac arrest increases the probability of SCD at follow-up [97]. Except resuscitated SCA, syncope is one of the strongest sudden death warning signs [14]. This observation now presses to the level of critical importance the proper distinction between vasovagal syncope and torsadogenic syncope less the trend towards early ICD therapy be accelerated even

further. A negative family history for SCD cannot be regarded as a predictor of favorable outcomes. On the other hand, a family history of SCA may not indicate increase risk for the affected relatives left behind.

A markedly prolonged QT interval (i.e. QTc > 600 ms) is associated with the greater risk for cardiac events but only a minority of LQTS patients manifest such a QTc [13, 38]. More recently, a QTc > 500 ms [85] or 530 ms [14] have been indicated as pro-arrhythmic cut-off values. Despite a discernible relationship between clinical risk and degree of QT prolongation, there is not a **ZERO RISK QTc**. Nevertheless, patients with LQTS but a QTc persistently <440 ms comprise a very low risk category [38].

T wave notching or humps are common in symptomatic patients and may be of prognostic significance [45]. QT dispersion >100 ms and a lack of shortening following a beta-blocker therapy are associated with high risk of recurrent cardiac events [52]. Beat-to-beat repolarization lability may identify patients with sudden cardiac death and predict arrhythmia-free survival [98]. Various methods quantifying T wave morphology have been developed based on observations that T wave morphology is a reflection of repolarization wavefront in the myocardium [99]. T wave spectral variance (TWSV) index, a new method to assess beat-to-beat variability of the T wave, revealed increased heterogeneity of repolarization in patients prone to both VT and VF [100]. Macroscopic T wave alternans (TWA) on the 12-lead ECG is a marker of severe electrical instability in LQTS [48, 49]. However, quantitation of the actual risk of sudden cardiac death associated with TWA is still uncertain and visible TWA is an infrequent sighting in the LQTS evaluation [101]. Nonetheless, it can be used appropriately as a marker of persisting electrical instability and risk despite current therapy. There appears to be no role nor justification for both children and adults for invasive electrophysiology studies in LQTS risk stratification as most LQTS patients are not inducible during programmed electrical stimulation [102].

Using catecholamine provocation with dobutamine instead of exercise stress testing, Nemec et al. [103] demonstrated that μ(mu)V-TWA occurred at lower heart rates in patients with LQTS than in healthy people. However, catecholamine-provoked μ(mu)V-TWA failed to identify high-risk subjects. In contrast, the investigators described and quantified macroscopic non-alternating, beat-to-beat lability in T wave amplitude and morphology during catecholamine stress testing with dobutamine [104]. This lability was quantified using a newly derived T wave lability index (TWLI) based on a determination of the root-mean-square of the differences in T wave amplitude at each isochronic point. The TWLI was significantly higher in LQTS and marked T wave lability (TWLI ≥ 0.095) was detected in all three LQTS genotypes (10/23), but in no control subjects. Importantly, all high-risk patients having either a history of out-of-hospital cardiac arrest or syncope plus at least one sudden death in the family had TWLI ≥ 0.095. Future studies are necessary to determine whether or not such catecholamine provoked T wave lability identifies patients harboring high-risk genetic substrates.

Treatment Recommendations

Today, there is uniform agreement that all symptomatic patients with LQTS require treatment because the risk of mortality without treatment is unacceptably too high. However, with the growing, but often unjustified attitude to move away from time tested beta blocker therapy to more aggressive and more definitive, sudden death preventative, implantable cardioverter defibrillator (ICD) therapy, the treatment plan for the symptomatic patient must be individualized. Here, the perceived risk-benefit ratios should be explained carefully to the patients. Given the potential morbidities associated with lifelong ICD therapy in a young person, the patients should be educated sufficiently to be able to have a say in the final decision. **There is near universal agreement that an episode of aborted/resuscitated cardiac arrest, whether occurring while on medical therapy or not, warrants an ICD.**

On the other hand, the necessity for treating **asymptomatic** patients is debated. In 1993, Garson et al. [38] reported that SCA was the sentinel event in 9 % of patients with LQTS. Furthermore, 12 % of patients who were asymptomatic at the time of

diagnosis later developed symptoms, including sudden death in 4 %. This provided a cogent argument for the universal treatment of all patients with LQTS, both symptomatic and asymptomatic. Priori et al. [105] suggested that all **young asymptomatic** patients should be treated because sudden death as the sentinel event has been documented too frequently. Ackerman [5] recommended that after a patient with LQTS has been diagnosed, all first-degree relatives must be screened, a cardiologist must be involved in the care of families with LQTS, symptomatic patients must be treated, and treatment options must be considered carefully in asymptomatic patients.

We recommend that pharmacotherapy be considered strongly in MOST ASYMPTOMATIC patients UNLESS:

1. 30 years old male with LQT1 and QTc < 480 ms or
2. QTc < 440 ms.

To be sure, many patients with LQTS harbor a long QT "dud" rather than a "ticking time bomb" and are destined for asymptomatic longevity. In fact, there are many LQTS patients who require **NO** therapy. However, until clinical testing reveals the virtually "No Risk" patient, near universal prophylactic beta blocker therapy remains the prudent recommendation. Debate will continue over when an asymptomatic patient is no longer young and has "out-grown" his/her LQTS risk such that no medical therapy is necessary. All LQTS investigators have a collection of patients who had their sentinel event occur in their 30s, 40s, 50s, and even 60s such that suggesting any age as a cut-off in the decision for prophylactic therapy in an asymptomatic patient seems arbitrary. Besides, this may be a mute point as the standard therapy for LQTS, i.e. beta blocker therapy, has been found to prolong life in older persons independent of its protective effect in LQTS providing an "excuse"/"rationale" for a universal treatment recommendation.

The ultimate goal for the treatment of LQTS is to prevent SCD secondary to a long QT heart that degenerates into polymorphic ventricular tachycardia and fails to spontaneously convert back to normal sinus rhythm. Currently, the treatment options for inherited LQTS include β(beta)-blocker therapy, implantable cardioverter-defibrillator (ICD), continuous pacing, left cardiac sympathetic denervation (LCSD) surgery, and genotype-directed therapy.

Pharmacotherapy

β(beta)-blocker therapy remains the front line therapy for most LQTS, particularly type 1 and type 2 LQTS [31, 97]. Although not in a randomized study, β(beta)-blockers appear to decrease mortality from 71 % in historical controls to 6 % in a treated group [106]. A study from the Pediatric Electrophysiology Society demonstrated that β(beta)-blockers were effective in decreasing symptoms, ventricular arrhythmias and sudden death [38]. A large retrospective study involving 869 LQTS patients of whom 69 % were symptomatic treated with β(beta)-blocker revealed a marked beneficial effect with a significant reduction in cardiac events including sudden death with mortality below 2 % [97].

It is generally thought that all beta-blockers are equally protective, with propranolol 2–4 mg/kg/day, nadolol 0.5–2.0 mg/kg/day, metoprolol 0.5–1.0 mg/kg/day, and atenolol 0.5–1.0 mg/kg/day being most commonly used. These are significant doses of beta-blockers and a commonly observed mistake is inadequate dosing (homeopathic doses). When prescribing different β-blockers, the total dose, dosing schedule, and adverse effects need to be considered. Propranolol and nadolol are nonselective β(beta)-blockers whereas atenolol and metoprolol are relatively cardioselective β(beta)-blockers. Propranolol has a short half-life and requires frequent dosing. Moreover, it causes side effects involving the central nervous system. Atenolol is more popular than propranolol in treatment of LQTS due to its fewer side-effects likely secondary to its decreased blood brain barrier penetration. However, one study suggested atenolol may not be sufficiently protective for the treatment of LQTS especially with once-a-day dosing, so they recommended twice-a-day dosing of atenolol if chosen [107].

In general, we recommend (i) either nadolol or propranolol in children, adolescents, and adults, (ii) liquid propranolol until able to swallow pills, (iii) nadolol or propranolol during pregnancy, and (iv) propranolol as the beta blocker of choice for LQT3 patients because of its concomitant late sodium current blocking properties. Although the data is limited, we have concluded that atenolol and metoprolol are not as protective as the other beta-blockers. At minimum, the practice of once-a-day dosing of atenolol is not pharmacokinetically justified.

Other pharmacotherapeutic strategies including calcium channel blockers and potassium channel openers should be considered unproven in LQTS and should not represent "stand alone" therapies. Patients with severe symptoms including appropriate ICD therapies refractory to both beta blocker therapy and LCSD are probably the only candidates for calcium channel blockers such as verapamil or potassium channel openers such as nicorandil.

Device Therapy – Pacemakers and Implantable Cardioverter-Defibrillators (ICDs)

Pacemaker Therapy

Viskin et al. [108] demonstrated that the majority of spontaneous arrhythmias in congenital LQTS are pause dependent (TdP is usually preceded by sudden decrements in cycle length). Overall, LQTS patients have slower resting heart rates than normal subjects, particularly in LQT3 patients. Moreover, β-blocker therapy can cause symptomatic bradycardia and sinus pauses. For these reasons, cardiac pacing in conjunction with β(beta)-blocker therapy may be beneficial in preventing pause-dependent TdP, especially in patients with LQT2 [109–111]. However, cardiac pacing should be considered as an adjunct to β(beta)-blocker therapy, not as a sole therapy, in treatment of LQTS patients who have pre-existing atrioventricular block or evidence of pause-dependent arrhythmias. Moreover, concomitant beta-blocker therapy and continuous pacing has failed to significantly reduce the sudden death risk in high risk patients [110]. In conclusion, cardiac pacing is a possible adjuvant therapy to β(beta)-blocker therapy in patients with bradycardia, atrioventricular block, pause-dependent TdP as seen in LQT2, or in LQT3 patients. In practice however, we have seldom implanted solely a pacemaker. Instead, if the patient is perceived to be at significant risk and a pacemaker appears indicated, an implantable defibrillator/pacemaker is the preferred device therapy.

Implantable Cardioverter-Defibrillators (ICD) Therapy

Contrary to the seemingly current and pervasive trend embracing ICD therapy as front-line therapy in LQTS, <10–20 % of the patients seen in our respective LQTS clinics have an ICD [112–114]. The vast majority are being managed successfully without an ICD. The ICD represents one of the twentieth centuries greatest medical advances and when used appropriately, it is both life saving and life changing. When used indiscriminately, it is only life changing and in some instances, dramatically so. Figure 27.7 summarizes the indications for ICD therapy in our LQTS clinics. There is virtually universal agreement with the recommendation for ICD therapy as secondary prevention following an aborted cardiac arrest [115–117].

In terms of the ICD as primary prevention, the various risk-benefit scenarios must be considered carefully. **The motivation for primary prevention ICD therapy is the correct recognition that beta blockers significantly reduce but do not eliminate the risk for sudden death** [14, 97]. Although emotionally compelling and currently cited as a class IIb indication for ICD therapy, there is no evidence to indicate that a positive family history of sudden death is an independent risk factor for those genetically affected family members and relatives who are still living [118]. Risk factors that warrant consideration of an ICD include (Figure 27.7) (i) syncope despite adequate beta blocker therapy, especially if syncope while on beta blockers and after LCSD [14], (ii) intolerance of primary pharmacotherapy, (iii) severe QT prolongation (QTc > 550 ms) and not LQT1 genotype [117, 119], (iv) JLNS especially

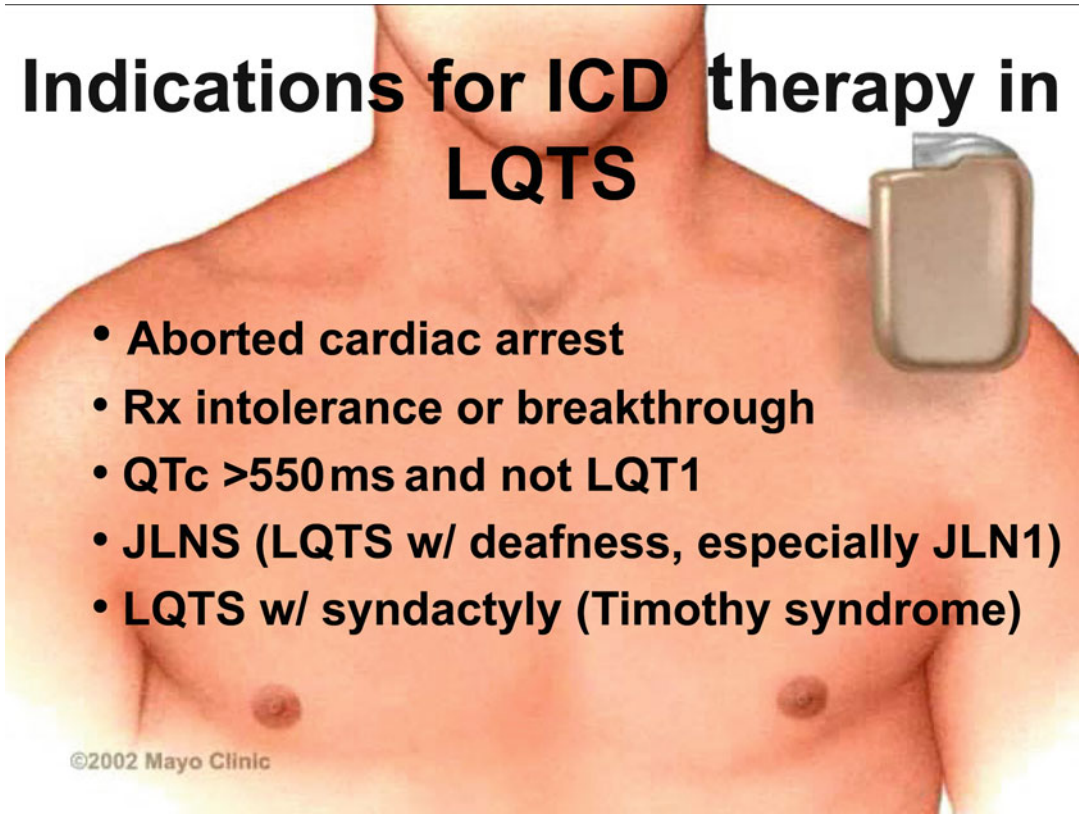

Figure 27–7. Summary of indications for ICD therapy in LQTS

patients with type 1 JLNS [86], and (v) LQTS with syndactyly (Timothy syndrome) [88]. In practice, if an ICD seems indicated, we generally proceed with a single lead system unless a need for pacing therapy has already been declared (i.e. pause-dependent TdP, severe bradycardia, etc.). The vast majority of our implanted devices are still single lead ICDs. In some cases, surgical LCSD might be considered an alternative to primary prevention ICD therapy especially for the patient with beta blocker intolerance or the next step in the patient receiving appropriate VF-terminating ICD therapies.

Left Cardiac Sympathetic Denervation (LCSD)

The concept of imbalance in cardiac sympathetic innervation in LQTS [120] (overactivity of the left stellate ganglion and decreased right stellate ganglion activity) contributed to the left cardiac sympathetic denervation (LCSD) surgery. Its main rationale was and is the unquestionable evidence that left sided cardiac sympathetic nerves have a very high arrhythmogenic potential [120, 121]. There has not been a randomized, controlled clinical trial to systematically evaluate the efficacy of LCSD. However, Moss and McDonald [121] first reported in 1971 a successful LCSD in a single symptomatic LQTS patients refractory to β(beta)-blockers and this was followed by Schwartz et al. in 1975; these two cases paved the way to the spreading use of LCSD in patients not fully protected by beta-blockers [48].

Recently, Schwartz et al. [122] reported the results of the LCSD in 147 LQTS patients with the average QTc of 543 ± 65 ms, 99 % of them were symptomatic and 48 % had experienced cardiac arrest. After LCSD, 46 % of these patients became asymptomatic, syncope occurred in 31 %, aborted cardiac arrest in 16 % and sudden death in 7 %. For high-risk patients with syncope

only prior to surgery, their 5-year mortality was only 3 % and was confined to those continuing to have a QTc > 500 ms 6 months after surgery. They concluded that the LCSD is associated with a significant, long term reduction in the frequency of aborted cardiac arrest and syncope and should be considered in all LQTS patients who experience syncope despite β(beta)-blocker therapy and in those who have arrhythmia storms and shocks with an ICD [34]. LCSD can be performed with a minimally invasive videoscopic approach and we have now performed nearly 100 videoscopic LCSDs at Mayo Clinic as of January 2012 [123].

In the absence of a head-to-head trial randomizing high risk patients to either ICD or LCSD therapy, a decision to proceed initially with LCSD therapy for the perceived high risk patient (primary prevention) rather than a prophylactic ICD will likely hinge on the surgical expertise with respect to this surgical denervation procedure [123]. Among experienced surgeons, mortality associated with LCSD should be near zero and morbidity (Horner's syndrome with ptosis of the left eyelid) should be <3 %. Compared to the complications associated with ICD therapy including inappropriate therapies, lead fractures, and infections, the overall co-morbidities are much less and the impact on quality of life is better with LCSD. Presently, among leading LQTS centers in the United States, LCSD remains reserved for patients having the most malignant expressions of their LQTS, typically patients experiencing multiple VF-terminating ICD therapies. However, indications in both of our LQTS Clinics now include (i) beta blocker intolerance (LCSD preferred over prophylactic ICD), (ii) beta blocker therapy breakthrough, and (iii) prophylactic LCSD for some high risk patients where ICD co-morbidities seem excessive.

Gene-Specific Therapy

Patients with LQT1

Due to the fact that life-threatening cardiac events occur during sympathetic activation in 90 % of cases [18], patients with LQT1 are protected effectively by the use of anti-adrenergic interventions. In LQT1, β(beta)-blocker therapy is the most effective [119]. If syncope recurs despite β-blocker therapy, LCSD is effective [122]. **The majority of patients with LQT1 will not require ICD therapy** [34].

Patients with LQT2

In LQT2, β(beta)-blockers are effective although more breakthroughs occur among patients with LQT2 compared to LQT1 [14, 97]. Attempts to augment serum potassium via supplementation and spironolactone therapy have been shown to attenuate the QT interval both acutely and long term [124, 125]. However, given the lack of outcome data and the extremely poor patient tolerability due to side effects, this strategy has gained little traction. The corollary is important however, namely that keen attention should be given during medical illnesses (vomiting and diarrhea) that could decrease serum potassium significantly thereby increasing the potential for an LQT-triggered cardiac event. The recent demonstration of a relatively LQT2-specific, pause-dependent TdP mechanism may prompt consideration for dual chamber ICD/PM therapy in the high-risk patient with LQT2 [111]. Presently, we generally advise single lead ICD therapy for the high-risk patient.

Patients with LQT3

The management of patients with LQT3 is complex and difficult. Fortunately, LQT3 accounts for only 5–10 % of the families seen in LQTS Clinics. Early evidence suggested that β-blocker therapy was not sufficient in patients with LQT3, because of a 14–17 % incidence of cardiac arrest/sudden death [18, 97, 126]. Subsequently, β(beta)-blockers appeared pro-arrhythmic in *in vitro* models of LQT3 [127]. However, such models need to be taken with caution before extrapolating to clinical reality. Unfortunately, based on earlier studies, that called attention to the excess lethality of cardiac events in LQT3 patients and the lesser efficacy of β-blocker therapy, many physicians, often motivated by an appropriate desire to offer the maximum protection for their patients, have developed a "knee-jerk" reaction to implant an ICD whenever they receive a genetic test result indicating

an LQT3 mutation. However, in 2009, after their follow up study on 33 LQT3 patients (four children with events during the first year of life, 11 asymptomatic or events later in life and no antiadrenergic therapy, and 18 asymptomatic or events later in life and on antiadrenergic therapy) cared for at their medical center in Pavia, Italy, Schwartz and colleagues first warned that a molecular diagnosis should never lead to an automatic decision to implant an ICD [112]. Accordingly, this study showed that LQT3 patients presenting with cardiac symptoms in the first year of life represented a group of patients with extremely high risk for sudden death, yet LQT3 patients asymptomatic in the first year of life and beyond had a very good prognosis when treated with an antiadrenergic therapy. Since this initial observation, recent unpublished data from the LQTS Registry indicates that β(beta)-blocker therapy reduces risk in LQT3 and does so to a similar degree as in LQT2. Consequently, for LQT3 patients without events in the first year of life, **β(beta)-blocker therapy, particularly propranolol, should be viewed as the initial treatment of choice.**

Sodium channel blockade represents a rational approach for a gene-specific therapy in LQT3, since most LQT3-causing mutations precipitate an increase in late sodium current via the Nav1.5 sodium channel. In an arterially perfused canine left ventricular wedge preparation pharmacologically mimicking LQT1-3, the sodium channel blocker mexiletine effectively reduced dispersion and prevented torsade de pointes in both LQT2 and LQT3 models [128]. Mexiletine also abbreviated the action potential duration of M cells more than that of epicardium and endocardium, thus diminishing transmural dispersion and the effect of isoproterenol to induce TdP in LQT1 models [129].

Clinically, Schwartz et al. [56] first demonstrated that mexiletine, significantly shortened the QTc and normalized the morphology of the T wave in patients with LQT3. Even though large numbers are still needed before reaching definitive conclusions and despite some anecdotal reports of mexiletine failures, the available data suggest that mexiletine (always in conjunction with beta blockers) has a significant QT shortening effect in most of these patients and can be extremely useful, especially in the management of newborns with life-threatening forms of LQT3. Besides mexiletine, flecainide has been studied in families with LQT3 and shown to attenuate the QTc also [130, 131]. However, flecainide also blocks the I_{Kr} current and in some patients with LQT3, flecainide has mimicked some of the Brugada syndrome patterns [132]. Anecdotal reports using ranolazine in LQT3 are appearing as well. Notably, propranolol is the only beta blocker with a degree of late sodium current blocking properties and as a result is the preferred beta blocker for patients with LQT3 [133, 134].

In practice, we assess the degree of QTc shortening produced by oral mexiletine and if significant (≥50 ms), we continue with mexiletine-based pharmacotherapy. However, although attenuating the QTc seems like an effective antiarrhythmic intervention, there is no long term outcome data that currently exists to demonstrate that late sodium current attenuation with either mexiletine, flecainide, or ranolazine confers survival benefit. Thus, for now, it is very reasonable to seriously consider concomitant ICD therapy for most patients with high risk LQT3. For asymptomatic infants and small children, the risks of an ICD likely outweigh the benefits. Here, a strategy of propranolol plus mexiletine, ± LCSD, monitoring, and an automatic external defibrillator (AED) may be more prudent.

Lifestyle Modification and Sports Participation in LQTS

Besides the aforementioned considerations regarding active therapy, there are several preventative health measures for the patient/family that receives a life changing diagnosis of LQTS. First, patients should be reminded of the importance of healthy sleep and dietary habits. A diet rich in potassium cannot hurt. In contrast, hypokalemia can potentially precipitate a LQT-related event. Therefore, careful attention to hydration and electrolyte replenishment during illnesses (vomiting, diarrhea, etc.) that can deplete potassium is prudent. A potassium supplement could be considered,

particularly for patients with LQT2, although it is difficult to push potassium levels harder without the concomitant use of medications that facilitate retention of potassium. Regarding supplements or complimentary alternative medicines, caution should be exercised with products containing amphetamine-like agents or cesium [135]. While a healthy weight should be sought, aggressively fast and excessive weight loss has been associated with QT prolongation and in theory could precipitate a cardiac event in a patient with congenital LQTS. Grapefruit juice, especially purple grapefruit juice, contains a chemical that inhibits the I_{Kr} potassium channel encoded by *KCNH2*, akin to the mechanism of nearly all FDA-approved medications associated with drug-induced QT prolongation [136].

Second, patients with LQTS in general and LQT2 in particular should be advised to remove/minimize sources of loud noise in their environment (such as alarm clocks and telephones) especially while sleeping [18, 20, 34]. Third, fever has emerged as a pro-arrhythmic trigger in LQTS, particularly LQT2, and therefore, antipyretics should be used to normalize core body temperature [137]. Fourth, for the patient/family with LQTS, the AED should be viewed as akin to the epinephrine pen for patients with life-threatening allergies. Fifth, patients with LQTS should have a disease identification card/bracelet with them at all times. It is important that adult family members are instructed on how to perform 'thump-version', which in LQTS, may terminate TdP, restoring sinus rhythm, prior to initiating CPR.

Perhaps most importantly, patients should consult their doctor before taking any medications. At a minimum, all patients with LQTS **MUST** avoid drugs known to aggravate the QT interval as a drug-mediated side effect (www.qtdrugs.org). Occasionally, the patient will need a medicine that is on this "QT Hit List" and the physician–patient must consider carefully the risks and benefits. If there is a reasonable alternative medication with similar efficacy, then the decision is simple. However, there are several scenarios that are not so straightforward including (1) treatment of asthma with albuterol, (2) treatment of concomitant depression with selective serotonin re-uptake inhibitors, and (3) treatment of concomitant attention deficit disorder with Ritalin or other ADHD therapies. In each case, the physician must weigh carefully the evidence for this potential side effect and determine, in the context of treating the whole person, what constitutes the best risk-benefit decision.

Finally, the single biggest lifestyle impact pertains to the issue of sports participation. For the patient diagnosed during his/her adolescent years, it is probably an underestimate to call this impact "huge". According to the 2005 Bethesda Conference and the ESC Guidelines, competitive sports should be prohibited in the patient with **symptomatic** LQTS even if treated with either pharmacotherapy or ICD therapy [138, 139]. The Bethesda Conference, but not the ESC, guidelines do suggest the possibility to liberalize the restrictions in patients with genotype positive but phenotype negative LQTS. Suffice it to say, this is an extraordinarily complex issue. If we took inventory of every activity of daily living associated with a LQT-triggered event seen in our LQTS Clinics, we would have to restrict virtually every activity that one can imagine: waking, eating, brushing teeth, watching TV, sleeping, etc.

At first glance, it may seem prudent to recommend the avoidance of "competitive" sports as competition brings together the key triggers that threaten the long QT heart, the "fight-flight-fright" response [140]. Further, such recommendations seem particularly appropriate in patients with LQT1 where exercise and activities such as swimming seem to be key arrhythmogenic triggers. However, there are known, predictable health outcomes for the obese, sedentary individual and encouraging an active, normal life for LQTS patients should be the goal. As our patients hear our admonitions to stay fit with "mild to moderate recreational physical activity", they astutely perceive the logical inconsistency behind prohibiting competitive sports while permitting recreational activities and wonder to what extent potential liability considerations are driving this recommendation. Ideally, we would prefer to see the risks and benefits laid out in excruciating detail and help each patient/family draw their own conclusions regarding a reasonable risk exposure.

Case Vignettes Revisited

Case #1 – Do I or Don't I Have Long QT Syndrome? That Is the Question

JPA is a 15-year-old male referred for a second opinion and possible ICD implantation following the diagnosis of LQT1. At a pre-participation sports physical, an innocent murmur was heard, an ECG was obtained and a QTc of 440 ms was noted. The patient fainted once in the setting of having his blood drawn. His family history was completely negative. No ECGs were performed on the parents. Holter monitoring revealed a QTc maximum of 501 ms occurring at 2:30 AM. An epinephrine QT stress test using the Mayo continuous infusion protocol demonstrated a 100 ms increase in the QTc during low dose epinephrine. Genetic testing revealed a rare amino acid substitution in the KCNQ1-encoded Kv7.1/I_{Ks} potassium channel localizing to the C-terminus of the channel. The patient was diagnosed with LQT1, placed on once-a-day atenolol beta-blocker therapy, restricted from all competitive sports, and a recommendation for sudden death primary prevention with an ICD has been rendered.

This case reflects the hazards of diagnostic miscues in the evaluation of LQTS. First, just on the clinical presentation, his QTc of 440 ms should represent normalcy with an odds ratio of about 1,000:1 rather than the capture of a patient with concealed LQTS. Second, ECGs of the parents of an index case should be viewed as obligatory in the evaluation of LQTS. Third, a Holter QTc > 500 ms is per se of very modest value. Fourth, using the Mayo epinephrine QT stress test, an increase in QTc can be totally normal. Again, the LQT1 profile with this test is a paradoxical increase in the **absolute QT interval**. Fifth, the genetic test must be interpreted carefully and incorporated into the entire diagnostic work-up. In this context, the genetic test was likely a false positive which can occur in approximately 5 % of healthy subjects.

Now, even if the diagnosis of concealed LQT1 were correct, the physician's management plan was equally amiss. First, atenolol may not be sufficiently protective in LQTS and certainly should not be dosed in a once-a-day fashion if the goal is for 24-h coverage. Second, the latest Bethesda Conference guidelines would support lifting of a competitive sports ban in patients with concealed LQTS. Finally, despite being all too common of a recommendation, prophylactic ICD therapy should be viewed as excessive for the management of concealed LQT1. Unfortunately, our LQTS Clinics are replete with this exact case scenario. In fact, 40 % of patients coming to Mayo Clinic's LQTS clinic with a diagnosis of LQTS have left without the diagnosis [141].

Case #2 – Oh Great, an Infant with LQT3, Now What Do I Do?

JMA is a healthy asymptomatic newborn female born to a mother with genetically proven LQT3. The family history is significant for a nocturnal sudden death involving a maternal aunt at age 40. The initial newborn ECG shows a QTc of 420 ms. Would you perform any additional tests or dismiss as normal?

First, a one time ECG is unreliable for a determination of LQTS. Second, once a LQTS genotype has been established in a family, a screening ECG can NOT exclude the diagnosis. Here, the infant should have the family-specific mutation test performed. If negative, then the evaluation is over and the infant can be dismissed as normal. If positive, however, then the approach to an infant with concealed LQT3 likely favors preventative measures like QT drug avoidance and monitoring only.

Case #3 – Doctor Please Do Something, My Defibrillator Keeps Shocking Me

LLA is a 32-year-old female with previously symptomatic LQT2, QTc of 550 ms, and a positive family history of multiple premature sudden deaths. Despite adequate beta blocker therapy with nadolol,

she continues to receive frequent ventricular fibrillation-terminating ICD therapies. What therapeutic strategy would you consider next?

Possible additional pharmacotherapeutic strategies include calcium channel blockers, mexiletine, ranolazine, nicorandil, and/or spironolactone with potassium supplementation. However, the most definitive therapy would be the left cardiac sympathetic denervation (LCSD) procedure, which can be performed extrapleurally, without opening the chest in less than 1 h. Performed correctly, this procedure is seldom associated with the Horner's syndrome and the degree of left ptosis, if present, is minimal (1–2 mm). For this malignant subset of LQTS, the potential benefit from denervation therapy is extremely high.

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28
Brugada Syndrome: Clinical and Genetic Aspects

Paola G. Meregalli, Hanno L. Tan, and Arthur A.M. Wilde

Abstract

Brugada Syndrome is characterized by a specific ECG pattern with ST segment elevation in the right precordial leads and is associated with a high risk of sudden cardiac death (SCD) at young age particularly in situations with an augmented vagal tone. SCD occurs due to ventricular tachycardia (VT)/fibrillation (VF). One of the most striking elements of this inherited syndrome is that no gross structural cardiac abnormalities are found in affected patients. Moreover, the penetrance of the disease is very variable, therefore only a minority of the affected patients will develop symptomatic arrhythmias. Not only the diagnosis, but especially the prompt identification of the patients at risk has become a real challenge for the cardiologists.

Although Brugada syndrome is quite rare, the worldwide interest around its pathophysiologic mechanism, risk stratification strategies and treatment is outstanding, as witnessed by the many publications in a relative short span of time.

As with other primary arrhythmias syndromes, the most fascinating aspect for the researchers is to be able to unravel the genetic background and to provide functional data of the mutant cardiac channel on conduction and repolarisation parameters. Cardiac susceptibility to develop arrhythmias will derive from these effects in combination with other factors, like the gender of the patient, the body temperature or the concomitant use of drugs. In the majority of the cases

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the cardiac voltage gated sodium channel is involved, still the genetic yield of the Brugada syndrome is low and more recent data focus the attention on other cardiac channels like the calcium channels. In both cases the clinical phenotype can display features of multiple arrhythmias syndrome among which the Long QT and the Lev-Lenègre syndromes in combinations with typical features of Brugada syndrome.

Keywords
Inherited arrhythmias • Sodium channel blockers • Genetic mutations • Conduction disorders • Implantable cardioverter defibrillator • Body temperature

Introduction

After its recognition as a distinct clinical entity [1], Brugada syndrome is increasingly recognized worldwide as an important cause of SCD at young age, in the absence of gross structural cardiac abnormalities. Patients affected with Brugada syndrome are at risk for SCD from fast polymorphic ventricular tachycardia (VT)/ventricular fibrillation (VF), especially at rest [2].

Brugada syndrome is characterized by a typical ECG pattern consisting of ST segment elevation in the right precordial leads and in leads positioned in the third intercostal space (Fig. 28.1) [3–5]. The large number of case reports and clinical/experimental studies published in the last 10–15 years about Brugada syndrome are testimony to its increasing weight and interest for its still not completely known aspects, such as the underlying pathophysiological mechanism [6, 7], its genetic background [8] and its prognosis and treatment [9, 10]. The pathophysiology of Brugada syndrome is very intriguing and the core of an international controversy [6, 7, 11], with two predominant theories regarding the typical ECG features and the genesis of the arrhythmias: (1) a repolarization disorder, i.e., unequal expression of the transient outward potassium current I_{to} between epicardium and the other transmural layers [12–15], or (2) a depolarization disorder [16–18], i.e., a delay in the onset and/or propagation of the action potential (AP) in the region of the right ventricle outflow tract (RVOT) [19–22]. In 2002 a First Consensus Report was published to define the diagnostic criteria for this syndrome [23]. Three repolarization patterns of ST segment elevation (with two different shapes: the coved and the saddle-back types) were recognized as potential manifestation of Brugada syndrome. The coved-type morphology (type I) is required for the diagnosis, while a saddle-back shaped ST elevation or a coved-type <1 mm (types II–III) are indeterminate forms that necessitate pharmacological challenge (Fig. 28.2) [23]. The diagnosis is posed when a type I ECG, spontaneously or after provocation with sodium channel blockers, is present in more than one right precordial lead in the absence of structural abnormalities, and in association with one of the following conditions: (1) documented VF or polymorphic VT, (2) a family history of SCD at young age or a type I ECG in family members, (3) otherwise unexplained syncope (4) inducibility of VT/VF with programmed electrical stimulation (EPS) [23, 24].

Of these criteria, only a history of documented VT/VF has clear prognostic implications. Spontaneous occurrence of a type I ECG also has prognostic implications, representing a condition with increased risk for malignant arrhythmias and, if present in conjunction with syncope (otherwise unexplained), conferring an indication for implantation of an implantable cardioverter/defibrillator (ICD) [25, 26]. In contrast, a positive family history of SCD does not confer a higher risk to the family members, remaining therefore, only a useful criterion for the diagnosis, but not for the prognosis of Brugada syndrome patients [10, 27]. Risk stratification and indications for ICD implantation are extensively discussed in the Second Consensus Report paper, published in 2005 [24]. Since then, new data emerged which support the notion that EPS is a poor event predictor [10]. Moreover, there is increasing recognition that, in most patients, the

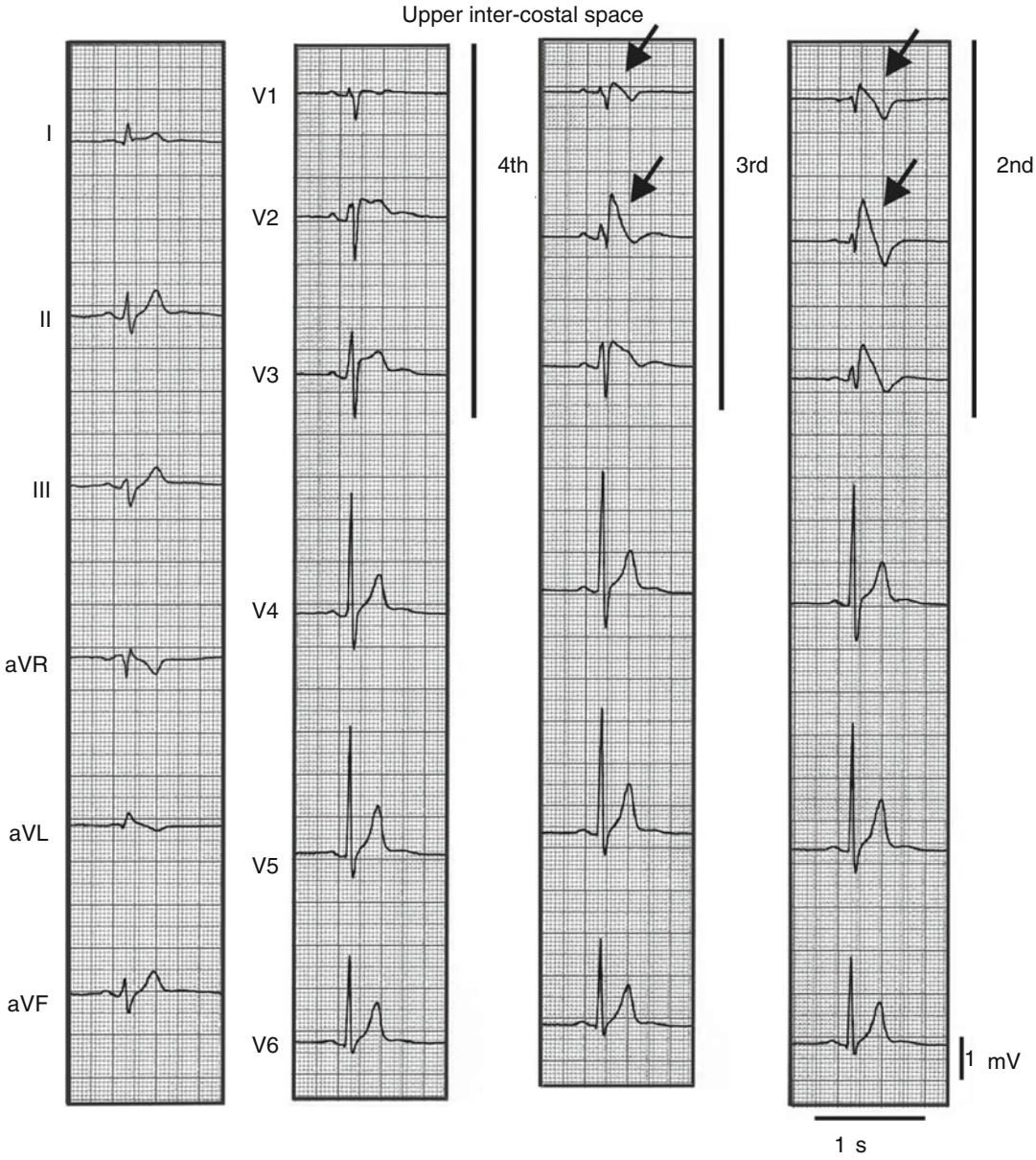

Figure 28–1. Four ECG traces of a resuscitated Brugada syndrome patient showing most severe ST-T abnormalities in leads positioned over the second and third intercostal space (*right two panels*) where a coved-type ECG is present (*arrows*). Intermediate ST-T abnormalities (saddleback-type) are recorded in the fourth intercostal space (leads V2–V3). Calibrations are given (Courtesy of Dr. Wataru Shimizu)

prognosis of Brugada syndrome is not as unfavorable as initially thought. This is leading to more carefulness in ICD implantation strategy. This topic and other important aspects about management in Brugada syndrome are reported in Chap. 29. Brugada syndrome is inherited as autosomal dominant trait. It was linked to mutations in the *SCN5A* gene, encoding the α-subunit of the cardiac sodium channel protein [28]. Up to now more than 200 *SCN5A* mutations correlating with the Brugada syndrome phenotype have been found [29] (see also http://www.fsm.it/cardmoc/) (Fig. 28.3). All Brugada syndrome-linked mutations cause a reduction in

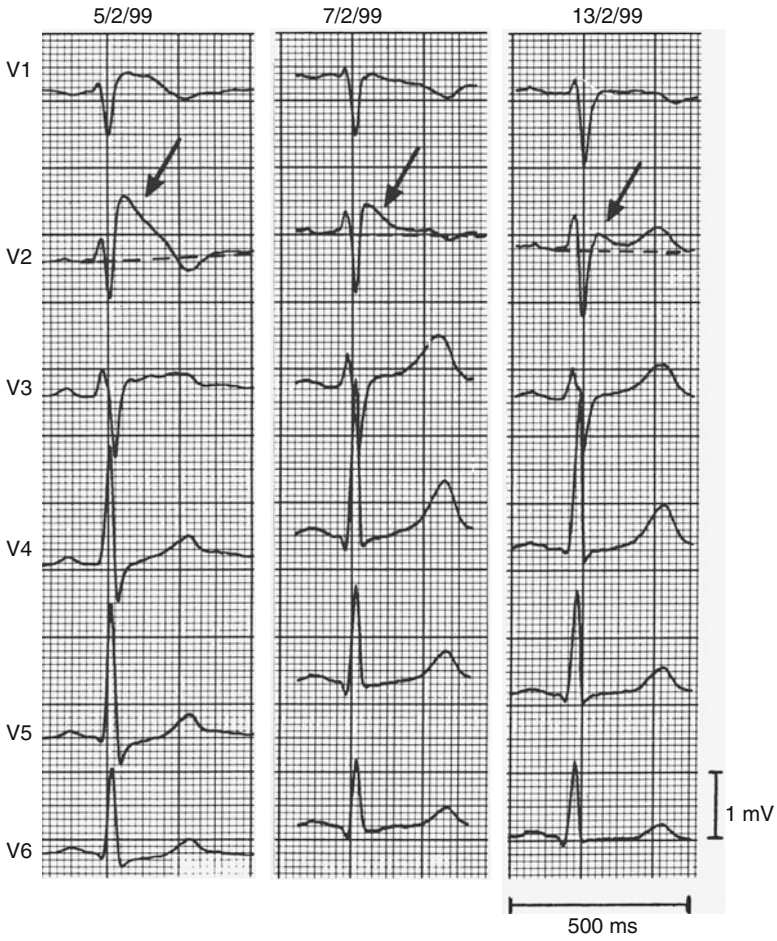

Figure 28–2. Precordial leads ECG of a resuscitated patient with three types of ST segment elevation described in Brugada syndrome. Within a few days the ECG changed from type I (*left panel*) to type II (*middle*) and type III (*right panel*). For explanation see text. The *arrows* indicate the J waves. Calibrations are given (Modified from Wilde et al. [23])

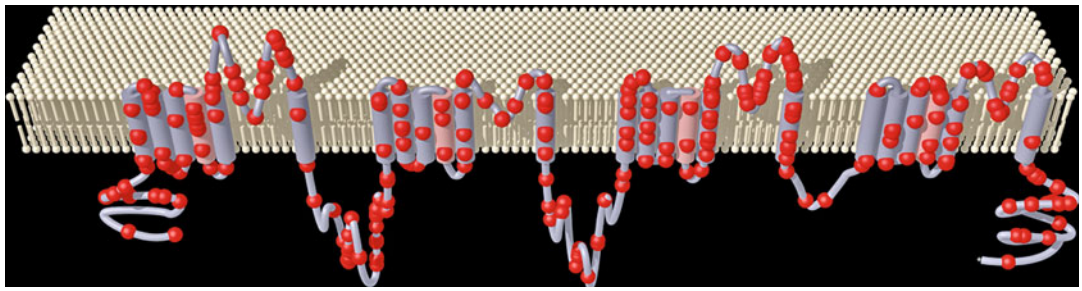

Figure 28–3. Representation of the α-subunit of the voltage gated SCN5A sodium channel showing the locations of the mutations associated with Brugada syndrome (*circle*) (Courtesy of Dr. Andre Linnenbank, Department of Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands)

sodium inward current (I_{Na+}) during phase 0 of the cardiac AP [30]. Interestingly, the type of *SCN5A* mutation may influence the severity of the phenotype [31], although genotype-phenotype correlation studies have been limited due to the low prevalence of mutation carriers and to the fact that only a few mutants have undergone functional studies. Also, the genetic background of an individual, i.e., the presence of polymorphisms (either on the same or other genes) that can act as modifiers, may explain the differences in severity of phenotype [32–34].

A mutation in the *SCN5A* gene is actually found in up to 22% of all Brugada syndrome cases [8]. Some investigators have reported that, in another 11–12% of the cases, mutations in calcium channel genes (*CACNA1C*, *CACNB2B* and *CACNA2D1*) are found [35, 36]. More rarely, genes encoding for sodium channel β-subunits or genes affecting transient outward current I_{to} current are involved [37–40]. Occasionally, the glycerol-3-phosphate dehydrogenase 1-like gene (*GPD1L*) is involved. Yet, with the exception of a large Dutch family carrying mutation 1795insD in the *SCN5A* gene [41] and of mutations in *GPD1L*, classic genetic linkage analysis in Brugada syndrome is lacking [42]. Furthermore, two groups showed that in several large *SCN5A*-related Brugada syndrome families, affected subjects (phenotype positive) did not carry the familial mutation (genotype negative) [43, 44]. For these reasons, interpretation of genetic data in Brugada syndrome patients requires extreme caution [8].

General Clinical Properties

Demography

Since its recognition as a distinct subgroup of idiopathic VF in 1992, Brugada syndrome is increasingly described worldwide, although its exact prevalence remains unclear and can vary significantly between different regions of the world [45, 46]. Moreover, the Brugada syndrome ECG in the medical literature is often used as synonymous of Brugada syndrome, which is not completely correct [47, 48], since more clinical criteria need to be fulfilled for the diagnosis in addition to ECG abnormalities [23]. The Brugada syndrome is endemic in East and Southeast Asia, where it underlies the Sudden Unexplained Nocturnal Death Syndrome (SUNDS) [49]. It is also particularly prevalent in Japan, the Philippines and Thailand, being the leading cause of nontraumatic sudden death among young men [50, 51]. In China and Korea, the reported incidence is lower [52–54]. In Europe, although Brugada syndrome is less prevalent than in Asia, it is still extensively described [25, 55], with exception of the Scandinavian countries. Its prevalence is estimated at 5–50 cases per 10,000 inhabitants [56, 57]. Conversely, occurrence of Brugada-type ECG and/or Brugada syndrome in the United States and Canada seems to be uncommon, but it might be under-recognized [58–60]. In Iran, the reported prevalence of the typical Brugada syndrome ECG among subjects presenting with palpitations is greater than in some European countries, but lower than in Japan [61]. Finally, Brugada syndrome is also described in North Africa, with a prevalence of 2 per 100,000 adults, although this data may represent an underestimation [62].

Clinical Characteristics and Types of Arrhythmia

The clinical presentation is highly heterogeneous and may include palpitations, dizziness, syncope, and (aborted) sudden death. In the areas where Brugada syndrome is endemic, there seem to be also a more severe clinical course with a high incidence of VT/VF at young age [63]. Still, most subjects in whom a Brugada syndrome type I ECG is found remain asymptomatic [10, 64]. Syncope is by far the most important symptom because its occurrence has a recognized prognostic value [10], especially when associated with spontaneous segment ST elevations [26]. An estimated 80% of patients with documented VT/VF have a history of syncope [55]. Syncope may be provoked by self-terminating VT and this may explain why patients experience agonal respiration at night after which they wake up [65–69]. Interpretation of syncope episodes requires caution since several descriptions point out that syncope in subjects with a type I Brugada syndrome ECG may also present with typical vasovagal features [70, 71]. Indeed, the prevalence of a positive head-up tilt test reached 35% in two small Brugada syndrome cohorts and was higher than in control subjects [72, 73]. This suggests that the impairment of the autonomic nervous system may play a role in triggering syncopal episodes in Brugada syndrome subjects. However, a protocol on management of syncope episodes in patients suspected to have Brugada syndrome is missing and future studies on this topic are certainly needed. In any case, episodes of syncope in a

young or middle aged subject should lead to screening for Brugada syndrome, especially when syncopes are recurrent and severe [74]. Arrhythmic events in Brugada syndrome can occur at all ages, from childhood to the elderly (range 0–77 years) [53, 75–77], with a peak around the fourth decade [78]. In children, clinically manifest Brugada syndrome is less prevalent than in adults [79], and a typical Brugada syndrome ECG pattern rarely appears before adolescence [80].

It has been estimated that Brugada syndrome causes 4–12% of all SCD, and up to 20% among patients without identifiable structural abnormalities [81]. However, in some families the clinical course is more benign [64]. Correct diagnosis of the disease by the index patient and prompt identification of (pre-symptomatic) affected family members is necessary in order to prevent more cases of SCD. An extensive familial examination of (aborted) sudden unexplained arrhythmic death (SADS) cases conducted at our institution unmasked inherited cardiomyopathies or channelopathies in up to 40% of the tested families [82]. According to recent reports, at least 10% of SADS families associate with Brugada syndrome [83]. Sudden death in Brugada syndrome results from fast polymorphic VT which usually originate from the right ventricular outflow tract (RVOT) [84], and degenerate into VF [1, 2, 85], whereas no significant variations in QTc intervals precede spontaneous VF episodes [1, 86]. Ventricular arrhythmias and (aborted) sudden death in Brugada syndrome- distinct from arrhythmogenic right ventricular cardiomyopathy (ARVC)- typically occur at rest when the vagal tone is augmented [87], and often at night [88, 89] or after large meals [90, 91]. During the night, Brugada syndrome patients suffer (more often than controls) from sleeping disorders with episodes of apnea, which can activate the parasympathetic nervous system and function as an arrhythmogenic trigger [92].

Clinical presentation with sustained monomorphic VT, although very uncommon, has also been described [93–95], also in one infant [96]. Data obtained from stored electrograms of ICDs have demonstrated that, although premature ventricular complexes (PVCs) in patients affected with Brugada syndrome are rare [97], their prevalence increases prior to spontaneous VF [86]. These PVCs appear to have the same morphology as the first VT beat, and different VT episodes are initiated by similar PVCs in the same subject [86, 98]. They show a left bundle branch block morphology [99] and endocardial mapping localized their origin in the RVOT [100]. Further confirmation of the role of these initiating PVCs and of the RVOT derives from the clinical benefit resulting from their elimination via catheter ablation [100]. More recently, a study in nine severely symptomatic Thai Brugada syndrome patients with recurrent ICD discharges upon VF demonstrated the presence of abnormal ventricular fragmentation at the epicardial site of the anterior RVOT region [101]. After ablation in this area, the ECG normalized in the majority of the patients and no recurrence of VT/VF occurred in 8/9 patients during a follow-up of 20 ± 6 months.

This elegant publication strongly supports the hypothesis that Brugada Syndrome is caused by conduction delay in the right ventricular outflow tract.

Supraventricular Tachyarrhythmias

Episodes of atrial flutter/fibrillation (AF) are often documented in Brugada syndrome [102–106] with a prevalence up to 30% [88, 107, 108]. This is abnormally high considering the young average age of the affected patients [109]. There seems to be no difference in occurrence of AF in *SCN5A* mutation positive patients compared with *SCN5A* mutation negative patients [110]. Given that a history of atrial arrhythmias correlates with VT/VF inducibility during EPS, and that ST segment elevation correlates with the onset of AF episodes [88], Brugada syndrome patients with paroxysmal atrial arrhythmias may constitute a population at higher risk with a more advanced disease state [109], but these data are still limited [111]. Importantly, it is known that the annual incidence of inappropriate ICD shocks due to supraventricular tachyarrhythmias in Brugada syndrome patients may be as high as 14% [112–114]. Therefore, correct management strategies aiming not only to avoid ICD implantation in low risk patients

but also to prevent inappropriate ICD discharges (drug-achieved and/or through accurate ICD programming) are mandatory [9, 115].

Gender Disparity

A salient property in the clinical manifestation of Brugada syndrome is the higher disease prevalence in males (70–80% of all affected subjects), particularly in regions where this syndrome is endemic, despite equal genetic transmission among both genders [50, 56, 78]. That a role in gender disparity could be played by sex hormones, in particular by testosterone, was suggested by the demonstration that castration attenuated ST elevations in two asymptomatic male Brugada syndrome patients [116] and by the revelation that men affected with Brugada syndrome have significantly higher levels of testosterone than age-matched control subjects [117]. Also, is has been shown that male subjects with Brugada-like ECG have a higher risk for prostate cancer, independently of their smoking habit, age or radiation exposure (the study population consisted of survivors of the Nagasaki atomic bomb) [118]. A possible explanation for this phenomenon, derived from clinical [119] and experimental studies [120, 121], is that sex hormones may modulate potassium currents (e.g., I_{to}) during the early repolarization phase of the cardiac AP.

Among affected patients, men and women differ in their clinical presentation. This is reflected in the greater amount of ST segment elevation in men (on average), while women show more pronounced conduction disorders in response to drug challenge [122]. Still, exceptions have been described [123]. Also, the prognosis seems to be worse in men in comparison with women [124], although male sex *per se* was not a statistically significant predictor of events in multivariate analysis in a large cohort of asymptomatic patients [10].

Genetic Aspects

In 1998 Brugada syndrome was linked to mutations in the *SCN5A* gene, encoding the pore-forming α-subunit of the human cardiac sodium channel protein [28]. Nowadays, 11 different genes are associated to the Brugada syndrome, with *SCN5A* still being the most important involved gene [8]. Yet, with the exception of a large Dutch family carrying mutation 1795insD in the *SCN5A* gene [41] and of mutations in the glycerol-3-phosphate dehydrogenase 1-like gene (*GPD1L*) [125], classic genetic linkage analysis to any of the invoked genes in Brugada syndrome is lacking.

The *SCN5A* gene is situated on chromosome 3p21 and encodes a large protein of 2016 amino acid residues [126]. Every α-subunit, which constitutes the major component of the cardiac Na^+ channel complex, contains four homologous domains, each composed of six segments (S1–S6) and assembled with four ancillaries β-subunits (cytoskeleton proteins) to form the voltage-dependent cardiac sodium channel. The S5–S6 segments and the p-loop between them form the inner pore of the channel, which is high selective for Na^+ ions. S4 segments act as the voltage sensor [127]. This channel belongs to a family with different isoforms and different biophysical properties according to its tissue distribution [8, 128]. In the heart it is responsible for the rapid initiating phase of the AP and thus plays an important role in impulse formation and propagation through the cardiac conduction system and muscle. The Na^+ channel is dynamic and undergoes rapid structural transformations in response to the voltage changes across the sarcolemma. This process is known as "gating". Upon membrane depolarization, the channel activates, allowing the opening of the pore. This increases channel permeability for Na^+ ions. The resulting inward current causes the rapid upstroke of the AP. After a few milliseconds, fast inactivation of the channel occurs, a state in which the pore cannot re-open. Membrane repolarization is necessary to allow the Na^+ channels to recover from inactivation into the resting state (closed state), from which they can re-open during the next cardiac cycle.

In the last years more than 200 *SCN5A* gene mutations (inherited Arrhythmia Database: http://www.fsm.it/cardmoc/) have been described in patients with the Brugada syndrome phenotype [29], alone or in combination with Long QT

Syndrome type 3 (LQT3) and/or progressive cardiac conduction defects (PCCD, also known as Lev-Lenègre disease), AF, atrial standstill and dilated cardiomyophaty, diseases in which *SCN5A* mutations may also be present (Fig. 28.3) [8, 129–132]. Of interest, some *SCN5A* mutations may cause a combination of Brugada syndrome and LQT3 or Lev-Lenègre disease within the same family or even within the same individual [41, 133, 134]. While LQT3 associated *SCN5A* mutations generally increase I_{Na}, those associated with Lev-Lenègre disease reduce it, similar to those in Brugada syndrome [131, 135].

For a comprehensive compendium of *SCN5A* mutations in Brugada syndrome see the recent work of Kapplinger et al. [29].

Functional studies of mutant channels have been performed, up to now, with mutant proteins from at least 25 different *SCN5A* mutations. The common effect of *SCN5A* mutations associated with Brugada syndrome is reduction in sodium inward current (I_{Na}), during phase 0 of the cardiac AP, resulting from failure of expression of the mutant sodium channel in the cell membrane (trafficking) or changes in its functional properties (gating), resulting from: (1) shift in the voltage and time-dependence of I_{Na} activation and/or inactivation; (2) enhanced entry into an intermediate state of inactivation from which the channel recovers more slowly; (3) accelerated inactivation [30, 131, 136, 137].

The reduction in I_{Na} caused by the mutant sodium channels in Brugada syndrome is in agreement with the clinical observation that sodium channel blockers accentuate ST segment abnormalities in affected subjects [138]. Moreover, this finding concurs with the demonstration that Brugada syndrome patients who carry a *SCN5A* mutation have significantly more conduction disorders than non-carriers [110, 139, 140]. Also, within the cohort of the carriers, mutations which result in premature truncation of the sodium channel protein (insertions/deletions, nonsense and splice site mutations) are associated with longer PR and QRS intervals than missense mutations in which the mutant sodium channel is able to reach the membrane and is, at least in part, functionally active [31]. Despite the increasing number of *SCN5A* mutations recognized in Brugada syndrome, the proportion of clinically diagnosed Brugada syndrome probands who carry a *SCN5A* mutation is estimated around 14–22% [8, 55, 141]. Few other genes linked to the Brugada syndrome phenotype are now discovered. Still, other candidate genes other than *SCN5A* are unlikely to be major casual genes in this syndrome [142, 143]. In 2002, a novel mutation in the glycerol-3-phosphate dehydrogenase 1-like gene (*GPD1L*) on chromosome 3p22-24 has been described, linked to the Brugada syndrome phenotype in a large family [125]. This gene encodes a protein of 351 amino acids whose function in the heart still remains unknown, but the mutant protein, when studied in cell lines, was responsible for a diminished inward sodium current, similar to the other *SCN5A* mutations in Brugada syndrome studied so far [30, 42]. Mutations in *GPD1L* may also be responsible for sudden death in neonates [144]. More recently, genetic and heterologous expression studies revealed loss-of-function missense mutations in the gene which encodes the α1 subunit of the L-type calcium channel (*CACNA1C*) and its auxiliary subunits (*CACNB2C* and *CACN2D1*) in Brugada syndrome patients [36]. Several of these affected subjects also presented QT intervals shorter than normal [35]. Also, mutations in the β-subunits 1 (*SCN1B*) and 3 (*SCN3B*) of the sodium channel were found to have an effect on I_{Na}, leading to Brugada syndrome [37, 40]. Lately, also genes encoding for the transient outward potassium current, *KCNE3* [145], *KCND3* [39] and *KCNE5* [38] were found to be associated with the Brugada syndrome phenotype. When the mutated *KCNE3* was co-transfected with *KCND3* in Chinese hamster ovary cells, this resulted in a significant augmentation in the amplitude of the I_{to} current, compared with the wild type [145]. Occasionally, mutations in the *MOG1* gene, a gene encoding for a protein that regulates the expression of the sodium channel on the cell membrane, could be involved [146].

Other genes still await discovery. Good candidates appear to be, besides all genes that modulate ion current amplitudes during the initial parts of the cardiac AP, genes which encode adrenergic receptors, cholinergic receptors, ion-channel interacting proteins, transcriptional factors and transporters [147–149].

Though *SCN5A* mutations account for only 14–22% of all affected probands [26, 55], genetic testing is recommended during work-up in Brugada syndrome to support the clinical diagnosis, to identify affected relatives and to better elucidate the genotype-phenotype relationship in Brugada syndrome. Still, interpretation of genetic results must be performed with extreme caution and should be restricted to specialized centers where there is a structured collaboration between the cardiologists and the geneticist and where probands and his/her family members can be followed on a regular basis.

It has been shown in a Japanese cohort that the presence of a *SCN5A* mutation may somewhat influence the clinical course and VF recurrence [150]. Moreover, our group found that the type of the *SCN5A* mutation influences the severity of the clinical phenotype: carriers of a truncating mutation presented more severe conduction disorders than missense mutation carriers [31]. Interestingly, larger studies did not support the presence of a *SCN5A* mutation in the guidance of management of Brugada syndrome patients [10, 151, 152]. The explanation for this discrepancy could be due to the low prevalence of mutation carriers and the lack of functional data for many of the involved *SCN5A* mutants. Notably, the genetic background of an individual other than the primary *SCN5A* mutation, i.e. the presence of polymorphisms (either on the same or in other gene loci) [153] that can acts as strong modifiers, may also explain the differences in severity of phenotype. This concept is shown for the common polymorphism H558R [34, 154, 155]. Rare *SCN5A* variants are also described in healthy subjects [29]. Interestingly, common polymorphisms in the promoter region of *SCN5A* (Hap B) were also found to be associated with interindividual variability of expression and therefore variability in conduction parameters [156, 157]. These concepts add complexity in understanding and managing affected subjects and their family members. In daily practice, clinicians will deal with subjects carrying the primary mutation and suffering from severe arrhythmias and family members carrying the same genetic mutation that might not develop any arrhythmia. Even more strikingly, two groups showed that in several large *SCN5A*-related Brugada syndrome families, affected subjects (phenotype positive) did not carry the familial mutation (genotype negative) [43, 44], indicating that other factors may play a more important role than appreciated so far For all mentioned reasons, interpretation of genetic data in Brugada syndrome patients requires extreme caution [8].

ECG Characteristics

ST Segment Elevation Pattern

Typical electrocardiographic abnormalities have represented, since its first description, the fundamental aspect in recognition of subjects affected by Brugada syndrome [1, 2]. Particular attention was given to the presence of a (incomplete) right bundle branch block (RBBB), accompanied by ST segment elevation in the right precordial leads, not related to ischemia, electrolyte imbalance, or structural heart disease [4]. At present, diagnosis of Brugada syndrome revolves around characteristic ST segment elevations in leads V1–V3 or in leads positioned at more cranial intercostal spaces, whereas the presence of a RBBB is no longer required [23].

Three ECG repolarization patterns were described as potential manifestations of Brugada syndrome: type I ECG, referred to as coved-type, is the one illustrated in 1992 during the first description of the Brugada syndrome and consists of >2 mm J point elevation, followed by a down-sloping ST segment and a negative T wave; type II ECG, called saddle-back type, also shows a elevated J point (>2 mm) with a gradually descending ST segment that does not reach the baseline and gives rise to a positive or biphasic T wave; type III ECG could be of any of the previously described morphologies and is characterized by a smaller magnitude of ST segment elevation (≤1 mm) (Fig. 28.2). The presence of a type I ECG is required for the diagnosis [23]. Important considerations and cautions in interpretation of the ECG in diagnosing Brugada syndrome have to be taken into account. Firstly, the ST segment in Brugada syndrome is typically highly dynamic, exhibiting profound day-to-day variation in amplitude and morphology, even within the same patient [158–160]. It can even

disappear temporarily. Clinical conditions of reduced cardiac excitability, like in the case of hyperkalaemia, can exaggerate ECG abnormalities in affected patients [161] and should be promptly recognized.

This aspect may contribute to possible bias and underestimation of the prevalence of Brugada syndrome. The presence of spontaneous ECG fluctuations measured on separate days in Brugada syndrome patients is associated with the highest risk of arrhythmic events and can be used as a non-invasive method for risk stratification, as well as the presence of late potentials on signal-averaged ECGs [162–164]. A spontaneous variation of ≥0.20 mV in right precordial leads ST segment elevation was found in affected patients who have had VF, but not in asymptomatic patients [165].

An inter-individual variation of the ST segment can also be observed between members of the same family who carry the same *SCN5A* mutation. The average magnitude of ST segment elevation does not differ, on average, between *SCN5A* mutation carriers and non-mutation carriers in Brugada syndrome [139]. In contrast, affected men have on average a higher degree of ST elevation than women [122]. Finally, many agents and conditions are reported to significantly influence ST segment elevation and therefore the risk of developing arrhythmias in Brugada syndrome. This phenomenon and its clinical implications are described further in this chapter.

Importance of Positioning of the Right Precordial Leads

The signature ST elevations in Brugada syndrome are usually observed in leads V1–V3, with rare occurrences in inferior or lateral limb leads [166–168]. More strikingly, leads positioned cranially from V1 and V2 in the third ($V1_{IC3}$ and $V2_{IC3}$) or second ($V1_{IC2}$ and $V2_{IC2}$) intercostal spaces often produce the most severe abnormalities, both in the presence and absence of pharmacological challenge [5, 169, 170], as also demonstrated with body surface mapping [3, 171]. This phenomenon emphasizes the crucial role of the RVOT area in the pathogenesis of Brugada syndrome [7]. The use of 87-lead body surface maps permitted to demonstrate that in 7/28 Brugada syndrome patients the typical ECG pattern was located at the level of the RVOT (second and third intercostals space), while conventional leads V1 and V2 registered only minimal ST segment elevation. Conversely, investigation of the more cranial leads in 40 control subjects did not reveal any significant ST elevation, neither at baseline, nor after disopyramide [3]. In 2006, we demonstrated that a more cranial positioning of the ECG leads increased the sensitivity of the flecainide testing in unmasking *SCN5A*-related Brugada syndrome [172]. These results were later on confirmed with the use of ajmaline in subjects with a family history of Brugada syndrome and/or SCD [173]. Although little is known about the specificity of the third intercostal leads and their prognostic information, we believe that ECG investigation in these more cranial leads should be performed whenever a case of Brugada syndrome is suspected [172, 174]. This topic should also be further addressed in a new Consensus Paper.

Other Electrocardiographic Parameters in Brugada Syndrome

Brugada syndrome has habitually been associated with the presence of right bundle brunch block, thought atypical because of the absence of a wide S wave in the left lateral leads [56]. Nowadays, the presence of a RBBB is no longer considered necessary for the diagnosis [23], though a widening of the QRS complex is frequently observed in patients affected by Brugada syndrome [172, 175]. Actually, signs of conduction defects are found at all levels (Fig. 28.4), particularly in patients carrying a *SCN5A* mutation [139, 140]: QRS axis deviation [1, 168, 176, 177], P wave enlargement [110, 172], and PQ prolongation, presumably reflecting prolonged His-Ventricular conduction time [1, 56, 139, 178]. Moreover, sinus node dysfunction [177, 179, 180] and AV node dysfunction [139, 181, 182] have been extensively reported. In contrast, QTc duration generally is within the normal range [23, 56, 109], but it may be occasionally prolonged [1]. A new clinical entity was discovered in 2007 represented by coexistence

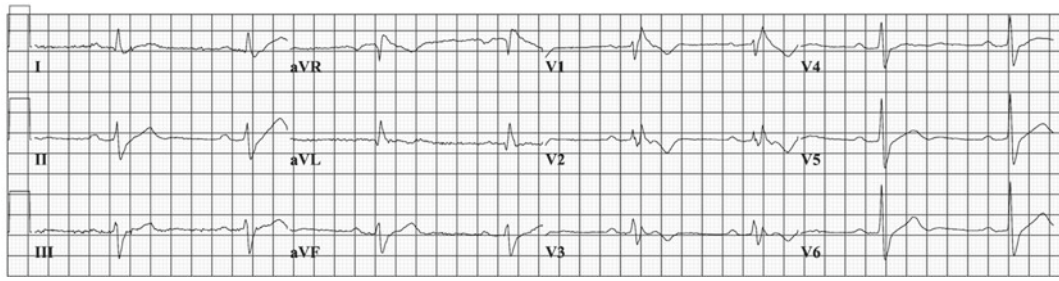

Figure 28–4. Twelve lead ECG recorded at basal condition in a 49 year old male subject who suffered aborted sudden death. In addition to ST segment elevation in V1, signs of conduction disorders are present, including sinus bradycardia, PQ interval prolongation, left QRS axis deviation, and widening of QRS complex with a RBBB configuration, suggestive for Brugada syndrome

of Brugada syndrome alterations and short QT interval, due to loss-of-function in calcium channels [35].

The presence of a *SCN5A* mutation significantly influenced the phenotype with more exhibition of clinically relevant conduction defects (first degree AV block, hemiblocks, complete RBBB, LBBB) in *SCN5A* carriers *vs* non carriers [139, 140], independently from the amount of ST segment elevation. Also, the type of *SCN5A* mutation influences the severity of conduction disorders in Brugada syndrome/PCCD patients [31]. Attention has also been paid to the recognition of other ECG criteria, in addition to the amount of J point elevation that may aid in identifying subjects at risk for sudden death. Five relatively new ECG parameters are: (1) S wave width in leads II and III, to be considered a mirror image of the electrical activity taking place in the RVOT [7]; these S waves were significantly wider in the individuals with a positive response to flecainide than in the negative responders [172]; (2) S wave width in lead V1 ≥ 0.08 s was shown to be a good predictor of arrhythmic events in Brugada syndrome patients [175] (3) baseline QRS width in lead V1 ≥ 110 ms significantly contributed to the prediction of a positive drug test [183]; (4) QRS width ≥120 ms on baseline ECG was associated with the occurrence of symptoms in a Japanese population [184]. The same cut-off value of prolongation of QRS duration on standard 12-leads ECG was already found to be a marker of arrhythmic risk and yielded a specificity and sensitivity of 70 and 52%, respectively, in identifying subjects with symptoms [185]; (5) fragmented QRS complex in V1–V3 or at the higher intercostal leads was found to be predictor of syncope in Brugada syndrome patients during 4 years follow-up [186].

Late Potentials

The presence of late potentials (LP) on signal-averaged ECGs (SAECG) has gained attention as a useful non-invasive method able to predict arrhythmic events in Brugada syndrome [17, 19]. LP are especially found in the anterior wall of the RVOT in symptomatic Brugada syndrome patients and can be exaggerated by infusion with flecainide [106]. They are generally regarded as delayed and disorganized ventricular activation (at the terminal portion of the QRS) and are related to a high risk of developing ventricular tachyarrhythmias [187]. The value of LP as predictors of arrhythmic events has been mainly tested in patients with structurally abnormal hearts, especially in studies of patients after a myocardial infarction [188]. In Brugada syndrome LP could represent delayed activation in the RVOT area [11], being in agreement with the depolarization disorder theory, or, according to some other authors, they may be an extension beyond the QRS of the second epicardial upstroke generated by a phase 2 reentry mechanism [12, 106].

Surely, SAECG detects a higher prevalence of LP in symptomatic patients, in comparison with asymptomatic patients [164, 189, 190]. Moreover, daily fluctuations in LP are more accentuated in symptomatic versus asymptomatic patients [162].

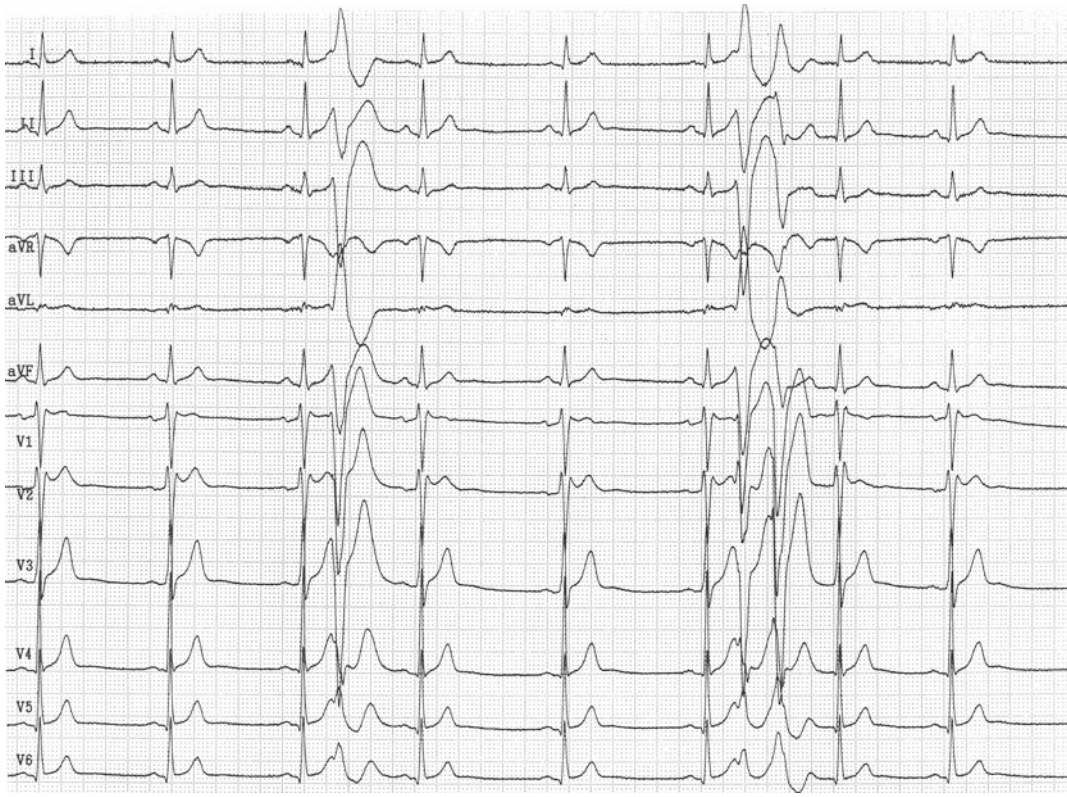

Figure 28–5. ECG recorded after intravenous infusion of 80 mg flecainide in a 45 year old male subject showing a saddle-back ST segment elevation in leads V1 and V2 (type II) and the appearance of premature ventricular beats, isolated and in couples, from the right ventricular outflow tract. The ectopic beats show a short coupling interval. Flecainide challenge was performed to pose the diagnosis of Brugada syndrome after an aborted sudden death

Drug Tests

Pharmacological challenges utilize intravenous administration of sodium channel blockers, i.e., class IA (except quinidine) and IC, but not class IB [191] antiarrhythmic drugs. They are used to unmask concealed forms of Brugada syndrome because of their capacity to provoke/exaggerate ST segment changes [138, 182, 192–194]. Provocation tests are required when ST segment elevation is not initially present or when type II or III ECG patterns are seen [23]. However, provocation challenges are not recommended and could be even harmful in the presence of a spontaneous type I ECG [24, 195].

The diagnostic yield and safety of such tests, when performed with ajmaline or flecainide, have been recently reported in genotyped populations. Ajmaline (1 mg/kg body weight; 10 mg/min) was the most powerful drug [196], with a higher sensitivity (80%) and specificity (94%) [44] than flecainide (2 mg/kg; max 150 mg) (sensitivity 77%, specificity 80%) (Fig. 28.5) [172]. In Japan drug tests are performed with pilsicainide, a so-called pure sodium blocker (class IC). The protocol and the stopping criteria of the test with pilsicainide are similar to the European protocols [197]. Data on sensitivity and specificity of the tests with pilsicainide are missing, while ECG changes upon this drug (either in the positive or in the negative responders including investigation in the high precordial leads) and the safety issue have been carefully evaluated [197, 198]. Drug provocation tests with flecainide, ajmaline or pilsicainide have proven to be safe in large series of patients when conducted according to the guidelines of the European Society of Cardiology [23], since VT/VF did not occur [177, 197]. In particular, drug

infusion must be discontinued when a type I ECG is reached or when PVCs/(non)sustained VT occur or when QRS duration increases by more than 30% of the basal value. If not discontinued, life-threatening ventricular tachyarrhythmias may develop (Fig. 28.4) [84, 195, 199]. Some authors suggested to abolish the stopping criterion of QRS prolongation during ajmaline administration because it may lead to an under recognition of positive cases [200]. Still, in their series, quite a high incidence of drug-induced arrhythmias was seen; moreover, more data is needed to evaluate whether such an aggressive protocol is leading to false positive results or not [201]. In patients with gross structural heart abnormalities, infusion with sodium channel blockers should also be avoided [202]. The presence of a *SCN5A* mutation seems to increase the risk of arrhythmias during infusion with sodium channel blockers [195].

Structural Abnormalities

One clinical characteristic of Brugada syndrome is the absence of gross structural abnormalities [1, 2]. Nonetheless, there is emerging evidence that Brugada syndrome may represent a mild form of right ventricle cardiomyopathy, not apparent with routine diagnostic tools [178, 203]. Similarities with arrhythmogenic right ventricular cardiomyopathy (ARVC) were pointed out especially by Italian researchers [178, 204] and were strengthened by the discovery of a *SCN5A* mutation in a family with ARVC [205]. On echocardiography, contraction of the RV was delayed by sodium channels blockade in Brugada patients compared with controls [16]. The sensitivity to detect slight structural abnormalities has become greater with electron beam CT scan and cardiac MRI. These methods have revealed RV wall motion abnormalities in at least three series of Brugada syndrome patients (these studies included control subjects) [206–209]. More recently, it has been shown that Brugada syndrome subjects show a significantly lower right ventricle ejection fraction on MRI then controls. Moreover, among affected subjects, the subgroup with spontaneous type I ECG also had significant RVOT enlargement and structural changes also of the left ventricle [210]. Notably, an Italian study had showed already in 2005 that in 4/18 Brugada syndrome patients who underwent biventricular endomyocardial biopsies, there were signs of right ventricular cardiomyopathy (n = 4), although the hearts appeared normal at non-invasive evaluation, including MRI [211] (no control group in this study). Interestingly, the presence of a *SCN5A* mutation was found in all of the four patients with cardiomyopathy-like changes on biopsy specimens. The remaining 14 heart specimens revealed changes compatible with myocarditis (n = 14). Furthermore, in 8/18 patients (45%), similar findings were also found in the left ventricle. Unlike this study, a German group found no evidence of inflammation or localized myocarditis in biopsies of Brugada syndrome patients. Still, they found fatty replacement in 4/21 patients in none of whom ARVC diagnostic criteria were fulfilled [212]. In addition, right ventricular fibrosis and epicardial fatty infiltration were documented in the explanted heart of a *SCN5A* mutation carrying young Brugada syndrome patient who experienced very many ICD discharges (129 appropriate shocks in 5 months) [21]. In this patient there were no clinically detected cardiac structural abnormalities, but it should be mentioned that MRI had not been performed due to ICD implantation, 10 years before cardiac transplantation. Fibrosis represents a substrate for conduction slowing, which is extensively found in Brugada syndrome. The amount of conduction disorders in affected patients is dynamic, as well as the degree of ST elevations, and is worse in the presence of type I ECG, compared with type II ECG or controls. The cell-to-cell transmission between myocardial cells seems to be impaired rather than the conduction times in the specialized conduction system [20]. These findings demonstrate a strong link between functional and structural abnormalities and also suggest the hypothesis that sodium channel mutations themselves are able to induce subtle structural derangements and myocardial cell death. This hypothesis has been elegantly tested in transgenic adult mice with *SCN5A* haploinsufficiency where a significant amount of cardiac fibrosis was found [213] and is supported by the clinical

Table 28–1. Abnormalities associated with Brugada-like ST segment elevation

Conditions that can lead to ST segment elevation, mimicking Brugada syndrome
Early repolarization syndrome [13]
Cocaine intoxication [215]
Acute myocardial infarction or isolated right ventricular infarction [216, 217]
Prinzmetal's angina [218, 219]
Hyperkalemia [220]; hypercalcaemia [221]
Acute pericarditis/myocarditis [222]
RBBB or LBBB and left ventricular hypertrophy [223]
Acute aortic dissection/acute pulmonary embolism [224, 225]
Arrhythmogenic right ventricular cardiomyopathy [204, 226]
Long QT syndrome type III [227]
Hypothermia [228]
Duchenne muscular dystrophy [229]
Friedreich's ataxia [230]
Various central and autonomic nervous system abnormalities [231, 232]
Mechanical compression of the RVOT by a mediastinal tumor [233]
Cardiac amyloidosis [234]
Hypothyroidism [235]

Table 28–2. List of medications to avoid in patients affected with Brugada syndrome

Medications to be avoided in Brugada syndrome patients
Sodium channel blockers
Class I anti-arrhythmic drugs (flecainide, ajmaline, propafenone, pilsicainide, procainamide, disopyramide, cibenzoline) [138, 182]
Local anesthetics (lidocaine, bupivacaine) [240]
Carbamazepine, phenothiazine [241]
Tricyclic and tetracyclic anti-depressants [241–246], including lithium [247]
Alpha adrenergic stimulation (norepinephrine, methoxamine) [248]
Medications to be used with caution in Brugada syndrome patients
β-adrenergic blockers [191, 248]
Calcium antagonists, non-dihydropyridines (verapamil, diltiazem) [248–250]
Nitrates [104]
General anesthetics/antagonism of anesthesia [52, 248, 251–253]
Muscarinic drugs (i.e. neostigmine) [52, 191, 248]

observation that certain *SCN5A* defects were associated with fibrosis in the conduction system and in the ventricular myocardium [214]. There is no end to complexity, since it is also acknowledged that in large Brugada syndrome families affected subjects might not even show the familial *SCN5A* mutation [43]. This work represents an eye-opener and leads to the hypothesis that genetic factors, other than alterations in the sodium channel gene, may also contribute to the occurrence of the disease.

Differential Diagnosis in Brugada Syndrome

A number of clinical conditions which are also accompanied by ST segment elevation should be carefully ruled out before the diagnosis of Brugada syndrome is made (Table 28.1). Relatively common causes of ST segment elevation include: (1) early repolarization syndrome [13]; (2) acute myocardial infarction, isolated right ventricular infarction or left ventricular aneurysm [216, 217, 236]; (3) Prinzmetal's angina, which may also coexist with Brugada syndrome [218, 219]; (4) electrolyte disturbances, such as hyperkalemia (when severe can lead to QRS prolongation and ST changes, resembling the Brugada coved-Type ECG) [220] and hypercalcaemia [221]; (5) acute pericarditis/myocarditis [222]; (6) RBBB or LBBB and left ventricular hypertrophy [223]; (7) ECG recorded after electrical cardioversion [23].

More rarely, ST segment elevation may occur under the following conditions: (1) acute pulmonary embolism/acute aortic dissection [224, 225]; (2) ARVC [204, 237]; (3) Long QT syndrome type III [227]; (4) hypothermia [228]; (5) Duchenne muscular dystrophy and Friedreich's ataxia [229, 230]; (6) central and autonomic nervous system abnormalities [231, 232]; (7) mechanical compression of the RVOT by a mediastinal tumor [233] (8) cardiac amyloidosis [234].

Influence of Drug Use on the Brugada ECG

A large number of drugs have been reported to unmask Brugada syndrome or evoke Brugada syndrome-like ECG characteristics. For an exhaustive up-to-date list see the webpage www.brugadadrugs.org [238]. Shortly, the most important are antiarrhythmic drugs (with sodium channel blocking efficacy), local anesthetics, antidepressive agents, psychotropic drugs and also substances like cocaine [239] and alcohol (Table 28.2) [93]. This group also includes cardiac anti-ischemic medication, such as calcium channel blockers or nitrates, and medications used to provoke/antagonize anesthesia [191, 248–250].

Finally, tricyclic or tetracyclic antidepressant medications as well as selective serotonin re-uptake inhibitors and lithium should be

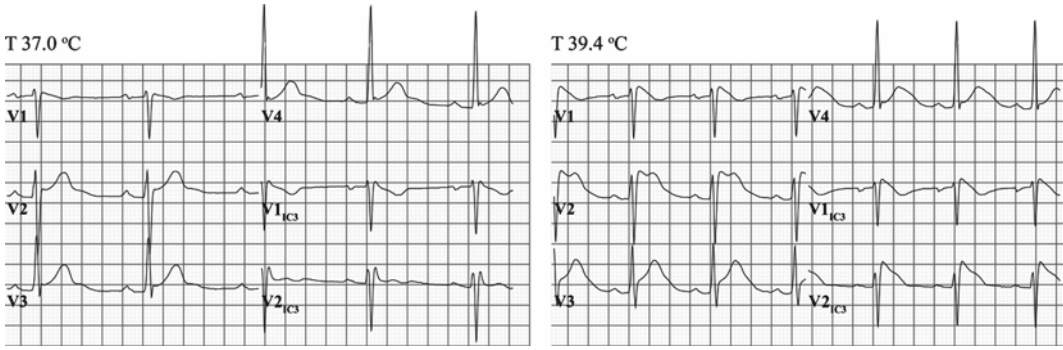

Figure 28–6. ECG recorded at normal temperature and during fever in a male subject affected with Brugada syndrome. Leads $V1_{IC3}$ and $V2_{IC3}$ are positioned cranially from V1 and V2, respectively, in the third intercostal space. This patient had multiple syncopes during fever with documented VF. Screening of *SCN5A* was negative. During fever, we recorded ST segment elevation with appearance of type I in leads V1, $V1_{IC3}$ and $V2_{IC3}$ and type II in lead V2 (*right panel*), while ECGs of the same patient during normothermia display only minimal ST segment elevation (*left panel*)

mentioned. All these drugs have been reported to cause a Brugada syndrome-like ST segment elevation [215, 241–245, 247] and tricyclic antidepressants have been reported to provoke VF, even when used in normal dosages [246].

Sodium channel blockers [182], α-adrenoreceptor agonists and cholinergic stimulation (increased vagal tone) provoke an augmentation of ST segment elevation, while α-adrenoreceptor blockade and β-adrenoreceptor stimulation with isoprenaline reduce the amount of ST segment abnormalities [19, 191]. Since accentuation of ST elevation immediately preceding episodes of VF has been extensively reported [19, 88, 104, 254], all these drugs also modulate susceptibility to arrhythmias.

Some clinically relevant aspects can be derived from these observations: (1) A variety of Na^+ channel blockers are utilized as diagnostic tool for unmasking concealed forms of Brugada syndrome [138, 255]; (2) Use of any Na^+ channel blocker and other medications capable to provoke ST elevation must be avoided in patients with Brugada syndrome (Table 28.2) [138, 182]. Particular attention must be also given to clinical management surrounding local or general anesthesia of patients affected with Brugada syndrome [52, 248, 251–253, 256]. For these reasons, it has become good clinical practice to provide all patients a list of the medication to avoid. Since avoidance of such medication does not require a big effort, and is not difficult to achieve, we recommend the same strategy also for the offspring, even when the children are younger than 16 years of age and have not undergone a drug provocation test (yet). In any case, is it advisable to do that until it is known whether they are also affected or not.

Influence of Body Temperature

A strongly relevant clinical characteristic of Brugada syndrome is that hyperthermia, e.g. fever, may also induce/aggravate ECG changes or provoke arrhythmias in a subset of affected patients. This is also known for other primary arrhythmia syndromes [257]. Several case reports revealed that febrile illness [76, 258–261] or prolonged contact with hot water [262] could precipitate arrhythmic events in Brugada syndrome patients. It is also our personal experience that asymptomatic Brugada patients with a normal basal ECG can, during an episode of fever, display typical ECG changes with different amounts of ST segment elevations up to the appearance of a type I pattern (Fig. 28.6). This phenomenon is also well illustrated by a systematic work of our group [263]. In this retrospective analysis, we found that in 4/22 (18%) of the symptomatic cases (SCD, syncope), there was fever at the time of occurrence of the symptoms and this was significantly more often than in the control population of non-Brugada syndrome survivors of cardiac arrest due to VT/VF at our institution. In three out of four of these cases, an

SCN5A mutation was present. Moreover, ECG analysis was performed in Brugada syndrome patients of whom we had both an ECG at normothermia and during fever. Fever-induced ECG changes in this group of patients were compared with the same changes in ten control patients (all adults, average age at fever 48 ± 19 years, comparable body temperature). The degree of ST segment elevation (maximal elevation between leads V1 and V2) increased significantly during fever ($p < 0.05$) only in the Brugada syndrome group. Controls reached a higher heart rate than Brugada syndrome patients at fever. Notably, in Brugada syndrome patients, mean PR and QRS durations were significantly increased during high body temperature, in comparison with normothermia. Conversely, during fever, both PQ and QRS durations, on average, decreased in the control group. Also, QTc significantly increased during fever in the Brugada syndrome patients while it did not change significantly in controls.

Body temperature represents also in other primary arrhythmia syndromes an important modulating factor in ECG patterns and arrhythmogenesis [264]. In 1999 Dumaine et al. discovered that the changes in Na^+ channel gating properties, induced by the *SCN5A* mutant T1620M, were more prominent at higher temperature (32 °C compared to room temperature) [265] which supports the notion that the consequences of possessing a certain *SCN5A* mutation or a mutation in other genes responsible for Brugada syndrome, can be manifested only during fever or in a hot climate. For this reason, appropriate treatment of fever illnesses is vividly recommended in all patients with Brugada syndrome. Also, activities and conditions that may provoke augmentation of the body temperature must be discouraged [149, 262].

Summary

Brugada syndrome has gained an enormous interest worldwide in the span of almost 20 years after its first description. It is characterized by typical ST segment elevations in ECG leads positioned over the right ventricle and more precisely the RVOT.

Attempts have been made to recognize ECG parameters, other than J point elevation in V1–V3, that could help in posing a correct diagnosis (positioning ECG leads cranially from V1 and V2) or identifying subjects at high risk (S wave width in V1, QRS duration in V2). Linkage to mutations in the *SCN5A* gene (in 14–22% of the Brugada syndrome patients) has represented the biggest step in understanding the pathogenesis of Brugada syndrome (with reduction in I_{Na} being the common denominator of the mutant proteins) and the recent discovery of other genes (β subunit of the sodium channel, components of the L type calcium channel, *GPD1L*, potassium channels, *MOG1*) has confirmed the genetic heterogeneity of this syndrome. For this reason, genetic screening cannot be considered the gold standard in Brugada syndrome; however, it represents a unique instrument to confirm the diagnosis, identify affected family members in a presymptomatic stage, and unravels genotype-phenotype relationships in Brugada syndrome and as such, is strongly recommended.

Despite major advances achieved, there are still unanswered issues revolving around its epidemiology, its unique pathogenesis/arrhythmogenesis, the role of the genetic mutations (either causal or cofactors), and its prognosis and treatment.

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29
Brugada Syndrome: Cellular Mechanisms and Approaches to Therapy

Charles Antzelevitch and Sami Viskin

Abstract

The Brugada Syndrome, introduced as a new clinical entity 20 years ago, has attracted great interest because of its prevalence and association with high risk of sudden death, especially in males as they enter their third and fourth decade of life. Consensus reports published in 2002 and 2005 focused on diagnostic criteria, risk stratification and approaches to therapy. More recently, the risk stratification approaches have been the subject of controversy and debate.

Over 21 years have transpired since the introduction of the concept of phase 2 reentry, the mechanism believed to underlie development of arrhythmogenesis in BrS. Thus, the entity initially introduced as "ST segment elevation and right bundle branch block (RBBB)", which came to be known as Brugada syndrome in 1996, evolved in the experimental laboratory and in the clinic along parallel but separate tracks. While the electrocardiographic and arrhythmic manifestations of BrS are well explained by abnormal repolarization in the right ventricular outflow track (RVOT), recent data have suggested conduction impairment in the RVOT as the basis for BrS, thus generating a debate as to the basis for the pathogenicity of the syndrome.

This review provides an overview of the clinical, genetic, molecular and cellular aspects of the Brugada syndrome and the various approaches to therapy.

Keywords

J Wave syndrome • Sudden cardiac death • Electrophysiology • Electrocardiography • Quinidine • Pharmacology

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Introduction

Twenty years have passed since Pedro and Josep Brugada introduced the syndrome of ST segment elevation and right bundle branch block (RBBB) associated with a high incidence of ventricular fibrillation (VF) as a new clinical entity [1]. Over 21 years have transpired since the introduction of the concept of phase 2 reentry (induced by sodium channel block), the mechanism believed to underlie development of

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Table 29–1. Diagnostic criteria for Brugada syndrome

	ST segment abnormalities in leads V1–V3		
	Type 1	Type 2	Type 3
J-point	≥2 mm	≥2 mm	≥2 mm
T-wave	Negative or isoelectric	Positive or biphasic	Positive
ST-T configuration	Coved type	Saddleback	Saddleback
ST segment (terminal portion)	Gradually descending	Elevated ≥1 mm	Elevated <1 mm

Modified from Wilde et al. [7], with permission from Oxford University Press

1 mm = 0.1 mV, the terminal portion of the ST-segment refers to the latter half of the ST-segment

arrhythmogenesis in this clinical syndrome [2, 3]. Thus, the entity, which in 1996 came to be known as the Brugada syndrome [4, 5], evolved in the experimental laboratory and in the clinic along parallel but separate tracks until the late 1990s [6].

The Brugada syndrome has attracted great interest because of its prevalence and association with high risk of sudden death, especially in males as they enter their third and fourth decade of life. A consensus report published in 2002 delineated diagnostic criteria for the syndrome [7, 8]. A second consensus conference report published in 2005 focused on risk stratification schemes and approaches to therapy [9, 10]. More recently, these risk stratification approaches have been the subject of controversy and hot debate [11–15]. This review provides an overview of the genetic, molecular and cellular aspects of the Brugada syndrome and the various approaches to therapy.

Clinical Characteristics

A brief description of clinical characteristics is included to provide a perspective for our discussion of mechanisms and therapy. This subject is more thoroughly dealt with in the preceding chapter. The Brugada syndrome is characterized by an ST segment elevation in the right precordial ECG leads and a high incidence of sudden death. Worldwide prevalence of BrS is estimated to be 1 in 10,000, but is much higher in Asian and Southeast Asian countries, reaching 5–10 in 10,000 [10, 16, 17] and much lower in some Eastern European countries like Denmark, with an estimated prevalence of 1.1 in 100,000 [18]. The average age at the time of initial diagnosis or sudden death is 40 ± 22, although the range is wide with youngest patient diagnosed at 2 days of age, and the oldest at 84 [19, 20].

The electrocardiographic manifestations of the Brugada syndrome are often concealed but can be unmasked by sodium channel blockers and febrile or vagal states [5, 21–27]. Three types of repolarization patterns in the right precordial leads are recognized (Table 29.1) [7, 8]. A Type 1 ST segment elevation is characterized by a coved ST-segment elevation ≥2 mm (0.2 mV). Only a Type I ST segment elevation with or without a negative T-wave is diagnostic of Brugada syndrome. Although present in the majority of BrS cases, a negative T wave is not necessary for the diagnosis of BrS [28]. Type 2 ST segment elevation has a saddleback appearance with a high take-off ST-segment elevation of ≥2 mm followed by a trough displaying ≥1 mm ST elevation followed by either a positive or biphasic T-wave. Type 3 ST segment elevation has either a saddleback or coved appearance with an ST-segment elevation of <1 mm. These three patterns may be observed sequentially in the same patient. The BrS ECG is generally very dynamic, varying over time. Using serial ECGs, Richter et al. found that among patients initially presenting with a spontaneous Type I ST segment elevation, the BrS phenotype is only seen on every third ECG [29], Type 2 and Type 3 ST segment elevation are not diagnostic of the Brugada syndrome. According to the 2005 consensus statement, Brugada syndrome is definitively diagnosed when a Type 1 ST-segment elevation is observed in more than one right-precordial lead (V1–V3), in the presence or absence of sodium channel blocking agent, and in conjunction with one or more of the following: documented ventricular fibrillation; polymorphic ventricular tachycardia; a family history of SCD

(<45 years old); coved type ECGs in family members; inducibility of ventricular tachycardia (VT) with programmed electrical stimulation; syncope; or nocturnal agonal respiration [7–10]. A recent study provided evidence that a Type I ST segment elevation or J wave in one rather than two right precordial lead may suffice to meet the criteria for diagnosis of BrS [30].

Diagnosis of Brugada syndrome is also considered positive when a Type 2 (saddleback pattern) or Type 3 ST-segment elevation is observed in more than one right precordial lead under baseline conditions and can be converted to the diagnostic Type 1 pattern upon exposure to sodium channel blocker (ST-segment elevation should be ≥2 mm). One or more of the clinical criteria described above should also be present. Drug-induced conversion of Type 3 to Type 2 ST-segment elevation is considered inconclusive for diagnosis of Brugada syndrome.

The ECG manifestations of the syndrome are often concealed, but can be unmasked using sodium channel blockers. Definitive diagnosis is difficult when the degree of basal ST-segment elevation is relatively small and the specificity of sodium channel blockers such as flecainide, ajmaline, procainamide, disopyramide, propafenone and pilsicainide [22, 31, 32] to identify patients at risk is uncertain. A comparison of intravenous ajmaline and flecainide in the same cohort of patients revealed that ajmaline is more effective in unmasking the syndrome [33]. Flecainide failed in 7 of 22 cases (32 %) unmasked by ajmaline. A greater inhibition of transient outward current (I_{to}) by flecainide renders it less effective than ajmaline. False positive as well as false negative responses have been reported with ajmaline, as well, although a false positive is difficult to prove [34, 35]. Itoh et al. demonstrated that a specific missense mutation in SCN5A, the gene that encodes the α subunit of the sodium channel, although responsible for the disease, prevented the ability of ajmaline to unmask the syndrome, due to loss of the ability of the drug to produce use-dependent inhibition of sodium channel current [35].

Most cases of Brugada syndrome display right precordial ST-segment elevation, although isolated cases of inferior lead [36, 37] or left precordial lead [38] ST-segment elevation have been reported in Brugada-like syndromes, in some cases associated with *SCN5A* mutations. In rare cases, ST segment elevation is observed in all precordial leads. The appearance of an early repolarization pattern, characterized by J point elevation, distinct J waves, slurring of the terminal part of the QRS and/or ST segment elevation, in the inferior or infero-lateral leads in patients with Type I ST segment elevation in the anterior leads has been associated with adverse prognosis [39–41]. This type of global ST segment elevation, manifesting in inferior, lateral and anterior leads, has been termed Type 3 Early Repolarization Syndrome (ERS) in a recent proposed classification of ERS [42].

Placement of the right precordial leads in a superior position (up to the second intercostal spaces above normal) can increase the sensitivity of the ECG for detecting the Brugada phenotype in some patients, both in the presence or absence of a drug challenge [43, 44] or during Holter recordings [45]. Studies are underway to ascertain whether the greater sensitivity is at the cost of a lower specificity and whether a Type I ECG in the elevated leads is as predictive of events as a Type I ECG the in the standard leads. A recent report demonstrates that as many as 1.3 % of normal Korean males display a Type 2, but not Type 1, ST segment elevation when the right precordial leads are recorded from a superior position [46].

A slight prolongation of the QT-interval is sometimes observed associated with the ST segment elevation [32, 47, 48]. The QT-interval is prolonged more in the right vs. left precordial leads, presumably due to a preferential prolongation of action potential duration (APD) in right ventricular (RV) epicardium secondary to accentuation of the action potential notch [49]. A QTc >460 ms in V2 has been shown to be associated with arrhythmic risk [50]. Depolarization abnormalities including prolongation of P-wave duration, PR and QRS intervals are frequently observed, particularly in patients linked to *SCN5A* mutations [51]. PR prolongation likely reflects HV conduction delay [47]. QRS fragmentation, defined as three or more spikes in the QRS complex, has been found to be an independent predictor of spontaneous arrhythmic events [52, 53].

In many cases arrhythmia initiation is bradycardia-related [54]. This may contribute to the higher incidence of sudden death at night in individuals with the syndrome and may account for the success of pacing in controlling the arrhythmia in isolated cases of the syndrome [55]. Makiyama and co-workers reported that loss-of-function SCN5A mutations resulting in Brugada syndrome are distinguished by profound bradyarrhythmias [56]. Related to this observation is the recent report by Scornik and co-workers [57] demonstrating expression of the cardiac sodium channel gene, *SCN5A*, in intracardiac ganglia. This interesting finding suggests that a loss of function mutations in *SCN5A* may not only create the substrate for reentry in ventricular myocardium, but may also increase vagal activity in intracardiac ganglia, thus facilitating the development of arrhythmias in patients with the Brugada syndrome. This finding may also explain the frequent association of vasovagal syncope with BrS [58].

A polymorphic ventricular tachycardia (VT) is most commonly associated with the Brugada syndrome. Monomorphic ventricular tachycardia (VT) is observed infrequently and is generally more prevalent in children and infants [59–64].

The majority of congenital Brugada syndrome patients are believed to possess a structurally normal heart, consistent with the notion that this is a primary electrical heart disease [65]. While fibrosis and myocarditis may exacerbate or indeed trigger events in patients with the Brugada syndrome, it seems clear that in the vast majority of cases these structural changes are unrelated to arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D).

Prognosis and Risk Stratification

Risk stratification of patients with the Brugada syndrome has been an issue of lively debate. It is generally accepted that Brugada syndrome patients presenting with aborted sudden death are at high risk for recurrence and that they should be protected by an implantable cardiac defibrillator (ICD). There is also little argument that a patient with Brugada syndrome presenting with syncope, particularly when the clinical history suggests an arrhythmic syncope (as opposed to typical vasovagal syncope), are at high risk. In contrast, risk stratification of asymptomatic patients has met with considerable debate [11, 15, 66–70]. Several invasive and noninvasive parameters have been proposed for identification of patients at risk of sudden death, including the presence of spontaneous Type 1 ST segment elevation, the characteristics of the S wave [71], wider QRS complexes [72], the presence of late potentials [73] or QRS fragmentation [52, 53] and inducibility of VT/VF using programmed electrical stimulation (PES) [74].

In 1998, Brugada et al. [75] reported that over a 34 month follow-up period, 27 % of previously asymptomatic patients experienced a first ventricular fibrillation or sudden cardiac death. This figure corresponds to an occurrence of life-threatening events of approximately 10 % per year. In 2002, with a mean follow-up of 27 ± 29 months, the same authors [66] reported that 8 % of previously asymptomatic patients had become symptomatic; an occurrence of a life-threatening event of 3.5 % per year. In 2005, Brugada et al. [68] reported that 6 % of asymptomatic patients displayed a first event during a mean follow-up of 42 ± 42 months, corresponding to an event rate of 1.7 % per year. This progressive decline in first event rate in previously asymptomatic patients most likely reflects a reduced severity of phenotypes referred to the Brugada registry in subsequent years. In contrast, Priori et al. in 2002 [67] reported that asymptomatic patients have a cumulative probability of 14 % for developing a cardiac arrest by age 40, corresponding to an natural history incidence of cardiac arrest of 0.35 % per year. In 2005, the same authors reported a first event rate of 3 % (4/132) over a 31 month follow-up period, corresponding to an event rate of 1 % per year [69].

The reason behind the disparity in the data generated by these two groups is not clearly evident. It was suggested by Brugada et al. [68] that the difference may be due to the inclusion by Priori and co-workers of patients with Type 2 and 3 ST segment elevation, which is not considered diagnostic of the Brugada syndrome [7–10]. Priori and co-workers argue that exclusion of Type 2 and 3 from the diagnosis of the syndrome can lead to missed diagnosis of the disease [69].

While this is clearly the case, it may be a rare occurrence, and the exclusion appears justified on the basis that it avoids a large number of false positive diagnoses. As a result of the failure to exclude individuals with Type 2 and 3 ST segment elevation, the European registry may contain many individuals who do not have the syndrome. However, a recent report by Eckardt and co-workers [70] suggests that other factors may be involved. These authors report that 1 out of 123 asymptomatic individuals with a Type 1 ECG (0.8 %) had a first arrhythmic event during a 40 ± 50 month follow-up. This translates into a first event rate of 0.24 % per year, considerably less than the other two registries. Thus, as additional data have become available, it has become clear that the prognosis of asymptomatic patients is associated with much less risk than initially perceived.

The large registry studies agree that Brugada syndrome patients at higher risk for the development of subsequent events are those presenting with a spontaneous Type 1 ST segment elevation or Brugada ECG and/or those with a previous VT/VF or Sudden Cardiac Death (SCD) [70]. The registries also agree that PES inducibility is greatest among patients with previous VT/VF or syncope. Approximately 1/3 of asymptomatic patients are inducible. In the Priori et al. [69] and Eckardt et al. [70] studies inducibility of VT/VF in asymptomatic patients was not associated with risk. The lack of association between inducibility and spontaneous VF in Brugada patients was also reported by a number of smaller studies, such as that of Kanda et al. [76].

In sharp contrast, Brugada et al. [77] found that the risk for developing VT/VF is considerably greater in patients who are inducible during PES, whether or not a Type 1 ST segment elevation was spontaneously present or whether or not they were symptomatic. The reason for the marked disparity in the predictive power of PES inducibility among the different studies is not immediately apparent. The discrepancies may be due to differences in patient characteristics and the use of multiple testing centers with non-standardized or non-comparable stimulation protocols [78]. Additional studies are clearly needed to further define risk stratification strategies for asymptomatic patients.

It is noteworthy that in experimental models of the Brugada syndrome involving the coronary-perfused wedge preparation, polymorphic VT is readily inducible with a single ventricular extrastimulus, but only when applied on the epicardial surface of the wedge. Inducibility is not possible or much more difficult when extrastimulation is applied to the endocardial surface. The shorter refractory period of epicardium allows extra-stimuli direct access to the vulnerable window across the ventricular wall, thus facilitating the induction of reentry. These relationships suggest that PES applied to the epicardium may provide a more accurate assessment of risk than the current clinical approach in which stimuli are applied to the endocardial surface. In support of this hypothesis, Carlsson et al. reported that a Brugada syndrome patient with recurrent syncope due to polymorphic ventricular tachycardia could not be induced with right ventricular endocardial stimulation. However, epicardial stimulation from a left ventricular site through the coronary sinus led to the development of polymorphic VT [79].

Gehi et al. [80] recently reported the results of a meta-analysis of 30 prospective studies that included 1,545 patients with a Brugada ECG to assess predictors of events. The meta-analysis suggested that a history of syncope or SCD, the presence of a spontaneous Type I Brugada ECG, and male gender predict a more malignant natural history. The findings however did not support the use of a family history of SCD, the presence of an *SCN5A* gene mutation, or electrophysiologic study to guide the management of patients with a Brugada ECG. A similar meta-analysis by Paul et al. [81] also concluded that the inducibility of VF in asymptomatic patients is of no prognostic value.

Since the publication of the meta-analyses by Gehi et al. and Paul et al., two large multicenter studies from Japan [82] and from Europe (the FINGER study) [83] were published. The Japanese study is important because it included only probands (330 probands of whom 154 were asymptomatic) and the FINGER study was important because the number of asymptomatic patients included (n = 654) was significantly larger than the number of asymptomatic patients included in the last published series by Brugada (n = 263) and even larger than the 457 patients

number compiled from 15 studies in the meta-analysis by Paul. Both studies used an EPS protocol that included two sites of stimulation [right ventricular apex (RVA) and outflow tract (RVOT)] with up to three extrastimuli, with the FINGER protocol using a minimal coupling interval of 200 ms, whereas the Japanese investigators limited the coupling interval of the extrastimulus only by ventricular refractoriness. This difference in the EPS protocol probably explains why the percentage of asymptomatic individuals with inducible VF was higher in the Japanese study than in FINGER (57 % vs. 37 %). The latter figure is very close to the 34 % inducibility rate reported by Brugada among their asymptomatic individuals.

In Finger, asymptomatic individuals with inducible VF did have more spontaneous arrhythmic events than non-inducible patients, but the event rate was very low for both groups (1.1 vs. 0.4 % event rate per year). Moreover, using multivariate analysis the EPS results were no longer (independent) predictors of outcome. Inducibility of VF was not a predictor of outcome among asymptomatic individuals in the Japanese study.

Makimoto et al. [84] recently reported that inducibility to VF was of prognostic value *only* when VF was induced with one or two extrastimuli and suggested that triple extrastimulation should be avoided to prevent false-positive results. These results, however, where not confirmed by the prospective PRELUDE study [53]; the induction of VF was of no prognostic value regardless of the protocol of extrastimulation used.

Genetic Basis

Brugada syndrome shows an autosomal dominant mode of inheritance. The first gene to be linked to the syndrome is SCN5A, the gene that encodes the α subunit of the cardiac sodium channel gene [85]. Figure 29.1 highlights the

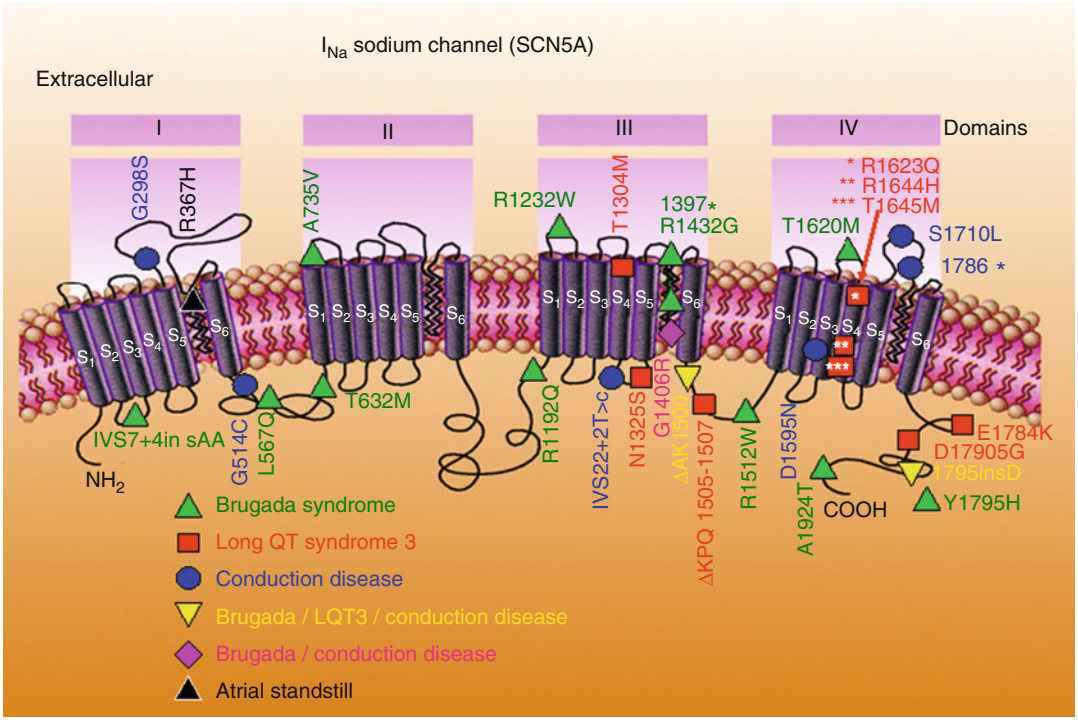


Figure 29–1. Schematic of SCN5A, the gene that encodes the α subunit of the sodium channel, illustrating mutations linked to Brugada syndrome, long QT3 syndrome, conduction disease and atrial standstill. Some mutations are associated with combined phenotypes (From Antzelevitch and Viskin [86]. Reprinted with permission from Springer)

diversity of SCN5A mutations associated with the Brugada syndrome. Of note, mutations in SCN5A are also responsible for the LQT3 form of the long QT syndrome and cardiac conduction disease. A number of mutations have been reported to cause overlapping syndromes; in some cases all three phenotypes are present [87]. Over 300 mutations in *SCN5A* have been associated with the syndrome in recent years [88].

Only a fraction of these mutations have been studied in expression systems and shown to result in loss of function. A number of mechanisms have been delineated for the reduction in sodium channel current (I_{Na}), including [89–93]: (1) failure of the sodium channel to express due to faulty protein synthesis and/or trafficking; (2) a shift in the voltage- and time-dependence of sodium channel current (I_{Na}) activation, inactivation or reactivation; (3) entry of the sodium channel into an intermediate state of inactivation from which it recovers more slowly or (4) accelerated inactivation of the sodium channel. In in vitro expression systems, the premature inactivation of the sodium channel is sometimes observed at physiological temperatures, but not at room temperature [94]. Acceleration of I_{Na} inactivation was still more accentuated at higher than physiological temperatures, suggesting that the syndrome may be unmasked, and that patients with the Brugada syndrome may be at an increased risk, during a febrile state [94]. A number of Brugada patients displaying fever-induced polymorphic VT have been identified since the publication of this report [23, 61, 95–102].

Mutation in the SCN5A gene account for approximately 11–28 % of Brugada syndrome cases [88]. A higher incidence of SCN5A mutations has been reported in familial than in sporadic cases [90]. It is important to recognize that a negative SCN5A result does not rule out this gene as a cause, since the promoter region, cryptic splicing mutations or presence of gross rearrangements are generally not part of routine investigation. A recent report by Hong et al. [103] provided the first report of a dysfunctional sodium channel created by an intronic mutation giving rise to cryptic splice site activation in SCN5A in a family with the Brugada syndrome. The deletion of fragments of segments two and three of Domain IV of SCN5A caused complete loss of function.

Evidence in support of the hypothesis that an SCN5A promoter polymorphism common in Asians modulates variability in cardiac conduction, and may contribute to the high prevalence of Brugada syndrome in the Asian population was recently advance by Bezzina and co-workers [104]. Sequencing of the SCN5A promoter identified a haplotype variant consisting of six polymorphisms in near-complete linkage disequilibrium that occurred at an allele frequency of 22 % in Asian subjects and was absent in whites and blacks. These findings demonstrate that sodium channel transcription in the human heart may vary considerably among individuals and races and be associated with variable conduction velocity and arrhythmia susceptibility [104].

BrS has in recent years been linked to mutations in ten other genes. Calcium channel genes mutations, including those in *CACNA1C* (Cav1.2, BrS3), *CACNB2b* (Cavß2b, BrS4) and *CACNA2D1* (Cavα2δ1, BrS9) are found in approximately 12–13 % of probands [105, 106]. Mutations in glycerol-3-phosphate dehydrogenase 1-like enzyme gene (GPD1L, BrS2), *SCN1B* (β1-subunit of Na channel, BrS5), *KCNE3* (MiRP2; BrS6), *SCN3B* (β3-subunit of Na channel, BrS7), *KCNJ8* (BrS8) and *KCND3* (BrS10) are more rare [107–112]. Mutations in these genes lead to loss of function in I_{Na} and I_{Ca}, as well as to a gain of function in I_{to} or $I_{K\text{-}ATP}$, *MOG1* was recently described as a new partner of $Na_v1.5$, playing a role in its regulation, expression and trafficking. A missense mutation in *MOG1* has also been associated with BrS (BrS11) [113, 114].

It is generally accepted that identification of specific mutations may not be very helpful in formulating a diagnosis or providing a prognosis. Mutations have been reported throughout the *SCN5A* gene and no hotspots have been identified. It is not clear whether some mutations are associated with a greater risk of arrhythmic events or sudden death. Genetic testing is recommended for support of the clinical diagnosis, for early detection of relatives at potential risk and particularly for the purpose of advancing research and our understanding of genotype-phenotype relations [115].

Cellular and Ionic Mechanisms Underlying the Development of the Brugada Phenotype

Transmural Cellular and Ion Channel Distinctions

Ventricular myocardium is known to be comprised of at least three electrophysiologically and functionally distinct cell types: epicardial, M and endocardial cells [116, 117]. These three principal ventricular myocardial cell types differ with respect to phase 1 and phase 3 repolarization characteristics. Ventricular epicardial and M, but not endocardial, cells generally display a prominent phase 1, due to a large 4-aminopyridine (4-AP) sensitive transient outward current (I_{to}), giving the action potential a spike and dome or notched configuration. These regional differences in I_{to}, first suggested on the basis of action potential data [118], have now been directly demonstrated in canine [119], feline [120], rabbit [121], rat [122], and human [123–126] ventricular tissues and cells. Differences in the magnitude of the action potential notch and corresponding differences in I_{to} have also been described between right and left ventricular epicardium [127]. Similar inter-ventricular differences in I_{to} have also been described for canine ventricular M cells [128]. This distinction is thought to form the basis for why the Brugada syndrome, a channelopathy–mediated form of sudden death, is a right ventricular disease.

The molecular basis for the transmural distribution of I_{to} has long been a subject of debate. The transmural gradient of I_{to} in dog has been ascribed to a transmural distribution of: (1) *KCND3* gene (Kv4.3), which encodes the α subunit of the I_{to} channel [129]; (2) *KChIP2*, a β subunit that co-assembles with $K_v4.3$ [130] and (3) *IRX5*, a transcriptional factor regulating KCND3 [131].

Myocytes isolated from the epicardial region of the left ventricular wall of the rabbit show a higher density of cAMP-activated chloride current when compared to endocardial myocytes [132]. I_{to2}, initially ascribed to a K^+ current, is now thought to be primarily due to the calcium-activated chloride current ($I_{Cl(Ca)}$) is also thought to contribute to the action potential notch, but it is not known whether this current, differs among the three ventricular myocardial cell types [133].

Recent studies involving canine ventricular myocytes have shown that calcium current (I_{Ca}) is similar among cells isolated from epicardium, M, and endocardial regions of the left ventricular wall [134, 135]. One study however reported differences in Ca^{2+} channel properties between epicardial and endocardial canine ventricular cells. In that study, I_{Ca} was found to be larger in endocardial than in epicardial myocytes (3.4 ± 0.2 vs. 2.3 ± 0.1 pA/pF). A low-threshold, rapidly activating and inactivating Ca^{2+} current that resembles the T-type current, was also recorded in all endocardial myocytes, but was small or absent in epicardial myocytes. The T-like current was comprised of two components: a Ni^{2+}-sensitive T-type current and a tetrodotoxin-sensitive Ca^{2+} current [136].

The surface epicardial and endocardial layers are separated by transitional and M cells. M cells are distinguished by the ability of their action potential to prolong disproportionately relative to the action potential of other ventricular myocardial cells in response to a slowing of rate and/or in response to action potential duration (APD)-prolonging agents [116, 137, 138]. In the dog, the ionic basis for these features of the M cell include the presence of a smaller slowly activating delayed rectifier current (I_{Ks}) [139], a larger late sodium current (late I_{Na}) [140] and a larger Na-Ca exchange current ($I_{Na\text{-}Ca}$) [141]. In the canine heart, the rapidly activating delayed rectifier (I_{Kr}) and inward rectifier (I_{K1}) currents are similar in the three transmural cell types. Transmural and apico-basal differences in the density of I_{Kr} channels have been described in the ferret heart [142]. I_{Kr} message and channel protein are much larger in the ferret epicardium. I_{Ks} is larger in M cells isolated from the right vs. left ventricles of the dog [128].

Cellular Basis for the Electrocardiographic J Wave

The presence of a prominent action potential notch in epicardium but not endocardium gives rise to a transmural voltage gradient during

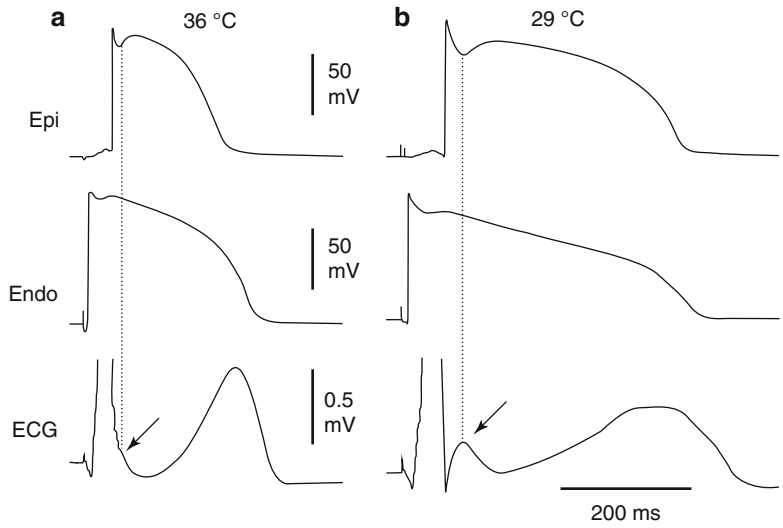


Figure 29–2. Hypothermia-induced J wave. Each panel shows transmembrane action potentials from the epicardial and endocardial regions of an arterially perfused canine left ventricular wedge and a transmural ECG simultaneously recorded. (**a**) The relatively small action potential notch in epicardium but not in endocardium is associated with an elevated J-point at the R-ST junction (*arrow*) at 36 °C. (**b**) A decrease in the temperature of the perfusate to 29 °C results in an increase in the amplitude and width of the action potential notch in epicardium but not endocardium, leading to the development of a transmural voltage gradient that manifests as a prominent J wave on the ECG (*arrow*) (Modified from Yan and Antzelevitch [4], with permission from Wolters Kluwer Health)

ventricular activation that manifests as a late delta wave following the QRS or what more commonly is referred to as a J wave [4] or Osborn wave. A distinct J wave is often observed under baseline conditions in the ECG of some animal species, including dogs and baboons. Humans more commonly display a J point elevation rather than a distinct J wave. A prominent J wave in the human ECG is considered pathognomonic of hypothermia [143–145] or hypercalcemia [146, 147].

A transmural gradient in the distribution of I_{to} is responsible for the transmural gradient in the magnitude of phase 1 and action potential notch, which in turn gives rise to a voltage gradient across the ventricular wall responsible for the inscription of the J wave or J point elevation in the ECG [118, 119, 148]. Direct evidence in support of the hypothesis that the J wave is caused by a transmural gradient in the magnitude of the I_{to}-mediated action potential notch derives from experiments conducted in the arterially-perfused right ventricular wedge preparation showing a correlation between the amplitude of the epicardial action potential notch and that of the J wave recorded during interventions that alter the appearance of the electrocardiographic J wave, including hypothermia, premature stimulation (restitution), and block of I_{to} by 4-aminopyridine (4-AP) (Fig. 29.2) [4].

Transmural activation within the thin wall of right ventricle (RV) is relatively rapid causing the J wave to be buried inside the QRS. Thus, although the action potential notch is most prominent in right ventricular epicardium, right ventricular myocardium would be expected to contribute relatively little to the manifestation of the J wave under normal conditions. These observations are consistent with the manifestation of the normal J wave in ECG leads in which the mean vector axis is transmurally oriented across the left ventricle and septum. Accordingly, the J wave in the dog is most prominent in leads II, III, aVR, aVF, and mid to left precordial leads V_3 through V_6. A similar picture is seen in the human ECG [147, 149]. In addition, vectorcardiography indicates that the J wave forms an

extra loop that occurs at the junction of the QRS and T loops [150]. It is directed leftward and anteriorly, which explains its prominence in leads associated with the left ventricle.

The first description of the J wave was in the 1920s in animal experiments involving hypercalcemia [146]. The first extensive description and characterization appeared 30 years later by Osborn in a study involving experimental hypothermia in dogs [151]. The appearance of a prominent J wave in the clinic is typically associated with pathophysiological conditions, including hypothermia [143, 149] and hypercalcemia [146, 147]. The prominent J wave induced by hypothermia is the result of a marked accentuation of the spike-and-dome morphology of the action potential of M and epicardial cells (i.e., an increase in both width and magnitude of the notch). In addition to inducing a more prominent notch, hypothermia produces a slowing of conduction which permits the epicardial notch to clear the QRS so as to manifest a distinct J wave. Hypercalcemia-induced accentuation of the J wave [146, 147, 152] may also be explained on the basis of an accentuation of the epicardial action potential notch, possibly as a result of an augmentation of the calcium-activated chloride current and a decrease in I_{Ca} [153]. Accentuation of the action potential notch also underlies the electrocardiographic and arrhythmogenic manifestations of the Brugada syndrome.

Repolarization Abnormalities as the Basis for the ECG and Arrhythmic Manifestations of BrS. Exaggeration of the J Wave Is the Basis for ST Segment Elevation in BrS

An increase in net repolarizing current, whether secondary to a decrease of inward currents or an increase of outward current, accentuates the action potential notch leading to augmentation of the J wave or appearance of ST segment elevation. A further increase in net repolarizing current can result in partial or complete loss of the action potential dome, leading to a transmural voltage gradient that manifests as accentuation of the J wave or an ST segment elevation [154–156]. In regions of the myocardium exhibiting a prominent I_{to}, such as the right ventricular epicardium, marked accentuation of the action potential notch leads to a augmentation of the J wave, manifesting as a coved-type ST segment elevation, which is diagnostic of BrS (Fig. 29.3a, b). Because the second action potential upstroke is delayed, epicardium now repolarizes after endocardium, causing inversion of the T wave. Note that although the ECG now displays the typical BrS morphology with a covered type ST segment elevation and negative T wave, no major arrhythmogenic substrates are present. The substrate for reentry, however develops with any additional outward shift of the net current active during the early phase of the action potential. This leads to loss of the action potential dome, thus creating a dispersion of repolarization between epicardium and endocardium as well as within epicardium, between the region where the dome is lost and regions at which it is maintained (Fig. 29.3c). Sodium channel blockers like procainamide, pilsicainide, propafenone, flecainide and disopyramide cause a further outward shift of current flowing during the early phases of the action potential and are thereby effective in inducing or unmasking ST segment elevation in patients with concealed J-wave syndromes [22, 31, 158]. Sodium channel blockers like quinidine, which also inhibits I_{to}, reduce the magnitude of the J wave and normalize ST segment elevation [154, 159]. Loss of the action potential dome is usually heterogeneous, resulting in marked abbreviation of action potential at some sites but not others. The dome can then propagate from regions at which it is maintained to regions where it is lost, giving rise to a very closely coupled extrasystole via phase 2 reentry (Fig. 29.3d) [3]. The phase 2 reentrant beat is capable of initiating polymorphic ventricular tachycardias (VT) or ventricular fibrillation (Fig. 29.3e, f).

Phase 2 reentry has been shown to occur when right ventricular epicardium is exposed to: (1) K+ channel openers such as pinacidil [160]; (2) sodium channel blockers such as

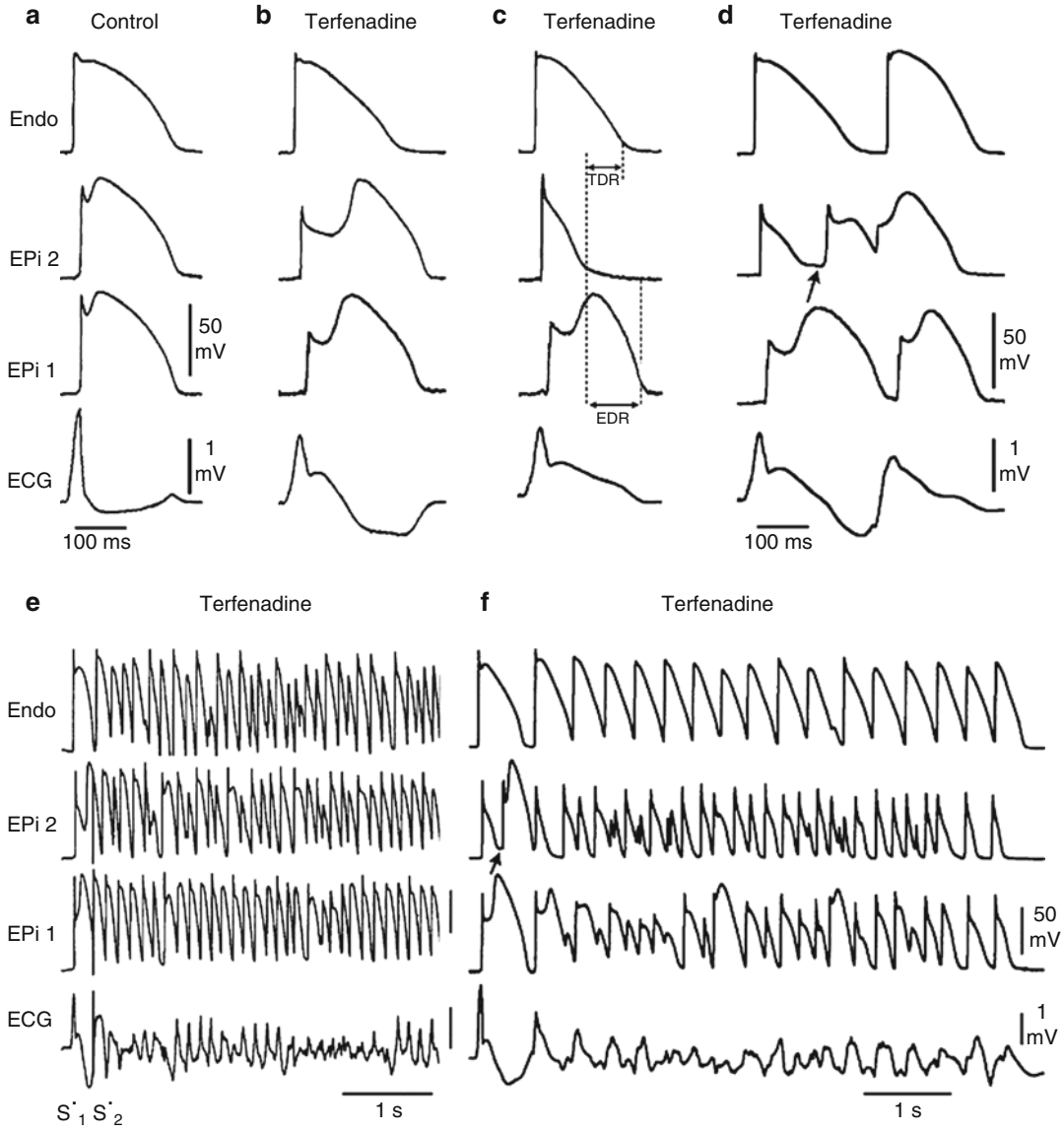


Figure 29–3. Cellular basis for electrocardiographic and arrhythmic manifestation of BrS. Each panel shows transmembrane action potentials from one endocardial (*top*) and two epicardial sites together with a transmural ECG recorded from a canine coronary-perfused right ventricular wedge preparation. (**a**) Control (Basic cycle length (BCL) 400 ms). (**b**) Combined sodium and calcium channel block with terfenadine (5 μM) accentuates the epicardial action potential notch creating a transmural voltage gradient that manifests as an exaggerated J wave or ST segment elevation in the ECG. (**c**) Continued exposure to terfenadine results in all-or-none repolarization at the end of phase 1 at some epicardial sites but not others, creating a local epicardial dispersion of repolarization (EDR) as well as a transmural dispersion of repolarization (TDR). (**d**) Phase 2 reentry occurs when the epicardial action potential dome propagates from a site where it is maintained to regions where it has been lost giving rise to a closely coupled extrasystole. (**e**) Extrastimulus (S1–S2 = 250 ms) applied to epicardium triggers a polymorphic VT. (**f**) Phase 2 reentrant extrasystole triggers a brief episode of polymorphic VT (Modified from Fish and Antzelevitch [157], with permission from Elsevier Limited)

flecainide [3]; (3) increased $[Ca^{2+}]o$ [153]; (4) calcium channel blockers such as verapamil; (5) metabolic inhibition [161]; and (6) simulated ischemia [162]. Evidence in support of a phase 2 reentrant mechanism in humans was provided by Thomsen et al. [163, 164].

Does Delayed Conduction Contribute to *the ECG and Arrhythmic Manifestations of BrS?*

While most investigations suggest that repolarization abnormalities underlie the pathophysiology of Brugada syndrome, recent studies suggest the possibility of delayed depolarization in the right ventricular outflow tract as a contributing mechanism to ST segment elevation or J waves associated with BrS [165, 166]. The repolarization vs. depolarization hypotheses controversy has been documented as a published debate [167].

Seemingly compelling data in support of delayed conduction in the RVOT as the basis for BrS was recently presented by Nademanee and co-workers [166]. Using an electrogram applied to the epicardial surface of the RVOT, these authors recorded late potentials, in some cases appearing as a continuous fractionated electrogram. This was interpreted as indicating delayed conduction over the anterior aspect of the RVOT epicardium. Catheter ablation over this abnormal region of the RVOT resulted in normalization of the Brugada ECG pattern over a period of up to 3 months and prevented VT/VF, both during electrophysiological studies, as well as spontaneous recurrence of VT/VF episodes. Signal averaged ECG (SAECG) recordings have also demonstrated late potentials in patients with the Brugada syndrome, especially in the anterior wall of the right ventricular outflow tract (RVOT) [168–174].

Although traditionally ascribed to conduction delays secondary to structural defects, in cases of BrS, late potentials of the type described by Nademanee et al. more likely arise from a delayed second upstroke of the epicardial action potential in the RVOT or to a concealed phase 2 reentry [19, 167, 170]. It is noteworthy that wall motion abnormalities have also been detected in BrS patients [175]. Although such contractile abnormalities are commonly considered pathognomonic of structural disease, in the setting of BrS, they could well be the result of loss of the action potential dome in the right ventricular epicardium [170, 176]. Loss of the dome would lead to contractile dysfunction because calcium entry into the cells would be greatly diminished and sarcoplasmic reticulum calcium stores would be depleted.

It has been suggested that the rate-dependence of the ST segment elevation in BrS may be helpful in discriminating between the repolarization and depolarization hypotheses [19]. If the ST segment elevation is due predominantly to delayed conduction in the RVOT, acceleration of the rate would be expected to further aggravate conduction and thus accentuate the ST segment elevation and the RBBB morphology of the ECG. If, on the other hand, ST segment elevation or the BrS sign is secondary to accentuation of the epicardial action potential notch, acceleration of the rate would be expected to normalize the ECG, by reducing the action potential notch and restoring the action potential dome in RV epicardium. This occurs because the transient outward current, which is at the heart of this mechanism, is slow to recover from inactivation and is less available at faster rates.

Although there are relatively few reports of the effects of pacing, most investigators in the field agree that there is a tendency for the ST segment elevation associated with Brugada ECGs to normalize during an increase in heart rate, consistent with the repolarization hypothesis [177–179]. There are however also reports of ST segment elevation or J point elevation with exercise. Amin et al. recently reported J point elevation in BrS patients (both SCN5A+ and SCN5A−) during exercise [180]. The principal difference between studies showing a decrease vs. increase of the J point in response to exercise appears to be the presence of a prominent ST segment elevation at baseline in the former.

Interestingly, in BrS cases in which ST segment elevation is accompanied by notching of the QRS, the latter suggesting major conduction delay in the RVOT, exercise-induced acceleration of rate leads to further fragmentation of the QRS, but to a *normalization* of the ST segment elevation in all right precordial leads [167]. These findings suggest that the ST segment elevation is due to a repolarization defect and not to the clearly apparent depolarization defect responsible for the fragmentation of the QRS. Fragmentation of the QRS is associated

with increased mortality and arrhythmic events in patients with coronary artery disease as well as patients with arrhythmogenic right ventricular cardiomyopathy and Brugada syndrome [181].

Kurita et al. placed monophasic action potential (MAP) electrodes on the epicardial and endocardial surfaces of the right ventricular outflow tract (RVOT) in patients with the Brugada syndrome and demonstrated an accentuated notch in the epicardial response, thus providing direct support for the repolarization hypothesis in humans [182, 183].

Additional support for the repolarization hypothesis derives from the demonstration that quinidine normalizes ST segment elevation and suppresses late potentials recorded in patients with Brugada syndrome [184–186]. These effects of the drug are presumably due to inhibition of I_{to} leading to reduction of the epicardial action potential notch and normalization of the repolarization heterogeneities. If the ST segment elevation or the late potentials were due to delayed conduction, quinidine-induced I_{Na} inhibition would be expected to accentuate their appearance.

Although there is no experimental model of BrS based on the depolarization hypothesis, several groups have developed a models of BrS based on the repolarization hypothesis [154, 157, 187–192].

Experimental models displaying major conduction delays have been developed by exposing wedge preparations to global ischemia [193, 194]. Progressive delay in these models leads to a gradual prolongation of the R wave and inversion of the T wave. The ECG at first glance resembles a BrS phenotype, but with closer inspection it is clear that the depolarization hypothesis does not lead to an ST segment elevation, but rather to an R wave prolongation. The marked conduction delay is due to a discontinuity in conduction in the deep subepicardium. Conduction under these conditions is exquisitely sensitive to rate, with acceleration leading to block and inexcitability. This is a common characteristic of ischemia-induced tombstone morphology of the ECG induced by coronary spasm [195], but is not characteristic of BrS A similar phenomenon is observed in right ventricular wedge preparations following exposure to hyperkalemic conditions [196].

Of note, magnetocardiograms recorded from patients with complete RBBB have been shown to generate currents from the RVOT to the upper left chest that are opposite from those recorded in patients with Brugada syndrome [197].

While the available data, both basic and clinical, point to the repolarization hypothesis, i.e., transmural voltage gradients that develop secondary to accentuation of the epicardial notch, at times leading to loss of the action potential dome, as the predominant mechanism underlying the Brugada syndrome ECG signature and arrhythmogenesis, there is no doubt that in some cases, particularly those associated with sodium channel loss of function, conduction slowing may contribute to the development of arrhythmias. There are also likely to be cases in which a conduction defect may predominate [198, 199].

Parasympathetic agonists like acetylcholine facilitate loss of the action potential dome [200] by suppressing I_{Ca} and/or augmenting potassium current. β(beta)-adrenergic agonists restore the dome by augmenting I_{Ca}. Sodium channel blockers also facilitate loss of the canine right ventricular action potential dome via a negative shift in the voltage at which phase 1 begins [2, 3]. These findings are consistent with accentuation of ST segment elevation in patients with the Brugada syndrome following vagal maneuvers or Class I antiarrhythmic agents as well as normalization of the ST segment elevation following β(beta) adrenergic agents and phosphodiesterase III inhibitors [4, 5, 201]. Loss of the action potential dome is more readily induced in right vs. left canine ventricular epicardium [127, 161, 202] because of the more prominent I_{to}-mediated phase 1 in action potentials in this region of the heart. This distinction is believed to be the basis for why the Brugada syndrome is a right ventricular disease.

T Wave Alternans

T wave alternans (TWA) is characterized by beat to beat alteration in the amplitude, polarity and/or morphology of the electrocardiographic T wave. TWA has been reported in

patients with the Brugada syndrome (BS) and is thought to be associated with an increased risk for development of VT/VF [158, 203–209]. Experimental studies suggest that T wave alternans is due to at least two cellular mechanism, including: (1) loss of the epicardial action potential dome on alternate beats (Fig. 29.4) and (2) concealed phase 2 reentry on alternating beats (Fig. 29.5) [157, 210–212]. T wave alternans developing after administration of sodium channel blockers has also been associated with a high risk of clinical VT/VF in patients with BrS [213].

Acquired Forms of Brugada Syndrome and Modulating Factors

The electrocardiographic and arrhythmic manifestations of the Brugada syndrome can be induced and modulated by a large number of agents and factors. The Brugada ECG can be unmasked or modulated by sodium channel blockers, a febrile state, vagotonic agents, α adrenergic agonists, β adrenergic blockers, tricyclic or tetracyclic antidepressants, first generation antihistaminics (dimenhydrinate), a combination of glucose and insulin, hyperkalemia, hypokalemia, hypercalcemia, and by alcohol and cocaine toxicity (Fig. 29.6) [5, 21, 22, 214–221]. These agents may also induce acquired forms of the Brugada syndrome (Table 29.2). An up-to-date list of agents to avoid in Brugada syndrome can be found at www.BrugadaDrugs.org.

Myocardial infarction or acute ischemia due to vasospasm involving the RVOT mimics ST-segment elevation similar to that seen in Brugada syndrome. This effect is secondary to the depression of I_{Ca}, inactivation of I_{Na} and the activation of $I_{K\text{-}ATP}$ during ischemia, and suggests that patients with congenital and possibly acquired forms of Brugada syndrome may be at a higher risk for ischemia-related sudden cardiac death [253, 255]. Although the coexistence of Brugada syndrome and vasospastic angina in the same patient is not rare, Chinushi et al. have failed to observe an enhanced susceptibility to VF nor a proarrhythmic effect of Ca-antagonist in this setting [256].

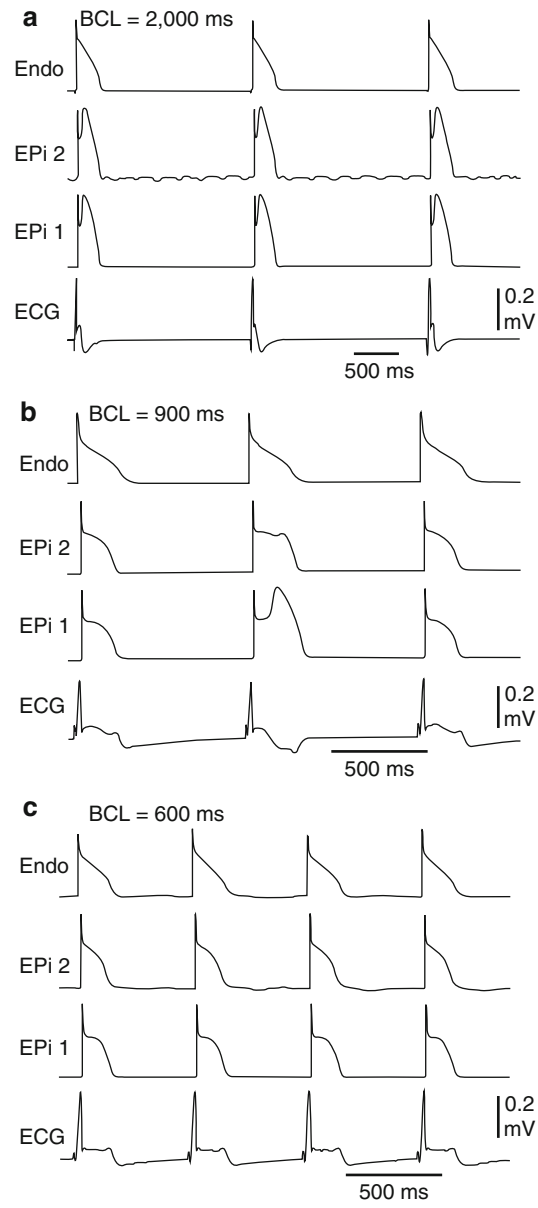


Figure 29–4. Verapamil (1 μM)-induced loss of the epicardial action potential dome in alternate beats causes T wave alternans in a canine arterially-perfused right ventricular wedge preparation. (**a**) At a BCL of 2,000 ms, endocardial and epicardial action potentials repolarize almost simultaneously, generating little or no T wave on the ECG. (**b**) Decreasing the cycle length to 900 ms induces heterogeneous loss of the epicardial action potential dome in alternate beats while the endocardial response remains constant, resulting in profound T wave alternans. (**c**) Decreasing the cycle length to 600 ms leads to homogeneous loss of the action potential dome on all beats, leading to ST segment elevation but no T wave alternans in the ECG (Modified from Fish and Antzelevitch [210], with permission from John Wiley and Sons)

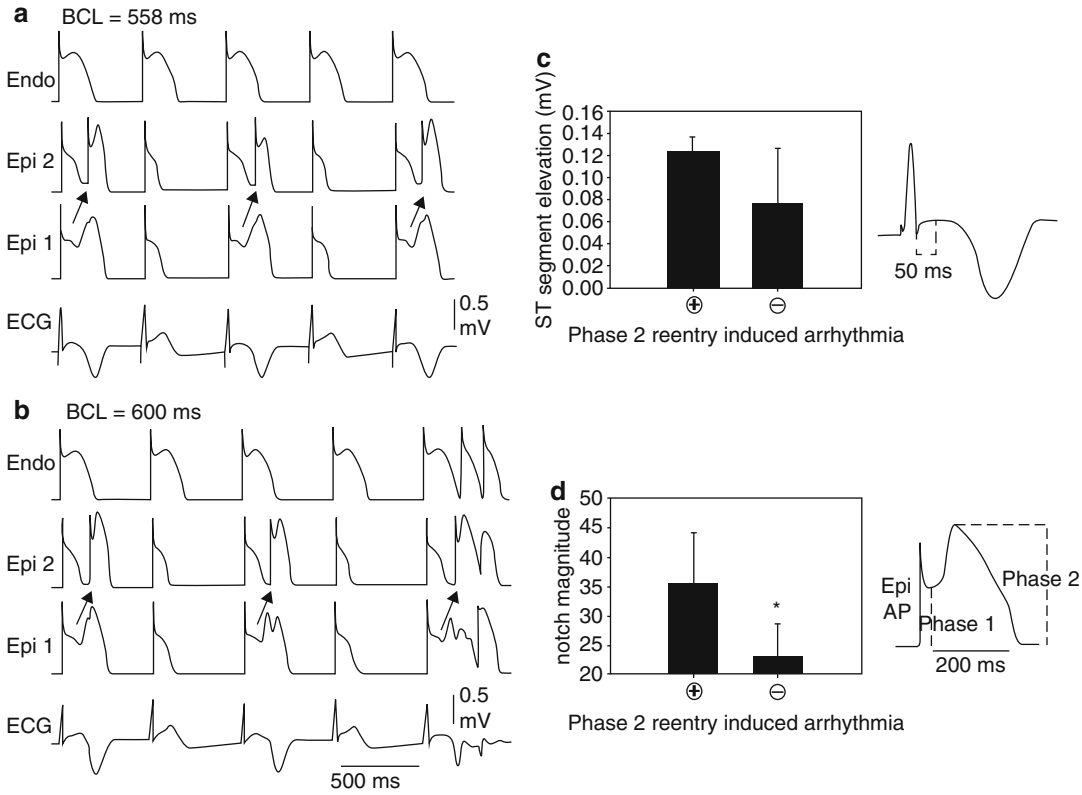


Figure 29–5. Verapamil (1 μM)-induced concealed phase 2 reentry in alternate beats leading to T wave alternans in the coronary-perfused right ventricular wedge preparation. (**a**) T wave alternans occurs as a result of concealed phase 2 reentry. The dome propagates from Epi 1 to Epi 2 on alternating beats while the endocardial response remains constant. The concealed phase 2 reentry results in a negative T wave. BCL = 558 ms. (**b**) Increasing the cycle length to 600 ms exaggerates the T wave alternans. The phase relationship between Epi 1, Epi 2, and Endo shifts slightly, allowing the previously concealed phase 2 reentry to propagate transmurally, leading to two extrasystoles. (**c**) The ST segment elevation, measured 50 ms after the end of the QRS, is greater during alternans secondary to concealed phase 2 reentry compared to alternans due to alternating loss of the epicardial action potential dome (n = 10 phase 2 reentry + and 12 phase 2 reentry – episodes, *p = 0.027). (**d**) The size of the epicardial action potential notch magnitude (ph2 amp – ph1 amp/ph 2 amp) at BCL 2,000 ms during control was significantly smaller in preparations not displaying phase 2 reentry-induced arrhythmias versus those that did (n = 5 for each category, *p = 0.025) (Modified from Fish and Antzelevitch [210], with permission from John Wiley and Sons)

Hypokalemia has been suggested to be a contributing factor in the high prevalence of Sudden unexpected nocturnal death syndrome (SUDS) in the Northeastern region of Thailand where potassium deficiency is endemic [220, 257]. Serum potassium in the Northeastern population is significantly lower than that of the population in Bangkok, which lies in the central part of Thailand where potassium is abundant in the food. Hypokalemia has been reported to induce VF in a 60 year old man who had asymptomatic Brugada syndrome, without a family history of sudden cardiac death [220]. This patient was initially treated for asthma by steroids, which lowered serum potassium from 3.8 mM on admission to 3.4 and 2.9 mM on the 7th day and 8th day of admission, respectively. Both were associated with unconsciousness. VF was documented during the last episode, which reverted spontaneously to sinus rhythm. Hypokalemia may exert these effects by increasing the chemical gradient for K^+ and thus the intensity of I_{to}.

VT/VF and sudden death in the Brugada syndrome usually occur at rest and at night. Circadian variation of sympatho-vagal balance, hormones and other metabolic factors are likely

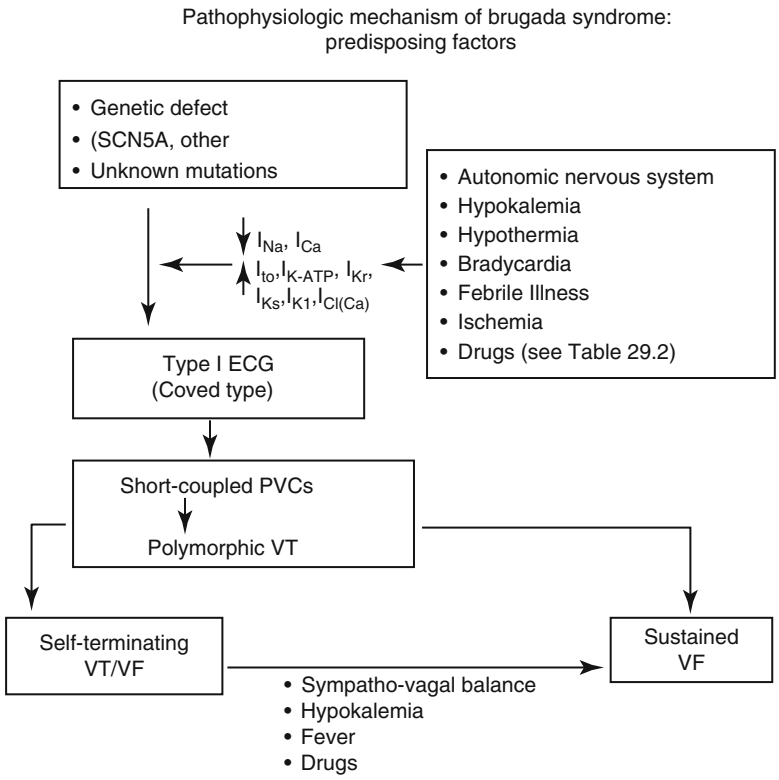


Figure 29–6. Factors predisposing to the electrocardiographic and arrhythmic manifestations of the Brugada syndrome (From Antzelevitch and Viskin [86]. Reprinted with permission from Springer)

to contribute to this circadian pattern. Bradycardia, due to altered sympatho-vagal balance or other factors, may contribute to arrhythmia initiation [26, 54, 55]. Abnormal ^{123}I-MIBG uptake in 8 (17 %) of the 17 Brugada syndrome patients but none in the control group was demonstrated by Wichter et al. [258]. Segmental reduction of ^{123}I-MIBG in the inferior and the septal left ventricular wall was observed indicating presynaptic sympathetic dysfunction. Of note, imaging of the right ventricle, particularly the RVOT, is difficult with this technique, so that insufficient information is available concerning sympathetic function in the regions known to harbor the arrhythmogenic substrate. Moreover, it remains unclear what role the reduced uptake function plays in the arrhythmogenesis of the Brugada syndrome. If the RVOT is similarly affected, this defect may indeed alter the sympatho-vagal balance in favor of the development of an arrhythmogenic substrate [154, 200].

The Thai Ministry of Public Health Report (1990) found an association between a large meal on the night of death in Sudden unexplained nocturnal death syndrome (SUNDS) patients [257]. Consistent with this observation, a recent study by Nogami et al. found that glucose and insulin could unmask the Brugada ECG [219]. Another possibility is that sudden death in these patients is due to the increased vagal tone produced by the stomach distention. A recent study by Ikeda et al. [24] has shown that a full stomach after a large meal can unmask a Type I ECG, particularly in Brugada syndrome patients at high risks for arrhythmic events, thus suggesting that this technique may be of diagnostic and prognostic value.

Accelerated inactivation of the sodium channel caused by *SCN5A* mutations associated with the Brugada syndrome has been shown to be accentuated at higher temperatures [94] suggesting that a febrile state may unmask the Brugada

Table 29–2. Drug-induced Brugada-like ECG patterns

I. Antiarrhythmic drugs
1. Na^+ channel blockers
   Class IC drugs (flecainide [22, 31, 169, 222, 223], pilsicainide [224, 225], propafenone [226–228]
   Class IA drugs (ajmaline [22, 229], procainamide [5, 22], disopyramide [5, 8], cibenzoline [204, 230]
2. Ca^{2+} channel blockers
   Verapamil [192, 210, 231]
3. β Blockers
   Propranolol intoxication [232]

II. Antianginal drugs
1. Ca^{2+} channel blockers
   Nifedipine, diltiazem
2. Nitrate
   Isosorbide dinitrate, nitroglycerine [233]
3. K^+ channel openers
   Nicorandil

III. Psychotropic drugs
1. Tricyclic and tetracyclic antidepressants [234–237]
   Amitriptyline[238, 239], nortriptyline [216], desipramine [214], clomipramine [215] loxapine
   Maprotiline [238]
2. Phenothiazine
   Perphenazine [238], cyamemazine, oxacarbazepine [240]
3. Selective serotonin reuptake inhibitors
   Fluoxetine [239]
4. Lithium [227, 240–246]

IV. Other drugs
1. Histaminic H1 receptor antagonists
   Dimenhydrinate [217]
   Diphenhydramine [247]
2. Cocaine intoxication [218, 248]
3. Alcohol intoxication
4. Anesthetics: propofol [249, 250], bupivacaine [251, 252]
5. Acetylcholine [253, 254]
6. Ergonovine [253]

syndrome. Indeed, several case reports have emerged recently demonstrating that febrile illness could reveal the Brugada ECG and precipitate VF [23, 95, 96, 259–261]. A report from Keller et al. [262] has identified a missense mutation, F1344S, in SCN5A in a patient with Brugada syndrome and fever-induced VT/VF. Expression of F1344S showed a shift in the voltage-dependence of activation, which was further accentuated at high temperatures mimicking fever. Thus fever may also cause a loss of function in I_{Na} by accelerating inactivation as well as producing a shift in the voltage-dependence of activation.

Anecdotal data point to hot baths as a possible precipitating factor. Of note, the Northeastern part of Thailand, where the Brugada syndrome is most prevalent, is known for its very hot climate. A study is underway to assess whether this extreme climate influences the prognosis of the disease.

Sex-Related Differences in the Manifestation of the Brugada Syndrome

Although the mutation responsible for the Brugada syndrome is equally distributed between the sexes, the clinical phenotype is eight to ten times more prevalent in males than in females. The basis for this sex-related distinction has been shown to be due to a more prominent I_{to}-mediated action potential notch in the right ventricular (RV) epicardium of males vs. females (Figs. 29.7 and 29.8) [263]. The more prominent I_{to} causes the end of phase 1 of the RV epicardial action potential to repolarize to more negative potentials in tissues and arterially perfused wedge preparations from males, facilitating loss of the action potential dome and the development of phase 2 reentry and polymorphic VT.

Clinical and basic studies suggest that testosterone plays an important role in ventricular repolarization. Ezaki and co-workers [264] recently demonstrated that ST segment elevation is relatively small and similar in males and females before puberty. After puberty, ST segment elevation in males, but not females, increased dramatically, more so in the right precordial leads and decreased gradually with advancing age. The effect of androgen-deprivation therapy on the ST segment was also evaluated in 21 prostate cancer patients. Androgen-deprivation therapy significantly decreased ST segment elevation. These results suggest that testosterone modulates the early phase of ventricular repolarization and thus ST segment elevation.

The proposed cellular mechanism for the Brugada syndrome is summarized in Fig. 29.9. Available data support the hypothesis that the

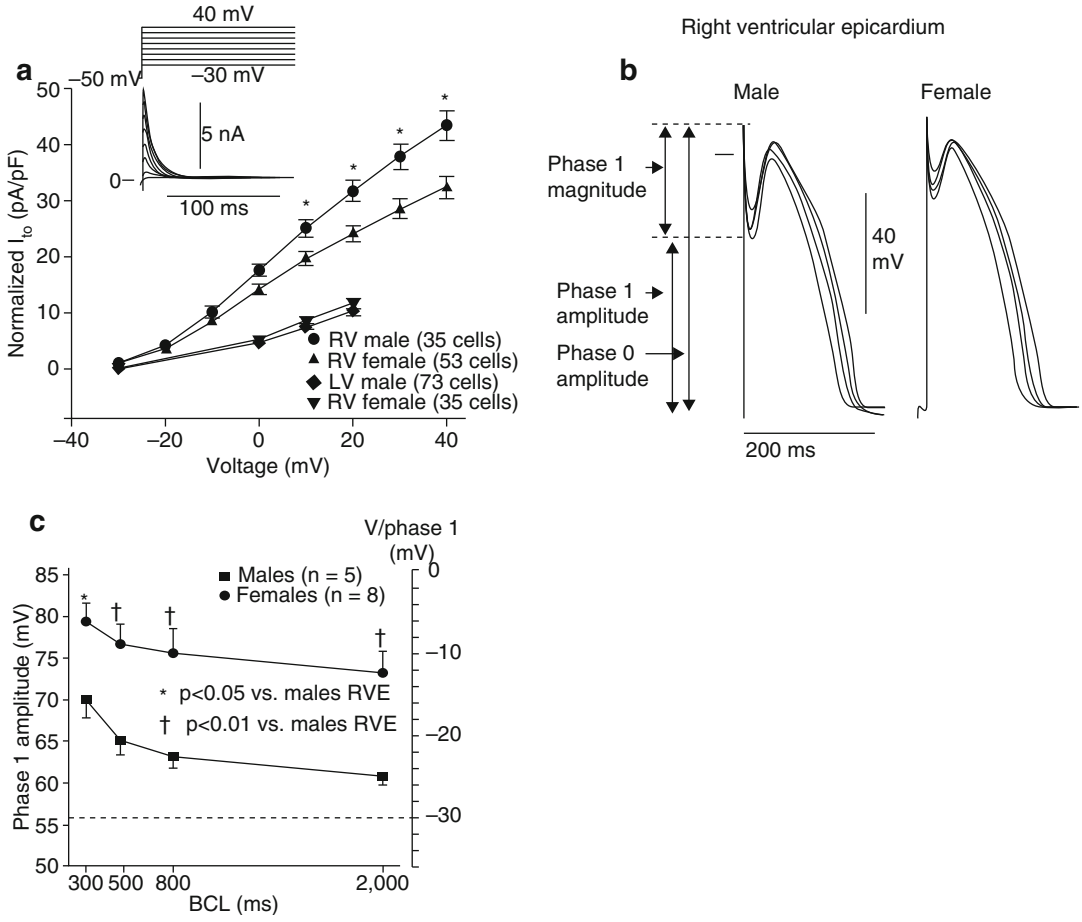


Figure 29–7. Sex-based and inter-ventricular differences in I_{to}. (**a**) Mean I-V relationship for Ito recorded from RV epicardial cells isolated from hearts of male and female dogs. **Inset**: Representative I_{to} current traces and voltage protocol. I_{to} density was significantly greater in male vs female RV epicardial cells. No sex differences were observed in LV. (**b**) Transmembrane action potentials recorded from isolated canine RV epicardial male and female tissue slices. BCLs 300, 500, 800, and 2,000 ms. (**c**) Rate dependence of phase 1 amplitude and voltage at end of phase 1 (V/phase 1, mV) in males (*solid squares*) vs females (*solid circles*) (Modified from Di Diego et al. [263] with permission from Wolters Kluwer Health)

Brugada syndrome results from amplification of heterogeneities intrinsic to the early phases of the action potential among the different transmural cell types. This amplification is secondary to a rebalancing of currents active during phase 1, including a decrease in I_{Na} or I_{Ca} or augmentation of any one of a number of outward currents. ST segment elevation similar to that observed in patients with the Brugada syndrome occurs as a consequence of the accentuation of the action potential notch, eventually leading to loss of the action potential dome in right ventricular epicardium, where I_{to} is most prominent. Loss of the dome gives rise to both a transmural as well as epicardial dispersion of repolarization. The transmural dispersion is responsible for the development of ST segment elevation and the creation of a vulnerable window across the ventricular wall, whereas the epicardial dispersion give to phase 2 reentry which provides the extrasystole that captures the vulnerable window, thus precipitating VT/VF. The VT generated is usually polymorphic, resembling a very rapid form of Torsade de Pointes.

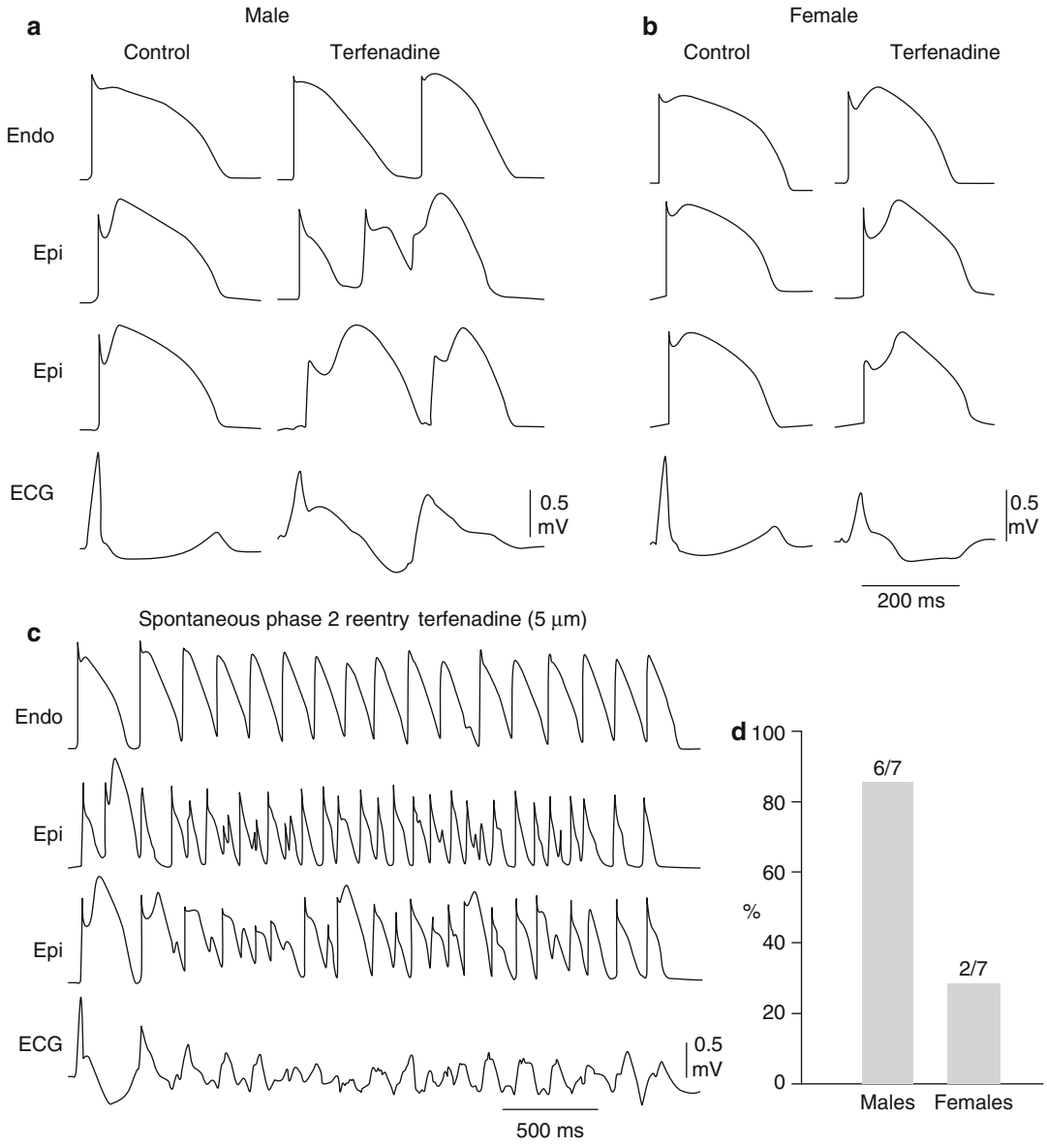


Figure 29–8. Terfenadine induces Brugada phenotype more readily in male than female RV wedge preparations. Each panel shows action potentials recorded from two epicardial sites and one endocardial site, together with a transmural ECG. Control recordings were obtained at a BCL of 2,000 ms, whereas terfenadine data were recorded at a BCL of 800 ms after a brief period of pacing at a BCL of 400 ms. (**a**) Terfenadine (5 uM)-induced, heterogeneous loss of action potential dome, ST-segment elevation, and phase 2 reentry (*arrow*) in a male RV wedge preparation. (**b**) Terfenadine fails to induce Brugada phenotype in a female RV wedge preparation. (**c**) Polymorphic VT triggered by spontaneous phase 2 reentry in a male preparation. (**d**) Incidence of phase 2 reentry in male (six of seven) vs female (two of seven) RV wedge preparations when perfused with 5 uM terfenadine for up to 2 h (Modified from Di Diego et al. [263], with permission from Wolters Kluwer Health)

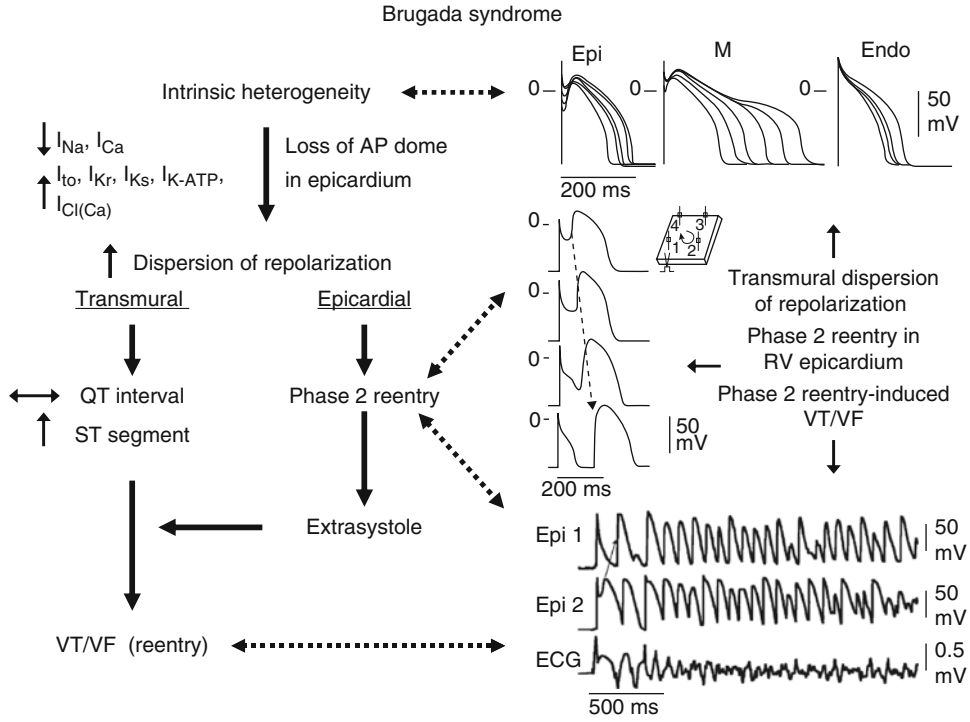


Figure 29–9. Proposed mechanism for the Brugada syndrome. A shift in the balance of currents serves to amplify existing heterogeneities by causing loss of the action potential dome at some epicardial, but not endocardial sites. A vulnerable window develops as a result of the dispersion of repolarization and refractoriness within epicardium as well as across the wall. Epicardial dispersion leads to the development of phase 2 reentry, which provides the extrasystole that captures the vulnerable window and initiates VT/VF via a circus movement reentry mechanism (Modified from Antzelevitch [265], with permission from Oxford University Press)

Approach to Therapy

Device Therapy

The only proven effective therapy for the Brugada syndrome is an implantable cardioverter defibrillator (ICD) (Table 29.3) [266, 283]. Recommendations for ICD implantation from the Second Consensus Conference [9, 10] are presented in Fig. 29.10 and are summarized as follows:

1. Symptomatic patients with a Type 1 ST segment elevation or Brugada ECG (either spontaneously or after sodium channel blockade) who present with aborted sudden death should receive an ICD as a Class I indication without additional need for electrophysiologic study (EPS). Similar patients presenting with related symptoms such as syncope, seizure or nocturnal agonal respiration should also undergo ICD implantation as a Class I indication after vasovagal syncope has been excluded on clinical grounds and non-cardiac causes of these symptoms have been carefully ruled out. The Task Force recommended that symptomatic patients undergo EPS only for the assessment of supraventricular arrhythmia. Electrophysiologic studies to guide quinidine therapy (that is, demonstrating that readily inducible VF at baseline becomes non-inducible after quinidine therapy) may be considered for symptomatic patients unwilling or financially incapable of undergoing ICD implantation [274].
2. The recommendation at the time was that asymptomatic patients with a Brugada ECG (spontaneously or after sodium channel block)

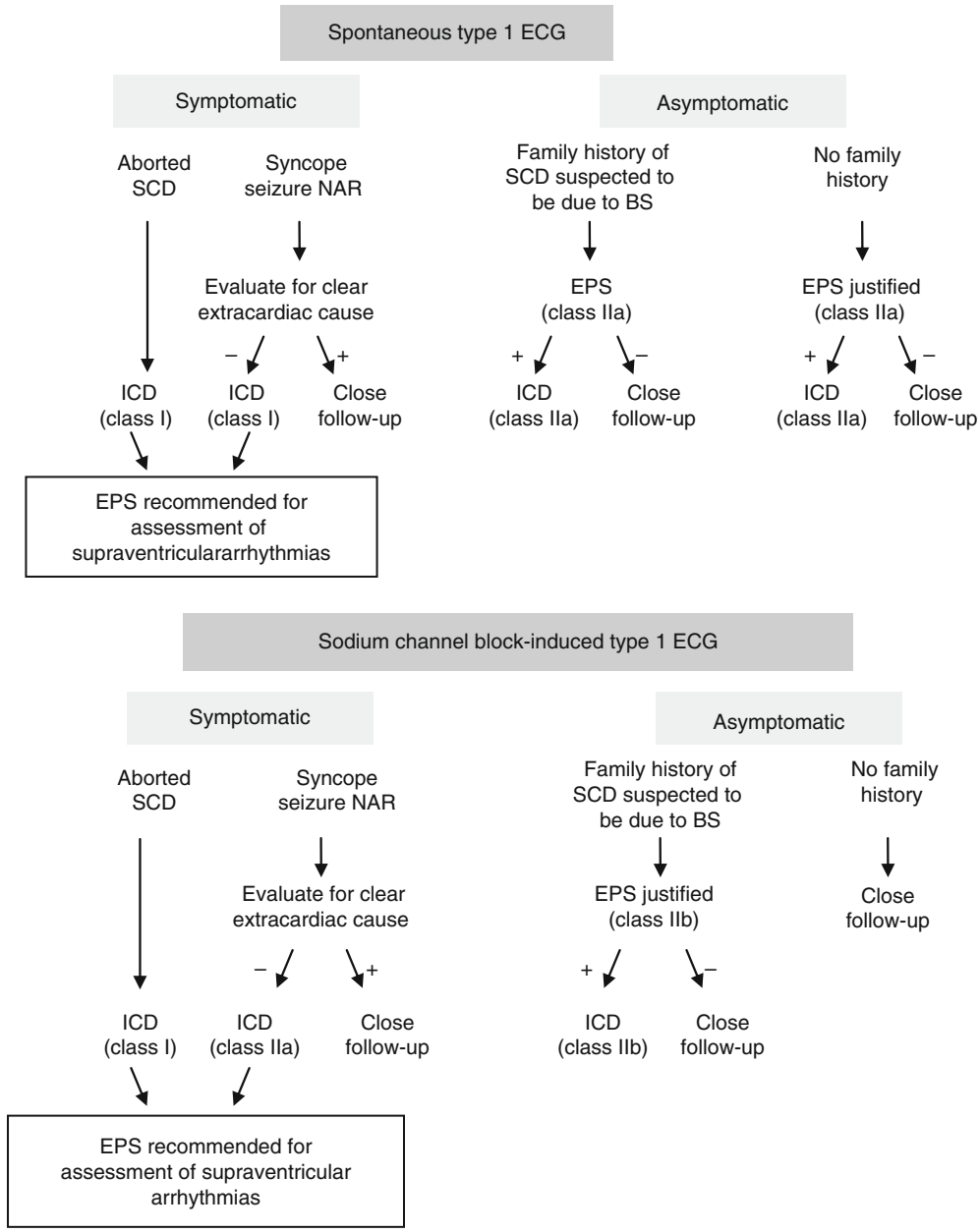
Table 29–3. Device and pharmacologic approach to therapy

Devices and ablation
ICD [266]
? Ablation or cryosurgery [166, 267]
? Pacemaker [268]
Pharmacologic approach to therapy
Ineffective
Amiodarone [75]
β Blockers [75]
Class IC antiarrhythmics
Flecainide [31]
Propafenone [226]
? Disopyramide [269]
Class IA antiarrhythmics
Procainamide [22]
Effective for treatment of electrical storms
β Adrenergic agonists – isoproterenol (Miyazaki et al. [5]; Shimizu et al. [31, 43]; Sharif-Kazemi et al. [270])
Orciprenaline (Kyriazis et al. [271])
Phosphodiesterase III inhibitors-cilostazol (Tsuchiya et al. [201])
Quinidine (Haghjoo et al. [272]; Sharif-Kazemi et al. [270])
Effective general therapy
Quinidine (Belhassen and Viskin [184, 185]; Alings et al. [273]; Belhassen et al. [274]; Belhassen et al. [275]; Yan and Antzelevitch [154]; Hermida et al. [276]; Mok et al. [62, 277]; Haghjoo et al. [272]; Probst et al. [63])
Phosphodiesterase III inhibitors-cilostazol (Tsuchiya et al. [201]; Kanlop et al. [278])
Effective in experimental models
I_{to} blockers
Quinidine (Yan and Antzelevitch [154])
4-aminopyridine (Yan and Antzelevitch [154])
Tedisamil (Fish and Antzelevitch [157]; Fish et al. [279, 280])
AVE0118 (Fish and Antzelevitch[157]; Fish et al. [279, 280])
β adrenergic agonist
Isoproterenol (Yan and Antzelevitch [154])
Dobutamine (de la Coussaye et al. [281])
I_{Na} agonist
Dimethyl lithospermate B (Fish et al. [282])

should undergo EPS if there is a family history of sudden cardiac death suspected to be due to Brugada syndrome. EPS was thought to be justified when the family history is negative for sudden cardiac death if the Type 1 ST segment elevation occurs spontaneously. If inducible for ventricular arrhythmia, an ICD was recommended as a Class IIa indication for patients presenting with a spontaneous Type I ST segment elevation and as a Class IIb for patients who display a Type I ST segment elevation only after sodium block challenge. More recent data have called these recommendations into question and suggest that it might be more appropriate to consider both as Class IIb indications. As discussed above, recent data seriously challenge the prognostic value of EPS for asymptomatic patients, calling for modification of these recommendations.

Although ICD implantation is the mainstay of therapy for the Brugada syndrome, implantation can be challenging in infants and is not an adequate solution for patients residing in regions of the world where an ICD is unaffordable. Also, ICD implantation in "asymptomatic high-risk patients" is not trivial, especially because the level of risk is still being debated (see Sect. "Prognosis and Risk Stratification" above). One should recall that in the Antiarrhythmics vs Implantable Defibrillators (AVID) trial, a multicenter study of ICD implantation for patients with heart disease and malignant arrhythmias, the risk for ICD-related complications serious enough to warrant re-intervention was 12 % [284]. The rate of serious complications from ICD implantation for patients with Brugada syndrome is likely to be higher than the 12 % reported for the patients in AVID, who were relatively old (65 ± 11 years) and had a 3-year mortality rate of 25 % [285]. Patients with Brugada syndrome are younger, have a very low risk for non-arrhythmic cardiac death and will remain at risk for ICD-related complications for many more years. In particular, the risk for potentially serious complications like infection or lead-insulation break leading to inappropriate ICD shocks, will increase over the years and after repeated ICD replacements. Indeed, Sacher et al. [286] reported a 28 % rate (8 % per year) of serious complications, including a 20 % risk of inappropriate shocks, in young patients with Brugada syndrome. Therefore, although the ICD represents the most effective way to prevent arrhythmic death in Brugada syndrome, pharmacologic and other solutions may be desirable as an alternative to device therapy for select cases [275] as well as for minimizing the firing of the ICD in patients with frequent events [9, 275, 287, 288].

Although arrhythmias and sudden cardiac death generally occur during sleep or at rest and have been associated with slow heart rates, a potential therapeutic role for cardiac pacing remains largely unexplored [289].



Class I: Clear evidence that procedure or treatment is useful or effective
Class II: Conflicting evidence concerning usefulness or efficacy
Class IIa: Weight of evidence in favor of usefulness or efficacy
Class IIb: Usefulness or efficacy less well established

Figure 29–10. Indications for ICD implantation in patients with the Brugada syndrome. *BS* Brugada syndrome, *EPS* electrophysiologic study, *NAR* nocturnal agonal respiration, *SCD* sudden cardiac death (From Antzelevitch et al. [10] with permission from Wolters Kluwer Health. From Antzelevitch et al. [9] with permission from Elsevier Limited)

Radiofrequency Ablation Therapy

Data concerning ablation therapy for BrS is very limited. Haissaguerre and co-workers [267] reported that focal radiofrequency ablation aimed at eliminating the ventricular premature beats that trigger VT/VF in the Brugada syndrome may be useful in controlling arrhythmogenesis. Haissaguerre also reported that regional endocardial ablation of RVOT areas normalizes the type I electrocardiogram in anecdotal cases [290].

The most extensive ablation study thus far reported involves 9 BrS patients with recurrent VF [166]. Electroanatomic mapping revealed fractionated late potentials in the anterior aspect of the RVOT epicardium. Ablation at these sites rendered VT/VF noninducible in seven of nine patients and normalization of the Brugada ECG pattern. Long-term outcomes (20 ± 6 months) were excellent, with no recurrent VT/VF in all patients. This report awaits confirmation by future studies.

Pharmacological Therapy

Because the mechanism underlying most cases of BrS, is thought to be due an net outward shift of the balance of current during the early phases of the action potential, the search for a pharmacologic treatment has been focused on rebalancing of the ion channel current active during the early phases of the epicardial action potential in the right ventricle so as to reduce the magnitude of the action potential notch and/or restore the action potential dome. Table 29.3 lists the various pharmacologic agents thus far investigated. Antiarrhythmic agents such as amiodarone and β blockers have been shown to be ineffective [75]. Class IC antiarrhythmic drugs (such as flecainide and propafenone) and Class IA agents, such as procainamide, are contraindicated because of their effects to unmask the Brugada syndrome and induce arrhythmogenesis. Disopyramide is a Class IA antiarrhythmic that has been demonstrated to normalize ST segment elevation in some Brugada patients but to unmask the syndrome in others [269].

The presence of a prominent I_{to} is fundamental to the mechanism underlying the Brugada syndrome. Consequently, the most prudent general approach to therapy, regardless of the ionic or genetic basis for the disease, is to partially inhibit I_{to}. Cardioselective and I_{to}-specific blockers are not currently available. 4-aminopyridine (4-AP) is an agent that is ion-channel specific at low concentrations, but is not cardioselective in that it inhibits I_{to} in the nervous system. Although it is effective in suppressing arrhythmogenesis in wedge models of the Brugada syndrome (Fig. 29.11) [154], it is unlikely to be of clinical benefit because of neurally-mediated adverse effects.

Quinidine is an agent currently on the market in the United States and other regions of the world with significant I_{to} blocking properties. Accordingly, we suggested several years ago that this agent may be of therapeutic value in the Brugada syndrome [291]. Quinidine has been shown to be effective in restoring the epicardial action potential dome, thus normalizing the ST segment and preventing phase 2 reentry and polymorphic VT in experimental models of the Brugada syndrome (Fig. 29.11) [154, 292, 293]. Clinical evidence of the effectiveness of quinidine in normalizing ST segment elevation in patients with the Brugada syndrome has been reported as well [184, 273, 275, 294]. Quinidine has also been reported to be effective in suppressing arrhythmogenesis in an infant too young to receive an ICD [63].

In a prospective study of 25 Brugada syndrome patients orally administered quinidine bisulfate (1,483 ± 240 mg), Belhassen and coworkers [184] evaluated the effectiveness of quinidine in preventing inducible and spontaneous ventricular fibrillation (VF). There were 15 symptomatic patients (7 cardiac arrest survivors and 7 with unexplained syncope) and 10 asymptomatic patients. All 25 patients had inducible VF at baseline electrophysiological study. Quinidine prevented VF induction in 22 of the 25 patients (88 %). After a follow-up period of 6 months to 22.2 years, all patients were alive. Of nineteen patients treated with oral quinidine (without ICD back-up) for 6–219 months (56 ± 67 months), none developed arrhythmic events. Administration of quinidine was associated with a 36 % incidence of side effects, principally diarrhea, which resolved

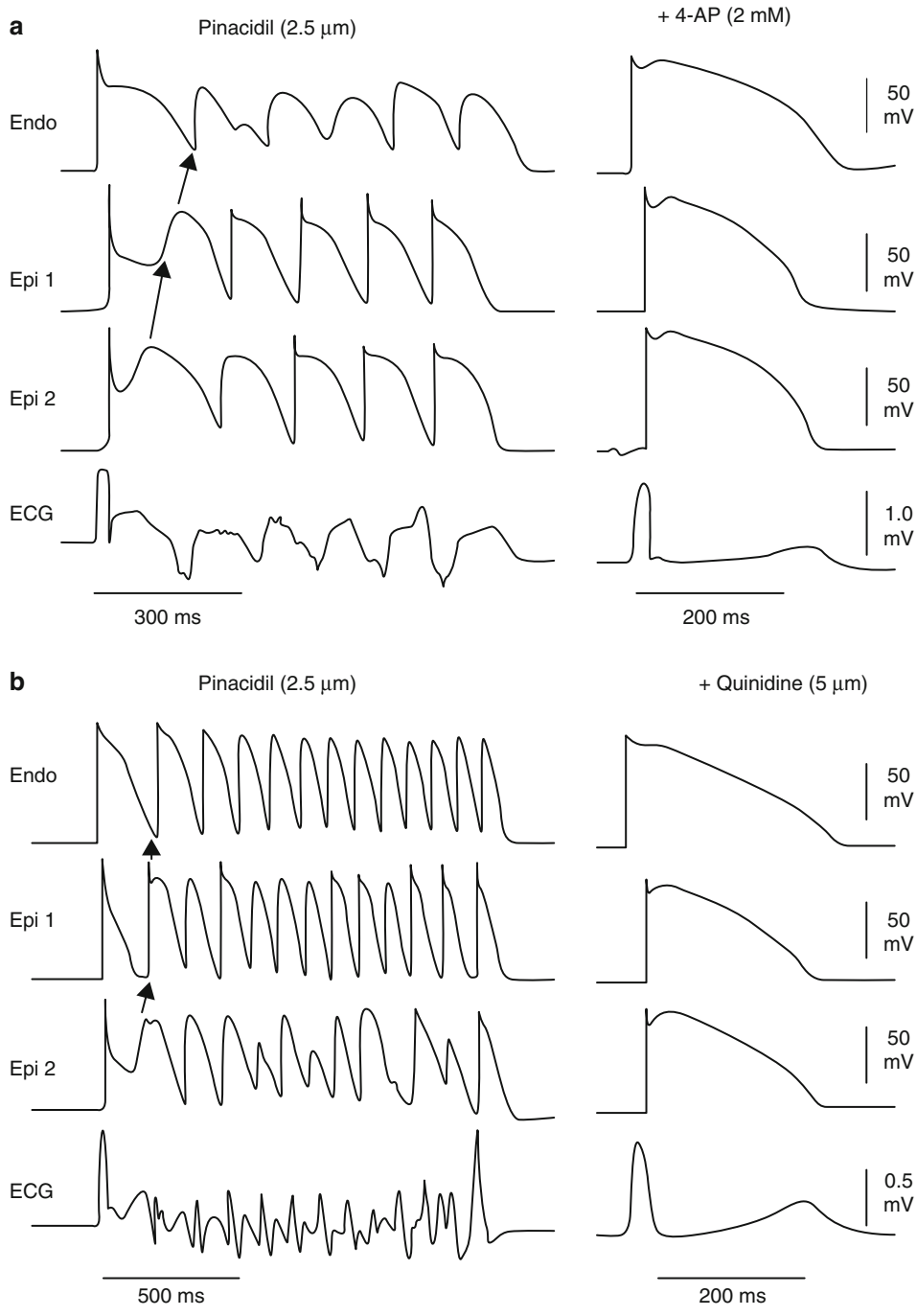


Figure 29–11. Effects of I_{to} blockers 4-AP and quinidine on pinacidil induced phase 2 reentry and VT in the arterially perfused RV wedge preparation. In both examples, 2.5 mM pinacidil produced heterogeneous loss of AP dome in epicardium, resulting in ST-segment elevation, phase 2 reentry, and VT (*left*); 4-AP (**a**) and quinidine (**b**) restored epicardial AP dome, reduced both transmural and epicardial dispersion of repolarization, normalized the ST segment, and prevented phase 2 reentry and VT in continued presence of pinacidil (From Yan and Antzelevitch [154], with permission from Wolters Kluwer Health)

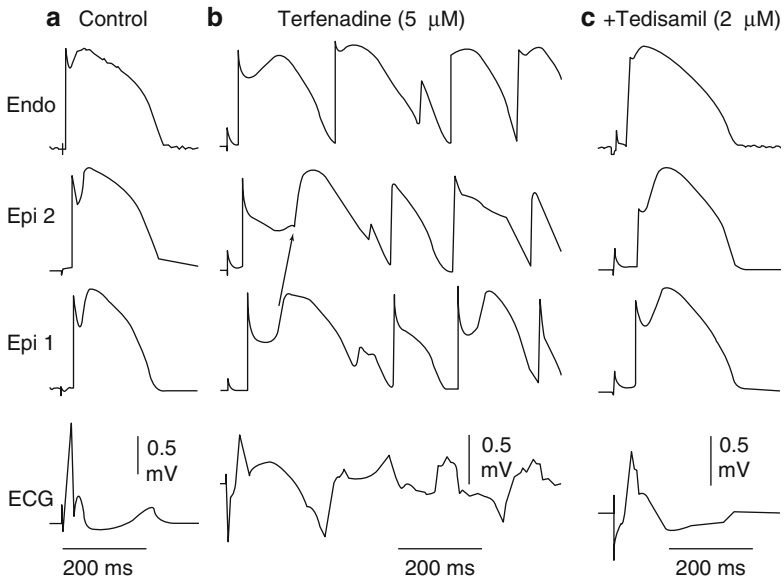


Figure 29–12. Effects of I_{to} block with tedisamil to suppress phase 2 reentry induced by terfenadine in an arterially perfused canine RV wedge preparation. (**a**) Control, BCL 800 ms. (**b**) Terfenadine (5 μM) induces ST segment elevation as a result of heterogeneous loss of the epicardial action potential dome, leading to phase 2 reentry which triggers an episode of poly VT (BCL = 800 ms). (**c**) Addition of tedisamil (2 μM) normalizes the ST segment and prevents loss of the epicardial action potential dome and suppresses phase 2 reentry induced and polymorphic VT (BCL = 800 ms) (From Antzelevitch and Fish [288], with permission from Springer)

after drug discontinuation. The authors concluded that quinidine effectively suppresses VF induction as well as spontaneous arrhythmias in patients with Brugada syndrome and may be useful as an adjunct to ICD therapy. They also suggested that EPS-guided quinidine therapy may be used *as an alternative* to ICD in cases in which an ICD is refused or unaffordable as well as when patients who are well informed about the risk and benefits of ICD and EP-guided quinidine therapy prefer medical therapy to device implantation [184]. Their results are consistent with those reported by the same group in prior years [274, 275] and more recently by other investigators [276, 277, 294]. A relatively small recent study by Mizusawa et al. [295] also showed that low-dose quinidine (300–600 mg) can prevent electrophysiologic induction of VF and has a potential as an adjunctive therapy for Brugada syndrome in patients with frequent implantable cardioverter defibrillator discharges.

There is a clear need for a large randomized controlled clinical trial to assess the effectiveness of quinidine, preferably in patients with frequent events who have already received an ICD. A prospective registry for empiric quinidine therapy for asymptomatic BrS patients was recently initiated [296]. The registry encourages empiric therapy with hydroquinidine hydrochloride 300 mg twice a day. Lower doses of quinidine have been associated with higher incidence of inducibility of TdP during repeated EPS [295]. These findings are consistent with the biphasic dose-response of quinidine to induce TdP, due to a greater inhibition of I_{Kr} with relatively low concentrations and enhanced late I_{Na} block at higher concentrations of the drug [297]. A clinical trial evaluating the number of appropriate ICD shocks in Brugada syndrome patients using quinidine vs. placebo has recently been initiated (QUIDAM study).

It is noteworthy that several manufacturer's of quinidine formulations have opted take these products off the market, prompting pleas for reconsideration of these policies in light of the need for quinidine therapy in Brugada and other syndromes, such as the short QT syndrome [298].

The quest for additional cardioselective and I_{to}-specific blockers is on-going. Another agent being considered for this purpose is the drug tedisamil. Tedisamil may be more potent than quinidine because it lacks the inward current blocking actions of quinidine, while potently blocking I_{to}. The effect of tedisamil to suppress phase 2 reentry and VT in a wedge model of the Brugada syndrome is illustrated in Fig. 29.12 [279].

Tedisamil and quinidine are both capable of suppressing the substrate and trigger for the Brugada syndrome via their inhibition of I_{to}. Both, however, also block I_{Kr} and thus have the potential to induce an acquired form of the long QT syndrome. Thus these agents may substitute one form of polymorphic VT for another, particularly under conditions that promote TdP, such as bradycardia and hypokalemia. However, the majority of patients with Brugada syndrome are otherwise healthy males, for whom the risk of drug-induced Torsade de Pointes is small [299]. This effect of quinidine is minimized at high plasma levels because at these concentrations quinidine block of I_{Na} counters the effect of I_{Kr} block to increase transmural dispersion of repolarization, the substrate for the development of Torsade de Pointes (TdP) arrhythmias [117, 297, 300, 301]. High doses of quinidine (1,000–1,500 mg/day) are recommended in order to effect I_{to} block, without inducing TdP.

Another potential therapeutic candidate is an agent reported to be a relatively selective I_{to} and I_{Kur} blocker, AVE0118 [280]. AVE0118 has been shown to normalize the ECG and suppress phase 2 reentry in a wedge model of the Brugada syndrome. This drug has the advantage that it does not block I_{Kr}, and therefore does not prolong the QT interval or have the potential to induce TdP. The disadvantage of this particular drug is that it undergoes first-pass hepatic metabolism and is therefore not effective with oral administration.

Drugs that increase the calcium current, such as β adrenergic agents like isoproterenol, are useful as well [154, 201, 302]. Isoproterenol or orciprenaline, sometimes in combination with quinidine, have been shown to be effective in normalizing ST segment elevation in patients with the Brugada syndrome and in controlling electrical storms, particularly in children [43, 271–273, 275, 277, 303, 304]. A new addition to the pharmacological armamentarium is the phosphodiesterase III inhibitor, cilostazol [201, 278], which normalizes the ST segment, most likely by augmenting calcium current (I_{Ca}) as well as by reducing I_{to} secondary to an increase in heart rate.

Another pharmacologic approach is to augment a component of I_{Na} that is active during phase 1 of the epicardial action potential. Dimethyl Lithospermate B (dmLSB) is an extract of Danshen, a traditional Chinese herbal remedy, which slows inactivation of I_{Na}, but only during a window of time corresponding to the action potential notch. This leads to increased inward current during the early phases of the action potential. Figure 29.13 shows the effectiveness of dmLSB in eliminating the arrhythmogenic substrate responsible for the Brugada syndrome in three different experimental models of the syndrome [282]. The Brugada syndrome phenotype was created in canine arterially-perfused right ventricular wedge preparations using either terfenadine or verapamil to inhibit I_{Na} and I_{Ca}, or pinacidil to activate $I_{K\text{-}ATP}$. Terfenadine, verapamil, and pinacidil each induced all-or-none repolarization at some epicardial sites but not others, leading to ST-segment elevation as well as an increase in both epicardial and transmural dispersions of repolarization. Under these conditions, phase 2 reentry developed as the epicardial action potential dome propagated from sites where it was maintained to sites at which it was lost, generating closely coupled extrasystoles and VT/VF. Addition of dmLSB (10 μM) to the coronary perfusate restored the epicardial action potential (AP) dome, reduced both epicardial and transmural dispersion of repolarization and abolished phase 2 reentry-induced extrasystoles and VT/VF in 9/9 preparations. Our data suggest that dmLSB may be a candidate for pharmacologic treatment of Brugada syndrome in cases in which an ICD is not feasible or affordable or as an adjunct to ICD use.

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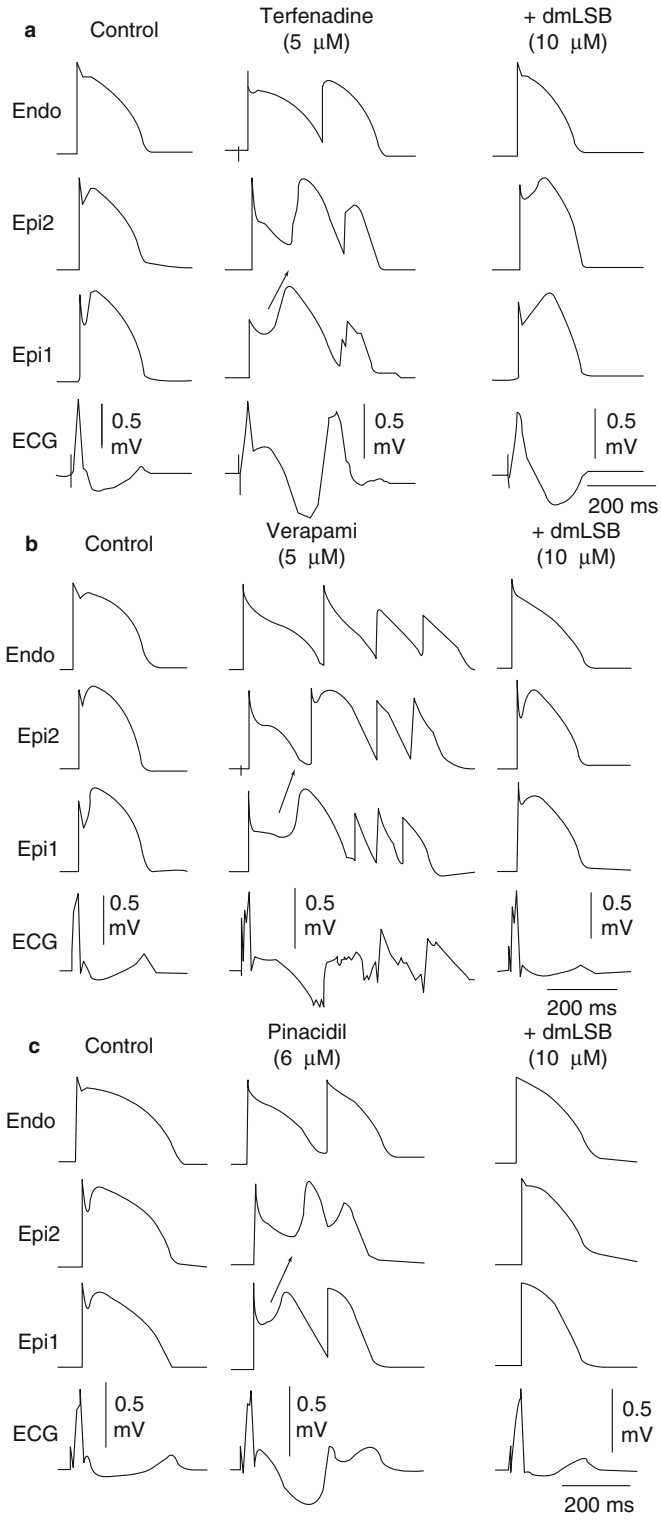

Figure 29–13. Effect of dmLSB to suppress the arrhythmogenic substrate of the Brugada syndrome in three experimental models. Phase 2 reentry was induced in three separate models of the Brugada syndrome. Terfenadine (5 μM, **a**), verapamil (5 μM, **b**), or pinacidil (6 μM, **c**) induce heterogeneous loss of the epicardial action potential dome and ST segment elevation. Phase 2 reentry occurs as the dome is propagated from Epi 1 to Epi 2, triggering either a closely coupled extrasystole or polymorphic ventricular tachycardia. In all three models, addition of dmLSB (10 μM) normalizes the ST segment and abolishes phase 2 reentry and resultant arrhythmias (From Fish et al. [282], with permission from Wolters Kluwer Health)

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Early Repolarization Syndrome: Epidemiology, Genetics, and Risk Stratification

Nicolas Derval, Frédéric Sacher, Ashok Shah, Sébastien Knecht, Mélèze Hocini, Pierre Jaïs, and Michel Haïssaguerre

Abstract

Early repolarization (ER) involving the inferolateral leads, previously considered a benign electrocardiographic (ECG) phenomenon, has recently been associated with sudden cardiac death. Although the mechanisms underlying early repolarization are unknown, ER is the latest of the primary electrical cardiac disease discovered to have significantly high prevalence in SCD cases and contribute to increased risk of death from cardioarrhythmic cause. Population-based studies have demonstrated an association between early repolarization and sudden death, primarily when the ECG demonstrates ≥0.2 mV of ST elevation. Risk stratification, especially for asymptomatic patient, remains challenging. However, clinical features as personal history of syncope, familial history of sudden cardiac death and characteristics of the J-wave (>0.2 mV, wide distribution, horizontal/descending ST segment) should be considered at higher risk.

Keywords

Sudden cardiac death • Early repolarization • Idiopathic ventricular fibrillation • Cardiac arrhythmia

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History: From Benign ECG Pattern to Malignant Primary Electrical Disease

Early Repolarization in the Past: A Benign and Normal ECG Variant

It was a wide- and a long-held belief that ER on ECG is not associated with any adversity. Most of the literature that described and stated the benignancy of ER pattern was found to have been published in a period between early 1950s and late 1970s [1–6]. The past literature comprised of observational studies involving 5–75 patients with a follow-up period ranging from 6 months to 26 years. Importantly, most of the studies did not have well-matched control group since their primary objective was to observe the

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ECG features of ER and not its long-term follow up. Obviously, when these studies are evaluated using current standards, the conclusions drawn from them do not seem to bear a strong impact. However, this long-held concept was reconsolidated by Klatsky et al. in 2003 in a study involving 73,088 patients who underwent voluntary health examination including ECG in Oakland, California, between 1983 and 1985 [7]. The objective of this study was to observe whether patients with ER were at increased risk of hospitalization for chest pain. The cardiologists, then, set aside the ECGs that had possible ER plus the next two consecutive ECGs which served as control. This yielded 2,234 ECGs which were photocopied and reinterpreted by the authors in 2000. Excluding the tracings with missing data and those judged as abnormal, finally 2,081 ECGs were analyzed including 670 ECGs showing ER. The investigators ascertained medical events during follow-up by computer search of databases through 1998 including hospitalizations and death certificate diagnoses. Outpatient diagnostic data were only available from 1995 to 1999 in 45,528 examinees. The authors concluded that the prevalence of ER in their cohort was 0.9 % (670/73,088) and the patients with ER were less likely to experience arrhythmias. The overall rate of hospitalization and outpatient visits was not higher than in the control population.

Early Repolarization in the Current Era: An Electrical Disorder Associated with Ventricular Fibrillation

During the past decade, ER was reported as the only "abnormal" finding in patients diagnosed with idiopathic ventricular fibrillation (VF) in more than ten clinical reports from around the globe [8–10]. Meanwhile, the potential arrhythmogenicity of ER was also demonstrated in experimental studies [11–13]. These observations indicated towards potentially non-benign nature of ER. A more definitive clinical evidence and a turning point in our perception towards ER came in 2007–2008 when pioneering work by Haïssaguerre et al. reported a high prevalence of ER in patients with idiopathic VF [14, 15]. ER was observed in 31 % (64/206) of idiopathic VF cases versus 5 % (21/412) of well-matched healthy subjects ($P < 0.001$). Furthermore, based on the data from implantable cardioverter-defibrillator, 64 idiopathic VF survivors with ER experienced higher VF recurrence than 142 VF survivors without ER (41 % vs. 23 %; $P = 0.008$). Subsequently, Rosso et al. compared the ECGs of 45 idiopathic VF cases with that of 124 age- and gender-matched control subjects and 121 young athletes and found that ER was more common among the patients with VF than among the control subjects (42 % vs. 13 %, $P < 0.001$) [16]. In another study by Nam et al., baseline ECGs of 11 out of 19 (57.9 %) patients with VF showed ER in contrast to 3.3 % of 1,395 controls representing the general population [17]. Derval et al. reported prevalence and characteristics of inferolateral ER pattern in a prospective registry of patient with unexplained cardiac arrest (CASPER). All patients in this registry underwent systematic and rigorous clinical testing to identify subclinical primary electrical disease. This allowed a strict definition of IVF, and therefore, a potentially more unbiased estimate of symptomatic ER pattern prevalence than in previous retrospective studies [14, 16, 17]. In this study, inferolateral ER pattern was present in 19 % of all cardiac arrest survivors, and in 23 % of those without concurrent diagnosis after thorough testing. Though case-control studies do not establish causation, strong evidence in favor of association between ER and VF-related sudden cardiac death emerged.

Tikkanen et al. systemically reported the long-term outcome of ER in general population [18]. The authors assessed the prevalence and prognostic significance of ER on routine ECG performed during a community-based investigational coronary artery disease study involving 10,864 middle-aged subjects. The mean follow-up was 30 ± 11 years with the primary end point of cardiac death and secondary end points of all-cause mortality and arrhythmic death. The prevalence of ER was 5.8 % in this cohort. Importantly, ER in the inferior leads was found to be associated with an increased risk of cardiac death (adjusted relative risk, 1.28; 95 % confidence interval [CI], 1.04–1.59; $P = 0.03$) in general population. J-point elevation in the lateral leads was of borderline

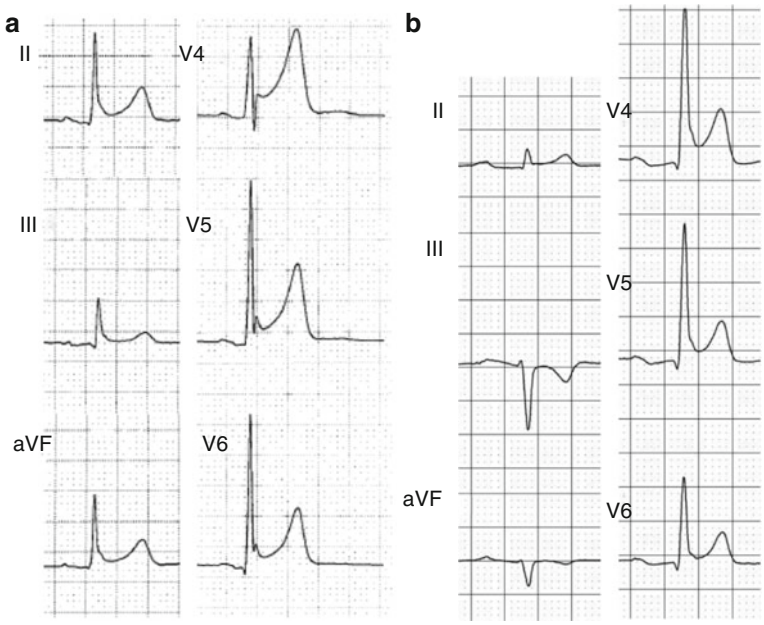

Figure 30–1. Different features of J wave elevation as notching (**a**) or slurring (**b**) in inferior or lateral leads in patients with VF

significance in predicting cardiac death and all-cause death. Moreover, the survival curves started to diverge 15 years after first ECG recording in the early 1980s and continued to diverge at a constant rate throughout the follow-up period, despite continued improvement in the treatment and prognosis of patients with cardiac disease during past two decades. Although authors retrospectively classified cardiac deaths into arrhythmic and non-arrhythmic categories, the results strongly challenge the long-held benignancy of ER.

On the other hand, 59 out of 630 subjects with ER in general population died of a proven arrhythmic cause over a mean period of 30 ± 11 years. Considering these data, the prevalence of so called malignant ER turns out to be one per ten cases with early repolarization pattern on ECG, proving that nine out of ten cases with ER on ECG should have been really benign. Also, the multivariate adjusted risk of all-cause mortality associated with presence of ER in any region and magnitude was not reported. Investigators of the population-based prospective cohort study MONICA/KORA (1,945 participants aged 35–74 years, representing a source population of 6,213 individuals) have also reported that ER pattern was associated with about a two to four-fold increased risk of cardiac mortality [19].

Definition of Electrocardiographic Early Repolarization

Early repolarization pattern (ER) is a common electrocardiographic (ECG) variant, characterized by J point elevation manifested either as QRS slurring (at the transition from the QRS segment to the ST segment) or notching (a positive deflection inscribed on terminal S wave), ST-segment elevation with upper concavity and prominent T waves in at least two contiguous leads [7, 20]. The J-point deflection occurring at the QRS-ST junction (also known as Osborn wave or J wave) was first described in 1938 and is seen in both extracardiac and cardiac conditions such as hypothermia, hypercalcemia, brain injury, hypervagotonia or spinal cord injury leading to loss of sympathetic tone, vasospastic angina, acute posterior myocardial infarction with occlusion of the left circumflex coronary artery and Brugada syndrome (BrS) besides recently described 'early repolarization syndrome' [21].

In the study by Haissaguerre et al., cases with ER had at least 0.1 mV J point elevation manifested as QRS slurring or notching in the two contiguous inferior and/or lateral leads (Fig. 30.1). The leads V1–V3 were not considered in the definition to exclude Brugada syndrome and Arrhythmogenic

Right Ventricular Cardiomyopathy [14]. To avoid confusion with the pattern commonly seen in highly trained athletes (J point elevation + ST elevation in V2–V4), the term "Inferolateral J wave elevation syndrome" is probably more appropriate for the ER associated with ventricular fibrillation.

Prevalence

The prevalence of ER in general population varies from under 1 to 13 %, depending on the age (predominant in young adults), the race (highest amongst black population), and the cut-off value for significant J point elevation (0.05 mV vs. 0.1 mV) [14, 16–19, 22, 23]. Using the same ECG criteria as reported by Haissaguerre et al., Tikkanen et al. reported the prevalence of ER as 5.8 % in a middle-aged population of 10,864 Finnish people. When we consider J point elevation ≥0.2 mV, the prevalence dropped to 0.33 % (0.7 % in the control group studied by Haissaguerre et al. [14]).

In the patients with documented idiopathic ventricular fibrillation and structurally normal heart, the prevalence of ER varies from 23 to 42 % [14, 16, 17, 23]. Prevalence rates up to 60 % have been reported in smaller studies [17].

Pathophysiology of Early Repolarization (Mechanism)

Insights from Experimental Studies

The exact mechanism for ER is still unknown. In 1991, Antzelevitch and colleagues first proposed that transmural differences in early phases of cardiac action potential (phases 1 and 2) are probably responsible for inscription of the electrocardiographic J wave [24]. Subsequently, they obtained direct evidence in support of this hypothesis in arterially perfused canine ventricular wedge preparations in 1996 [25]. Briefly, arrhythmogenic platform is created by disproportionate amplification of repolarizing current in the epicardial myocardium due to a decrease in inward sodium or calcium channel currents or an increase in outward potassium currents mediated by I_{to}, $I_{K\text{-}ATP}$, $I_{K\text{-}Ach}$ channels. The trigger and substrate for development of phase 2 reentry and VT/VF eventually emerge from the transmural dispersion in the duration of cardiac action potentials.

Insights from Genetic Testing

Because, ER was not associated with increased risk of SCD until recently, the genetic markers differentiating benign and arrhythmic forms of ER have not been identified. The importance of the genetic background in ER has recently been suggested by Haïssaguerre et al. when they showed that 16 % of cases with VF and ER have a family history of SCD [14].

Given the high frequency of the genetic background underlying the early repolarization pattern in the population, it is probably polygenic and influenced by environmental factors. One could hypothesize that common variants contribute to the ECG pattern of ER and that a combination of such variants or the cosegregation of common and rare variants leads to the malignant form of ER. Large multicentric studies using state-of-the-art genomics approaches on large cohorts with malignant forms of ER patients should lead to the identification of the underlying molecular bases in this lethal arrhythmia.

As described previously, rare monogenic forms of ER have been reported using a candidate gene approach. ER on ECG suggests a shift in transmural voltage gradient between epicardium and endocardium as a causal mechanism. An increase in I_{to}, I_{Kr}, I_{Ks}, I_{KACH}, I_{KATP} current or a decrease of sodium I_{Na} and/or calcium I_{CaL} current could lead to this phenomenon.

Following these hypotheses, a candidate gene approach on 156 probands allowed the identification of a rare variant in *KCNJ8*, responsible for the pore-forming subunit of the I_{KATP} channel, in a 14 year old girl who was resuscitated following an episode of sudden death due to VF with early repolarization syndrome [26]. Her coronary angiogram with ergonovine injection, MRI, and flecainide and isoproterenol challenge tests were normal. She experienced >100 episodes of recurrent VF unresponsive to beta-blockers, lidocaine/mexiletine, verapamil, and amiodarone. Recurrences of VF were associated

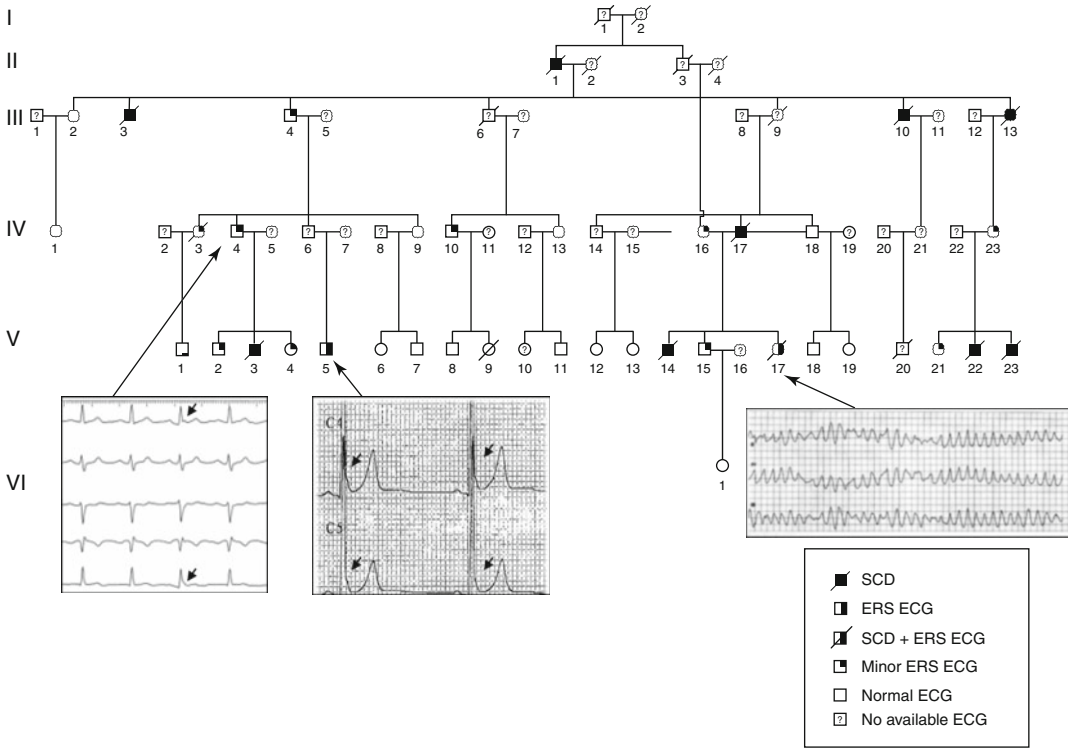

Figure 30–2. Pedigree chart of 66 members with family history of electrocardiographic early repolarization syndrome (*ERS ECG*) and sudden cardiac death (*SCD*)

with massive accentuation of the early repolarization pattern at times mimicking acute myocardial ischemia. Coronary angiography during an episode with 1.2 mVJ/ST elevation was normal. Isoproterenol infusion acutely suppressed electrical storms, while quinidine eliminated all recurrences of VF and restored a normal ECG which has persisted over a follow-up of 65 months. The precise pathophysiological mechanism is still been studied using in vitro reexpression of the mutant channel. Implication of KCNJ8 as a novel J-wave syndrome susceptibility gene has been confirmed by recent work of Medeiros-Domingo et al. [27]. They have search for KCNJ8 mutation in 101 unrelated subjects with (87 BrS phenotype and 14 ER pattern). Six hundred healthy individuals were also examined to assess the allelic frequency for all variants detected. One BrS case and one ERS case hosted the identical missense mutation S422L, which was reported previously [26]. KCNJ8-S422L involves a highly conserved residue and was absent in 1,200 reference alleles. This mutation seems to lead to a marked gain of function in the cardiac K(ATP) Kir6.1 channel and might represent a novel pathogenic mechanism for the phenotypic expression of both BrS and ERS.

Burashnikov and colleagues identified a missense variant in the β(beta)2 subunit of the cardiac L-type calcium channel in patients with early repolarization syndrome. Expression studies for this variant are not available as yet [28].

In parallel familial studies, several large pedigrees with malignant ER forms with autosomal dominant pattern of inheritance have been identified. In each of these families the prevalence rates of SCD and ER are higher than in the general population. A strong genetic background has been suspected in a large pedigree of 66 members with 11 (16.6 %) SCDs and two individuals with ER pattern (Fig. 30.2). Seven out of 11 SCDs occurred in individuals less than 35 years of age. Also, 11 (16.6 %) asymptomatic individuals have an early repolarization pattern. Furthermore, all the disease transmitters were identified to have variable expression of ER on

ECG (slight notch at the end of the QRS). An ECG showing ER and VF before sudden death is available only for patient V-17. In a second pedigree, individuals across three generations suffered sudden cardiac death and five out of eight siblings have early repolarization pattern (data not shown). Classical genetic linkage analysis assuming an autosomal dominant form of inheritance of ER should allow identifying the disease-causing gene.

These data suggest an association between ER and SCD not only at a population level but also at a familial level demonstrating that early repolarization ECG pattern could be considered as a malignant syndrome associated with a high risk of SCD in some families.

Using two large, population-based cohorts (Framingham Heart Study (FHS) and Health 2000 Survey (H2K)), Noseworthy et al. have found higher prevalence of ER pattern among siblings (recurrence risk ratio of 1.89) suggesting a heritable basis of ER pattern in the general population [29]. In another large family-based cohort (1,877 individuals from 505 white nuclear families representative of the British general population), Reinhard et al. have found that offspring of parents with ER have a 2.5-fold increased risk of presenting with ER on their ECG [30].

Altogether these results strongly suggest that ER is a heritable phenotype. The familial pattern undermines the need for systematic familial screening for the identification of ER to limit the risk of sudden death within the family.

Early Repolarization or Delayed Depolarization: A Controversy on the Origin of J Wave

Although the J wave is synonymously used with early repolarization abnormality, the mechanistic evidence elucidating the inscription of J wave on surface ECG is incomplete. Basic investigators propose the inscription of J wave as coincident with phase 1 of cardiac action potential in the epicardial region of the ventricular myocardium which precedes phase 1 in the endo- and mid-myocardial cells generating an early gradient in the repolarization currents within the ventricles, thereby justifying J wave as an early repolarization phenomenon [24, 25]. In accordance with it, some clinical investigators concluded that J wave should be considered as a repolarization phenomenon rather than late depolarization because of its slower inscription, spontaneous/rate dependant fluctuation in morphologic pattern (increased pattern at slow heart rate, decreased pattern at faster heart rate) or amplitude in the face of stable QRS complexes, and amplitude varying concurrently with ST segment. These investigators did not find late potentials on high-amplification electrocardiography and invasive endocardial mapping further reinforcing their view [14].

In a recent work on deciphering the pathophysiology of J wave in ER syndrome, 22 idiopathic VF patients were monitored for 24 h using a newly developed signal-averaging system to record late potentials (depolarization marker), T-wave alternans and QT dispersion (repolarization markers) [31]. Frequency-domain heart rate variability (HRV), which reflects autonomic modulation, also was assessed. The incidence of late potentials in 7/22 (32 %) patients with VF and ER was higher than in the remaining 15 VF patients without ER (86 % vs. 27 %, $P = .02$). In contrast, repolarization markers did not differ between the two groups. Moreover, dynamic changes in late potential parameters (fQRS, RMS_{40}, LAS_{40}) were observed and were pronounced at night time only in the patients with VF and ER and high-frequency components (vagal tone index) on frequency-domain HRV analysis were associated with J waves in VF patients ($P < .05$). These investigators concluded that since idiopathic VF patients with ER had a high incidence of late potentials showing circadian variation with night ascendancy, J waves due to ER may be more closely associated with depolarization abnormality and autonomic modulation than with repolarization abnormality. Recently, Roten et al. have studied the effect of Ajmaline on J wave in three groups of patients: 31 with Early Repolarization (symptomatic and asymptomatic); 21 with type 1 Brugada syndrome and 22 controls [32]. They showed that Ajmaline significantly decreased mean J wave amplitude in ER group from 0.2 ± 0.15 mV at baseline to 0.08 ± 0.09 mV ($p < 0.001$). QRS width prolonged significantly in all three groups, but prolongation was significantly less in ER group (+21 ms) compared to Br group (+36 ms;

$p < 0.001$) or controls (+28 ms; $p = 0.010$). They conclude that these results indicate different pathogenesis for ER and BrS. The altered terminal QRS vector probably is responsible for the decrease in J wave amplitude in ER, although a specific effect of ajmaline on J waves cannot be excluded.

Relationship Between J Wave Elevation and Ventricular Fibrillation

Amplitude of J Wave

Amplitude of J wave is more important in VF patients compared to controls (2.15 ± 1.2 mm in IVF vs. 1.05 ± 0.2 mm) [14]. Tikkanen et al. reported higher relative risk of cardiac death with a J point elevation of 0.2 mV (RR-3.03 – CI:1.88–4.90, $p = 0.001$) compared to 0.1 mV (RR-1.30 – CI:1.05–1.61, $p = 0.02$) [18].

Spontaneous Dynamicity

Since most of the VF episodes cannot be predicted clinically in patients with sporadic episodes of VF, it is difficult to get further insights into the role of ER in the mechanism of VF. However, some of the patients with VF experience electrical storm during hospitalization unraveling the dynamics of ER in VF arrhythmogenesis. Haïssaguerre et al. performed serial ECGs during electrical storm (including frequent ventricular ectopy and episodes of ventricular fibrillation) in 16 subjects and all patients showed consistent and marked increase in the amplitude of J wave during the period of storm when compared with baseline pattern (from 2.6 ± 1 mm to 4.1 ± 2 mm, $P < 0.001$) [33]. Besides spontaneous accentuation of the J wave amplitude preceding electrical storm, spontaneous beat to beat fluctuation in the morphologic pattern of ER was also observed [14]. Out of 11 patients with VF and ER reported by Nam et al., five patients experienced VF storm during their stay in intensive care unit. ECGs recorded within 30 min of the VF storm exhibited global appearance of J waves [17]. These dynamic ECG features of ER appeared spontaneously for a transient period around the VF storm revealing the presence of a functional "substrate" for arrhythmia. Derval et al. reported a high degree of spontaneous fluctuation of J wave in all cases, such that at least 1 ECG from the index hospitalization showed no evidence of significant ER in 58 % of patients (11 of 19 patients with ER and IVF; Fig. 30.3). These major fluctuations were not explained by heart rate fluctuation [23].

Correlation Between J Point Location and Arrhythmia Origin

We mapped patients with ER and VF targeting the ventricular ectopy initiating the VF. In patients with ER recorded in inferior leads alone, all ectopies originated from the inferior left ventricular wall. In the subjects with widespread global ER, as recorded in both inferior and lateral leads, ectopy originated from multiple regions [14]. These findings prove that ER abnormality may be either limited to a single region in the ventricles or can extend beyond it to involve more than one region simultaneously. Whether J wave truly represents an abnormality of repolarization or not is still debated, but, these findings help towards localizing ER as an abnormality involving distal Purkinje tissue, its innervated myocardium or the Purkinje-myocardial junctions [34].

Electrocardiographic Phenotypes Associated with Favourable Outcome

Tikkanen et al. have collected and analysed ECG from large group of athletes (from Finland, $n = 62$; and US, $n = 503$) and in general population (middle aged, Finnish population; $n = 10{,}864$). ST segment after J wave were classified as horizontal/descending or rapidly ascending/upsloping [35]. Main findings were: (1) In athlete, ST segment in rapidly ascending/upsloping in the majority of cases; (2) Infero-lateral ER patterns are not associated with uniformly increased risk of arrhythmic death in a middle-aged general population. Subjects with ER patterns associated with horizontal/descending ST segment after J-wave had higher risk of sudden arrhythmic death (age- and sex adjusted HR 1.43; 95 % CI 1.05–1.94) than subjects without ER. Subjects with rapidly ascending/upsloping ST segment did not have an elevated

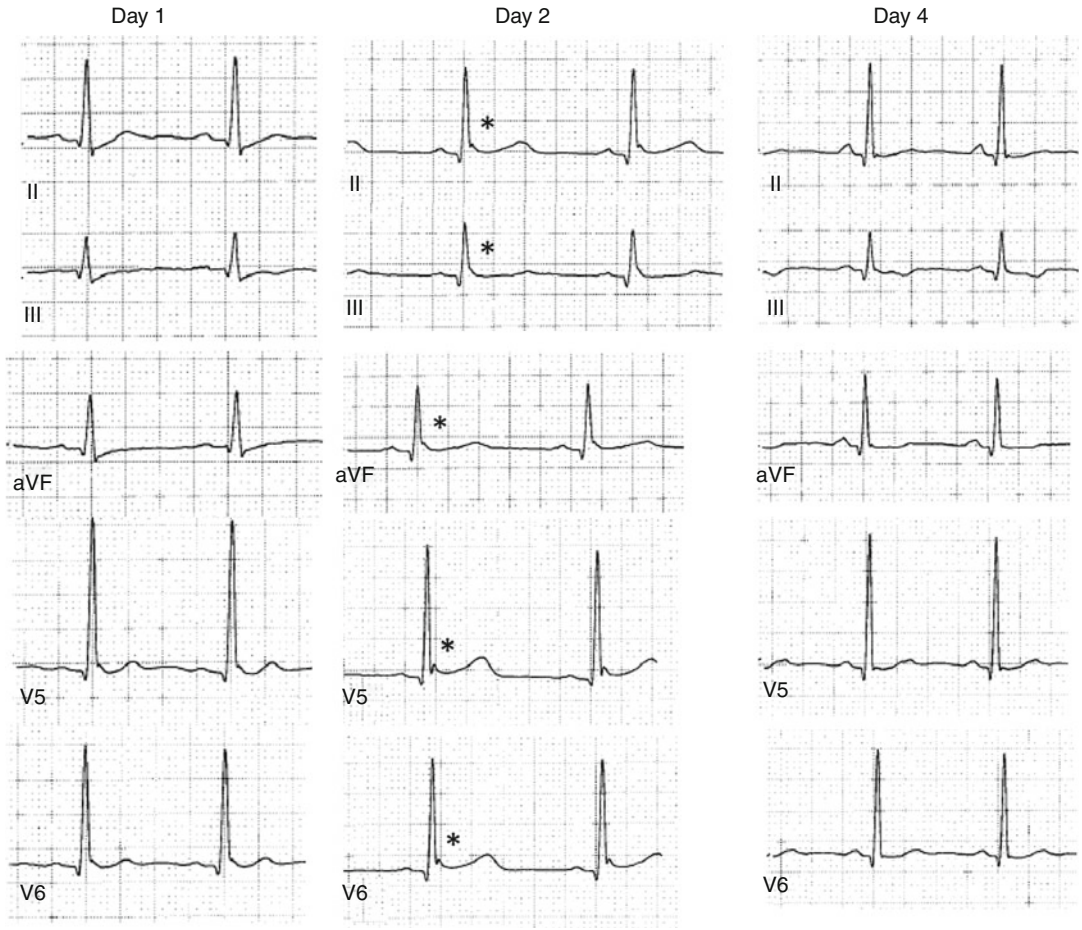

Figure 30–3. Day-to-day fluctuation of ER pattern in an IVF patient. The ECG recorded at different times after cardiac arrest demonstrates fluctuation of J-wave amplitude (*) with typical Type I ER pattern (day 2), type II ER pattern (day 1) and no evidence of ER pattern day 4 after the cardiac arrest

risk for arrhythmic death (adjusted HR 0.89; 95 % CI 0.52–1.55). (3) No difference between prognostic significance of notching and slurring of ER patterns emerge from their study. They conclude that the highest risk of event occurred in patients with ER pattern combining: inferior location, high amplitude (above 0.2 mV), and a dominant horizontal or descending ST segment after J-wave.

Risk Stratification

As described above, although ER is a common entity, unexplained sudden cardiac arrest in young adults is very rare. Some investigators addressed this issue by using the Bayes' law of conditional probabilities. Rosso et al. claimed that the presence of J-wave in a young adult would increase the probability of VF from 3.4:100,000 to 11:100,000 which is a negligible rise. They, therefore, concluded that the incidental discovery of J-wave on routine screening should not be interpreted as a marker of "high risk" for sudden death because the odds for this fatal disease would still be approximately 1:10,000 [16]. Now, the million dollar question is: "how to differentiate subjects with 'high risk' ER from the so-called benign ER?"

Clinical Features

In such a situation, we consider that close follow-up should be offered to the patients with ER and unexplained syncope or a family history of

unexplained sudden death. Abe et al. reported that the prevalence of ER in 222 patients with syncope and no organic disorder was 18.5 %, which is almost ten times that in 3,915 healthy controls (2 %) [31]. Therefore, the possibility of ER-associated syncopal episodes cannot be excluded in at least some of these patients. The genetic basis of ER is still largely unknown. Also, in patients with VF and ER, positive family history of sudden death was not significantly higher than in those without ER (16 % vs. 9 %, $P = 0.17$) [14]. Nevertheless, it does not imply that family history is not an important aspect of history-taking in ER patients.

Magnitude of J Wave

In the study by Tikkanen et al., subjects with J-point elevation of more than 0.2 mV on inferior leads not only bore a higher risk of death from cardiac causes (adjusted relative risk, 2.98; 95 % CI, 1.85–4.92; $P < 0.001$) as compared with J point elevation of more than 0.1 mV, but also had a markedly elevated risk of death from arrhythmia (adjusted relative risk, 2.92; 95 % CI, 1.45–5.89; $P = 0.01$) [18]. This finding indicates that the magnitude of J-point elevation could be a discriminator of risk. However, this study did not provide the sensitivity and specificity of this measure in predicting the endpoint-events. In accordance with this finding, Haïssaguerre et al. also found that the magnitude of J wave elevation in case group was significantly higher than that in control subjects (2.0 ± 0.8 mV vs. 1.2 ± 0.4 mV, $P < 0.001$) [14]. Derval et al. also reported a higher magnitude of J-wave in patient with ER pattern and purely Idiopathic VF than in patient with ER pattern and another substrate for VF (0.25 ± 0.12 vs. 0.13 ± 0.05; $p = 0.02$) [23]. It is worthy to note that J point elevation of more than 0.2 mV seems rare in normal population. In 630/10,864 subjects with ER identified by Tikkanen et al., 0.33 % of total population had J wave elevation of more than 0.2 mV [18]. However, it is also necessary to point out that the magnitude of J wave elevation can fluctuate even without drug provocation or exercise. This means low magnitude of J wave should not be considered as a static entity. It can potentially get augmented. Unfortunately, there is no reliable provocation test to augment ER in infero-lateral leads currently.

Distribution of J Waves

In normal subjects, most of the ER is confined to inferior leads, lateral leads (I/aVL) or left precordial leads. As reported by Tikkanen et al., out of 630 subjects with ER, only 16 subjects (2.5 %) had ER in both the inferior and lateral leads [18]. Sinner et al. found that ER pattern was associated with about a two to four-fold increased risk of cardiac mortality and inferior localization of ER pattern was associated with a particularly increased risk [19]. Focusing on patients with VF, Haïssaguerre et al. found that 46.9 % of patients with VF and ER had ER in both inferior and lateral leads [14]. Derval et al. found a larger number of involved leads (4.1 ± 1.1 leads vs. 2.8 ± 0.8; $p = 0.01$) in patient with ER pattern and Idiopathic VF than in patient with ER pattern a concurrent substrate for VF (0.25 ± 0.12 vs. 0.13 ± 0.05; $p = 0.02$) [23]. Similarly, global presence of ER was observed in none of the 46 subjects with ER without VF (selected from among 1,395 individuals from the general population) but in 45.5 % of patients with ER who developed VF [17].

Morphology of J Waves

Recently, Merchant et al. compared the baseline ECGs between nine patients with VF/VT and ER (so-called malignant ER group) and 61 age- and gender-matched controls with normal ER (so-called benign ER group). The results demonstrated that QRS notching was more prevalent among cases than controls in leads V4 (44 % vs. 5 %, $p = 0.001$), V5 (44 % vs. 8 %, $p = 0.006$) and V6 (33 % vs. 5 %, $p = 0.013$). They concluded that left precordial terminal QRS notching is more prevalent in malignant variants of ER than in benign cases and could be used as a tool for risk stratification of subjects with ER. However, the case number in this study is small and it includes three patients with idiopathic monomorphic VT without VF [36].

ST Segment Pattern

As described above, ST-segment morphology associated with ER may help to stratify patients with and without an increased risk of arrhyth-

mic death in a middle-aged population [35]. Rapidly ascending ST segments after the J-point (dominant pattern in young athlete), seems to be a benign variant of ER, whereas horizontal/descending ST segment seems more malignant.

Invasive Induction of Ventricular Fibrillation

Induction of VF was attempted in 132 VF patients from two different ventricular sites and up to three extrastimuli with shortest coupling interval of 209 ± 30 ms. The patients with ER did not show significantly higher inducibility than those without ER (16/47 vs. 17/85, $P = 0.07$) [14]. Moreover, a low rate of VF inducibility (34 %) in the patients with ER Syndrome (VF + ECG pattern) makes electrophysiologic study less sensitive in risk stratification of asymptomatic patients. Summary is presented in Table 30.1.

Table 30–1. Clinical characteristics of ER pattern patients at high vs. low risk

		High risk	Low risk
Clinical history		Syncope	Asymptomatic
Familial history		Unexplained cardiac arrest	No
J-wave	Amplitude	≥0.2 mV	<0.2 mV
	Distribution	Wide, involving inferior wall	Lateral V5–V6
	Pattern	Notch	–
ST segment		Horizontal/descending	Rapidly ascending/ upsloping

Management of Ventricular Fibrillation Associated with Early Repolarization

Patients with VF and ER have shown higher incidence of VF recurrence than VF patients without ER (43 % vs. 23 %; p < 0,001) during 5 years of follow-up. Moreover, out of 122 patients with VF and ER, 33 (27 %) patients experienced more than three episodes of VF and 16 experienced electrical storm (≥3 VF/24 h). Based on these observations, the patients with VF and ER may be the candidates for treatment over and above an implantable defibrillator. In this population, acute control of arrhythmia could be achieved by deep sedation and/or isoproterenol infusion. Quinidine was effective in preventing the recurrence during follow-up. In all case, oral quinidine led to dramatic

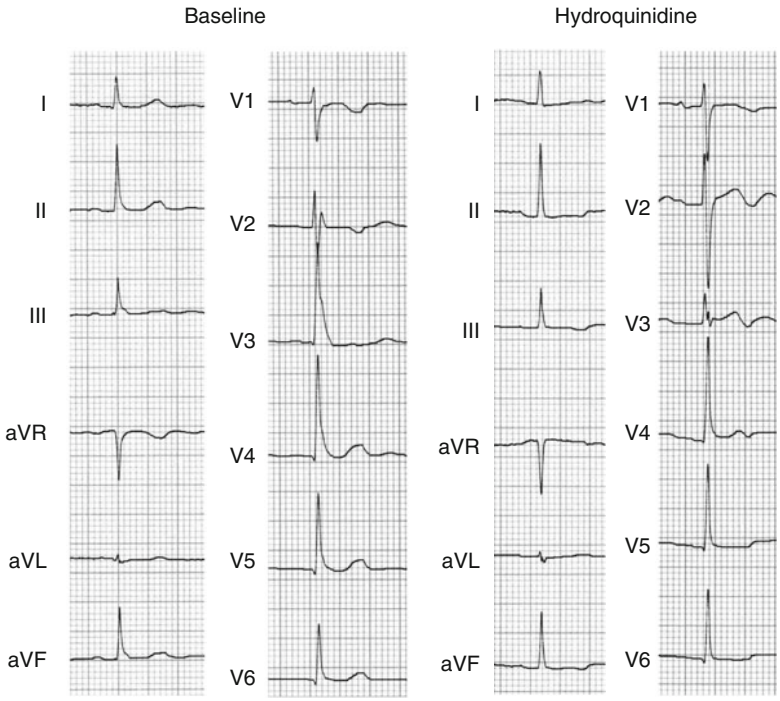

Figure 30–4. Effect of oral Hydroquinidine in a young patient with recurrent IVF. *Left*: Baseline ECG of survivor of IVF. Note the slurred-type ER pattern present in inferolateral leads. *Right*: Almost disappearance of inferolateral ER pattern under oral hydroquinidine

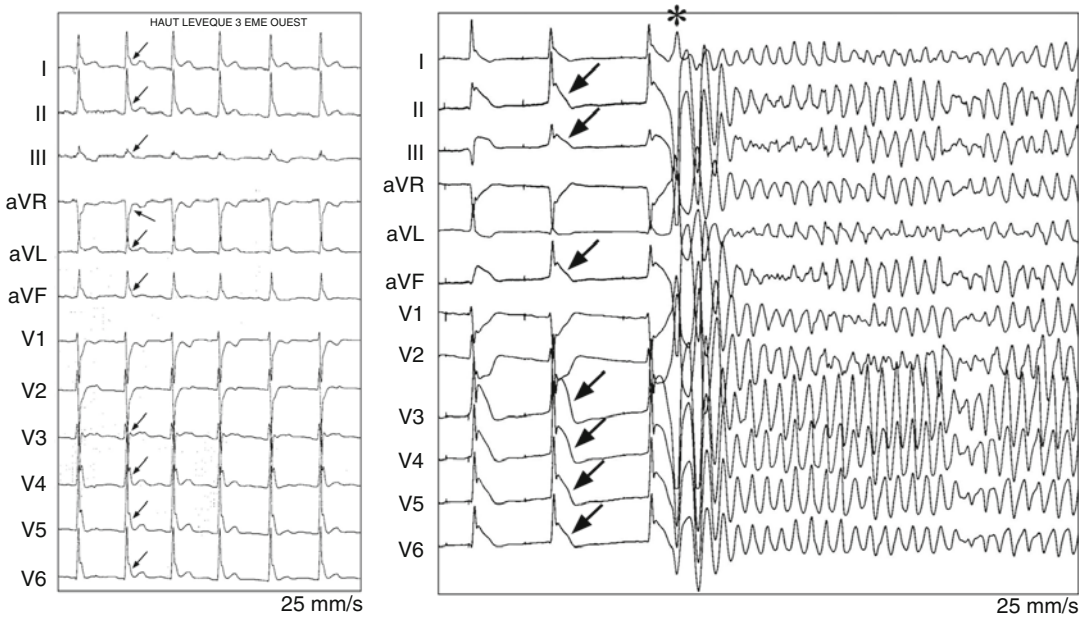

Figure 30–5. Extreme fluctuation of ER pattern in patients with recurrent VF epidodes. *Left*: the baseline electrocardiogram (ECG) of a survivor of sudden cardiac arrest (Female, 16 years/old). All known structural heart diseases and primary electrical diseases, including long/short QT syndrome, Brugada syndrome and catecholamine sensitive ventricular tachycardia were excluded in this patient. The only significant finding on the ECG was a prominent J wave (marked with *thin arrow*) as can be seen in almost all the leads other than V1 and V2. *Right*: During the electrophysiological study, an episode of spontaneous ventricular fibrillation (*VF*) was triggered by a ventricular ectopy with very short coupling interval (marked with *asterisk*). Note, the J wave in the inferior and precordial leads (marked with *bold arrow*) gets significantly augmented prior to the occurrence VF than at the baseline. Sweep speed, 25 mm/s

reduction of J-wave amplitude and sometimes to complete normalization of ECG (Fig. 30.4). Interestingly ER pattern was closely linked to the period of occurrence of arrhythmia and was helpful to monitor the efficacy of drug therapy. At the time of arrhythmia occurrence, ER was more pronounced than during the period without arrhythmia (Fig. 30.5). Amiodarone, beta-blockers and Class IC drugs were ineffective in establishing control of recurrent VF [33].

Catheter ablation of the ectopy initiating the VF could be another potential modality in the management of VF patients with ER who fail to respond to drugs. In a small number of patients who underwent invasive mapping of the ectopy, six out of eight subjects with ER in the inferior leads, ectopy originated from the inferior left ventricular wall. In the remaining two patients with inferolateral ER, ectopy originated from multiple regions. Catheter ablation eliminated all ectopies in five subjects [14].

J Wave Syndromes

Other conditions which display J wave elevation, include acquired (myocardial ischemia, hypothermia, hypercalcemia, etc.) and inherited (Brugada syndrome) disorders. As for variants of long QT syndrome, different types of J wave elevation are responsible for malignant ventricular arrhythmia due to different molecular and/or regional abnormalities. ER has been reported to be present in 12 % of Brugada Syndrome patients. The significance of this association is unclear. Two studies which looked at the outcome in these patients reported mutually conflicting results (no difference vs. increased risk) [37, 38].

Even if some similarities exist between the ER and the Brugada Syndrome, some major differences favour ER syndrome as a distinct entity (Table 30.2). Firstly, both ECG patterns were considered as normal variant before the 90s [1, 39]. Secondly, similar mechanism was

Table 30–2. Similarities and differences between early repolarization syndrome and Brugada syndrome

Characteristic	Early repolarization syndrome	Brugada syndrome
Gender preponderance	Male	Male
Age at diagnosis	Around 40 years	Around 40 years
Situation favoring arrhythmia	Rest/sleep	Rest/sleep
Effective therapy in VF storm	Isoproterenol/quinidine	Isoproterenol/quinidine
Autonomic influence	Vagal tone	Vagal tone
ECG localization	II, III, aVF – V5, V6 – I, aVL	V1–V3
ST pattern	J-wave (slur/notch)	Coved type
Sodium channel blocker effect	Reduction	Accentuation
VF sources	Left ± right ventricle	Right ventricle
Genetic mutations	Unknown	SCN5A, GPD1-L, CACNA1C, CACNB2B

Table 30–3. Management of patients with inferolateral ER

In the settings of resuscitated SCA	In the settings of syncope	In asymptomatic patients
ER syndrome	ER syndrome or ER pattern	ER pattern
ICD	Characteristics of syncope	Characteristics of ECG ER attern
±Isoproterenol in case of arrhythmic storm	Characteristics of ECG ER pattern	No pharmacological test yet
±Quinidine in case of recurrence	Familial history of SCD	No specific recommendations
Familial screening	Clinical follow-up, ILR, ICD in rare cases	

ICD implantable cardiac defibrillator, *SCD* sudden cardiac death, *ILR* internal loop recorder

experimentally demonstrated by Yan and Antzelevitch to explain J wave elevation in both syndromes [25]. Both syndromes are associated with ventricular arrhythmias at rest (±vagal period) predominantly in young males in their late 30s or early 40s. Finally, recurrent VF associated with these syndromes responds similarly to isoproterenol acutely and quinidine as a long term secondary prevention therapy [40, 41]. As regards to the differences between the two syndromes, Brugada syndrome differs from the ER syndrome in terms of regional electrocardiographic location (V1–V3 vs. infero-lateral leads) and morphology of J-point elevation (coved-type vs. slurring/notching). No conduction disorders were found in any patients with VF and ER pattern whereas they have been commonly found in Brugada Syndrome. Sodium channel blockers provokes or accentuates the ECG pattern in Brugada syndrome, it does not alter or attenuates (ajmaline) the ER pattern. To conclude, a mutation in SCN5A has been found in 20 % of patients with Brugada Syndrome but in none of the 135 patients with ER syndrome and VF who have been genotyped. Only sporadic variants of KCNJ8, CACNA1C, CACNB2B have been reported in patients with ER and VF [26, 28].

Conclusion

Current scientific evidence drawn from a large cohort of subjects with long-term follow up suggest that J wave elevation in the infero-lateral leads, is not always benign. There is high prevalence of early repolarization in the patients experiencing first, recurrent and stormy episodes of idiopathic ventricular fibrillation. We necessarily recommend careful evaluation of patients having early repolarization associated with unexplained syncope, family history of sudden cardiac death or idiopathic ventricular arrhythmias (Table 30.3). It is particularly important to pay attention in case of J wave >0.2 mV, global J wave and notched J wave in the left precordial leads.

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31
Catecholaminergic Polymorphic Ventricular Tachycardia

Nian Liu, Carlo Napolitano, and Silvia G. Priori

Abstract

The control of calcium fluxes in the heart is a key function that affects both excitability and contractility. At least two components of this pathway can generate inherited arrhythmias syndromes the release of calcium from its intracellular store, the sarcoplasmic reticulum and the transmembrane flux of calcium controlled by voltage-gated calcium channels. This chapter deals with inherited arrhythmias due to genetic defects of the proteins controlling sarcoplasmic reticulum calcium release, namely the catecholaminergic polymorphic ventricular tachycardia (CPVT). Two proteins, ryanodine receptor (RyR2) and calsequestrin (CASQ2) have been involved in the patogenesis of CPVT, a disorder characterized by adrenergically mediated bidirectional and/or polymorphic VT a structurally normal heart. The clinical presentation encompasses exercise- or emotion-induced syncopal spells and a distinctive pattern of reproducible, stress-related, bidirectional VT in the absence of structural heart disease. This chapter provides an overview of the structural organization and the function of calcium releasing proteins. We also describe the current understanding of the pathways leading form mutations to the clinical phenotype, which, in several cases, has driven (or it is driving) the development of more effective therapies.

Keywords

Inherited arrhythmias • Sudden death • Intracellular calcium handling • Cardiac channelopathies

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In 1975, Reid et al. [1] described a phenotype characterized by bidirectional ventricular tachycardia (VT) precipitated by effort and emotional stress in a 6-year-old girl with no evidence of any structural abnormality of the heart. Later on, in 1978 and 1995, Coumel et al. [2] and Leenhardt et al. [3] reported a series of cases in familial as well as in sporadic forms and introduced the term catecholaminergic polymorphic ventricular tachycardia (CPVT) to refer to a disease characterized by adrenergically mediated bidirectional and/or polymorphic VT in the absence of cardiac pathology. The clinical presentation encompasses exercise- or emotion-induced syncopal spells and a distinctive pattern of reproducible, stress-related, bidirectional VT in the absence of structural heart disease. The discovery of CPVT genes marks the inception of a line of experimental and translational investigations targeted to the understanding of the arrhythmogenic role of intracellular calcium handling in genetic but also in acquired disorders [4].

Genetics of CPVT

According to the initial systematic analyses of CPVT families an autosomal dominant inheritance was suggested [3]. Following the initial linkage study [5] that mapped the 1q42-q43 region as the locus for dominant CPVT, we performed candidate gene analysis [4] and identified Ryanodine receptor (RyR2) mutations in four families. These data have been shortly after confirmed by Laitinen et al. [6].

In 2001, Lahat et al. [7] described a recessively inherited CPVT phenotype and mapped the disease locus to a 16-megabase interval on chromosome 1p13-21. Subsequently the same group [8] showed a missense mutation in a highly conserved region of the calsequestrin 2 gene (*CASQ2*) as the cause of the autosomal recessive form.

To date, only 15 CASQ2 mutations are known, and this genetic variant is considered rare (see below). Finally in 2007 Bhuiyan et al. identified a novel autosomal recessive form of CPVT and mapped on chromosome 7p14-22, however the gene remains unknown [9].

Genetic Testing

The yield of CPVT genetic testing is in the range of 55–70 % [10, 11]. More than 150 RyR2 mutations have been reported and some were detected in patients with idiopathic ventricular fibrillation, and sudden infant death syndrome [12]. The majority of RyR2 mutations cluster in four regions of the protein (Fig. 31.1). Based on this observation some Authors proposed to limit genetic testing to those "critical" regions. However, based on our large mutation repository and literature analysis there appears that

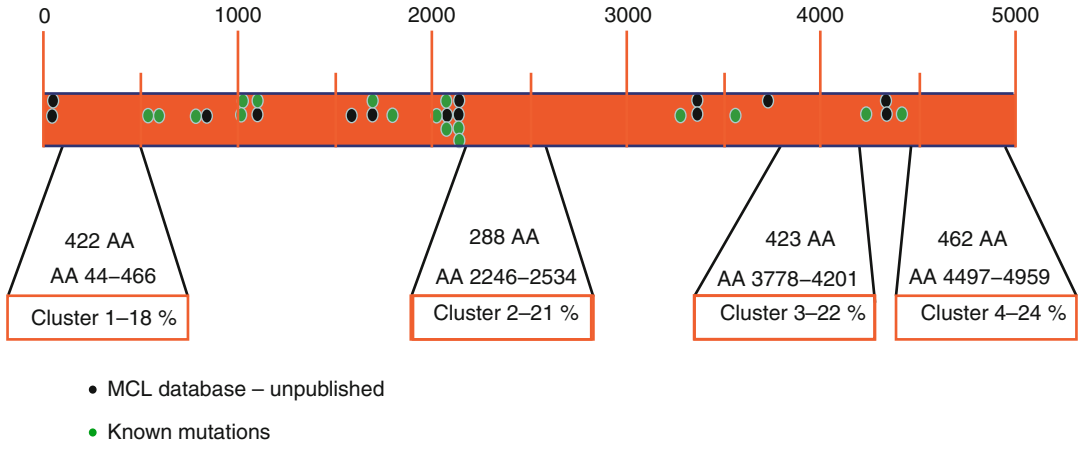

Figure 31–1. RyR2 mutational clusters. Clusters with frequent mutations are depicted with their location along RyR2 amino acid (*AA*) sequence. Percentage of known (published) mutation foe each cluster is also reported. Mutations outside the canonical clusters are depicted as *dots* in the figure. Each dot represents a unique mutation. *Green dots* represent published mutation while *black dots* represent unpublished mutation form our database. *MCL* Molecular Cardiology Laboratories, Pavia, Italy

approximately 20 % of mutations occur outside the clusters (Fig. 31.1). Thus the analysis of the entire open reading frame of the RyR2 gene is often required. There are no clues for cluster-specific phenotype.

According to a recent consensus document [12], only the analysis of RyR2 is recommended on a routine basis. CASQ2 screening is indicated in the presence of possible recessive inheritance and also in sporadic CPVT RyR2-negative patients since compound heterozygosity is possible [13].

Pathophysiology

RyR2 channels are normally activated by Ca^{2+} entering the cell through the voltage-dependent L-type Ca^{2+} channels, causing the release of Ca^{2+} from the sarcoplasmic reticulum (SR) into the cytosol, a mechanism known as Ca^{2+}-induced Ca^{2+} release (CICR) (Fig. 31.2) [14]. The hypothesis that arrhythmias in CPVT are initiated by delayed afterdepolarizations (DADs) and triggered activity had been advanced since the identification of the first RyR2 mutations. The

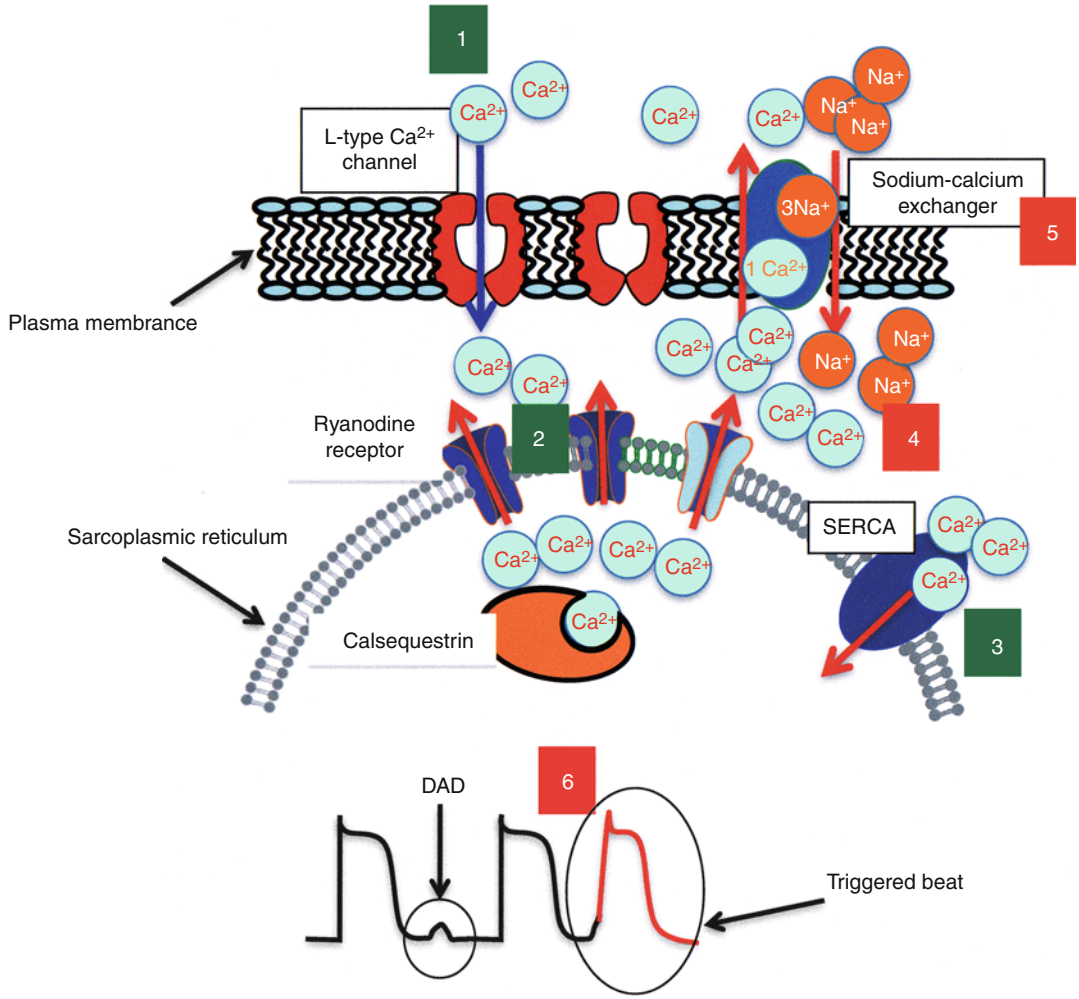

Figure 31–2. Cartoon illustrating the steps of calcium induced calcium release process (*green numbers*) and the cellular pathophysiology of CPVT (*red numbers*). (*1*) Calcium ions enter the cell through L-type channels during phase 1–2 of the action potential and trigger the release of a large amount of calcium form the sarcoplasmic reticulum (*2*) which allows contraction. After calcium is freed form actin-myosin complex it is transported back to the sarcoplasmic reticulum by the SERCA pump (*3*). When diastolic calcium leak occurs due to RyR2 or CASQ2 mutations the cell must get rid of the cytosolic overload (*4*) and the sodium-calcium exchanger is activated (*5*). The exchanger removes one calcium ion every three sodium ions entering the cell. Therefore it generates a depolarizing current (transient inward current) which underlies delayed afterdepolarizations (*DAD*); if large enough, DADs can trigger an action potential (*6*)

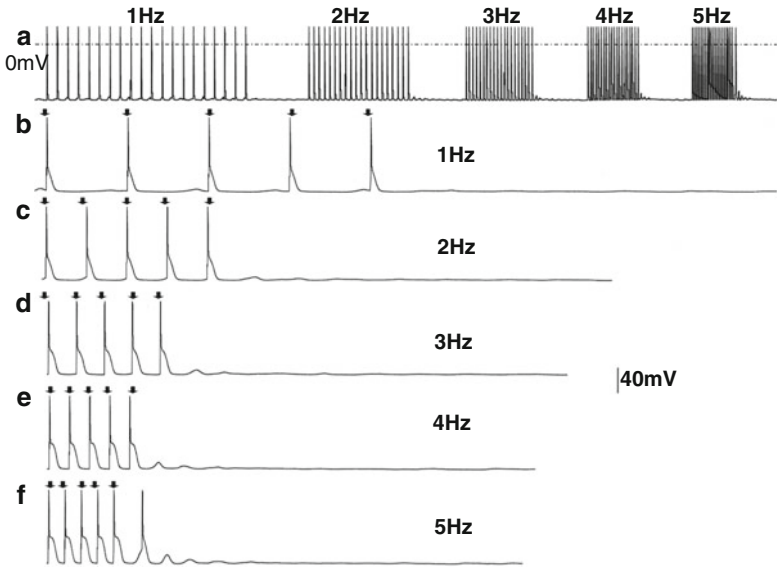

Figure 31–3. (**a**) Action potentials recorded from a RyR2$^{R4496C+/-}$ myocyte stimulated at 1–5 Hz are shown: DADs develop when pacing is interrupted. (*Panels* **b**–**f**) The last five driven action potentials at each pacing frequency and DADs and triggered activity developing when pacing is discontinued are shown. Note that DAD amplitude increases and DAD coupling interval decreases at faster pacing frequencies. At 5 Hz, (*panel* **f**) one triggered beat develops

hypothesis was proven correct with the availability of the conditional knock-in mouse model carrier of the RyR2$^{R4496C+/-}$ that develops the typical bidirectional VT [15] and DADs/triggered activity (Fig. 31.3) [16].

Pathophysiology of RyR2 Mutations

Jiang et al. [17, 18] directly determined the impact of a number of CPVT RyR2 mutations on the sensitivity of single RyR2 channels to luminal Ca^{2+} activation using single channel recordings in planar lipid bilayers. In vitro expression of many RyR2 mutations showed a consistent behavior characterized by an enhanced response of the channel to luminal Ca^{2+} activation while only few of them seem to affect the response of the RyR2 channel to cytosolic Ca^{2+}, or both cytosolic and luminal sensitivity [19].

The increased sensitivity is the cause of a reduced threshold for the so-called Store Overload Induced Calcium Release (SOICR). In a nutshell the macroscopic effect of mutations is that of lowering the threshold for spontaneous calcium release from the sarcoplasmic reticulum. Thus, upon adrenergic activation (which physiologically increases SR calcium content), it becomes easier for SR calcium release to occur [20] and to generate delayed afterdepolarization and triggered activity during electrical diastole (Fig. 31.4) [21].

The molecular mechanisms for such calcium sensitization are complex and still incompletely understood. Two major hypotheses have been proposed: (A) defective interaction between protein domains controlling channel gating (unzipping) or (B) the unbinding of FKBP12.6 that is believed to be a RyR2 stabilizer [22–24]. This latter hypothesis is questioned by the evidence that K201, a 1,4-benzothiazepine derivative (formerly called JTV 519) which enhances the binding of FKBP12.6 to RyR2, is unable to prevent triggered activity and arrhythmias in RyR2$^{R4496C+/-}$ mice [16]. More recently Guo et al. reported that 10–20 % of endogenous myocyte RyR2s have FKBP12.6 associated and PKA-dependent RyR2 phosphorylation has no significant effect on binding of either FKBP12 or 12.6 to RyR2 in myocytes [25]. Those data suggested that FKBP12.6 would not be the key point for the arrhythmogenesis of CPVT.

It is known that Purkinje fibers are more susceptible to Ca^{2+} overload than ventricular muscle possibly because of their greater sodium load and longer action potential duration. Recently two independent groups reported that Purkinje fibers isolated from RyR2$^{R4496C+/-}$ mice display a greater propensity to develop intracellular Ca^{2+} handling disorder than ventricular myocytes, suggesting that focally activated arrhythmias might originate in the specialized electrical conducting cells of the His-Purkinje system in CPVT

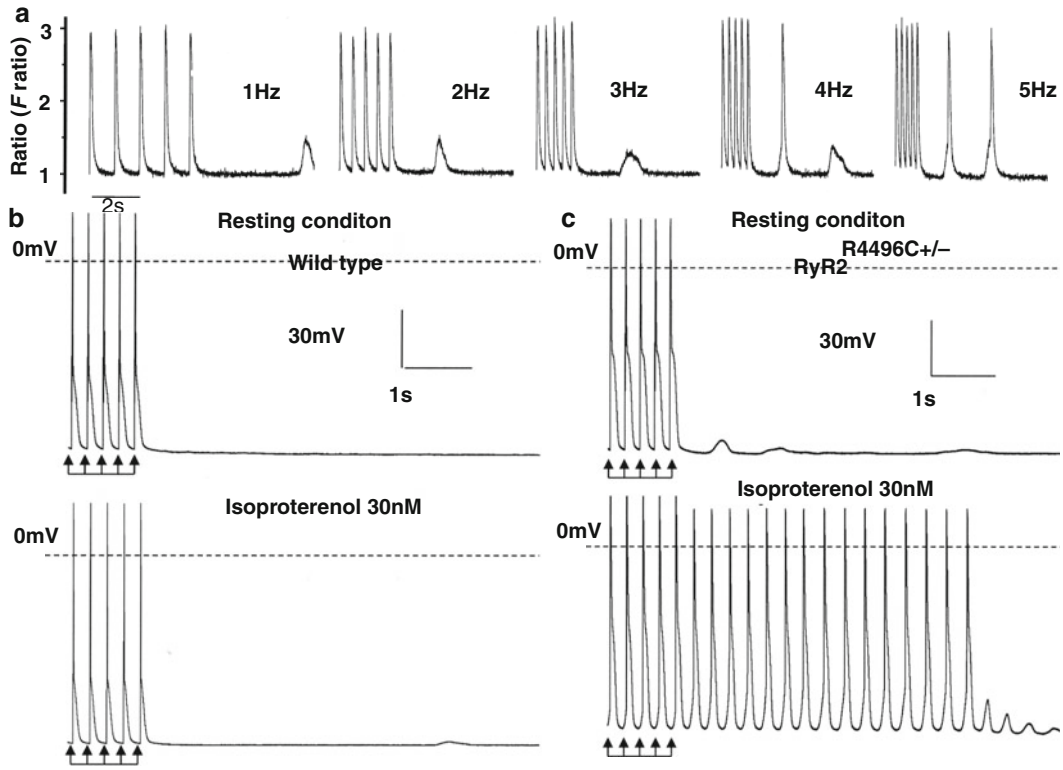

Figure 31–4. (**a**) A Fura-2 loaded RyR2$^{R4496C+/-}$ myocyte in presence of isoproterenol (30 nM), spontaneous Ca^{2+} release showed positive frequency dependent manner. Action potential recording from a WT myocyte (*panel* **b**) and a RyR2$^{R4496C+/-}$ myocyte (*panel* **c**) in the absence (*top panel*) and in the presence (*bottom panel*) of isoproterenol (30 nM). *Arrows* indicate the last five paced action potentials

[26, 27]. More interestingly epicardial mapping study in RyR2$^{R4496C+/-}$ hearts revealed that the bidirectional morphology of VT is caused by alternating firing of triggered activity from the right and left bundle [28].

Pathophysiology of CASQ2 Mutations

The *CASQ2* protein serves as the major Ca^{2+} reservoir within the SR and it is part of a protein complex that contains the ryanodine receptor. In the presence of 50 % loss of calsequestrin (in heterozygous carriers) a normal clinical phenotype is observed; thus suggesting that the heart can tolerate quite severe CASQ2 reductions. It has been demonstrated that the underlying mechanism for the CASQ2-CPVT is associated with impaired SR Ca^{2+} buffer and defects of CASQ2-dependent regulation of RyR2 channel open probability. More recently various transgenic mouse models harboring CASQ2 mutations have been developed [29–31]. Those mouse models exhibit severe CPVT phenotype and demonstrate marked-to-complete reduction of CASQ2 protein levels [29, 30]. Therefore reduced CASQ2 expression appears to represent a common feature of autosomal recessive CPVT. Interestingly this effect may induce a number of abnormal adaptive responses, such as increasing RyR2 and calreticulin, decreasing triadin and junction [29–31]. More interestingly, despite lack of cardiac hypertrophy and fibrosis in youth mice, electron microscopy has revealed ultrastructural remodeling in SR, including enlarged SR volume and widened jSR dimension, suggesting ultrastructural cardiomyopathy for autosomal recessive CPVT [29, 31]. Isolated ventricular myocytes with CASQ2 mutation present DAD as well as early afterdepolarization due to abnormal Ca^{2+} leak from RyR2 [29]. The pathophysiology of CPVT is schematically represented in Fig. 31.3.

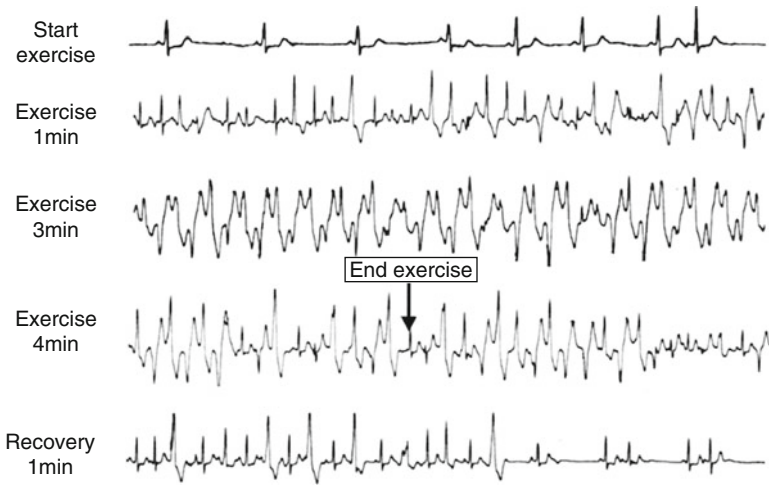

Figure 31–5. Exercise stress test in a patient with polymorphic VT and RyR2 mutation. Ventricular arrhythmias are observed with a progressive worsening during exercise. Typical bidirectional VT develops after 3 min of exercise with a sinus heart rate of approximately 120 bpm. Arrhythmias rapidly recede during recovery

Clinical Management

Clinical Manifestations

Syncope, triggered by exercise or emotional stress, is often the first manifestation of CPVT [3, 10]. These symptoms are accompanied by family history of sudden death in approximately 30 % of patients. The mean age at first event is between was 7 and 12 years [3, 10, 32]. Increasing evidences show that sudden cardiac death can be the first manifestation of the disease [10, 11] and that CPVT may be a cause of sudden infant death syndrome [33] and idiopathic ventricular fibrillation [34].

Electrocardiogram

The resting ECG of CPVT patients is normal with the exception of prominent U waves and mild sinus bradycardia in some patients [3, 32]; the CPVT phenotype manifests preferentially upon adrenergic stimulation with the onset of a reproducible pattern of ventricular arrhythmias. Exercise stress test usually elicits isolated atrial and ventricular extrasystoles at heart rate between 95 and 110 beats per minute [3, 10]. The complexity and frequency of ventricular arrhythmia progressively worsen with increase of workload (Fig. 31.5). The typical ventricular tachycardia (VT) observed in CPVT is the so-called bidirectional VT: an alternating QRS axis morphology with a rotation of 180° on a beat-to-beat basis.

Diagnosis

Exercise or emotion-induced syncope in a patient with a normal electrocardiogram (normal QT interval) and without structural abnormalities, should always prompt the suspect of CPVT. Exercise stress test is the most important tool for diagnosis since the bidirectional or polymorphic VT may be reproducibly elicited during physical activity in most of the patients. In patients showing polymorphic (and not bidirectional) VT, the progressive worsening of arrhythmias with exercise is to be considered as diagnostic for CPVT. Holter monitoring can also be useful especially in young children in whom it might be difficult to reach high heart rate during treadmill test or for patients particularly sensitive to emotional stress.

Interestingly, the bidirectional VT is a common finding in patients with in Andersen syndrome [35]. This disease is a variant of the Long QT syndrome (also called LQT7) caused by loss of function mutations of the *KCNJ2* gene encoding for the cardiac inward rectifier potassium current. The reasons for this apparent "phenocopy arrhythmia" are still unclear but differential diagnosis between the two conditions should be considered and it has clinical relevance since *KCNJ2* mutation are often benign and only exceptional cause of sudden cardiac death.

Programmed electrical stimulation to assess inducibility is of no value (either diagnostic or prognostic) in CPVT as patients are largely not inducible. Ventricular arrhythmia may be induced

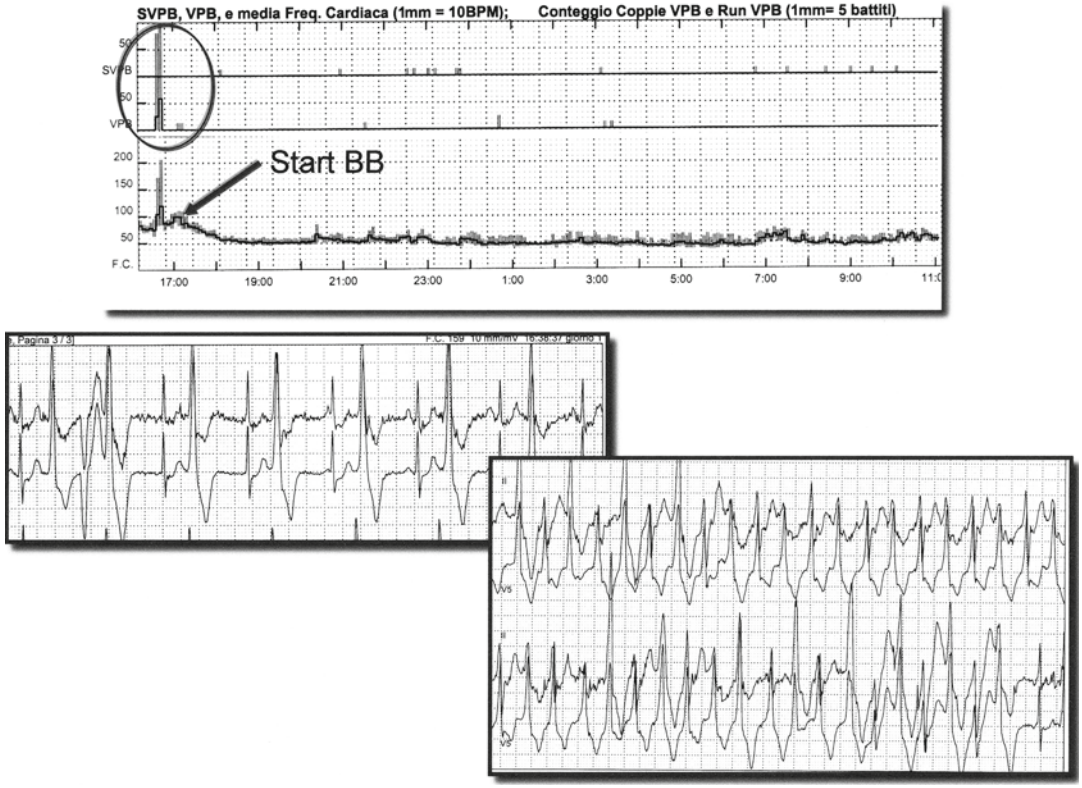

Figure 31–6. Holter monitoring and beta blocker therapy in CPVT. The *upper panel* shows the trend of arrhythmias and heart rate. When beta blocker therapy (nadolol 1.5 mg/kg) is started (*arrow*) hear rate decreases and the incidence of arrhythmias dramatically decreases. *Lower panels* show ventricular bigeminy and ventricular tachycardia recorded in the first hour before beta blockers

by isoproterenol or epinephrine infusion and it may be useful for diagnostic purposes [10, 36].

Treatment

Chronic treatment with beta-adrenergic blockers can prevent recurrent syncope in the majority of CPVT patients (Fig. 31.6). Nadolol at a daily dose of 1–2.5 mg/kg/day is the drug used at our center but propranolol can also be considered. Less experience is available with other beta blockers; in our experience metoprolol and atenolol are less effective due to their weaker effect in blunting exercise-induced heart rate increase. It is also important to be aware that dosage should be always individualized to achieve the best possible control of arrhythmias during exercise stress testing. Individual tolerability of the drug should be always considered. In our experience, some patients may be treated with up to 3.5–4 mg/kg/day of nadolol without clinically relevant side effects and increased protection from arrhythmic events. However, it is important to be aware that some patients still develop repetitive ventricular arrhythmias during exercise stress test even with maximal tolerated dose of beta blocking agents (see below).

Overall, agreement exists on the fact that beta-blockers reduce the occurrence of cardiac events and post pone the induction of VTs during exercise stress testing however incomplete protection from sudden cardiac death has been reported; indeed effectiveness of beta blockers is in the range of 60–85 % in the reported series [3, 10, 36, 37]. This suggests that the implant of an ICD should be considered for prevention of a cardiac arrest in CPVT patients in whom severe ventricular arrhythmias (sustained VT or rapid VT) are still observed while on beta-blocker therapy. In our study, 50 % of patients with the ICD received an appropriate shock to terminate ventricular tachyarrhythmias during 2 years follow-up [10].

Some studies explored the therapeutic value of left cardiac sympathetic denervation (LCSD) in patients with CPVT [38, 39] having cardiac events and/or malignant VT despite full dose β-blocker therapy. In few cases the treated patients remained asymptomatic and VT could only be induced at high workloads.

There is a potential threat in the use of ICD in CPVT. Cases have been reported (SGP personal communication) in which ICD shocks trigger the onset of post-shock TVs, probably due to the catecholamine release secondary to the pain and acute emotional stress due to shock itself. Thus, it is important to ensure that even when an ICD is provided the maximally tolerated doses of beta-adrenergic blockers should be recommended.

Verapamil, a Ca^{2+} channel antagonist, has been shown to directly inhibit the function of ryanodine receptor [40]. In 2003, Sumitomo et al. [36] reported that acute administration verapamil did not completely suppress CPVT, however the endurance time was prolonged and the number of CPVT episodes decreased; all three patients treated with verapamil survived during the follow up period. Subsequently, Swan et al. [41] also observed that intravenous administration verapamil could decrease the prevalence of ventricular arrhythmia in all patients with CPVT during exercise stress test. Thus, verapamil may be at least partially effective in CPVT for acute suppression of arrhythmias. No data are available for chronic administration.

More recently, Watanabe et al. [42] reported that flecainide, a class Ic sodium channel blocker, may also exert a direct effect on the ryanodine receptor and could inhibit DADs and triggered activity in CASQ2 knock-out mouse model. Our study in $RyR2^{R4496C+/-}$ mice showed that flecainide indeed exerts an antiarrhythmic effect but the mechanism is through the increase the threshold for triggered activity via its sodium channel blocking property [43]. Despite conflicting experimental results, preliminary observations on the use of flecainide in the clinical setting are encouraging [44]. A clinical trial is now ongoing to assess the potential beneficial effect of flecainide in CPVT (http://clinicaltrials.gov:NCT01117454). At present the use of flecainide can be attempted as an adjunction to beta-blockers in cases of poor control of arrhythmias

Summary

Catecholaminergic polymorphic ventricular tachycardia is a highly lethal form of arrhythmogenic disorder characterized by exercise or emotional induced polymorphic ventricular tachycardia in the absence of detectable structural heart disease. Clinical evaluations, such as exercise stress test, and genetic screening are extremely useful tools for early identification and diagnosis. Beta adrenergic blockers are first line therapy for CPVT and in patients with incomplete suppression of sustained or rapid VT implantation of an ICD is indicated.

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32
Andersen-Tawil and Timothy Syndromes

Martin Tristani-Firouzi and Susan P. Etheridge

Abstract

This chapter summarizes two relatively new ion channelopathies, Andersen-Tawil and Timothy Syndromes. Both disorders are pleiotropic in nature, with multiple clinical manifestations outside the cardiovascular system. While both Andersen-Tawil and Timothy Syndromes are disorders of ventricular repolarization, their unique clinical phenotype distinguishes them from the traditional forms of Long QT Syndrome (LQTS). The degree of QT prolongation in each disorder is directly related to the contribution of the disordered ion channel to the various phases of the cardiac action potential. This chapter discusses the most recent genetic, cellular and clinical data underlying these unique ion channelopathies.

Keywords

Ion channel • Channelopathy • Inherited arrhythmia • Sudden death • Bidirectional ventricular tachycardia • QT prolongation

This chapter summarizes two relatively new ion channelopathies, Andersen-Tawil and Timothy Syndromes. Both disorders are pleiotropic in nature, with multiple clinical manifestations outside the cardiovascular system. While both Andersen-Tawil and Timothy Syndromes are disorders of ventricular repolarization, their unique clinical phenotype distinguishes them from the traditional forms of Long QT Syndrome (LQTS). The degree of QT prolongation in each disorder is directly related to the contribution of the disordered ion channel to the various phases of the cardiac action potential. This chapter discusses the most recent genetic, cellular and clinical data underlying these unique ion channelopathies.

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Andersen-Tawil Syndrome

In 1971, Andersen reported a series of patients with periodic skeletal muscle paralysis, ventricular ectopy and dysmorphic features, the triad

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of clinical manifestations known as Andersen's Syndrome [1]. Prolongation of the QT interval was incorporated as an important cardiac manifestation in subsequent larger studies of this disorder [2]. The syndrome was renamed Andersen-Tawil Syndrome (ATS) in recognition of the exceptional contributions of the clinical neurologist Dr. Rabi Tawil. Within the cardiovascular field, this disorder remained obscure until mutations in the K channel gene *KCNJ2* were identified as a cause of ATS [3]. *KCNJ2* encodes the inward rectifier K channel Kir2.1, a component of the inward rectifier current I_{K1}. I_{K1} provides substantial repolarizing current during the most terminal repolarization phase of the cardiac action potential and is the primary conductance controlling the diastolic membrane potential [4].

Clinical Manifestations of Andersen-Tawil Syndrome

The clinical features of ATS represent a spectrum of phenotypic manifestations encompassing the skeletal muscle and cardiac systems, in addition to craniofacial and skeletal anomalies. A major obstacle in the clinical diagnosis of ATS is the high degree of phenotypic variability and non-penetrance. The full triad of clinical features (ventricular arrhythmias, periodic paralysis and characteristic dysmorphic features) is present in 58–78% of mutation-positive patients [5], while between 32 and 81% manifest involvement of two of the three organ systems [3, 5, 6]. Nonpenetrance ranges from 6 to 20% of mutation-positive individuals [5, 6]. In an interesting report, a large ATS family presented with sex-specific clinical manifestations. In this kindred, females manifested ventricular arrhythmias, while affected males had periodic paralysis. None of 41 mutation carriers expressed the complete triad of ATS features [6]. These sex-specific clinical findings, however, appear to be specific for this particular family in that the same mutation was reported in other individuals who manifested the typical ATS triad [7]. Recently, the ATS phenotype was expanded to include several consistent craniofacial features, dental and skeletal anomalies that were observed in all members of a small cohort, irrespective of cardiac or neuromuscular findings [8]. These new findings help to clinch the clinical diagnosis when cardiac or skeletal muscle findings are absent.

ATS is an autosomal dominant or sporadic disorder of ventricular repolarization manifested by mild QTc interval prolongation, but marked prolongation of the QUc interval [5, 9]. Prominent U waves are commonly described. Zhang and colleagues recently reported distinct ECG findings unique to ATS that included prolongation of the T wave downslope, wide T-U junction and high amplitude, broad U waves [9]. Arrhythmias in ATS patients include frequent premature ventricular contractions (PVCs), bigeminy and polymorphic ventricular tachycardia (VT) [5]. Typically, VT is nonsustained and bidirectional in nature with heart rates in the 130–150 range. The degree of ventricular ectopy is quite variable between subjects, but up to 50% of all beats may be ventricular in origin [8]. While tachycardia burden is often high in ATS subjects [10], degeneration into lethal ventricular arrhythmias is relatively uncommon [11]. For example, *torsades de pointes* was documented in only 3 of 96 *KCNJ2* mutation-positive individuals [9].

The frequent ventricular ectopy typical of ATS is notoriously difficult to suppress with pharmacological therapy [10, 12]. In the experience of the authors, beta-blockers are consistently ineffective at suppressing ventricular ectopy. Our recent clinical experience suggests that flecainide may be effective in reducing the frequency of ventricular ectopy and runs of bidirectional VT in both children and adults with ATS. Given that many patients with frequent ventricular ectopy are entirely asymptomatic, one wonders whether therapy is indicated in most patients. However, identifying the subset of ATS patients at risk for life-threatening arrhythmias remains a daunting task [10, 12] primarily due to the paucity of data in the literature. In light of the absence of known criteria, any patient with runs of rapid polymorphic VT or symptoms such as syncope, should be considered a candidate for placement of an implantable cardioverter-defibrillator (ICD). In patients who are not a candidate for ICD placement, we recommend flecainide as the first line therapy. We typically initiate therapy in the inpatient setting where continuous ECG telemetry is available.

The Molecular Correlate of I_{K1}

The inward rectifier potassium current I_{K1} is the major determinant of the resting membrane potential in the heart and participates in the most terminal phase of action potential repolarization [4]. I_{K1} is conducted by homo- and/or hetero-tetrameric channels formed by coassembly of the Kir2.x subfamily of proteins (Kir2.1, Kir2.2 and Kir2.3). Message and protein expression studies indicate that Kir2.1 is the most abundant subfamily member in ventricular tissue [13, 14]. The finding that mutations in *KNCJ2* cause human disease (but not the genes enconding Kir2.2 and 2.3) further underscores the pivotal role of Kir2.1 as a primary component of I_{K1}.

The Cellular Basis of Andersen-Tawil Syndrome

Nearly all the *KCNJ2* mutations described to date cause dominant-negative suppression of Kir2.1 channel function. A minority of mutations alter channel assembly and trafficking, with resultant accumulation of subunits within the endoplasmic reticulum and Golgi apparatus [15]. Most mutant subunits co-assemble with wild-type subunits and traffic appropriately to the cell surface, but fail to function normally. The mechanism underlying this abnormal function is an altered sensitivity of the mutant channel to the membrane-delimited second messenger phosphatidylinositol 4,5-bisphosphate (PIP_2), an essential activator of most inward rectifier K^+ channels [16]. Nearly one-half of all reported *KCNJ2* mutations occur at residues known to be important for PIP_2-channel interaction, supporting the idea that reduced PIP_2 binding is critical to the pathogenesis of ATS [7]. Recently, the effects of ATS mutations were studied at the atomic level by crystallizing the N-terminal and C-terminal cytoplasmic domains of Kir2.1. Arg218 and Glu303 in the C-terminus form charged and polar interactions with neighboring residues Thr309 and Arg312 [17, 18]. ATS mutations at Arg218 and Glu303 destabilize these interactions and likely render the channel insensitive to the activating effects of PIP_2 [17, 18]. To date, there is no clear genotype–phenotype relationship in ATS. No single mutation described to date has a higher likelihood of unstable arrhythmias.

How does reduced Kir2.1 channel function lead to arrhythmia susceptibility? Selective I_{K1} blockade in feline purkinje fibers results in action potential prolongation and an increased frequency of spontaneous action potentials [19]. In an elegant in vivo study, gene transfer of a Kir2.1 dominant-negative construct resulted in QT prolongation in adenoviral-transfected guinea pigs as well as spontaneous action potentials in isolated ventricular myocytes [20]. Both studies support the idea that a reduction in I_{K1} leads to the generation of spontaneous ventricular activity. The cellular consequences of reduced I_{K1} have been studied using in silico approaches. Reductions in I_{K1} initially cause mild prolongation of the most terminal foot of the cardiac action potential [21]. Greater reductions in I_{K1} result in the generation of spontaneous action potentials that are triggered by the Na^+/Ca^{2+} exchanger [5, 21, 22]. This is unlike the traditional forms of LQTS whereby prolongation of the plateau phase results in L-type Ca^{2+}-triggered early after-depolarizations. Computer simulations of reduced I_{K1} in virtual left ventricular tissue reveal an increase in action potential duration across the ventricular wall, without an increase in transmural dispersion of repolarization [21]. Thus, the low frequency of *torsades de pointes* arrhythmia in ATS patients may be a consequence of the lack of transmural dispersion of repolarization in the setting of reduced I_{K1}, despite the fact that action potential duration is prolonged. The in silico data are consistent with data from the isolated canine wedge preparation using $BaCl_2$ as a model of ATS [23]. Interestingly, the contribution of I_{Kr} to phase 3 repolarization increased dramatically in the setting of computer-simulated I_{K1} reductions [21]. The consequences of this observation are that patients with ATS may be particularly susceptible to changes in I_{Kr} (such as Herg channel blockers or decreased $[K^+]_o$) given the baseline reduced repolarization reserve and the dependence upon I_{Kr} as a substitute for reduced I_{K1}.

Timothy Syndrome

The first case of what we now call Timothy Syndrome (TS) was reported in 1992 in a German report describing an infant with 2:1

atrioventricular block, prolonged QT interval, syndactyly and subsequent sudden death [24]. The association between severe LQT and syndactyly was further defined in small cohort of three patients, two of whom died of ventricular fibrillation [25]. Mutations in the known LQTS genes were excluded in this population as well as mutations in the coding sequence of the cardiac L-type calcium channel $Ca_v1.2$. Subsequently, novel alternatively spliced $Ca_v1.2$ isoforms were described and a mutation in a splice variant was identified in a cohort of TS patients [26].

Clinical Manifestations of Timothy Syndrome

TS is a rare, primarily sporadic disorder that affects the development of multiple organ systems. Although originally described as severe LQT and syndactyly, the ascertainment of additional subjects revealed a more complex clinical constellation, including congenital heart disease, developmental delay (including autism), abnormal dentition and facial dysmorphic features [26]. One-hundred percent of TS subjects display QT prolongation, syndactyly, baldness at birth and small teeth [26]. The vast majority of subjects display 2:1 AV block in the neonatal period as a consequence of severe LQT. As the sinus rate slows down with advancing age, 1:1 AV conduction ensues. Consistent with the severe degree of QT prolongation, these individuals are at high risk for sudden cardiac death. The pleiotropic manifestations of this disease are a consequence of the wide expression pattern of the alternatively spliced variant of $Ca_v1.2$, including heart, brain, gastrointestinal system, lungs, immune system and smooth muscle [26]. Two cases of atypical TS were recently described (TS2) notable for the absence of the cardinal feature of syndactyly. As discussed below, mutations in the primary $Ca_v1.2$ transcript were identified in this subset of patients [27].

Molecular and Cellular Basis of Timothy Syndrome

A remarkable feature of TS is its molecular homogeneity; all of the original cases occurred as the result of the identical, de novo missense mutation in exon 8A (G406R), an alternatively spliced variant of $Ca_v1.2$. In the heart, approximately 23% of calcium channels are encoded by the exon 8A variant, with 77% encoded by exon 8 [26]. In the heterozygote state, only 11.5% of $Ca_v1.2$ channels carry the G406R mutation; however this causes sufficient calcium channel dysfunction to produce severe QT prolongation. The de novo predilection for the G406R exon 8A mutation is likely related to the high incidence of infant mortality, making its inheritance rare. In TS2, which is characterized by the absence of syndactyly, two mutations in the dominant splice variant exon 8 were identified, G402S and G406R [27]. All TS mutations are localized to the most terminal portion of the S6 transmembrane domain in Domain I.

L-type calcium channels display two forms of inactivation; a calcium-dependent and voltage-dependent inactivation. Expression of TS mutant $Ca_v1.2$ channels in heterologous expression systems revealed that mutant channels displayed near complete loss of voltage-dependent inactivation, while maintaining calcium-dependent inactivation [26, 27]. Computer simulations of the effect of reduced $Ca_v1.2$ voltage-dependent inactivation in the heterozygous state revealed marked action potential duration prolongation and the development of delayed afterdepolarizations (DADs) [26, 27]. These general findings were corrobated in subsequent, more detailed *in silico* analyses [28, 29]. Further analysis of TS1 mutants was performed in virally transfected adult rat ventricular myocytes. This model confirmed a defect in voltage-dependent inactivation, but also implicated increased activity of CaMKII in promoting excessive calcium release from the sarcoplasmic reticulum [30]. Calcium overload resulted in increased activity of the sodium–calcium exchanger (NCX) with secondary development of DADs. Thus, the cellular basis of arrhythmia in TS is reduced voltage-dependent $Ca_v1.2$ inactivation, leading to sustained inward calcium current during the plateau phase of the cardiac action potential, marked action potential prolongation and subsequent QT prolongation. The sustained inward calcium current leads to secondary DADs, ventricular arrhythmias and sudden death, facilitated by increased activity of CaMKII.

What do the TS mutations tell us about the fundamental biophysical gating processes of L-type calcium channels? The molecular mechanisms underlying voltage- and calcium-dependent inactivation in L-type calcium channels are not completely understood, however, distinct regions of the channel appear to differentially regulate each process. The cytoplasmic linker between domain I and II (I-II linker) likely represents an important structural determinant of voltage-dependent inactivation [31]. The I-II linker is proposed to function as a "lid" or inactivating blocking particle that occludes the inner mouth of the channel pore. The TS mutation G406R is located in the S6 C-terminal region of Domain I and likely influences the ability of the adjacent I-II linker to participate in channel inactivation. Substitution of the glycine at position 406 with charged, polar or hydrophobic residues also impairs channel inactivation, implying that glycine is an absolute requirement for voltage-dependent inactivation [27]. Glycine is a flexible amino acid and functions as a hinge in other ion channels. G406 may function as a hinge that allows proper bending of the intracellular inactivation gate.

Although mutant $Ca_v1.2$ channels do not inactivate properly, they remain sensitive to L-type calcium channel blockers with potencies in nanomolar concentrations [26]. This would suggest that calcium channel blockers may prove to be useful therapeutic strategies for TS. Indeed, in a recent case report, Jacobs and colleagues describe a TS2 patient (G402S) in whom verapamil successfully reduced the incidence of ventricular arrhythmias [32]. Over time, the patient developed symptomatic atrial fibrillation that responded favorably to treatment with the nonspecific ion channel blocker ranolazine [33]. Interestingly, in a subsequent report, verapamil did not reduce ventricular arrhythmias but in fact, increased arrhythmias in a TS1 patient [34]. The differential effects of verapamil may be a consequence of the distinct location of the mutation or an isoform-specific effect.

The initial reports of TS described a severely affected cohort with a high incidence of sudden cardiac death and developmental defects. More recently, a less severe phenotype was described in patients mosaic for the TS1 mutation [34]. Mosaicism refers to the presence of genetically distinct populations of somatic and germ-line tissues, with tissue-to-tissue variations that may not follow Mendelian rules of inheritance. This observation raises the possibility that TS phenotype may not be as dismal as previously reported. In addition, some of the previously described *de novo* TS mutations may represent cases of parental mosaicism and warrant careful genotyping of tissue other than peripheral blood lymphocytes.

Summary

The identification of the molecular bases of ATS and TS has advanced our understanding of the fundamental biophysical properties of Kir2.1 and $Ca_v1.2$ channels, respectively. Discerning the cellular mechanisms of these disorders has provided additional insight into the clinical manifestations of each disorder. Kir2.1 channels provide repolarizing current during the most terminal phase of the cardiac action potential. Reduced Kir2.1 channel function causes mild prolongation of action potential duration, resulting in mild QT prolongation. Disrupted $Ca_v1.2$ inactivation results in sustained inward calcium current during the plateau phase, causing marked action potential prolongation and secondarily, marked QT prolongation. Thus, the degree of QT prolongation in each disorder is directly related to the contribution of the disordered ion channel to the various phases of the cardiac action potential. The identification of the molecular basis of TS also allowed for tailoring of treatment strategies. The sensitivity of mutant $Ca_v1.2$ channels to traditional calcium channel blockers paved the way for a limited trial of verapamil to control ventricular arrhythmias. An activator of Kir2.1 channels remains to be discovered, however, such an agent could prove beneficial in the treatment of ATS.

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Short QT Syndrome

Preben Bjerregaard and Ihor Gussak

Abstract

Short QT Syndrome (SQTS) is a rare form of channelopathy with a moderately high risk of SCD, but the syndrome is not well-defined and information about long term follow-up still very scarce. A short QT interval is the main component of the syndrome, but because of an abnormal relationship between the QT interval and the RR interval in SQTS patients, the short QT interval in such patients is often only apparent at heart rates close to 60 bpm. Since routine ECGs are often taken at heart rates faster than that, many patients with SQTS may be missed.

Many mutations have been found responsible for SQTS, but in published families a mutation has only been found in one of every four, who has been genetically tested. Most diagnoses are therefore based upon the clinical presentation, which in 90 % of the cases has included a family member with SCD. The treatment of choice is an implantable defibrillator.

Keywords

Short QT interval • Short QT syndrome • Sudden cardiac death • Cardiac channelopathies • Potassium channel mutations • Atrial fibrillation • Implantable defibrillators

Introduction

Since the discovery of Short QT Syndrome (SQTS) less than 12 years ago [1], a lot have been learned about the latest member of the so-called channelopathies, mainly from case reports and research in a few laboratories and clinical centers especially dedicated to this syndrome. Despite the fact that the total number of published cases has just reached 100, the most important aspects of the syndrome have by now been investigated, latest by the addition of a clinical follow-up study of patients with SQTS (Table 33.1) [2–18]. The population of patients with SQTS will continue to grow as evidenced by the fact, that the first patient found with the syndrome has recently given birth to a child, who also has the syndrome. Most impressive is the list of mutations that has been found, in some cases in only a single individual, and the functional testing of these mutations in patch-clamp experiments and *in silico* has greatly improved

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Table 33–1. Time table for "*First Time Events*" in the short history of short QT syndrome

1999	Discovery of family with SQTS (Bjerregaard P, 1999, personal communication)
2000	Paper describing SQTS (Gussak et al. [1])
2003	High incidence of SCD in families with SQTS (Gaita et al. [2])
	ICD treatment of patients with SQTS (Schrimpf et al. [3])
2004	Mutation found in families with SQTS (Brugada et al. [4])
	Pharmacological treatment of patients with SQTS (Gaita et al. [5])
	Experimental model of SQTS (Extramiana et al. [6])
2005	Review article of mechanism, diagnosis and treatment of SQTS (Bjerregaard et al. [7])
	Successful prevention of SCD by an ICD in patient with SQTS (Schimpf et al. [8])
	SQTS presenting as bradycardia *in utero* (Hong et al. [9])
	SQTS website: shortqtsyndrome.org (Bjerregaard and Collier [10])
2006	Publication of the prevalence of a very short QT interval in the general population (Gallagher et al. [11])
2007	Overlap syndromes of SQTS and Brugada Syndrome (Antzelevitch et al. [12])
	Mutation linking SQTS to SIDS (Arnestad et al. [13])
	Population study of prevalence and prognostic significance of a short QT interval (Anttonen et al. [14])
2008	Animal model: Zebrafish with SQTS (Hassel et al. [15])
2009	Safety issue warning regarding drug induced shortening of the QT/QTc interval (Holbrook et al. [16])
2011	Diagnostic criteria to facilitate clinical recognition of SQTS (Gollop et al. [17])
	Long-term follow-up of patients with SQTS (Giustetto et al. [18])

our understanding of the electrophysiology behind the short QT interval. It is still a challenge to make the diagnosis with certainty and even more of a challenge to rule it out in patients with borderline low QT interval.

Definition and Terminology

By its own definition, any clinical syndrome is a combination of signs and symptoms that occur together and characterize a particular abnormality. In this context, short QT syndrome (SQTS) is best defined as an inheritable, primary electrical heart disease, that is characterized by (*a*) a short QT interval (Fig. 33.1) and (*b*) paroxysmal atrial and/or ventricular tachyarrhythmias resulting from an accelerated cardiac (atrial and ventricular) repolarization due to congenital (genetically heterogeneous) cardiac channelopathies. It requires exclusion of patients with secondary short QT interval [19], and without documentation of associated arrhythmogenic complications in the patient or the patient's family a short QT interval is only an

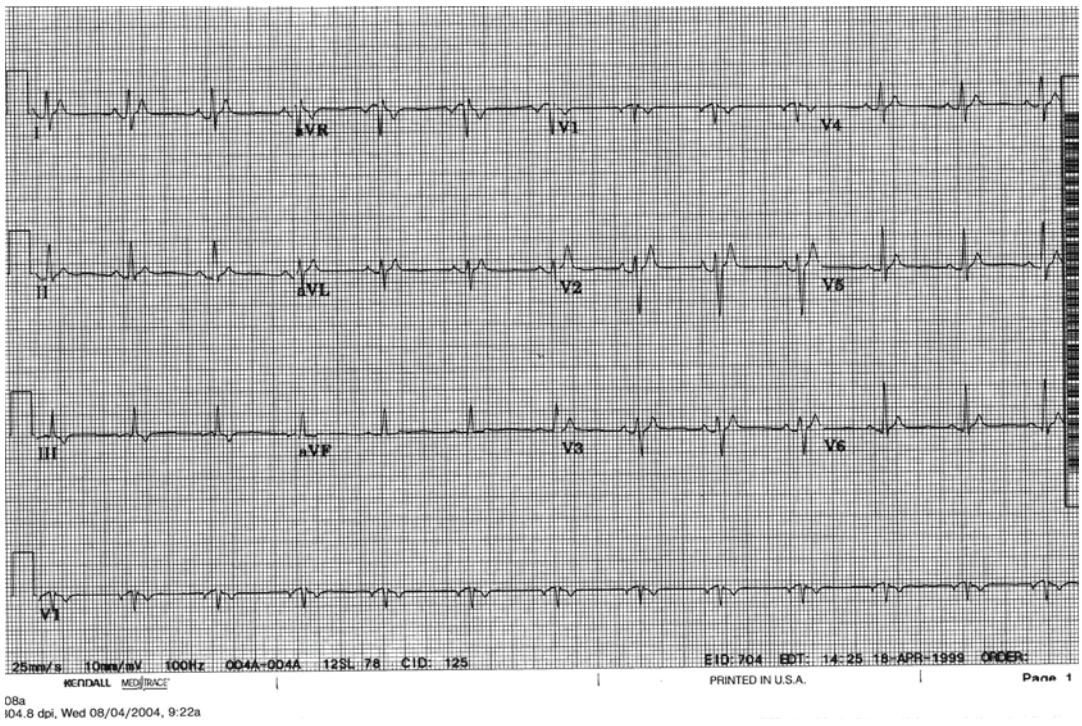

Figure 33–1. Twelve-lead ECG from a patient with SQTS based upon a short QT interval and paroxysmal atrial fibrillation. The ECG shows sinus rhythm at a heart rate of 75 beats/min. The QT interval is 240 ms

ECG abnormality. Unexplained sudden cardiac death (SCD) in a family with members having SQTS would normally be considered a manifestation of SQTS unless proven otherwise.

Before the discovery of SQTS there were no well-defined lower limit for the QT interval, and normal limit for the QT interval was usually given only as an upper limit. Based upon data from Rautaharju et al. study from 1992 of the QT interval in 14,379 healthy individuals, the lower limit for the QT interval (two standard deviations below the mean predicted value) at a heart rate of 60 bpm was 360 ms [20]. More recent studies of apparently normal people have found variable results for the lower limit of QTc probably influences by the population studied and the method used for measuring the interval [11, 14, 21–25], but arguments could be made for a lower limit of the QTc interval of ≤360 ms for men and ≤370 ms for women realizing that these are approximate figures and not a great help in finding patients with SQTS. In our review of the worldwide population of patients with SQTS in 2008 [26] we found among 49 published cases the mean QT interval to be 282 ± 63 (range: 210–340) ms and the mean QTc to be 305 ± 42 (range: 248–345) ms. In a 2011 follow-up study from Europe [18] of 49 patients the QTc was 314 ± 46 ms. In recent years some patients with longer QT intervals have been published as having SQTS (*vide infra*). Since the QT interval in patients with SQTS varies very little with changes in heart rate [7, 27, 28], it is important to realize, that normal correction formulas for the QT interval at heart rates greater than 60 bpm will grossly overestimate, what the OT interval will be at a heart rate of 60 bpm. ***Paradoxically, in patients with SQTS the QTc varies with HR. The faster the HR the longer the QTc and at heart rates 80–90 bpm many patients with SQTS will have a normal QTc.*** At heart rates much above 60 bpm, as often seen in children, there is, therefore, a risk of missing patients with SQTS, when QT correction formulas are used. ***Therefore, SQTS cannot be defined by QTc. In order to get a reliable assessment of the QT interval in a patient suspected of having a short QT interval and possibly SQTS every effort should be made to get an ECG at a heart rate as close to 60 bpm as possible, even by using a drug if necessary.*** The lack of adaptation of QT interval to change in HR in patients with SQTS has been suggested as a diagnostic tool in work up of patients suspected of having SQTS. It is likely, however, that anybody with a short QT interval will have a QT/RR slope lower than people with normal QT intervals. More research in this area is needed before it can be recommended as a reliable diagnostic tool especially in borderline cases, where it would be most needed. Another common finding in the ECG of patients with SQTS in particularly in precordial leads, is tall, peaked T waves, most commonly symmetrical, but in patients with *KCNJ2* mutation asymmetrical with a steep descent [29, 30]. Some studies have pointed out, that the T_{peak}–T_{end} interval and T_{peak}–T_{end}/QT ratio is prolonged in patients with SQTS compatible with an augmented transmural dispersion of repolarization [6, 18].

Since the discovery of SQTS and the significance of a short QT interval the question has been: "when does a short QT interval become clinically significant?"

The first indication of the prognostic significance of a relatively short QT interval was from a study in 1993 by Algra et al. [31]. Out of 6,693 patients who underwent 24 h Holter monitoring and followed for 2 years, patients with a QTc < 400 ms had a 2.4-fold increase in sudden death rate compared to patients with a QTc of 400–440 ms. This was slightly more than patients with a QTc > 440 ms, who had a 2.3-fold increase. The authors argued for the possibility of their finding being a true pathophysiological phenomenon with a relative short QT interval possibly leading to life-threatening arrhythmias.

Evidence that shortening of the QT interval may play a role for the occurrence of idiopathic VT was provided by Fei and Camm in 1995 [32]. Twenty-four hour Holter monitoring was used to detect 60 episodes of monomorphic repetitive ventricular tachycardia in ten patients. Analysis of three consecutive QT intervals immediately before the onset of ventricular tachycardia found these QT intervals significantly shorter than the intervals measured 40 min before at the same heart rates (342 ± 34 vs. 353 ± 35 ms, $P < 0.001$). Of the 60 episodes the QT intervals were shortened in 45 (75 %) compared to the intervals 40 min earlier. The shortening was explained by

sudden parasympathetic withdrawal leading to sympathetic predominance and thereby QT shortening. The shortening was suggested by the authors to play an important role in the pathogenesis of idiopathic VF.

In 1999 we presented a case of paradoxical shortening of the QT interval to 216 ms during severe transient bradycardia in a child with recurrent cardiac arrest and discussed deceleration-dependent shortening of the QT interval as a trigger of arrhythmic events [33]. We proposed activation of Ik_{Ach} due to an unusually high vagal discharge to the heart as a possible mechanism responsible for both slowing of the heart rate and shortening of the QT interval.

Visken et al. [34] compared ECGs of 28 patients with idiopathic VF (17 men and 11 women, age 31 ± 17 years) to those of 270 age- and gender-matched healthy controls. They found that the QTc of males with idiopathic VF was shorter than the QTc of healthy males (371 ± 22 ms vs. 385 ± 19 ms, P = 0.034), and 35 % of the male patients had QTc < 360 ms (range 326–350 ms) compared to only 10 % of male controls (345–360 ms). However, no such differences were found among women. They suggested that QTc intervals shorter than 360 ms might entail some arrhythmic risk.

The limited data available seem to indicate, that the extent of QT interval shortening is associated with the probability of an adverse outcome. In the largest study so far by Giustetto et al. [18] the QTc interval was 300 ± 20 ms in ten patients with cardiac arrest and 309 ± 19 ms in 19 patients with no such event. In our review of the world-wide population of patients with SQTS [26], in 16 patients with SCD or aborted SCD the QT intervals were 271 ± 33 ms compared to 291 ± 55 ms in 26 patients without SCD, aborted SCD and atrial fibrillation. In seven patients with atrial fibrillation only, the QT intervals were 265 ± 49 ms. From these data it is also apparent that the great majority of patients published so far with SQTS have had very short QT/QTc intervals, but because of sporadic cases with somewhat longer QT intervals [12, 28] we are not at a point where the diagnosis can be based upon the duration of the QT interval alone. Even though recently published proposed diagnostic criteria for the diagnosis of SQTS [17] was met by some criticism [35, 36], they very well points out features besides the QT interval, that has to be taken into consideration when making a diagnosis of SQTS, such as clinical and family history in addition to genotyping if possible.

Table 33–2. Gene mutations associated with SQTS

Mutation	Gene	# Families	# Patients
Gene mutations associated with SQTS			
N588K	*KCNH2*	5	≥12
T618I	*KCNH2*	2	≥2
E50D	*KCNH2*	1	2
V307L	*KCNQ1*	1	1
V141M	*KCNQ1*	2	2
I274V	*KCNQ1*	1	1 (SIDS)
D172N	*KCNJ2*	1	2
Gene mutations associated with Short QT and ST-segment changes			
R1135H	*KCNH2*	1	3
S481L	*CACNB2*	1	6
G490R	*CACNA1C*	1	2
A39V	*CACNA1C*	1	1
S755T	*CACNA2D1*	1	3

Genetic Basis of SQTS

SQTS is a genetically heterogeneous disease. In the study by Giustetto et al. [18] the yield of genetic screening in SQTS was 23 % of the investigated index patients in their study. So far a genetic mutation has been found in at least 22 patients from 13 families with SQTS [4, 9, 13, 29, 37–40], and in 15 patients from five families with a short QT interval and ST-segment changes (Table 33.2) [12, 41, 42]. Patients in the later group have ST changes in precordial leads somewhat similar to Brugada Syndrome patients either spontaneously or by provocation, and since there is no clear basis on which to select one syndrome over another, they have been looked upon as a distinct clinical entity. With QTcs ranging from 330 to 360 ms the QT interval in these patients has generally not been as short as reported for patients with only SQTS. Initially mutations in patients with SQTS were found in three genes (*KCNH2*, *KCNQ1* and *KCNJ2*) encoding for potassium channels and the respective syndromes were called SQT1, SQT2 and SQT3 based upon the chronology of their discovery.

In the first two families from Europe with SQT1 reported in 2004 by Gaita et al. [2] two different

missense mutations (C1764A and C1764G) were later discovered and found to result in the same amino acid change (N588K) in the S5-P loop region of the cardiac I_{Kr} channel *KCNH2* (HERG) [4]. Within the same year the first patient with SQT2 was reported, when a mutation (V307L) in the *KCNQ1* gene encoding the I_{Ks} channel KvLQT1 was found in a 70 year old male with idiopathic ventricular fibrillation and a short QT interval [38]. Another *KCNQ1* mutation was later found in two unrelated patients with bradycardia *in utero* and born with atrial fibrillation and high degree atrio-ventricular block in addition to a very short QT interval [9]. Genetic testing showed a missense mutation, G to A substitution at nucleotide 421 (g421a). This mutation results in substitution of valine by methionine at position 141 (V141M) adjacent to a previously described S140G mutation for familial AF [43]. Finally, in 2007 a third gain-of-function mutation (I274V) was found in *KCNQ1* in a patient with SIDS [44].

In 2005 in an Italian family a *KCNJ2* gene mutation was found in a 5-year old girl with a short QT interval (SQT3). Genetic analysis by Priori et al. led to the identification of a single base pair substitution (G514A) in *KCNJ2*, resulting in an amino acid change from aspartic acid to asparagine at position 172 in the Kir2.1 potassium channel (I_{K1}) [29]. From these initial findings an interesting concept emerged, that LQTS and SQTS had closely related genetic basis and could be considered "allelic diseases". Indeed, all three SQTS genes were known to also cause LQTS. Since then eight additional mutations responsible for shortening of the QT interval have been found (Table 33.2).

Cellular Basis of Arrhythmogenesis in SQTS

Mutations effecting potassium channels all leads to a gain-of-function. There is general agreement that gain-of-function from the N588K-HERG mutation in patients with SQT1 leading to an increase in the repolarizing currents active during phase 2 and 3 of the AP stems from severely compromised inactivation. McPate et al. found a ~ +60 mV positive-shift in voltage dependence of I_{HERG} inactivation [45]. Not all cells are affected to the same degree and Cordero et al. found that ventricular Purkinje cells were minimally affected [46]. Grunnet et al.'s patch clamp experiments showed that the biophysical characterization of the short QT mutation HERG-N588K was compatible with a mixed gain-and loss-of-function [47]. The most prominent loss-of-function property was reduced tail currents, but also slower activation and faster deactivation kinetics leading to reduced ability to conduct current at the end of repolarization. The authors pointed out that in patients carrying HERG-N588K the loss-of-function of repolarization current and diastolic HERG current might be at least as pro-arrhythmic as the gain-of-function of plateau current. All information on the likely consequences for I_{Kr} -kinetics of N588K-HERG mutation comes from studies of the HERG1a isoform. Recent evidence suggests, however, that native cardiac I_{Kr} may not be comprised of HERG1a alone, but rather of HERG1a heteromerically expressed with an alternative transcript, HERG1b, an isoform with a truncated N-terminus. This lead McPate el al. to conduct a study to determine the effects of the N588K-HERG SQT1 mutation on co-expressed HERG1a/1b channels [48]. Their data showed that the inactivation-attenuation effects of the N588K mutation were markedly greater when co-expressed HERG1a and 1b were studied, than when HERG1a alone was studied. The study also confirmed the differential effect the N588K-HERG mutation has on current during ventricular and Purkinje AP's as initially suggested by data from the study by Cordeiro et al. [46] and McPate et al. [45].

Another mutation, which effects the plateau phase of the action potential is V307L-KCNQ1 seen in SQT2 patients. Bellocq et al. did conventional patch-clamp experiments using COS-7 cells and showed faster activation at more negative potentials for V307L channels compared to wild-type (WT) while kinetics for deactivation were similar [38]. Computer simulations with findings from the patch-clamp experiments incorporated clearly showed diminished AP duration favoring the association of a short QT interval with the V307L-KCNQ1 mutation.

Harchi et al. using perforated-patch voltage-clamp recordings (Chinese Hamster Ovary cells) at 37 °C of whole-cell current with epicardial ventricular AP waveform carried by co-expressed *KCNQ1* and *KCNE1* showed a marked (−36 mV) shift in half-maximal activation for V307L compared to WT-KCNQ1 [49]. In contrast to Bellocq et al. [38], they also found a significant slowing of current deactivation. They also looked at the effect of the mutation on atrial cells and found peak repolarising current significantly augmented for V307L-KCNQ1 compared to WT for both ventricular and atrial AP commands, consistent with an ability of the V307L mutation to increase repolarising I_{Ks} in both regions. Although atrial fibrillation was not reported for the patient in whom the V307L-KCNQ1 mutation was first identified [38], SQTS patients especially with the N508K-HERG mutation do experience atrial fibrillation at a higher incidence than expected by chance alone [18]. Atrial fibrillation was also part of the clinical picture in patients with the V141M-*KCNQ1* mutation [9, 26]. These patients presented with bradycardia *in utero* and were born with a short QT interval and atrial fibrillation with a very slow heart rate suggesting high degree AV block. The inability to restore sinus rhythm in one of the patient and the inability to maintain sinus rhythm for more than a few hours in the other patient suggested possibly sick sinus syndrome as well. Hong et al. [9] injected oocytes from *Xenopus laevis* with WT or *V141M-KCNQ1* cRNA with or without KCNE1 cRNA and 2–3 days later exposed them to two electrode voltage clamp recordings. The V141M mutation did not noticeably alter the gating of KCNQ1 channels expressed alone in oocytes. The WT-*KCNQ1*/*KCNE1* channels exhibited a voltage-dependent threshold of activation near −50 mV and activated very slowly. In sharp contrast, the *V141M-KCNQ1*/*KCNE1* channel current developed instantly at all voltages tested, consistent with the interpretation that these channels were constitutively open. Computer modeling showed decrease in peak voltage and shortening of APD consistent with shortening of the QT interval. The effect of the V141M gain of function mutation was also modeled in a computer model of rabbit sinoatrial node cells. The results indicated that the enhanced outward I_{Ks} causes cessation of spontaneous activity and a stabilization of the resting membrane potential at a level positive to the normal maximum diastolic potential of these cells. The exact mechanism behind the bi-nodal dysfunction seen in these patients needs, however, further exploration. A third mutation in *KCNQ1* has been considered a possible cause for sudden infant death syndrome. In a patient with sudden infant death Arnestad et al. [13] found a 1,274 V-*KCNQ1* mutation and Rhoades et al. [44] presented electrophysiological data from patch clamp recordings (Chinese hamster ovary cells) showing that I274V-*KCNQ1* in the presence of *KCNE1* causes gain of function in I_{Ks} characterized by increased current density, faster activation, slower deactivation, and accumulation of instantaneous current during repeated stimulation. To test the hypothesis that I274V may promote a short QT syndrome phenotype, computerized modelings of ventricular action potentials were performed comparing WT-Iks to heterozygous I274V-Iks. At all cycle length the AP was shorter for I274V-Iks supporting the prediction that I274V-KCNQ1 will cause a short QT phenotype, and, therefore, may be a plausible explanation for sudden death in an infant carrying this mutation.

SQT3 patients are characterized by a mutation in the KCNJ2 gene [29]. Whole-cell patch-clamp studies of the heterologously expressed human D172N channel have demonstrated larger outward I_{K1} than the WT at potentials between −75 and −45 mV, with the peak current being shifted in the former with respect to the later (WT, −75 mV; D172N and −65 mV). Co-expression of WT and mutant channels to mimic heterozygous condition of the proband has yielded an outward current that is intermediate between WT and D172N. The authors hypothesized that the tall and asymmetrical T-waves with an exceedingly rapid terminal phase seen in these patients and not seen in SQT1 or SQT2 patients might be related to a more sudden acceleration of the final phase of action potential repolarization in patients with the D172N mutation.

Shortening of refractoriness is one of the key elements in the re-entry mechanism behind many tachy-arrhythmias and likely the main reason for the increased propensity to atrial and ventricular fibrillation seen in SQTS, but as pointed out, it has also been shown that the abbreviation of the action potential can effect

different cells differently leading to dispersion of refractoriness as an additional arrhythmogenic factor [6, 46, 50]. It is conceivable, however, that the mechanisms that lead to electrical instability and eventually results in VF in patients carrying mutations in HERG or KvLQT1 would be different from those resulting from gain-of-function substitutions in Kir2.1. The discrepancy between heart rate and QT interval duration is most pronounced during bradycardia and at least two observations suggest that the potential for developing life-threatening tachy-arrhythmias is highest at slow heart rates. In the study by Sun et al. [40] all four patients from a family with SQTS dying suddenly, died during sleep, and the first patient reported saved from VF by an ICD also had the episode at night during sleep. However, in the follow-up study by Giustetto et al. it was not possible to find a uniform trigger for arrhythmic events since cardiac arrest and syncope occurred both at rest and during effort [18].

Mutations effecting L-type calcium channels all lead to a loss-of-function due to a trafficking defects with a decrease in amplitude of the inward calcium current causing both a short QT interval and various degrees of ST-segment changes [12, 41, 42]. Similar overlap syndromes among channelopathies are well known from long QT syndrome and Brugada syndrome [51] and recently also early repolarization syndrome and short QT syndrome. Watanabe et al. [52] studied the later combination in three cohorts: (1) 37 SQTS patients (12 new patients with QTc ≤ 330 ms plus an arrhythmic event and/or genetic mutation, and 25 patients with SQTS from the literature), (2) 44 control cohort with QTc ≤ 330 ms and no symptoms, and (3) 185 control cohort with normal QTc and no sign or symptoms of heart disease. They found the prevalence of early repolarization to be 65 % in cohort 1, 30 % in cohort 2 and only 10 % in cohort 3. In a multivariable model early repolarization was associated with arrhythmic events in the SQTS cohort whereas neither QT nor QTc duration were associated with arrhythmic events, and they concluded that there is a high prevalence of early repolarization in patients with SQTS, and early repolarization may be useful in identifying risk of cardiac events in patients with SQTS.

Electrophysiologic Findings

Except for sporadic case reports the main information about electrophysiology studies in patients with SQTS comes from two studies. Watababe et al. [52] did not report any detailed results from programmed stimulation of 18 patients with SQTS, but stated there was no difference in inducibility of ventricular tachyarrhythmias between patients with arrhythmic events (73 %) and those without arrhythmic events (72 %). In the long-term follow-up study by Giustetto et al. [18] 28 patients underwent an electrophysiologic study. The ventricular effective refractory periods at the right ventricular apex were shortened and varied between 140 and 200 ms (mean: 166 ± 21 ms). No difference was found between patients with a history of cardiac arrest or syncope and those without. VF was induced in 16 patients (57 %), in seven by mechanical induction during catheter positioning. The atrial effective refractory periods also were shortened and ranged between 120 and 200 ms (mean: 163 ± 22 ms). Atrial fibrillation was induced in 36 %. Patients with HERG mutation had shorter refractory periods than those without. From these data it is apparent that an electrophysiologic study is not useful in predicting cardiac arrest having a sensitivity of only 37 %.

Clinical Manifestations and Clinical Course

It was an article from 2003 by Gaita et al. [2] that brought attention to the high incidence of SCD in families with short QT interval. Both families were later found to have a *HERG* gene mutation. In a family from Italy six members had died suddenly. Of those six, one had documented short QT interval. Two living members also were known to have short QT interval. In the other family, which was from Germany, three members had died suddenly, one with documented short QT interval in an ECG taken prior to death. Two members of that family with short QT interval were alive. The most comprehensive report about clinical manifestations of SQTS can be found in the recently published follow-up study from Giustetto et al. [18] of 53 patients with SQTS.

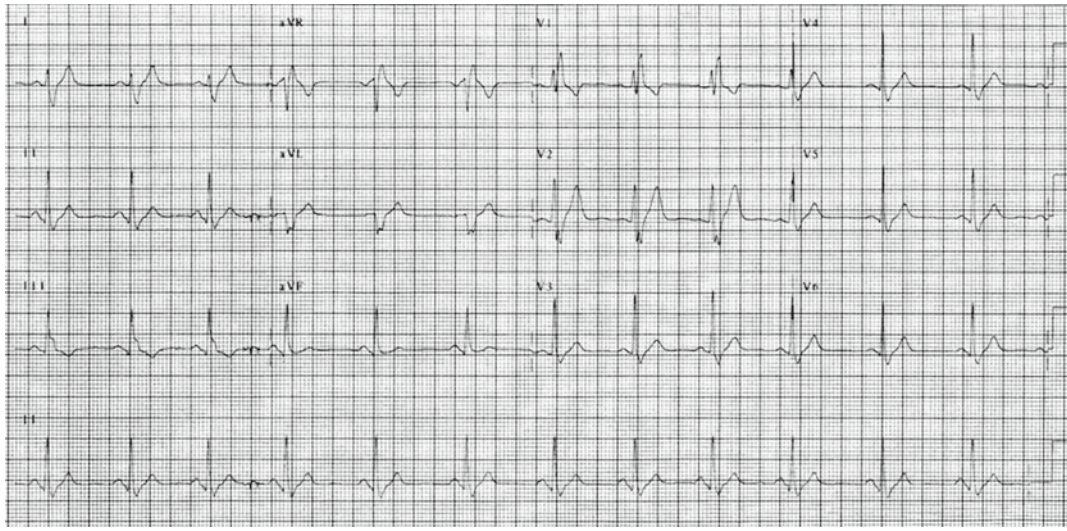

Figure 33–2. Twelve-lead ECG from asymptomatic female with SQTS based upon a short QT interval and sibling with aborted SCD due to SQTS. The ECG shows sinus rhythm at a heart rate of 81 beats/min, RBBB and R axis deviation. The QT interval varies between different leads from 320 to 360 ms. Twenty-four-hour Holter monitoring showed a QT/RR slope of 0.1 both during the day and during the night suggesting minimal variability in QT interval with changes in heart rate. The person had an ICD implanted for primary prevention of SCD

Almost 90 % of the patients had a personal or familial history of SD, and 33 (62 %) had symptoms at presentation: 4 had died suddenly, 13 had an aborted SCD, 8 had syncope and 13 palpitations. Cardiac arrest had a similar prevalence in males and females (35 % vs. 30 %), but in patients carrying a *HERG* mutation a greater proportion of affected females (55 % vs. 18 %; $p = 0.04$) and a higher prevalence of atrial fibrillation (36 % vs. 3.6 %; $p = 0.02$) were observed compared with non-*HERG* patients. The high incidence of atrial fibrillation in patients with *HERG* mutation in this study is in accordance with observations in the first family with SQTS, where all four members with SQTS and a HERG mutation had atrial fibrillation [39]. A few cases of SQTS have been reported in children. Morphett JAM [53] reported a 3 week-old neonate who presented with episodes of apnea. The ECG showed QT-interval of 210 ms. During ECG monitoring sinus node dysfunction in terms of sinus bradycardia, sinus arrest and atrial and ventricular standstill was observed. The case was interpreted as SIDS with SQTS in which sinus node dysfunction was an important aspect of the pathophysiology. In the study by Giustetto et al. [18] one of the patients had a history of aborted SCD at the age of 8 months (mutation unknown). These two cases combined with the finding of a gain-of-function *KCNQ1* mutation in a patient with SIDS [13], suggest that SQTS is a possible explanations for sudden death in infants. Atrial fibrillation with high degree AV block and a heart rate mostly in the 40s has been found in two babies with bradycardia *in utero* and a V141M-*KCNQ1* mutation [9, 26]. DC cardioversion was unable to restore persistent sinus rhythm suggesting a sick sinus node as well as AV nodal disease. Conduction system disease has also been present in other patients with SQTS. Left anterior hemiblock was observed in two asymptomatic subjects with SQTS and RBBB in two other patients, including a 25-year old sister to a patient with SQTS and aborted SCD (Fig. 33.2) [26]. She also had left posterior hemiblock with a QRS duration of 140 ms and the QT interval at a heart rate of 54 beats/min was 340 ms. The occurrence of this type of conduction disturbance in young adults is unexpected and suggests that conduction system disease may be part of the clinical picture of SQTS and possibly related to specific gene mutations. The two patients with left anterior hemiblock both had a HERG mutation [18], where as the mutation in patients with RBBB and RBBB + LPH is unknown.

During a follow up of 47 patients over 64 ± 27 months by Giustetto et al. [18] there were

no death. Among 24 patients with an ICD there were two, who was successfully defibrillated and three who had episodes of non-sustained ventricular tachycardia. Among 14 previously asymptomatic patients who received no treatment there was one who had a syncopal episode. Two patients who had previously had syncope, but opted for no treatment were asymptomatic during follow-up. If we assume that the two patients who were appropriately shocked by the defibrillator would have died without being treated, the incidence of SCD in the study by Giustetto et al. [18] is compatible with an intermediate risk of SCD in a mixed population with SQTS of approximately 0.8/100 pt-yrs. Just like in patients with LQTS [54] it is very likely that factors such as gender, QT-interval duration, type of mutation and a history of syncope will all have an impact upon the risk for the individual SQTS patient.

Treatment

The Implantable Cardioverter-Defibrillator (ICD)

Patients with SQTS belong to a category of patients at high risk of sudden cardiac death who are all candidates for prophylactic ICD implantation as long as features for individual risk stratification are not known and the benefit from medical treatment not proven. A characteristic finding in some patients with SQTS is tall peaked T waves, which may lead to double counting of the ICD and inappropriate shocks. This was encountered in four of the very first SQTS patients who received an ICD [3], but not been a clinical problem since then because of the ability to program ICDs in such a way that it can be avoided. Other complications to ICD treatment in patients with SQTS was presented in the long-term follow-up study from the European SQTS registry [18]. Among 24 patients who received an ICD 14 (58 %) had complications. As already mentioned four had T wave oversensing, but there were no recurrences following reprogramming of the ICD. Four had inappropriate shocks during supraventricular tachycardias, four patients needed ICD lead replacement: three because of lead fracture and one because of infection of the ICD system. One patient had early replacement of the ICD because of a recall. Finally, there was one patient who had severe psychological distress from having an ICD. During the approximately 5 years of follow-up, two patients were successfully defibrillated. One had initially presented with cardiac arrest and the other with syncope.

Pharmacological Therapy

Antiarrhythmic therapy in patients with SQTS has mainly been necessary for paroxysmal atrial fibrillation and as prophylaxis against ventricular tachycardia or fibrillation in patients with an ICD in order to reduce the number of shocks to a minimum. In the study by Giustetto et al. [18] hydroquinidine was initially used in 22 patients, but in ten it had to be discontinued because of poor compliance in six, no QT prolongation in two and gastrointestinal side-effects in two. Twelve patients (three with history of cardiac arrest, three with history of syncope and six previously asymptomatic) took hydroquinidine for a mean period of 76 ± 30 months without having any arrhythmic events. The use of quinidine was based upon limited data from previous studies. In 2004 Gaita et al. [5] had tested Flecainide, Sotalol, Ibutilide and Hydroquinidine in six patients with SQTS. Flecainide, Sotalol and Ibutilide did not produce any significant QT prolongation. Only hydroquinidine prolonged the QT interval from 263 ± 12 to 362 ± 25 ms with prolongation of the ventricular effective refractory period to >200 ms and VF was no longer inducible. The slight prolongation of the QT interval following Flecainide was mainly due to QRS prolongation. The lack of QT prolongation following selective I_{Kr}-blocking agents like Ibutilide and Flecainide suggested that the (N588K) mutation in the KCNH2 channel in these patients with SQT1 might have caused loss of some of the physiologic regulatory mechanisms, and the ion channel was no longer sensitive to a drug that normally has a specific action on it. Quinidine was recommended as the drug of choice for medical therapy while Flecainide because of some increase in the effective refractory period could be the second choice. Wolpert et al. [27] later performed exercise testing of quinidine in three patients with SQTS caused by a mutation in HERG and showed that the

linear relationship between QT_{peak} and increasing heart rate seen in normal persons did not exists in patients with SQTS and the slope of QT_{peek} in these patients was much less steep than in a control group. Quinidine was, however able to bring the HR/QT relationship in patients with SQTS close to normal. Recently Pirro et al. [55] reported a 5-year old child with SQTS and an N588H-*HERG* mutation who had been on hydroquinidine since she was 9 days old. She was followed by frequent ECG and plasma concentration of hydroquinidine with a target QTc interval >360 ms and a plasma concentration between 0.6 and 2.0 μg ml^{-1}. No cardiac symptoms or major side effects were observed during follow-up. Sun et al. tested the effect of quinidine and Sotalol on the mutant T618I-HERG channels expressed in HEK 293 cells and found for both drugs a much smaller loss of inhibitory effect than previously shown on N588K-HERG channels suggesting that SQTS patients with the T618I mutation may not be resistant to these drugs. Disopyramide is another drug considered as therapy for SQTS patients. McPate et al. [56] used whole-cell patch clamp recordings from Chinese Hamster Ovary cells expressing HERG with a N588K mutation to demonstrate, that the HERG-blocking potency of disopyramide was reduced only 1.5-fold. Since other studies had shown that Quinidine's blocking effect of N588K-HERG channels was reduced 5.8-fold and Sotalol's 20-fold, the study provided a rational basis for further evaluation of disopyramide as a treatment for SQTS. There are, however, at this point only a few case reports about the clinical effect of disopyramide in patients with SQTS and the results have been mixed [18, 57, 58]. Other drugs used sporadically with some success includes amiodarone [59], and propafenone [7, 60], but as with any of the other drugs, there are just not enough patients with SQTS to make drug testing possible.

Concluding Remarks

Everything stated about SQTS has to be seen in light of the fact that patient number 100 was published just recently and the fact that most of our knowledge stems from patients who have very short QT and QTc intervals (<340 ms) [18, 26]. Much more information is needed before our current knowledge about SQTS can be applied to patients with longer, but still short QT intervals, regarding risk of arrhythmic events and heritability, and we will have to wait quite a bit longer before more specific criteria for diagnosing and treating this syndrome can be made. The importance of a short QT interval in the setting of other arrhythmia syndromes such as Brugada Syndrome and early repolarization syndrome is still unknown. In the meantime it would seem prudent that work up of patients with a short QT interval is done in consultation with centers with special interest and knowledge of QT interval related diseases in order to avoid some of the diagnostic miscues observed in patients with congenital long-QT syndrome [61]. If possible, genetic testing should always be done whenever SQTS is suspected. If it is positive, it is a great help in making the diagnosis, also in family members. Clinical situations with secondary shortening of the QT interval are rare, but needs to be ruled out [19]. It is important to take several ECGs at different heart rate and especially as close to 60 bpm as possible and attempts should be made to evaluate the QT/RR relationship either by stress-testing or Holter monitoring. Minimal change in the QT-interval reflecting in an increase in QTc with an increase in heart rate is typical for SQTS. The presence of very tall and peaked T waves especially in precordial leads favors a diagnosis of SQTS, and the same is true for very short atrial and ventricular refractory periods obtained by an electrophysiologic study. The diagnostic and prognostic value of VF or VT induction by programmed electrical stimulation is questionable, but induction of VF by simple positioning of the electrode-catheter in the RV is a phenomenon rarely seen in a normal heart, but a common occurrence in patients with SQTS [7, 18]. Once the diagnosis of SQTS is made the treatment of choice is an ICD. Medical treatment with QT prolonging antiarrhythmic drugs may offer some protection against SCD and several drugs have shown some therapeutic benefit and so far without any proarrhythmic side effect in SQTS patients. They, therefore, could be alternatives to an ICD in patients who do not want to have an ICD or in small children where implantation of

an ICD may have a high risk. Non-cardiac side effects to quinidine and difficulties in getting the drug anymore may, however, hinder its use. Other anti-arrhythmic drugs which have been used in isolated patients with SQTS includes disopyramide, amiodarone, propafenone and sotalol.

Even though the incidence of life-threatening ventricular tachy-arrhythmias during follow up of patients with SQTS so far has been low, it is important to realize that most of the patients published have been relatively young and only followed for a few years. The risk important to these patients is the risk during a lifetime, and that may be quite high.

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34
Progressive Cardiac Conduction Disease

Jean-Jacques Schott, Flavien Charpentier, and Hervé Le Marec

Abstract

Cardiac conduction disease (CCD) is a common medical problem, with about 3 million people worldwide with pacemakers and 600,000 implanted each year. Despite its prevalence, very little is known about the molecular pathogenesis of CCD in the general population. Therefore, investigators have turned to cases of syndromic and isolated familial conduction system disease to gain insights.

Isolated familial CCD can result from a diverse set of genetic mutations rather than a single unifying cause or pathway. Mutations in ion channels *SCN5A*, *SCN1B* and *TRMP4* encoding respectively the pore forming cardiac sodium channel Nav1.5, the Nav1.5 auxiliary regulatory subunit and a monovalent-permeable, nonselective cation channel have been identified in several families. The disease-causing mechanisms are loss of function in abnormal *SCN5A* and *SCN1b* channel whereas abnormal TRPM4 channel impairs endocytosis and leads to elevated TRPM4 channel density at the cell surface corresponding to a gain-of-function mechanism. Abnormal cell-to-cell communication due to a missense mutation in *GJA5* encoding Connexin 40 also causes CCD in rare cases.

Besides isolated CCD, many diseases such as dilated or restrictive, cardiomyopathies associate cardiac conduction defects. Mutations in *Nkx2.5*, a cardiac specific transcription factor, Lamin A/C, a gene encoding a nuclear membrane protein and *PRKAG2*, a gene encoding a protein kinase subunit also lead to CCD.

In addition to Mendelian diseases caused by rare variants with strong effects, common variants can also impact electrocardiographic traits in the general population. Multiple associations identified loci to pathways with established role in ventricular conduction, transcription factors and calcium-handling proteins.

Altogether, CCD appears to be a phenotypical and genetically heterogeneous condition. The number of ways that the conduction system may fail speaks to the underlying complexity of this highly specialized tissue that is only now beginning to be unraveled by genetic investigations.

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Introduction

Cardiac conduction defect (CCD) is a serious and potentially life-threatening disorder [1] and belongs to a group of pathologies with an alteration of cardiac conduction through the atrioventricular (AV) node, the His-Purkinje system with right or left bundle branch block and widening of QRS complexes. Ultimately CCD can lead to complete atrioventricular block (AV block) and cause syncope and sudden death [1, 2]. Originally CCD was considered as a structural disease of the heart with anatomic changes in the conduction system underlying abnormal impulse propagation. In a substantial number of cases however, conduction disturbances are found to occur in the absence of anatomical abnormalities. In these cases functional rather than structural alterations appear to underlie conduction disturbances. These functional defects are called 'primary electrical disease of the heart [3–6]. The pathophysiological mechanisms underlying CCD are diverse but the most frequent form of CCD is a degenerative form also called Lenègre-Lev disease [7, 8] (idiopathic bilateral bundle-branch fibrosis) and represents a major cause of pacemaker implantation worldwide. Pedigrees showing clustering of CCD have been described suggesting autosomal dominant inheritance with either early or late onset of atrioventricular conduction disease.

This chapter aims to review the clinical features of isolated cardiac conduction defects and to focus on the most recent genetic discoveries.

First Description of Cardiac Conduction Defect

Morgagni [9] in the seventeenth century was probably the first to report recurrent fainting episodes in a man to a simultaneous observed slow pulse rate. In the nineteenth century Adams [10] and Stokes [11] made similar observations. The first known report of an Adams-Stokes attack combined with ECG recordings came from van den Heuvel [12] who described a case of congenital heart block. Lenègre [7] and Lev [8] combined ECG, clinical observations and detailed *post mortem* studies of the heart whereby they proved their direct relationship in the 1960s. The names of Lenègre and Lev became synonymous with Progressive Cardiac Conduction Defect (PCCD). Lenègre-Lev disease is characterized by progressive alteration of cardiac conduction through the His-Purkinje system with right or left bundle branch block and widening of QRS complexes, leading to complete atrioventricular block and causing syncope and sudden death [13–15]. In both diseases a sclerodegenerative process causes fibrosis of the His Purkinje system. The severity and extent of the fibrotic lesions differ in Lev and Lenègre descriptions. For Lenègre histological studies identified diffuse fibrotic degeneration of the common trunk, the proximal and distal portions of the right and left branches and the His bundle, the sinus node and the AV node remained unaffected whereas for Lev the sclerodegenerative abnormalities affect the specialized conduction system and the fibrous skeleton of the heart [13–15].

Therefore PCCD is considered as a primary degenerative disease or an exaggerated aging process with sclerosis affecting only the conduction tissue even if an inherited component may be involved.

Cardiac Conduction Defect Is an Inheritable Disease

The earliest reports by Morquio [16] and Osler [17] describe familial clustering of disturbance in cardiac conduction. Although most reports of

heart block have concerned affected siblings (most of which may represent cases of congenital complete heart block due to circulating autoantibodies in the mother with lupus), families of progressive forms multiple generations have been reported to prove dominant inheritance [18–20]. Gazes et al. [21] report conduction disturbances occurring in three or four generations. In most of the affected cases the heart block was of second degree with episodes of third-degree (complete) AV block.

In the past decade positional cloning approaches and more recently genome wide association studies have allowed to map and identify the first genes causing autosomal dominant PCCD in large pedigrees.

Clinical and Molecular Description of Familial Progressive Cardiac Conduction Defects

Transient Receptor Potential Cation Channel, Subfamily M, Member 4 Gene (TRPM4) Associates with Familial Conduction Disease

Combrink et al. [22] described a very large Afrikaner pedigree in which the mother had Right Bundle Branch Block (RBBB) and died at the age of 35 years from a Stokes-Adams attack. Of her four children, three had RBBB. The mother's parents both died suddenly in their 30s. One of her brothers was suspected to have a cardiac conduction disturbance; another had dextrocardia, while three other siblings were apparently normal. Steenkamp [23] described a South African family in which 6 of the 17 studied members showed of rhythm or conduction disturbance. Brink and Torrington [24] suggested that the disorder, referred as type I progressive familial heart block (PFHB), is prevalent in South Africa and is the same disorder reported by Combrink et al. and Steenkamp. Type I heart block in their description tends to have the pattern of a RBBB and/or left anterior hemiblock, manifesting clinically when complete heart block supervenes with syncopal episodes or sudden death. In two studies, van der Merwe et al. [25, 26] provided follow-up information on the kindred reported by Brink and Torrington [24] and documented progression of the disorder (Fig. 34.1a). In the mean time, they reported a second type of CCD (PFHB type II) in which the onset of complete heart block is associated with narrow complexes at an atrioventricular nodal level [24]. Electrocardiographic changes were bundle branch disease and AV nodal disease with an AV block and an idionodal escape rhythm.

In 1978, Stephan [27] reported a large Lebanese family. Among the 209 patients included in the study, 31 were diagnosed with conduction defects and three were implanted with a pacemaker. Within the family, conduction defects were mostly RBBB (12 patients), incomplete RBBB (seven patients), RBBB with left axis deviation (six patients), RBBB with right axis deviation (four patients) and complete AV block (two patients). The same authors reported in 1997 a follow-up of a family spanning five generations composed of 47 patients with major CDD and 36 patients with minor CCD (Fig. 34.1b) [28]. Conduction defects in this family were diagnosed early in life and were progressive in 5–15 % of the patients evolving to complete AV block.

In 1995, the disease locus in both the Lebanese and the South African family reported previously was mapped to chromosome 19q13.2-13.3 [29, 30]. The penetrance of the disease appears variable in the Lebanese families whereas almost complete in the South African family.

In 2009, Kruse et al. [93] identified the disease causing mutation in the large South African Family in *TRPM4* gene. The mutation (*TRPM4* c.19G->A) predicted the amino acid substitution p.E7K in the TRPM4 amino terminus. This gene encodes a member of the melastatin subfamily of transient receptor potential (TRP) channels. The encoded protein is a monovalent-permeable, nonselective cation (CAN) channel [31, 32] and comprises six predicted transmembrane spanning helices (TM1–TM6), a cytoplasmic N- and C-terminal domain, and a pore (P) region between TM5 and TM6, thus, resembling the membrane topology of voltage-gated cation channels [33]. Although this channel is regulated by (Ca^{2+}), it is not permeable for Ca^{2+}. The opening probability of the TRPM4 channel increases during depolarization because of an intrinsic voltage-sensing mechanism [34].

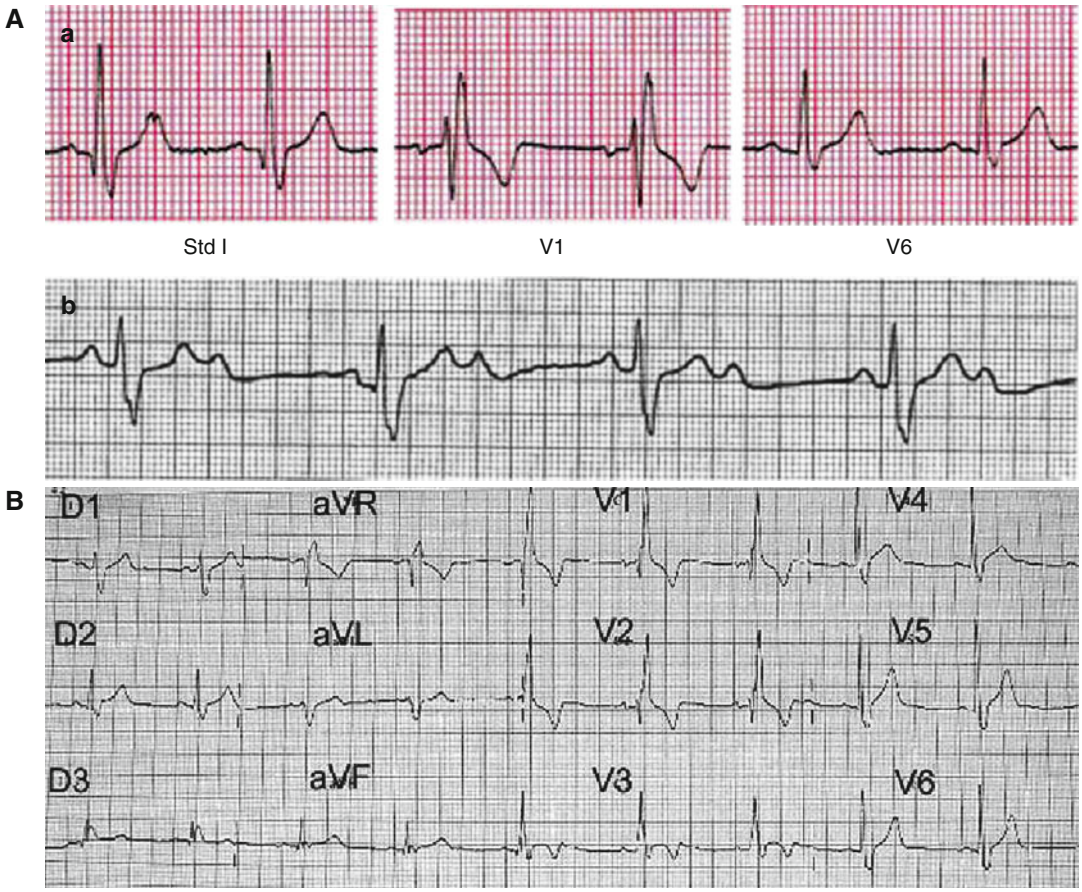

Figure 34–1. Cardiac phenotype of PFHBI patients. (**A**) ECG from the large Afrikaner family. (*a*) Sinus rhythm with a RBBB in an 8-year-old asymptomatic boy on a standard 12-lead ECG, with leads Std I, V1, and V6 shown. (*b*) ECG showing 2:1 atrioventricular node block (atrial rate, 76 bpm; ventricular rate, 38 bpm) with a broad QRS complex on Holter monitoring in a 54-year-old man who had recently become symptomatic. (**B**) ECG of the Lebanese family showing a complete RBBB (cRBBB) with a right-axis deviation (From Kruse et al. [93]. With permission from American Society for Clinical Investigation)

TRPM4 channels are a likely molecular correlate of the cardiacCa^{2+}-activated CAN channel, which plays a key role in regulating the driving force for Ca^{2+} entry in cardiac cells [31, 35]. Quantitative analysis of TRPM4 mRNA content in human cardiac tissue showed the highest expression level in Purkinje fibers. Cellular expression studies showed that the c.19G->A missense mutation attenuated deSUMOylation of the TRPM4 channel. The resulting constitutive SUMOylation of the mutant TRPM4 channel impaired endocytosis and led to elevated TRPM4 channel density at the cell surface corresponding to a gain-of-function mechanism underlying this type of familial heart block.

In 2010 Lui et al. identified the causal gene in the large Lebanese family in the same *TRMP4* gene (p.Ala432Thr) and reported two additional mutations in two smaller French families (p. Arg164Trp, and p.Gly844Asp) [36]. Altogether, clinical features in the three families were: two cases of LAD, ten cases of complete RBBB, 16 cases of incomplete RBBB, 15 cases of complete RBBB with LAD, six cases of complete RBBB with RAD, seven cases of AVBc and no cases of isolated RAD. A remarkable feature in these three families was that no family members had an incomplete or complete left bundle-branch block (LBBB). All three mutations result in an increased

current density. This gain of function is due to an elevated TRPM4 channel density at the cell surface secondary to impaired endocytosis and deregulation of SUMOylation.

All together these findings highlight endocytosis as a new mechanism of ion channel density control for electrical disturbances in the heart.

Following the identification of mutations in the *TRPM4* gene in the four pedigrees described above, Stallmeyer et al. screened 160 unrelated patients with various types of inherited cardiac arrhythmic syndromes [37]. Among the 45 cases with RBBB or AVB eight rare *TRPM4* variant were indentified. RBBB appeared to be the leading electrocardiographic phenotype (26.3 %) and 11.5 % of cases with AVB were associated with rare *TRMP4* variant. Among the eight variant, two (p.G844D and A.432 T) have been previously identified by Hui et al.; five segregated in small families and three were identified in sporadic cases. In two families the probands presented congenital conduction defects (p.K914R; p.Y790H) whereas 3 families presented a progressive form of conduction defect (p.P970S; p. G844D). In all cases *TRMP4* mutations were associated with a wide spectrum of severity. Of interest p.G844D mutations has now been reported in one French and two German unrelated families and no *TRPM4* mutations were identified in patients presenting sinus node dysfunction, Brugada syndrome, or long-QT syndrome.

Cardiac Specific Sodium Channel Nav1.5 Causes Progressive Cardiac Conduction Defect

In 1999, Schott et al. reported an independent large multigenerational pedigree presenting PCCD [38] and identified a mutation in the cardiac sodium channel Nav1.5.

Familial and clinical investigation in the large Lenègre pedigree started after the identification of a member with right bundle branch block (RBBB) and syncope, a brother with RBBB and a sister with complete AV block and syncope. Among the 65 family members included in the study, 15 had clinical and electrocardiographic abnormalities [39]. Familial investigation found all types of CCD in the family (Fig. 34.2). RBBB was present in five, left bundle branch block (LBBB) in two, left anterior or posterior hemiblock in three, and long PR interval (more than 210 ms) in eight members. None had a structural heart disease. Five members had received a pacemaker implantation because of syncope or complete AV block. A typical example of progressive development of conduction defect in a same patient is shown in Fig. 34.3. Electrocardiograms recorded in 1982, 1998, and 2000 show progressive increase in QRS duration (QRS: 130, 140, and 172 ms).

Long-term follow-up of several affected members demonstrated that their conduction defects increased in severity with age. The age of the patients who participated to the study ranged from 15 to 81 years. We plotted conduction parameters in relation to age (Fig. 34.4a). Whatever the age, averaged and filtered P-wave, PR, and QRS duration were longer in affected patients than in unaffected. There was a shift in the regression line for P-wave and PR duration toward higher values in affected patients, whereas the slopes, expressed in ms per year, were comparable in the two groups. In contrast, the QRS duration evolved differently in relation to age between the two groups. There was a more pronounced QRS lengthening with age in affected than in unaffected patients. In addition, an age-dependent variability in the QRS duration was evidenced in the affected group.

These data demonstrate that conduction was already abnormal early in life in the absence of specific conduction defects, which are never observed before the age 40 years. In the family, young affected patients have ECG parameters considered within 'normal limits'. Using the presence of selective conduction defects as a selective criteria, penetrance is compete only late during aging.

Molecular genetics excluded the chromosome 19 locus and linkage analysis mapped the disease locus on chromosome 3 near *SCN5A* gene encoding the cardiac specific sodium channel Nav1.5. *SCN5A* was considered as a candidate gene and direct sequencing of affected members identified a splice donor site mutation in exon 22 of *SCN5A* gene (IVS.22+2T->C) in 25 affected members [38]. The abnormal transcript is predicted to an in-frame skipping of exon 22 and an impaired

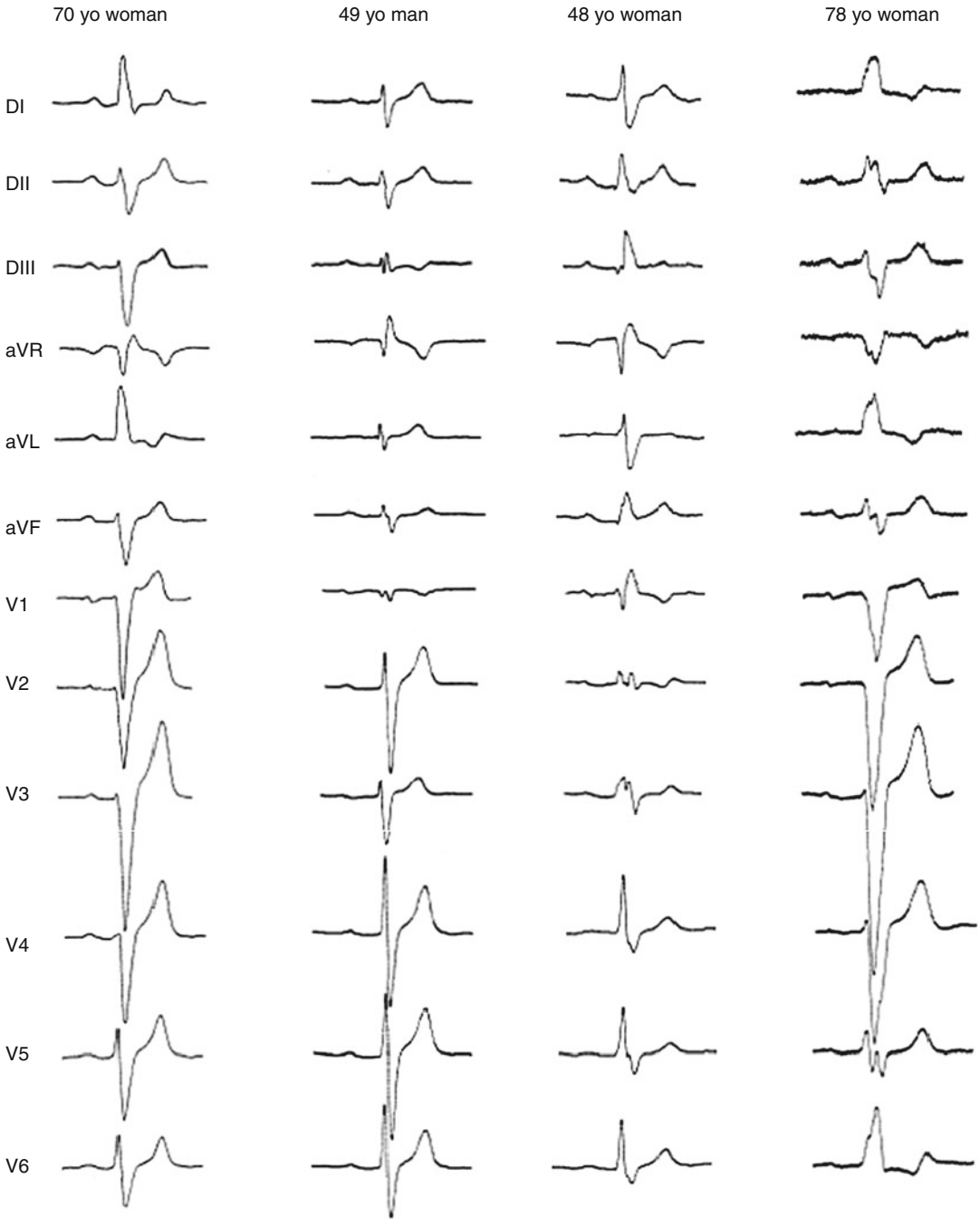

Figure 34–2. Examples of electrocardiogram patterns in *SCN5A* affected members. Patient I was a 70-year-old woman with parietal block and left-axis deviation (heart rate [HR]: 64 beats/min, P: 144 ms, PR: 215 ms, QRS: 160 ms). A pacemaker was implanted because of several episodes of syncope. Patient 2 was a 49-year-old man with a parietal block and undetermined axis (HR: 54 beats/min, P: 150 ms, PR: 244 ms, QRS 128 ms). Patient 3 was a 48-year-old woman with right bundle branch block (HR: 64 beats/min, P: 153 ms, PR: 205 ms, QRS: 172 ms). Sudden widening of QRS complexes and occurrence of 2–1 AV block during exercise led to pacemaker implantation. Patient 4 was a 78-year-old woman with left bundle branch block (HR: 59 beats/min, P: 157 ms, PR: 248 ms, QRS: 196 ms). A pacemaker was implanted after two episodes of syncope

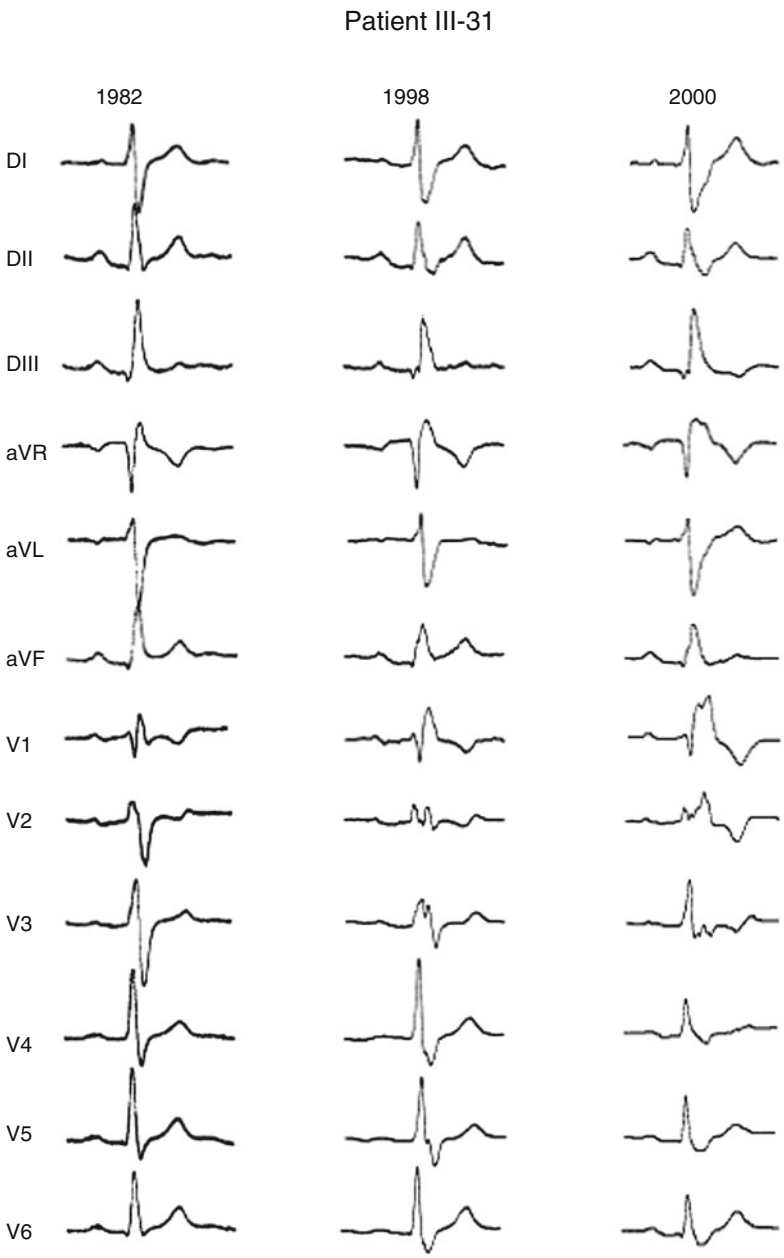

Figure 34–3. Serial electrocardiograms performed in 48-year-old woman *SCN5A* positive PCCD patient showing progressive development of conduction defect. Electrocardiograms recorded in 1982, 1998, and 2000 show progressive increase in QRS duration (QRS: 130, 140, and 172 ms)

gene product missing the voltage-sensitive DIIIS4 segment. *In vitro* exon-trapping experiments of the mutated *SCN5A* IVS.22+2T->C gene confirmed skipping of exon 22 [41]. Wild-type and exon 22-deleted Nav1.5 channels were expressed in COS-7 cells. Current traces show that Lenègre disease results from a loss-of-function mutation disease. Immunostaining in cells transfected with exon 22-deleted *SCN5A* suggests that the protein is correctly processed to the cell membrane.

Together with the first description of Lenègre-disease *SCN5A*-causing mutation, a second *SCN5A*-5280 del G frameshift mutation was described in a Dutch family with non-progressive conduction defect [39]. The proband presented after birth with an asymptomatic first-degree AV block associated with RBBB (PR interval and

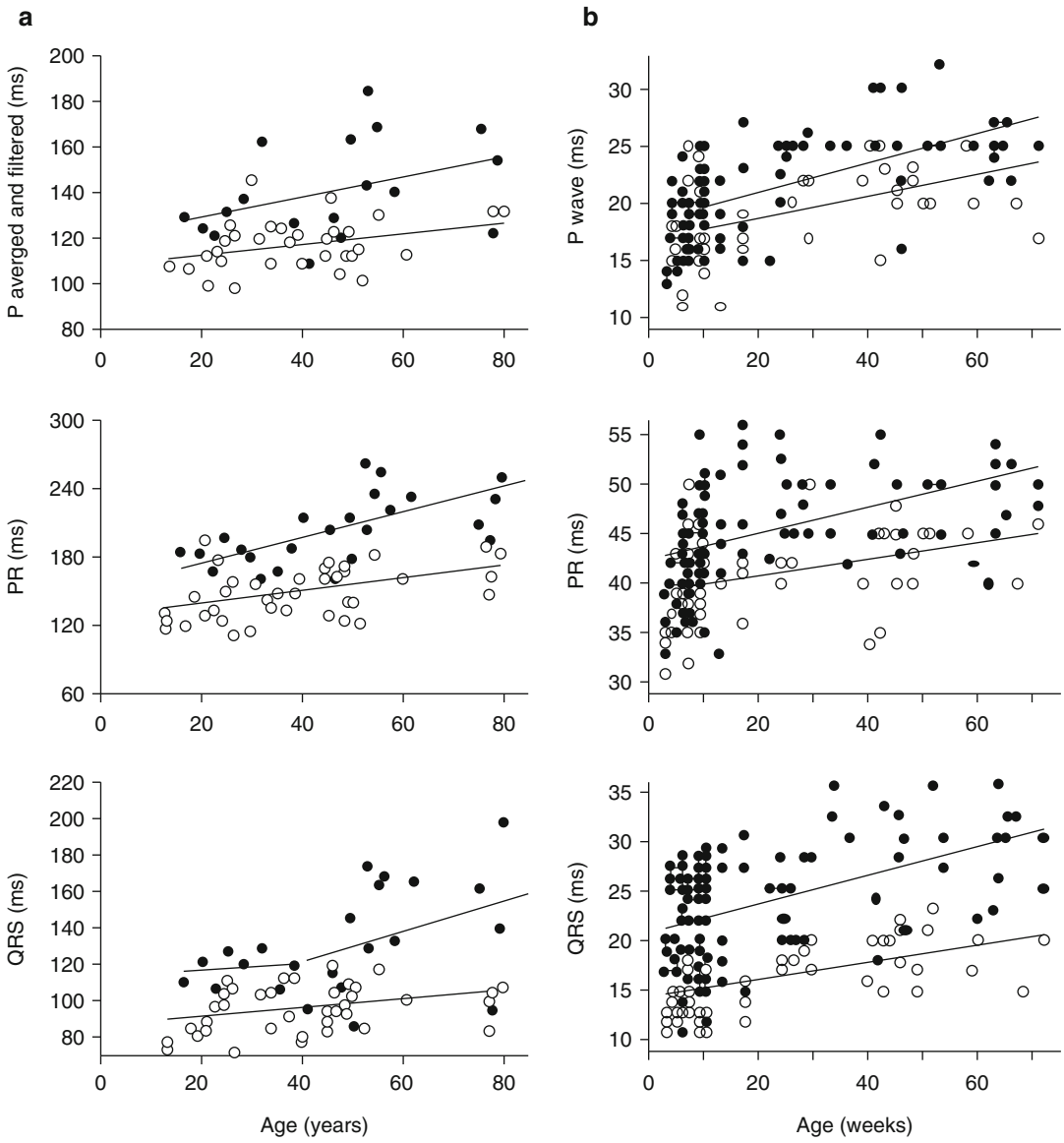

Figure 34–4. Evolution with aging of conduction parameters in humans and in a scn5a$^{+/-}$ knock-out mouse model. (**a**) Evolution with aging of conduction parameters in affected (*filled symbols*) and unaffected subjects (*open symbols*). *Top panel*: Averaged and filtered P-wave duration. *Middle panel*: PR duration. *Bottom panel*: QRS duration. In *top* and *middle panel*, data were fitted with a linear regression analysis. In *bottom panel*, assessment of the residuals showed that the linear model was poorly adapted to fit the relation between QRS duration and aging in affected members. The variance was significantly different before and after the age of 40 (ratio variance test; $p < 0.001$) was indicative of a threshold effect of age. (**b**) Effects of age on P-wave duration, PR interval duration, and QRS interval duration in WT (*open symbols*) and Scn5a+/– (*filled symbols*) mice

QRS duration: 200 and 120 ms, respectively). Three brothers were asymptomatic, one of which with RBBB (QRS duration: 110 ms). The asymptomatic mother had an unspecified conduction defect (QRS duration: 120 ms).

Altogether, a supposed 50 % reduction of sodium current is tolerated to some extent. The effect of the mutation only becomes evident later in age, when conduction in the heart becomes impaired because of the naturally occurring

aging process. Interestingly the normal aging process usually involves sclerosis, although evidence is emerging (as described in the next section of this chapter) that sclerosis is enhanced in carriers of loss-of function *SCN5A* mutations.

A Scn5a+/– Mouse Model Mimics Lenègre Disease

A mouse model with targeted disruption of *Scn5a* has been established by Andrew Grace's group from the University of Cambridge [40]. In this model, exon 2 of *SCN5A* gene was replaced with a splice acceptor (SA)-*Gfp*-PGK-neomycin cassette. Homozygous knockout mice die during embryonic development due to severe defects in ventricular morphogenesis whereas heterozygote (*Scn5a*+/−) mice show normal survival. In the initial report, it was shown that I_{Na} was reduced by about 50 % in isolated ventricular myocytes from 8 to 10 week-old heterozygote mice as compared to wild-type mice. It was also shown that 8–10 week-old Scn5a+/− mice have several cardiac defects including decreased atrial and atrio-ventricular conduction, as well as increased inducibility of ventricular arrhythmias. Following this initial report, the model was further analyzed to investigate the potential pathophysiological mechanisms involved in SCN5A-related PCCD [41, 42, 43]. ECG recording and activation mapping on Langendorff-perfused hearts in 4–71 weeks old mice showed that ventricular conduction was also decreased and that the ventricular phenotype worsened with age (Fig. 34.4b). In young Scn5a+/− mice, conduction velocity was only affected in the right ventricle. In old mice, right ventricular conduction defect worsened and was in addition associated with conduction defect in the left ventricle. The age-dependent increase in conduction defect was associated with the occurrence of fibrosis in ventricular myocardium. In some aspects, this phenotype resembles Lenègre-Lev disease, although in the later fibrosis was found to be limited to the conduction system area [44]. Whether fibrosis is a direct consequence of Nav1.5 loss of function or results from desynchronized ventricular contractile activity is still an open question. Whatever its triggering mechanism is, fibrosis, as well as associated redistribution of connexin 43 expression, most likely contribute to the age-dependent degradation of conduction in the mouse model (Fig. 34.5). The hypothesis that SCN5A-related inherited PCCD in human also results from a primary decrease in Na^+ current and a secondary progressive fibrosis with aging is unlikely to be clarified, but it is worth noting that fibrosis has been found in patients with the *SCN5A*-related Brugada syndrome [45, 46]. In this context, Scn5a+/− mice represent a promising tool for testing preventive therapies as an alternative to pacemaker implantation.

Other Familial Forms of Cardiac Conduction Defects Linked to *SCN5A* Mutations

Since its first description in 1999, many new *SCN5A* mutations causing progressive or congenital CCD have identified, sometimes associated with dilated cardiomyopathy or arrhythmias.

In 2001, Tan et al. [47] provided a functional characterization of a *SCN5A* mutation that causes a sustained, isolated conduction defect with pathological slowing of the cardiac rhythm. ECG findings reported bradycardia, AV-nodal escape, broad P wave, long PR and wide QRS. By analyzing the *SCN5A* coding region, they reported a *SCN5A*-G514C mutation in five affected family members. Biophysical characterization of the mutant channel showed abnormal voltage-dependent 'gating' behavior.

In 2002, Wang et al. [48] reported two new *SCN5A* mutations that result in AV conduction block presented during childhood. Molecular genetic studies revealed a first *SCN5A*-G298S mutation in a proband with progressive AV block (QRS = 135 ms at age 9 and QRS = 133 ms at age 20). A second *SCN5A*-D1595N mutation was identified in a proband with complete heart block at the age of 12 years. The functional consequences of the two mutations are impaired fast inactivation but not sustained non-inactivating current. The mutations reduce the sodium cur-

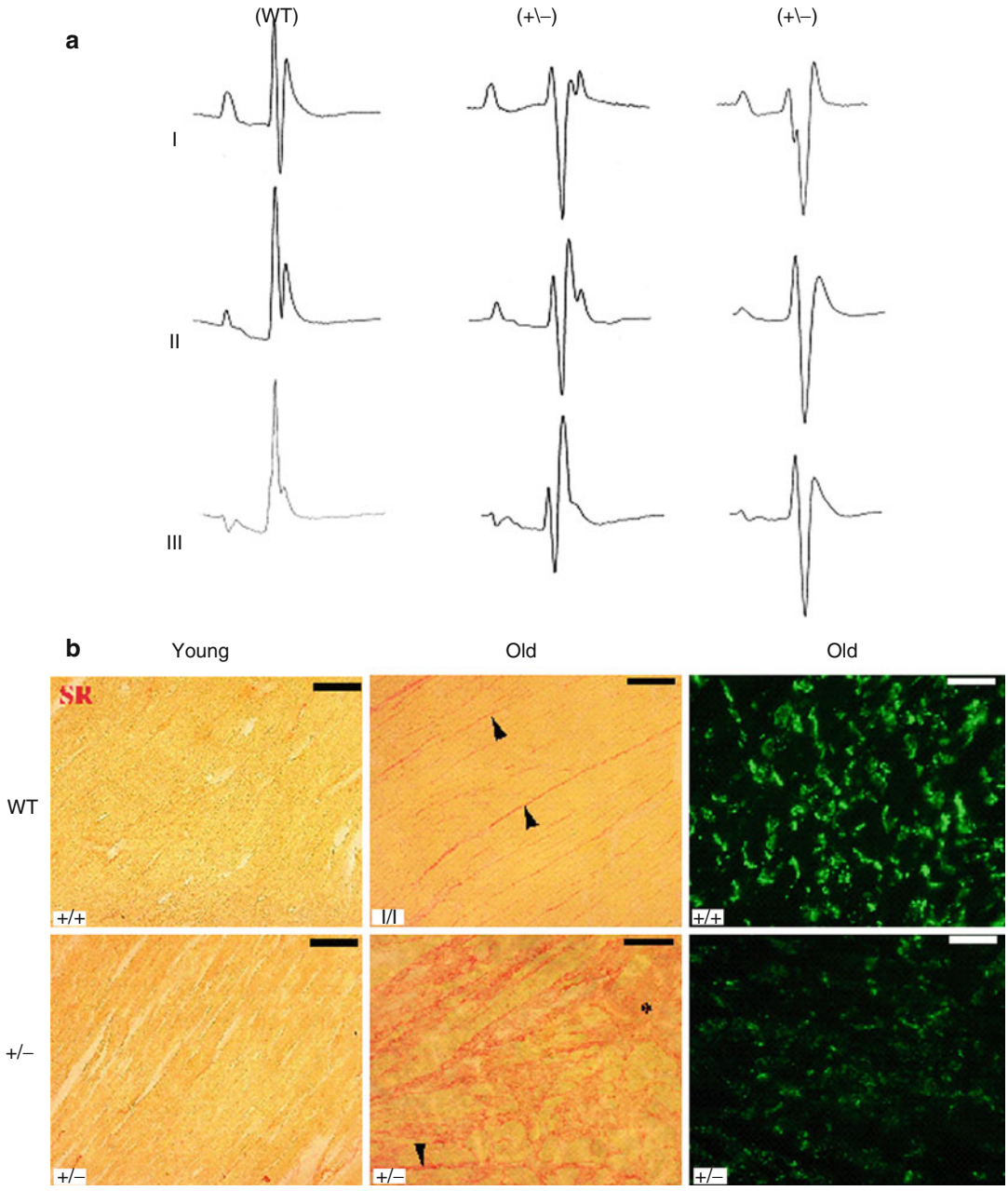

Figure 34–5. Surface ECG recordings and histology on WT and Scn5A +/– mice. (**a**) Surface ECGs in WT and Scn5a+/– mice. Representative ECG recordings (leads I, II, and III) in a WT and two different Scn5a+/– mice. (**b**) Ventricular fibrosis and expression of connexin 43. Ventricular fibrosis (in *red*) in young mice (*left*) is virtually absent in WT, which leaves the expression pattern of Cx43 unaffected. Fibrosis in old WT animals is increased in the interstitium between the muscle fibers whereas locally massive fibrosis is observed in Scn5A+/– old animals. Fibrosis is accompanied by a down regulation and redistribution of Cx43 in old Scn5A+/– mice, whereas Cx43 in old WT hearts appears unaffected. Bar = 50 μm in pictures showing Cx43 and 100 μm in those showing Sirius *red*

rent density and enhance slow inactivation components.

In 2003, Bezzina et al. [49] reported a family in which the proband born in severe distress with irregular wide complex tachycardia. An older sister died at 1 year of age from severe conduction disease with similarly widened QRS-complexes. Mutational analysis in the proband demonstrated compound heterozygosity for a nonsense *SCN5A*-W156X mutation, inherited from the father, and a *SCN5A*-R225W missense mutation, inherited from the mother. Expression studies showed that the W156X mutation is a loss of function mutation whereas the R225W mutation leads to a severe reduction in current density and is also associated with gating changes. Histological examination of the heart from the deceased sibling revealed changes consistent with a dilated type of cardiomyopathy and severe degenerative abnormalities of the specialized conduction system. These morphological changes may have occurred secondary to the sodium channel abnormality and contributed to the severity of the disorder in this individual.

In 2006, Niu et al. [50] reported a novel *SCN5A*-W1421X mutation in a four-generation family with cardiac conduction abnormalities and several cases of sudden death. Most family members who carry this W1421X mutation have developed major clinical manifestations. However, in a 73-year-old grandfather, who carried the *SCN5A*-W1421X mutation, a second *SCN5A*-R1193Q variant was identified. This patient has remained healthy and presents only minor electrocardiographic abnormalities, whereas most of his siblings, who carried a single mutation (W1421X), had died early or had major disease manifestations. This observation suggests that the R1193Q mutation has a complementary role in alleviating the deleterious effects conferred by W1421X in the function of the *SCN5A* gene. This report provides a good example to explain the mechanism of penetrance of genetic disorders.

The work of Viswanathan et al. [51] provides a second illustration of phenotypic modulation by an *SCN5A* polymorphism. They identified a novel mutation *SCN5A*-T512I, in a 2-year-old boy diagnosed with second-degree AV conduction block. The T512I mutation, when heterologously expressed, causes hyperpolarizing shifts in activation and inactivation, and enhances slow inactivation. However, the common *SCN5A*-H558R polymorphism also found in this child, has no effect on wild-type sodium current but attenuates the gating effects caused by the T512I mutation. The polymorphism entirely restores the voltage-dependent activation and inactivation voltage shifts caused by the T512I mutation, and partially restores the kinetic features of slow inactivation. This mutation and the H558R polymorphism were both found in the same allele of the child with isolated conduction disease, suggesting a direct functional association between a polymorphism and a mutation in the same gene.

Several reports identified *SCN5A*-D1275N mutation associated with CCD and various cardiac arrhythmias or structural cardiomyopathies. In 2003, Groenewegen et al. [52] reported a *SCN5A*-D1275N mutation co-segregating with two connexin 40 genotype [(−44 (G-->A) and +71 (A-->G)] in familial atrial standstill (AS). *SCN5A*-D1275N channels show only a small depolarizing shift in activation compared with wild-type channels. The authors proposed that, although the functional effect of each genetic change is relatively benign, the combined effect of genetic changes eventually progresses to total atrial standstill. More recently, a *SCN5A*-L212P mutation associated with the same connexin 40 polymorphisms combination was reported in another atrial standstill family [53].

In 2004, McNair et al. [54] reported a large family diagnosed with CCD associated with sinus node dysfunction, arrhythmia, right and occasionally left ventricular dilatation and dysfunction. Screening family members for mutations identified a *SCN5A*-D1275N defect. This finding expands the clinical spectrum of disorders of the cardiac sodium channel to cardiac dilation and dysfunction and supports the hypothesis that genes encoding ion channels can be implicated in dilated cardiomyopathies. In 2005, Olson et al. [55] reported a *SCN5A*-D1275N mutation associated to susceptibility to heart failure and atrial fibrillation.

In 2006, Laitinen-Forsblom et al. [56] reported a five family member with atrial arrhythmias and intracardiac conduction defects. Interestingly left ventricle dilatation was also seen in one individ-

ual and three individuals had a slightly enlarged right ventricle. Premature death due to stroke occurred in one subject, and two other members had suffered from stroke at a young age. Direct sequencing disclosed a *SCN5A*-D1275N mutation. This alteration was present not only in all six affected individuals, but also in two young individuals lacking clinical symptoms.

Sodium Channel Beta1 Subunit Mutations Associated with Cardiac Conduction Disease

Regulatory proteins of cardiac sodium channels Nav1.5 are logical candidates for CCD. Sodium channels are multisubunit protein complexes composed not only of pore-forming α subunits but also of multiple other protein partners including auxiliary function-modifying β subunits. The β1 transcript arises from splicing of exons 1–5 of *SCN1B* gene and a second transcript has been described, arising from splicing of exons 1–3 with retention of a segment of intron 3 (termed exon 3A), leading to an alternate 3' sequence (β1B transcript).

Watanabe et al. screened 44 probands with conduction disease for mutations in *SCN1B* and β1B transcript [57]. They reported 1 *SCN1B* and 2 β1B mutations in three kindred's with conduction abnormalities.

A missense mutation, c.259G->C in exon 3, resulting in p.Glu87Gln within the extracellular immunoglobulin loop of the protein was identified in a Turkish kindred affected by conduction disease. None of the families had a history of syncope, sudden cardiac death, or epilepsy. The proband was a 50-year-old woman who presented with palpitations and dizziness and had complete left bundle branch block. Electrophysiologic analysis revealed a prolonged His-ventricle interval of 80 ms and inducible atrioventricular nodal reentrant tachycardia; complete atrioventricular block occurred following atrial programmed stimulation and during induced tachycardia. Her brother was found to have bifascicular block (right bundle branch block and left anterior hemiblock), whereas their mother had a normal ECG.

Two nonsense mutations in the same codon were identified in two other families. A c.536G A in exon 3A in a French kindred affected with Brugada syndrome and conduction disease. This mutation results in p.Trp179X and is predicted to generate a prematurely truncated protein lacking the membrane-spanning segment and intracellular portion of the protein. The French proband was a 53-year-old man who presented with chest pain but had normal coronary angiography and echocardiography. ECG revealed ST segment elevation typical of Brugada syndrome as well as conduction abnormalities, including a prolonged PR interval of 220 ms and left anterior hemiblock. A baseline ECG in his brother, who had no history of palpitations or syncope, showed left anterior hemiblock and minor ST segment elevation suggestive of Brugada syndrome (type II saddleback) abnormalities. Their sister had a normal ECG but her son was found to have right bundle branch block and type II Brugada syndrome after flecainide challenge.

A different nonsense mutation, c.537G A in exon 3A resulting in p.Trp179X, affecting the same codon as in family 2, was identified in a Dutch kindred. The Dutch proband was a 17-year-old girl who had right bundle branch block and a prolonged PR interval of 196 ms on ECG; echocardiography was normal and a flecainide test for Brugada syndrome was negative. Her father had a normal ECG.

Both the canonical and alternately processed transcripts were expressed in the human heart and were expressed to a greater degree in Purkinje fibers than in heart muscle, consistent with the clinical presentation of conduction disease. Sodium current was lower when Nav1.5 was coexpressed with mutant β1 or β1B subunits than when it was coexpressed with WT subunits. These findings implicate *SCN1B* as a disease gene for human arrhythmia susceptibility.

Connexin 40 Mutation Associated with a Malignant Variant of Progressive Familial Heart Block Type-1

Cardiac myocyte excitability in atria, His-Purkinje system and ventricles is largely determined by the properties of voltage-gated Na channels. Once activated, excitatory currents rapidly propagate to neighbouring cells via low-

resistance intercellular channels called gap junctions, which facilitate the synchronous contraction of the heart. Connexins, or gap junction proteins, are a family of structurally-related transmembrane proteins that assemble to form vertebrate gap junctions. Connexins are four-pass transmembrane proteins with both C and N cytoplasmic termini, a cytoplasmic loop (CL) and two extra-cellular loops, (EL-1) and (EL-2). Connexins are assembled in groups of six to form hemichannels, or connexons, and two hemichannels then combine to form a gap junction.

Makita et al. evaluated 156 probands of diverse ethnic backgrounds with clinical diagnosis of PFHBI and identified a germline heterozygous missense mutation in exon 2 of the Cx40 gene, *GJA5*.

The proband, an 11-year-old male at time of death, was first referred for evaluation when he was 6 years-old because of ECG abnormalities. Although asymptomatic at that time, his ECG showed advanced atrioventricular (AV) block, complete left bundle branch block (LBBB) and left axis deviation (Fig. 34.6). The proband died suddenly 5 years later during exercise. The proband's younger sister shares the Cx40-Q58L mutation. She is asymptomatic with a QRS duration at the upper limit of normal, left axis deviation that has been progressive and QRS notch. These findings are consistent with impaired intraventricular conduction. The mother died suddenly at age 30, after delivering the second child. An ECG on record, obtained when she was 16 years old, was similar to that of the proband. In addition, a ventricular tachycardia was recorded during the recovery phase of an exercise stress test.

The *GJA5* mutation (c.173 A>T) caused an amino acid substitution (glutamine (Q) replaced by leucine (L)) at position58 in Cx40 (Cx40-Q58L) [58]. Heterologous expression experiments revealed that this novel mutation (Cx40-Q58L) significantly impaired the ability of Cx40 to form gap junction channels. Confocal microscopy showed that the Cx40-Q58L mutant but not the wild type (WT) failed to form plaques at sites of cell-cell apposition. Co-expression experiments indicated that Cx40-WT protein provided only partial rescue of the Cx40-Q58L cellular phenotype. Cells expressing both WT/Q58L showed intermediate conductance (15.4 ± 3.7 nS, $n = 16$) between WT (28.8 ± 3.6 nS, $n = 16$, $p < 0.001$) and Q58L (0.28 ± 0.11 nS, $n = 14$, $p < 0.001$).

Of interest other studies have shown somatic mutations of Cx40 or Cx43 in patients with idiopathic atrial fibrillation [59, 60] those mutations were confined to the atria, and conduction abnormalities in the ventricles or His-Purkinje system were not observed. A second recent publication by Norstrand et al. associated a *de novo* missense mutation in connexin 43 (E42K-Cx43) with Sudden Infant Death [61]. The E42K-connexin43 demonstrated a trafficking-independent reduction in junctional coupling, *in vitro* and a mosaic pattern of mutational DNA distribution in deceased cardiac tissue, suggesting a novel mechanism of connexin 43-associated sudden death.

SCN5A Overlap Syndrome

Cardiac sodium channel dysfunction caused by mutations in the *SCN5A* gene is associated with a number of rare arrhythmia syndromes, including long-QT syndrome type 3 (LQT3), Brugada syndrome, conduction disease, sinus node dysfunction, and atrial standstill, which potentially lead to fatal arrhythmias in relatively young individuals. Although these various arrhythmia syndromes were originally considered separate entities, recent evidence indicates more overlap in clinical presentation and biophysical defects of associated mutant channels than previously appreciated. Various *SCN5A* mutations are now known to present with mixed phenotypes, a presentation that has become known as "overlap syndrome of cardiac sodium channelopathy." In many cases, multiple biophysical defects of single *SCN5A* mutations are suspected to underlie the overlapping clinical manifestations [62].

One of the first reports on this entity was provided by Bezzina et al. with the description of the *SCN5A*-1795insD+/− mutation in a large multigenerational family presenting with extensive variability in type and severity of symptoms of sodium channel disease [63]. Heterogeneous

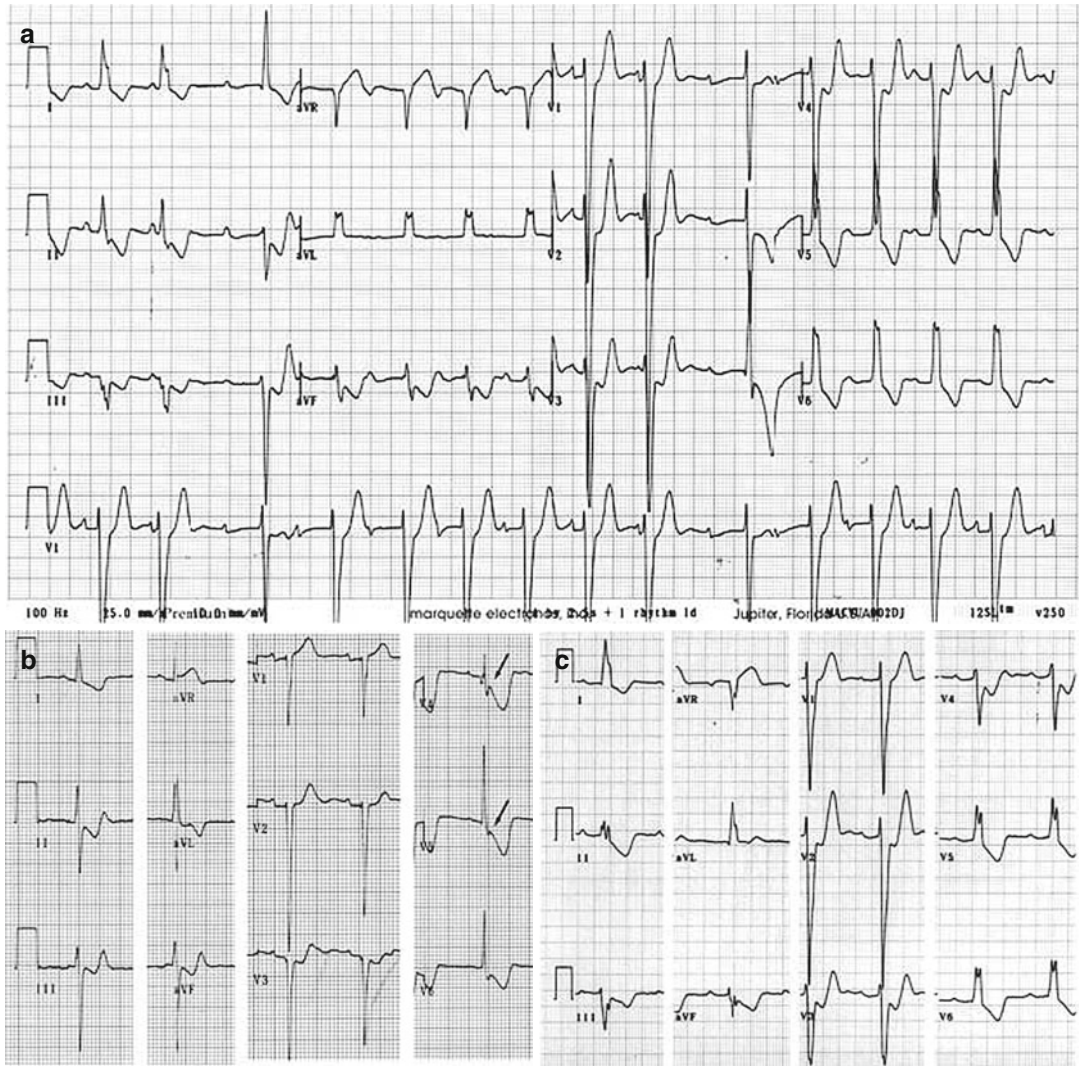

Figure 34–6. ECG of Connexin 40 PCD patients. (**a**) ECG of proband at age 6 showing advanced AV block, complete LBBB and left axis deviation. Patient died suddenly 5 years later. (**b**) ECG of proband's sister at age 6 showing QRS duration at upper limit of normal, left axis deviation that has been progressive, and QRS notch in V4 and V5 (*arrows*) consistent with impaired intraventricular conduction. (**c**) ECG of proband's mother at age 16 showing complete LBBB and left axis deviation. She died suddenly at age 30

biophysical properties of this and other *SCN5A* mutations underlying the mixed disease expressivity are now increasingly recognized. Other determinants of sodium channel disease expressivity, including genetic background, concomitant disease and environmental factors, are suspected but still largely unknown [64, 65]. Identification of these modifiers will prove essential for improved diagnosis and risk stratification in patients with sodium channelopathies.

Lenègre Disease and Brugada Syndrome: An Overlap Syndrome?

Brugada syndrome (BS) is a genetically inherited arrhythmogenic disorder characterized by an ECG pattern of ST segment elevation in the right precordial leads and an increased risk of sudden cardiac death resulting from episodes of polymorphic ventricular tachyarrhythmias

and fibrillation [66]. Loss-of-function mutations in the *SCN5A* gene account for about 20 % of BS [67].

An intriguing overlap exists between Lenègre disease and (BS): (i) decreased availability of the sodium channel accounts for both clinical entities; (ii) we have previously reported a family in which the same c.G1408R-*SCN5A* mutation leads to BS or Lenègre disease depending on the familial branches [65]; (iii) in a preceding work, we and others have shown that BS *SCN5A*-linked probands have an impaired cardiac conduction when compared to BS patients not related to *SCN5A*. Finally, sodium channel blockers have been shown to induce a more pronounced QRS widening of Brugada patients [68, 69].

Probst et al. characterized cardiac conduction defect in relation to aging in a cohort of 78 individuals carrying a *SCN5A* mutation associated with BS in their pedigree [70]. In this population, the penetrance of spontaneous Brugada syndrome phenotype is low (36 %), whereas the penetrance of conduction defects is high (76 %), consistent with the recent report by Hong et al. [71] *SCN5A* BS-type mutation carriers exhibit various degrees of cardiac conduction defects. This phenotype is similar to that of hereditary Lenègre disease. As in Lenègre disease, we found various types of conduction defects in *SCN5A* BS-type mutation carriers, although with clear predominance of RBBB and parietal block. When conduction parameters were plotted in relation to age, PR and QRS interval durations were longer in gene carriers than in non-carriers regardless of age. The interaction between age and PR interval is not significant, whereas the interaction between age and QRS interval is highly significant, showing a progressive alteration of intraventricular conduction (Fig. 34.6).

In this study, the conduction defects worsen with age in the *SCN5A* BS-type mutation carriers regardless of their BS phenotype and lead in five cases to pacemaker implantation. Thus, the conduction phenotype of BS-type and Lenègre-type *SCN5A* mutation carriers is undistinguishable. This positive correlation between the age and the progression of the conduction defect was found at a single time point, and only a follow-up of the patients during a long period of time could definitively prove the progressive evolution of the conduction defects with aging. Longitudinal follow-up has been achieved in the *Scn5aA*+/− mouse and firmly demonstrates slow progression of the conduction defect with aging.

Nevertheless, Lenègre disease and Brugada syndrome remain distinct entities with a different arrhythmic risk. Indeed, with programmed electrical stimulation, we were unable to provoke VF or ventricular tachycardia in gene carriers without a BS phenotype, whereas 33 % of the investigated patients with the Brugada phenotype experienced polymorphic ventricular tachycardia or ventricular fibrillation. These observations, despite an overlap of Lenègre and BS, further support the concept that Lenègre disease differs from BS not only because of the absence of ST-segment elevation, but also because the risk for life-threatening ventricular arrhythmias remains a major characteristic of BS.

Conduction Defects in Other Syndromes

Besides isolated PCCD, many diseases such as dilated or restrictive cardiomyopathies associate cardiac conduction defects [72, 73].

Clinical and Molecular Description of Lamin A/C Mutations in Dilated Cardiomyopathies Associating Conduction Defects

Over the last several decades, it has become increasingly clear that the cause of many 'idiopathic' dilated cardiomyopathies is genetic. Familial dilated cardiomyopathy (FDC) is now thought to account for up to 50 % of Idiopathic Dilated cardiomyopathy (IDC) patient. Most of these cases (>90 %) are thought to show autosomal dominant inheritance, although X-linked and autosomal recessive forms have been identified [74].

More than 20 different genes have been identified in patients with FDC. These include genes that affect sarcomeric proteins, cytoskeletal proteins, nuclear proteins, ion channel proteins, and mitochondria [75, 76]. Although up to 50 % of patients with IDC may have FDC by history, a genetic test may identify the cause in only a small minority of patients.

The exception to this is lamin A/C mutation. Several mutations in *LMNA* were identified in five families with DCM with conduction system disease by Fatkin et al. in 1999 [77] and Lamin A/C mutations are thought to be the cause in up to 10 % of FDC cases [78].

Lamin A and C are type V intermediate filament proteins found in the nuclear membrane or lamina [79, 80]. They are expressed in terminally differentiated somatic cells and found in multiple different tissues, including skeletal and cardiac muscle. The protein is composed of a conserved rod domain with a globular head and tail region. The functions of lamin A and C are incompletely understood, but it is thought that they are important in maintaining nuclear architecture.

The earliest cardiac finding in patients with lamin A/C deficiency is usually conduction system disease. In a meta-analysis of 299 carriers of a lamin A/C mutation, 18 % of patients less than 10 years of age had evidence of delayed intracardiac conduction. In patients over 30 years of age, 92 % had conduction system disease, with 44 % requiring pacemaker placement [79].

In early stages, patients have a characteristic electrocardiogram with low amplitude P waves, prolonged PR interval, but a relatively normal QRS complex. By age 50, over 60 % of patients have symptoms of cardiac heart failure. Meune et al. [81] found that, in a small cohort of patients with known lamin A/C defects, 42 % of patients experienced SCD.

Clinical and Molecular Description of Secundum Atrial Septal Defects Associating Atrioventricular Conduction Defects

Developmental aspects of the cardiac conduction system are complex and play a crucial role in the pathophysiology of CCD. Although several genes involved in mature conduction system function have been identified, their association with development of specific subcomponents of the cardiac conduction system remains challenging. Several transcription factors, including homeodomain proteins and T-box proteins, are essential for cardiac conduction system morphogenesis and activation or repression of key regulatory genes. In addition, several transcription factors modify expression of genes encoding the ion channel proteins that contribute to the electrophysiological properties of the conduction system and govern contraction of the surrounding myocardium.

Four families with secundum atrial septal defects and atrioventricular conduction defects [82], were found by Schott et al. [83] to show an autosomal dominant pedigree pattern and linkage to 5q35, and to have, in affected individuals, mutations in the *CSX* gene which encodes the cardiac-specific homeobox transcription factor NKX2-5. Since, numerous mutations in *NKX2.5* have been reported in isolated or familial forms of congenital heart defects [84–86].

Finally, mutations in *PRKAG2* gene encoding a non-catalytic, gamma-2 subunit of a AMP-activated protein kinase, were described in patients with the Wolff-Parkinson-White syndrome, a disease characterized by ventricular preexcitation, atrial fibrillation and CCD [87].

Genetic Variant Influencing Conduction Parameters in the General Population

In addition to Mendelian diseases caused by rare variants with strong effects most often located in coding region of the genome, common variants can also impact electrocardiographic traits. In the past decades genome wide association studies have identified hundreds of such variant in the general population but also in cohort of patients affected by common diseases [88]. Several studies have focused on identifying such variants impacting conduction parameters.

A genome-wide association study of electrocardiographic time intervals in 6,543 Indian Asians identified association of a nonsynonymous SNP, rs6795970, in *SCN10A* ($P = 2.8 \times 10(-15)$) with PR interval, a marker of cardiac atrioventricular conduction. These data have been successfully replicated in 6,243 Indian Asians and 5,370 Europeans for prolonged cardiac conduction (longer P-wave duration, PR interval and QRS duration, $P = 10(-5)$ to $10(-20)$). *SCN10A* encodes Na(V)1.8, a sodium channel [89].

A genome-wide association meta-analysis in 40,407 individuals of European descent from 14 studies, with further genotyping in 7,170 additional Europeans, identified 22 loci associated with QRS duration ($P < 5 \times 10–8$). These loci map in or near genes in pathways with established roles in ventricular conduction such as sodium channels, transcription factors and calcium-handling proteins, but also point to previously unidentified biologic processes, such as kinase inhibitors and genes related to tumorigenesis (Fig. 34.7). As reported by Chambers et al. *SCN10A* is the most significantly associated locus in this study. In mice *Scn5a* is expressed in the ventricular conduction system, and treatment with a selective SCN10A blocker prolongs QRS duration [90].

A third association study in ~10,000 individuals identified several genome-wide significant associations (with $P < 1.6 \times 10–7$). They identified one locus for heart rate (*MYH6*), four for PR interval (*TBX5, SCN10A, CAV1* and *ARHGAP24*) and four for QRS duration (*TBX5, SCN10A*, 6p21 and 10q21). They tested for association between these loci and subjects with selected arrhythmias in Icelandic and Norwegian case–control sample sets. We observed correlations between *TBX5* and *CAV1* and atrial fibrillation ($P = 4.0 \times 10–5$ and $P = 0.00032$, respectively), between *TBX5* and advanced atrioventricular block ($P = 0.0067$), and between *SCN10A* and pacemaker implantation ($P = 0.0029$) [91].

Conclusion

Progressive CCD also called Lenègre-Lev disease is a frequent disease with an expected increasing prevalence given the general aging of the population. PCCD is a heritable heterogeneous condition of mostly unknown origin. It is likely that the most common form of Progressive CCD has a mutifactorial origin, combining environmental and genetic factors. However, in some cases, familial PCCD with a monogenetic origin has been described. Today four genes (*SCN5A*, *TRPM4*, *SCN1B* and *GJA5*) have been identified in progressive cardiac conduction defects. Since its original description in 1999, many *SCN5A* mutations leading to either progressive or non-progressive CCD have been reported and the spectrum of *TRMP4* mutation is expanding with evidence for further genetic heterogeneity [92]. Genotype-phenotype studies reveal that, besides variable penetrance generally described in almost all monogenic diseases, *SCN5A* mutation-carriers, exhibit a wide phenotypic spectrum, associating structural

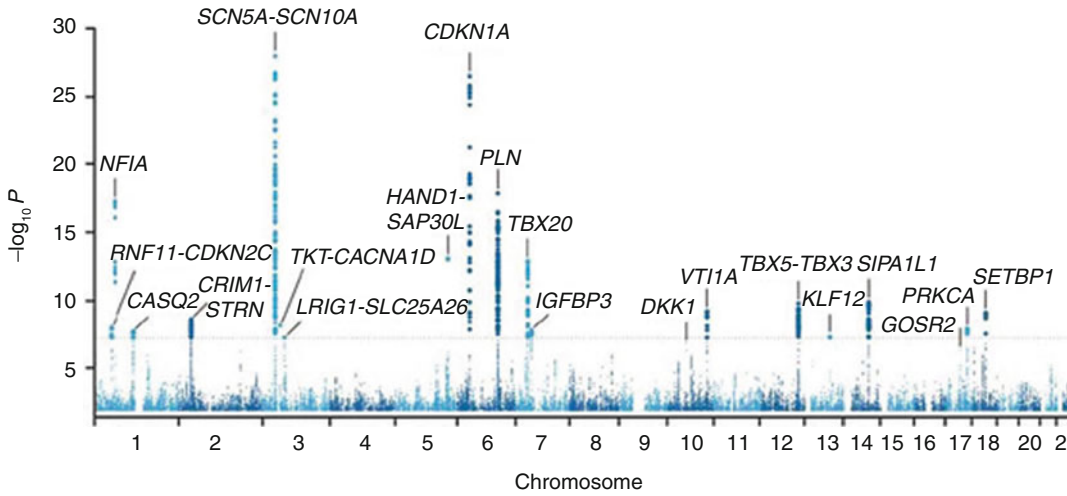

Figure 34–7. GWAS Manhattan plot showing the association of SNPs with QRS interval duration in a GWAS of 40,407 individuals. The *dashed horizontal line* marks the threshold for genome-wide significance ($P = 5 \times 10–8$). Twenty loci (labeled) reached genome-wide significance (From Nona et al. [90]. Reprinted with permission from Nature Publishing Group)

cardiomyopathies and arrhythmias. The study of a scn5a-haploinsufficiency mouse model mimicking Lenègres disease, provides a unique opportunity to understand the underling pathophysiological bases of Lenègre disease. It already appears that the disease causing mechanism due to a single channel defect is a consequence of a complex remodeling process and not just a slowing of the propagation of the electrical impulse through the conduction system. Increased fibrosis in *Scn5A* heterozygous mice reconciles the original description of Lenègre and Lev describing a combination of aging and fibrosis as an explication of the progressive alteration in conduction velocity.

Today pacemaker implantation constitutes the only therapeutic treatment to prevent sudden death in PCCD. It is conceivable that, in a near future, once the precise pathophysiological mechanism for PCCD identified, less invasive approaches will become available improving patient management.

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Genetics of Atrial Fibrillation and Standstill

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Abstract

Atrial fibrillation (AF) is the most common arrhythmia and is associated with an unfavorable prognosis. Monogenetic forms of AF, however, represent a rare AF subtype. Although the identified mutations in affected family members have large effects, they do not seem to play a major role in more common AF present in the majority of the patients. The majority of patients have AF in association with concomitant (cardiovascular) conditions. In the last few years increasing data have been reported supporting the notion that there is a genetic component to more common AF. Recently, GWAS of the common AF phenotype have been successful in identifying three genetic loci associated with AF.

In this chapter we will focus on single mutations causing familial forms of AF, and discuss single nucleotide polymorphisms associated with AF in the general population, and lastly, we will discuss the genetic aspects of atrial standstill. Identification of the genes that play a role in the initiation of the arrhythmia may give new insights into the development of AF, and eventually lead to novel therapeutic options. Also, early recognition of patients at risk may, eventually, prevent AF and reduce morbidity and mortality.

Keywords

Atrial fibrillation • Atrial standstill • Genetics • Familial • Risk factor • Mutation • Single nucleotide polymorphism

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia and is associated with an unfavorable prognosis [1, 2]. Since 1943, it has been recognized that familial clustering of AF occurs. Monogenetic forms of AF, however, represent a rare AF subtype. Although the identified mutations in affected family members have large effects, they do not seem to play a major role in more common AF present in the majority of the patients. The majority of patients have AF in association with concomitant (cardiovascular) conditions [3]. Although most people with AF have one or more identifiable risk factors, not every person with the same risk factors will however develop AF [4]. Hence, a substantial proportion of the variability in AF-risk remains unexplained. In the last few years increasing data have been reported supporting the notion that there is a genetic component to more common AF. Several population-based studies have demonstrated that AF is heritable [5–8]. Unraveling the genetic contribution underlying this common AF phenotype is challenging. Common AF is believed to be a complex trait, in which the interplay between multiple low penetrant genetic variants and environmental factors determine AF risk. In general, a single genetic variant underlying common AF exerts only a minor effect. This hampers the detection of novel genetic variants. Numerous candidate gene studies have been published, however, most results could not be replicated in additional cohorts [9]. Fortunately, advances in genotyping technology, and the advent of genome wide association studies (GWAS) have rapidly increased our understanding of the genetic contribution of many complex traits. Recently, GWAS of the common AF phenotype have been successful in identifying 3 genetic loci associated with AF [10–12].

Identifying individuals with genetic defects predisposing to AF may have important implications. Identification of the genes that play a role in the initiation of the arrhythmia may give new insights into the development of AF, and eventually lead to novel therapeutic options. Also, early recognition of patients at risk may, eventually, prevent AF and reduce morbidity and mortality [13].

In this chapter we will focus on single mutations causing familial forms of AF, and discuss single nucleotide polymorphisms associated with AF in the general population, and lastly, we will discuss the genetic aspects of atrial standstill.

Monogenetic Forms of Atrial Fibrillation

Lone Atrial Fibrillation

Loci Associated with AF

Although some 70 years ago a hereditary form of AF was recognized [14], in 1997 Brugada et al. were the first to identify linkage to a locus on chromosome 10q22-q24 for familial AF in three different Spanish families [15]. In these families, AF segregated as an autosomal dominant disease with high penetrance. In a three-generation family, 10 of 26 living family members were affected. Mean age was 18 years and nine had permanent AF, seven of them being asymptomatic. In two of them an increase of the left ventricular diameters, with left ventricular ejection fractions of 51 and 54 %, respectively, were observed. All others had no echocardiographic abnormalities. One patient had died suddenly at the age of 36 years. In the other two families, all nine affected persons had permanent AF and were asymptomatic without any echocardiographic abnormality. The causative gene has not been identified yet. Sequencing several candidate genes, including genes encoding α(alpha)- and β(beta)-adrenergic receptors and G–protein receptor kinase, in or in the vicinity of the locus on chromosome 10q22-q24 did not reveal any mutations, and could therefore putatively be excluded as causative genes.

Darbar et al. [8], however, could not confirm linkage to chromosome 10q22-q24 in three other families with familial paroxysmal AF and rapid ventricular response, and in one AF family with a slow ventricular response. In contrast to the other three families, AF in the latter family was often asymptomatic. Eventually, four out of ten patients of this family developed a junctional

rhythm, and five out of ten patients left ventricular enlargement with a low-normal left ventricular ejection fraction. The latter finding suggests that the phenotype of the fourth AF family is characterized by an additional cardiomyopathy and atrial conduction disease. Linkage with the AF-associated 3p22-p25 region (which contains the voltage-gated α(alpha)-subunit of the cardiac sodium channel (*SCN5A*) gene) and the *LMNA* gene, associated with familial AF, conduction system disease and dilated cardiomyopathy was excluded [8, 16, 17]. Nevertheless, clinicians should be aware of the development of conduction disturbances or a cardiomyopathy in (young) patients with familial AF and a slow ventricular response. It also highlights the importance of adequately describing the phenotype in studies searching for genes underlying arrhythmias, e.g. AF. The second family in the study by Darbar et al. is completely different from the first one with a different clinical outcome. This suggests at least two distinct mechanisms (and possible associated genes) involved in AF in these families.

In a large family with autosomal dominantly inherited lone AF (AF without apparent associated (non) cardiovascular conditions) [18], eight individuals with AF were identified [19]. In this family, AF started as paroxysmal AF in younger individuals, and became permanent in older family members. The age of onset was variable, ranging from young to elderly family members. The left ventricular function was normal except for one individual who had a left ventricular ejection fraction of 40 % while in AF with a rapid ventricular response. Remarkably, two other individuals of this family had a history of peripartum cardiomyopathy but no AF. The disease locus was mapped to chromosome 6q14-q16, but the causative gene has not been identified yet. Both the locus found by Brugada et al. [15] and this locus [19] overlap with known loci for familial autosomal dominant dilated cardiomyopathy on chromosome 10q21-23 and 6q12-q16, respectively [20–22]. The exact relationship between AF and cardiomyopathy in this family remains to be investigated. Moreover since four of the affected women had 17 pregnancies without signs of peripartum cardiomyopathy.

Potassium Channel Mutations

In 2003 Chen et al. [23] published data of a large Chinese family with autosomal dominantly inherited lone AF. AF was permanent in all patients. The causative mutation (p.S140G) was located in the *KCNQ1* gene on chromosome 11p15.5. The *KCNQ1* gene encodes the pore-forming α subunit of the cardiac I_{Ks} potassium channel (KCNQ1/KCNE1), and the KCNQ1/KCNE2, KCNQ1/KCNE3, KCNQ1/KCNE4 [24] and the KCNQ1/KCNE5 potassium channels [25]. Functional analysis of this mutation revealed a gain-of-function effect on the KCNQ1/KCNE1 and the KCNQ1/KCNE2 currents, thereby reducing the action potential duration and the effective refractory period in atrial myocytes, which in turn may set the stage for initiation and maintenance of AF. Although I_{Ks} and KCNQ1/KCNE2 are also expressed in the ventricle, the QT interval in the affected AF individuals was prolonged rather than shortened (in nine of 16 patients the QT interval was between 460 and 530 ms). The clinical importance of this mutation may be limited, since it could not be confirmed in six additional Chinese families and 19 sporadic idiopathic AF patients, nor in an unselected group of 141 AF patients in the United States of America (largely of Northern European descent) [26].

Recently, Das et al. [27] identified a *KCNQ1* mutation (p.S209P) in the third transmembrane region after linkage analysis in a family with autosomal dominant AF. Fourteen family members were studied; six of them had AF, with a mean age at onset of 32 years (range 16–59 years). Family members with AF had a longer mean QRS duration (100 ms), but no prolonged corrected QT interval (423 ± 15 ms). The electrophysiological properties of this mutant KCNQ1 channel were different; the channel activated faster, deactivated slower, and a hyperpolarizing shift in the voltage activation curve occurred. Also, some of the mutant channels are constantly open, resulting in a net increase in I_{Ks} current.

The group of Chen et al. [28] also identified a mutation (p.R27C) of the *KCNE2* gene in 2 Chinese families with lone AF. The *KCNE* gene family encodes small proteins that function as β(beta)-subunits of several voltage-gated cation

channels, such as the KCNQ1-KCNE2 channel, which produces background potassium current [29]. The age at diagnosis, between 40 and 60 years, was higher than observed in the family with the *KCNQ1* mutation. Most patients had symptomatic paroxysmal AF and also frequent premature atrial complexes. Left atrial size and left ventricular ejection fraction were within normal limits. Functional analyses revealed a gain-of-function effect resulting in both inward and outward KCNQ1-KCNE2 potassium currents leading to a shortening of the action potential duration, which again may trigger and perpetuate AF.

Xia et al. [30] studied 30 unrelated Chinese kindreds with familial AF. They identified one family in which a missense mutation (p.V93I) in the *KCNJ2* gene, encoding the Kir2.1 potassium channel, was present in all affected family members. These subjects had no underlying heart disease and a normal QT interval. The Kir2.1 channel is responsible for the I_{K1} inward rectifier current. Expression of this mutant in COS-7 and human embryonic kidney cells resulted in a shortening of the action potential duration and effective refractory period caused by a gain in function of Kir2.1. The investigators analyzed this gene in another 154 patients with lone AF but no mutations were detected [30]. Additionally, they studied the *KCNQ1*, *KCNH2*, *SCN5A*, *ANK-B* and *KCNE1-5* genes in the 30 families, which did not reveal additional mutations. However it is not clear whether the population studied overlaps population described before [28]. In 96 American patients with familiar AF no mutations in the *KCNJ2* gene could be identified [31].

In another family with lone AF, Olson et al. [32] identified a mutation in the *KCNA5* gene (Table 35.1). This gene encodes for the atrial specific Kv1.5, potassium channel, responsible for the ultrarapid delayed rectifier current (I_{Kur}). A heterozygous nonsense mutation (c.1123G > T) in exon 4 of *KCNA5*, resulting in a premature stop codon at residue 375 (p.E375X) was found. This mutation results in I_{Kur} loss of function by a dominant negative effect on Kv1.5. Thus, the atrial action potential prolongs and early afterdepolarizations may develop. The p.E375X mutation was not found in 540 unrelated control subjects. Yang et al. [33] screened 120 unrelated kindreds for mutations in 12 established AF susceptibility genes (*KCNQ1*, *HERG*, *SCN5A*, *Ankyrin-B*, *KCNJ2*, *KCNE1*, *KCNE2*, *KCNE3*, *KCNE4*, *KCNE5*, *KCNA5* and *GJA5*), and found three mutations in the *KCNA5* gene; p.T527M was found in two AF families, and p.A576V and p.E610K in two other AF families. Electrophysiological work revealed loss of function of the mutant KCNA5 channels influencing the ultra-rapid activating delayed rectifier potassium current. A similar approach was used in a cohort of 580 AF patients of Vanderbilt University. Yang et al. [34] screened candidate ion channel genes (*KCNQ1*, *KCNE1-5*, *KCNJ2*, *KCNA5*, *SCN5A* [α(alpha)- and β(beta)-subunits], L-type Ca^{2+}) as well as some candidate non-ion channel genes including *GJA5*, and *NPPA*. A deletion of 33 basepairs in the coding region of the *KCNA5* gene in two Caucasian probands was found. Both probands had a family history of AF, for one proband four other family members with AF were available, and all were mutation carriers. The mutation results in deletion of 11 amino acids in the N-terminus of the protein. This proline-rich region is involved in tyrosine kinase regulation of I_{Kur} and reduces the potassium current, but makes the channel kinase-resistant. Tyrosine kinase stimulation is a response to numerous factors, including atrial stretch. So, the physiologic response of I_{Kur} to tyrosine kinase stimulation is potassium current suppression, but the mutant channel may fail this response, and may cause action potential shortening and increase the susceptibility of AF.

Finally, Ravn et al. [35] sequenced the *KCNE5* gene in a cohort of 158 patients with AF. In one individual, without a familial history of AF, they found a missense mutation (p.L65F) in the *KCNE5* gene leading to a gain of function of I_{Ks}.

Sodium Channel Mutations

Besides changes in atrial action potential duration, such as caused by the above mentioned mutations in atrial potassium channels, also alterations in atrial conduction may lead to predisposition to AF.

Laitinen-Forsblom et al. [36] identified a mutation in the *SCN5A* gene in a large Finnish

Table 35–1. Monogenic forms of lone AF

Gene/chromosome	MOI	Nr. Fam	Type AF	Age	Ethnicity	HR	(A)symp	TCM	QTc (ms)	Mechanism
I. No locus and gene identified										
FAF 1-3 [8]	AD	3	PAF	38–51	?	High	symp	n = 2	Normal	?
FAF 4 [8]	AD	1	PAF	37	?	Slow	asymp	No	↑ in 2	?
II. Locus identified										
10q22-q24 [15]										
FAF 1	AD	1	CAF, (PAF)	18	Cauc	na	asymp	No	na	?
FAF 2-3	AD	2	CAF	2–46	Cauc	na	asymp	No	na	?
6q14-q16 [19]	AD	1	PAF, CAF	21–72	Cauc	na	na	No[a]	na	?
III. Gene identified										
KCNQ1 [23]	AD	1CAF	>5	Chin	na	na	na	50 % ↑		I_{Ks} ↑
KCNQ1 [27]	AD	1	PAF, CAF	30	Cauc	na	symp	No	Normal	I_{Ks} ↑
KCNE2 [28]	AD	2	PAF	>40	Chin	High	symp	No	Normal	I_{Ks} ↑
KCNE5 [35]	?	1[b]	CAF	59	Cauc	na	na	No	Normal	I_{Ks} ↓
KCNJ2 [30]	AD	1	PAF, CAF	50–58	Chin	63–118	symp	No	Normal	I_{K1} ↑
KCNA5 [32]	AD	1	PAF	30–40	?	na	symp	No	na	I_{Kur} ↓
KCNA5 [33]	?	1[b]	PAF, CAF	23–52	Chin	na	na	No	na	I_{Kur} ↓
KCNA5 [34]	AD	1	PAF	18–48	Cauc	na	symp	No	na	I_{Kur} ↓
SCN5A [36][c]	AD	1	PAF/AFL	12–20	Cauc	na	symp	No	Normal	Excitability ↓
SCN5A [39]	AD	1	PAF	41	Cauc	na	symp	No	Normal	Excitability ↓
SCN5A [40]	AD	1	PAF, CAF	26–51	Jap	68–88	symp	No	Normal	Excitability ↓
SCN1B [41]	?	1[b]	PAF	35–58	Multi	na	na	No	Normal	Excitability ↓
SCN2B [41]	?	1[b]	PAF	35–58	Multi	na	na	No	Normal	Excitability ↓
SCN3B [42]	?	1[b]	PAF, CAF	30	Cauc	61–86	na	No	na	Excitability ↓
NUP155 [43][d]	AR	1	CAF	Young	Uru	High	symp	Some	Normal	?
GJA5 [45]	?	1[b]	PAF	41	?	na	symp	na	na	Dispersed conduction
GJA5 [46]	AD	1[b]	PAF, CAF	45	Chin	73	na	na	na	Dispersed conduction
GJA5 [47]	AD	1[b]	PAF, CAF	45	Chin	73	na	na	na	Dispersed conduction
NPPA [48]	AD	1	PAF, CAF	40	Cauc	na	symp	n = 5	na	?
ANK2 [49]	?	2	PAF	11–79	?	45–88	na	No	Prolonged	?

AD autosomal dominant, *AF* atrial fibrillation, *AFL* atrial flutter, *(A)* symp (a) symptomatic, *CAF* chronic/permanent AF, *Cauc* Caucasian, *Chin* Chinese, *FAF* familial AF, *Fam* number of families, *HR* heart rate, *Jap* Japanese, *MOI* mode of inheritance, *na* not available, *PAF* paroxysmal AF, *TCM* tachycardia induced cardiomyopathy, *Uru* Uruguay, *?* unknown

[a]One patient had a reversible tachycardiomyopathy, one carrier had peripartum cardiomyopathy but no AF

[b]No families but subjects

[c]Three carriers suffered from stroke

[d]Multiple sudden deaths occurred at very young age

family with cardiac conduction defects and atrial arrhythmias (Table 35.1). Six of 44 family members were affected, of which five had a pacemaker implanted due to conduction defects and/or bradycardia before the age of 20. Five of these six subjects suffered from various atrial arrhythmias including paroxysmal AF and atrial flutter. Three suffered from stroke between the age of 20–30 years. None had symptoms before their teens. All six affected individuals had a normal left ventricular ejection fraction although the left ventricle and right ventricle were enlarged in one and three individuals, respectively. A mutation (D1275N) in the *SCN5A* gene was found in the six affected individuals as well as in two young asymptomatic family members. One of these two carriers had an abnormally wide QRS complex (116 ms). The other was only 12 years old at the time of analysis. The p.D1275N mutation has also been reported in a family with atrial standstill [37] (see below) and in different families with dilated cardiomyopathy [16, 17]. Although atrial standstill due to the p.D1275N mutation has been demonstrated in this Dutch family, it occurred only in combination with polymorphisms in the promoter and untranslated region of the *GJA5* gene, encoding connexin 40 [37]. Although p.D1275N did not seem to cause serious channel dysfunction when studied in heterologous expression systems, Watanabe et al. [38] found in a mouse model that the p.D1275N mutation produced extensive

channel dysfunction, and a cardiomyopathy phenotype. It is currently unknown why this mutation expresses these different phenotypes and whether the phenotype described in the Finnish family may be considered to be true lone AF.

Ellinor et al. [39] screened the *SCN5A* gene in a cohort of 57 probands with a familial history AF. They found a mutation (p.N1986K) in one family (two affected members available for analysis) with AF, leading to a loss of function of the sodium channel.

Makiyama et al. [40] studied a Japanese family with autosomal dominant segregated lone AF. Six out of 14 family members had AF; one additional family member had premature atrial beats, but no AF. They found a missense mutation (p.M1875T) in the *SCN5A* gene, again leading to a loss of function of the sodium channel.

Watanabe et al. [41] screened an AF cohort for mutations in the sodium channel β-subunit genes *SCN1B*, *SCN2B*, *SCN3B*, and *SCN4B*. A cohort of 480 individuals with AF, including 118 with lone AF and 362 patients with AF and cardiovascular disease was sequenced. They discovered two mutations in *SCN1B* (p.R85H, p.D153N), and two mutations in *SCN2B* (p. R28Q, p.R28W). In three of four mutation carriers, the ECGs showed saddleback-type ST-segment elevation in the right precordial leads. Expression studies demonstrated that the mutant *SCN1B* and *SCN2B* reduced the sodium current.

In Denmark, Olesen et al. [42] studied 192 unrelated lone AF patients, and sequenced the entire coding sequence and splice junctions of *SCN3B* and *SCN4B*. Three non-synonymous mutations p.R6K, p.L10P, and p.M161T, were detected in the *SCN3B* gene. Electrophysiological studies revealed that all mutations caused a reduction in sodium channel current.

Nucleoporin Mutation

Nucleoporin, encoded by the *NUP155* gene, is a major part of the nuclear pore complex [43]. The nuclear pore complex mediates bidirectional transport of mRNAs and proteins between the nucleus and the cytoplasm, potentially regulating the expression of important atrial genes, and subsequently increases the susceptibility of AF. Oberti et al. [44] identified a locus on chromosome 5p13 in autosomal recessively inherited neonatal AF. The affected children showed a rapidly progressive disease: AF with early onset at the fetal stage, neonatal sudden death, ventricular arrhythmias and cardiomyopathy (Table 35.1). The heterozygous parents, both without AF, could be identified by a broad P wave on the electrocardiogram. In 2008, the investigators were able to find the causative homozygous mutation (p.R391H) in the *NUP155* gene. These findings provide a novel non-ion-channel mechanism for the pathogenesis of AF, however the exact molecular mechanism linking the nuclear pore complex to AF is still unknown. Whether this disease is caused primarily by AF or, more likely, by an underlying cardiomyopathy remains to be determined.

Gap Junction Protein Mutations

The cardiac gap-junction protein connexin 40, encoded by the *GJA5* gene, is expressed selectively in the atria and coordinate the electrical activation of the atrial myocardium. Gollob et al. [45] sequenced the *GJA5* gene, encoding connexin 40, in cardiac tissue and lymphocytes in 15 unrelated patients with lone AF. In three patients a novel heterozygous missense mutation was identified in cardiac tissue alone (somatic mutation). However, in one patient the mutation (p.T286G) was present in both cardiac tissue and lymphocytes (germ-line mutation). This indicates that somatic mutations may underlie AF, but inheritable germ-line *GJA5* mutations may also occur. Analysis of the expression of the mutant proteins revealed impaired intracellular transport or reduced intercellular electrical coupling, which may result in anisotropic atrial conduction, promoting reentry. Yang et al. [46] sequenced the coding region of the *GJA5* gene in 126 Chinese unrelated probands with familial AF. A heterozygous mutation, c.145C > T (p.Q49X) was detected in one proband. This nonsense mutation was present in all relatives with AF of the carrier.

The same group published another report on 218 Chinese unrelated probands [47]. It

is unknown whether this study includes the same patients as the other report [46]. Three heterozygous missense *GJA5* mutations, p.V85I, p.L221I, and p.L229M, were found in three of 218 AF families, and co-segregated with AF. These results indicate that in some patients lone AF may have a genetic basis confined to the diseased myocardial tissue.

Atrial Natriuretic Peptide Mutation

Atrial natriuretic peptide, encoded by the *NPPA* gene, is a well-known circulating hormone regulating intravascular blood volume by stimulating natriuresis, diuresis, and vasodilatation. In addition, via cGMP signaling pathways, atrial natriuretic peptide has been shown to modulate ion channel currents in cardiac myocytes leading to shortening of the atrial refractory period. Also anti-apoptotic effects of atrial natriuretic peptide have been described.

Hodgson-Zingman et al. [48] described a family of European ancestry with autosomal dominant AF. Eleven of 21 family members had AF, five of whom presented with a tachycardiomyopathy. In all affected family members a heterozygous two-basepair deletion (c.456-457delAA) in exon 3 of the *NPPA* gene was found. This frameshift mutation leads to an extension of the reading frame, translating to a mutant protein that includes the normal atrial natriuretic peptide plus a carboxyl terminus of 12 residues. In all heterozygous affected family members increased concentrations of mutant atrial natriuretic peptide were found. The exact mechanism linking the frameshift mutation in *NPPA* to the phenotype AF needs to be elucidated.

Ankyrin-B Genetic Variants

Membrane adapter protein ankyrin-B, encoded by the *ANK2* gene, is expressed in atrial cardiomyocytes, ventricular cardiomyocytes, and cardiac Purkinje conduction fibers. Ankyrin-B is known to target ion channels, transporters, and signaling molecules like Na/K ATPase, Na/Ca exchanger, inositol 1,4,5 trisphosphate (Ins*P*3) receptor. Loss-of-function mutations in the ANK2 gene may affect cardiac conduction, the atrial and ventricular rhythm, and the sinus and atrioventricular nodes.

Cunha et al. [49] investigated 2 previously published large families with severe sinus node dysfunction associated with the *ANK2* locus [50], and found that individuals with loss-of-function mutations in *ANK2* developed early-onset AF. In additional experiments with atrial myocytes a shortened action potential was found, and loss of L-type Ca^{2+} current. Furthermore, Cav1.3 was found to function as ankyrin-B–binding target. In ankyrin-$B^{+/-}$ atrial myocytes, Cav1.3 protein expression and membrane function were reduced. Cav1.3 may be a potential molecular mechanism underlying the association between Ankyrin-B mutations and early-onset AF.

AF in the Setting of Other Monogenetic (Cardiac) Conditions

In certain individuals, AF is also related to other monogenic (cardiac) diseases including dilated cardiomyopathy [16, 20, 22, 51, 52], hypertrophic cardiomyopathy [53], peripartum cardiomyopathy [54, 55], arrhythmogenic right ventricular cardiomyopathy [56–58], skeletal myopathies [59–61], long QT syndromes [62–64], short QT syndromes [65–67], Brugada syndrome [68], familial amyloidosis [69], congenital cardiac abnormalities [70, 71], and preexcitation syndromes [72]. In some of these conditions, AF will occur secondary to left ventricular dysfunction rather than to primary atrial disease [73]. These associations are beyond the scope of this chapter and are not further discussed.

Genetic Variants Associated with Common AF

Heritability of AF in the Community

AF predominantly occurs in the setting of concomitant (cardiovascular) conditions, in particular, hypertension, coronary heart disease, valve disease and heart failure. Genetic differences may also contribute to this more common form of AF, explaining why some individuals will suffer repeatedly from AF, while others never will. Data from population-based studies suggest that familial clustering and heritability of AF is

widespread and that the common AF phenotype has a significant genetic component [5, 6].

Fox et al. [5] demonstrated that parental AF not only increases the risk for offspring AF in lone AF, but also in the more common forms of AF in the setting of structural diseases. In the Offspring cohort of the Framingham Heart study, they found that 681 out of 2,243 persons (30 %) had at least one parent with documented AF. During follow-up, 70 of these 681 persons (10 %) developed AF. If AF was present in at least one parent, the risk of offspring AF increased by 1.85 (95 % confidence interval 1.12–3.06, $p = 0.02$) when compared to the absence of parental AF. As expected, the results were stronger if only younger parents and offspring (<75 year) without a previous myocardial infarction, heart failure, or valvular disease were assessed (odds ratio 3.17, 95 % confidence interval 1.71–5.86, $p < 0.001$). More recently, Lubitz et al. [74] studied the predictive value of familial AF. In 1,185 of the 4,421 individuals (27 %) from the Original and Offspring Cohorts a first-degree relative was documented with AF. Individuals with a first-degree relative with AF more frequently developed AF during follow up than individuals without documented AF in family members. This association (hazard ratio 1.40, 95 % confidence interval 1.13–1.74, $p = 0.002$) was not altered by adjustment for traditional AF risk factors such as age, sex, body mass index, systolic blood pressure, treatment for hypertension, PR interval, heart murmur, or heart failure. Assessment of AF in a first-degree relative younger than 65 years was associated with a very slight increase in predictive accuracy compared with traditional risk factors. Arnar et al. [6] described similar findings in a cohort of 5,269 inhabitants of Iceland. In this study, 4,195 patients (80 %) with AF were related to others with AF. The risk of AF for first-degree relatives of a family member with AF was 1.77 (95 % confidence interval 1.67–1.88, $p < 0.001$), when compared with individuals in the general population. In patients younger than 60 years of age the risk for AF increased to 4.67 (95 % confidence interval 3.57–6.08, $p < 0.001$).

The Danish Twin Registry [75] reported on the risk of AF in monozygotic and dizygotic twins when their co-twin had documented AF. They studied 1,137 same-sex twin pairs in which one or both were diagnosed with AF. The risk of AF was two times higher for a monozygotic twin whose co-twin has AF in comparison to the risk for a dizygotic twin.

Polymorphisms Associated with Common AF

Candidate Gene Association Studies

Numerous candidate gene association studies have been published on common genetic variants that may increase risk of AF (Table 35.2). In general, the candidate genes studied in these reports were previously detected in monogenetic forms of AF. So, some of the underlying mechanisms underlying AF seem similar in both monogenetic AF and more common AF.

Potassium Channel Genetic Variants

Alterations in potassium channel function may affect the action potential duration and the effective refractory period in atrial myocytes. The electrophysiological changes may be important for the development of AF.

Lai et al. performed a case control study in 108 consecutive non-familial AF patients from Taiwan with any underlying disease but excluding patients with hyperthyroidism [76]. Mean age was 63 years and a history of hypertension was present in 52 % of the patients. More than 80 % of the patients had permanent AF. Matching was performed regarding sex, age, left ventricular dysfunction and valvular disease. An association between the *KCNE1* (encoding the minK β(beta)-subunit of I_{Ks} potassium channel) 38G allele and AF was observed. The odds ratios for AF in patients with 1 and 2 *KCNE1* 38G alleles were 2.16 (95 % confidence interval 0.81–5.74) and 3.58 (95 % confidence interval 1.38–9.27), respectively, compared to patients without the *KCNE1* 38G allele. Recently, the functional role of this polymorphism has been demonstrated [77].

Fatini et al. [78] likewise found an association between the presence of the 38G *minK* allele and AF in 331 Caucasian patients. Additionally, they studied several polymorphisms in the endothelial nitric oxide synthase (*eNOS*) gene including

c.894G > T nucleotide exchange in exon 7, a T/C exchange in the promoter region (−T786C), and the *eNOS* 4a/4b polymorphism. Only *eNOS* -T786C was significantly more prevalent in patients with AF when compared to healthy volunteers. Surprisingly, the combined presence of *minK* 38G and *eNOS* -786C resulted in a stronger predisposition for AF than the *minK* variant alone.

Ravn et al. [79] investigated the 97T-polymorphism of the *KCNE5* gene. This gene is located on the X-chromosome and encodes an inhibitory β(beta)-subunit, MiRP4, of the repolarizing cardiac potassium channel KCNQ1. When compared to 158 patients with AF, a greater proportion of the 96 healthy control subjects was carrier of the 97T-allele (15.2 % vs. 29.2 %). The OR for AF if a patient did not have a copy of the 97T-allele was 2.17 (95 % CI 0.89–5.28) in men and 1.94 (95 % CI 0.80–4.75) in women. This difference may be explained by the localization on the X chromosome. The exact mechanism by which this gene changes atrial electrophysiology has not been investigated yet.

Sinner et al. [80] analyzed 1,207 individuals with AF and 2,475 controls in Germany and genotyped 40 single nucleotide polymorphisms in the *KCNH2* (*HERG*) gene, encoding the α-subunit of the I_{Kr} ion channel, important for both ventricular and atrial repolarization. They found 5 polymorphisms associated with AF, of which one (the common K897-allele of the *KCNH2*-K897T variant) was replicated in an additional cohort of 536 individuals with AF and 1,781 controls. The odds ratio of the association between the K897-allele and AF was 1.25 (95 % CI 1.11–1.41, P = 0.0003). A case–control study performed by Wang et al. [81] included 297 individuals with AF and concomitant cardiovascular diseases, and the same number of controls. They genotyped four single nucleotide polymorphisms in the *KCNH2* gene region. Single nucleotide polymorphism rs1805120, located in exon 6 of the *KCNH2* gene, was associated with an increased risk of AF (odds ratio 1.45, 95 % confidence interval 1.09–1.93, P = 0.012). The underlying causal mechanism linking these common polymorphisms in *KCNH2* to AF needs to be elucidated.

Sodium Channel Genetic Variants

In contrast to potassium channel alterations it is less clear which effects loss-of-function sodium channels exert. Reduced sodium channel functionality may lead to decreased excitability of atrial myocardium.

Chen et al. [82] performed targeted genotyping of a common loss-of-function p.H558R polymorphism of the *SCN5A* gene in 157 patients with early-onset lone AF, and 314 matched controls. In comparison to the controls, the R558 allele was more common in AF patients (30 versus 21 %, P = 0.002), resulting in an odds ratio for AF of 1.6 (95 % confidence interval 1.2–2.2). Darbar et al. [83] genotyped the *SCN5A* gene for the presence of mutations or rare variants in cohort of 375 AF patients, 118 with lone AF and 257 with AF in the setting of heart diseases. In ten probands (2.7 %), eight novel variants not found in the control population (0 %, P = 0.001) were discovered, that were predicted to disturb cardiac sodium channel function. In 6 families with more than one affected family member, the variant segregated with AF. In addition, 11 rare missense variants in 12 probands (3.2 %) that have previously been described in patients with inherited arrhythmia syndromes (congenital long QT syndrome, Brugada syndrome) were found in this cohort.

Gap Junction Protein Genetic Variants

Loss of gap junction protein function may lead to dispersed conduction and increase the vulnerability for AF.

Firouzi et al. [84] reported that a *GJA5* gene, encoding connexin 40, promoter polymorphism (−44AA genotype) is linked to an increased coefficient of spatial dispersion of refractoriness and an increased risk of AF (Table 35.2). They investigated the coefficient of dispersion, defined as the standard deviation of all local mean fibrillatory intervals expressed as a percentage of the overall mean fibrillatory interval, in 30 unrelated adults. These patients had no structural heart disease and underwent an electrophysiological study because of the presence of an accessory pathway (n = 27) or atrioventricular nodal reentrant tachycardias (n = 3). Of these

subjects, 14 had prior documented sporadic episodes of AF whereas 16 had no history of AF. The AF patients suffered a mean of 1 (range 1–5) previous episode of AF with a median duration of 1 h (range 15 min to 3 h). The AF free interval before the study was 148 days (range 9–365 days). The prevalence of the minor connexin 40 allele (−44A) and −44AA genotype was significantly higher in subjects with increased dispersion (n = 13) compared to patients with a normal coefficient of dispersion (n = 17, p = 0.00046 and p = 0.025, odds ratio 6.7 and 7.4, respectively). Subjects with the −44AA genotype had a significantly higher coefficient of dispersion as compared to those with the −44GG genotype. Finally, all subjects with an increased dispersion had a history of AF compared with only 1 subject with a normal coefficient of dispersion. The mechanism is at present unknown. It may be surmised that an abnormal gap junction distribution may cause abnormal conduction with increased anisotropy. By creating a substrate, this may result in an increased propensity for AF.

Juang et al. [85] studied two single nucleotide polymorphism (alleles at position −44 and +71) in the *GJA5* region, in 173 patients with AF and concomitant cardiovascular conditions, and compared the prevalence of polymorphisms to controls (n = 232). Polymorphism −44A and +71G were linked to each other, and patients with the −44A *and* +71G haplotype more frequently had AF. The −44A *and* +71G haplotype was associated with lower promoter activity in atrial myocytes.

Wirka et al. [86] tested eight single nucleotide polymorphisms of the *GJA5* gene for their association with AF in atrial tissue of 61 AF patients. The A-44G polymorphism, described by Firouzi et al. [84], was not associated with decreased connexin 40 mRNA levels, but another common polymorphism (rs10465885) located in the TATA box of an alternative *GJA5* promoter was strongly associated with connexin 40 mRNA expression (P < 0.0001) in human atrial tissue. Both polymorphisms were tested for association with lone AF in 384 cases and 3,010 controls. rs10465885 was associated with the AF phenotype (odds ratio 1.18, P = 0.046), and confirmed in a meta-analysis including two other lone AF case–control cohorts (odds ratio 1.16, P = 0.022). The A-44G polymorphism was not associated with lone AF in any of these cohorts.

Thibodeau et al. [87] studied atrial tissues and lymfocytes of 10 unrelated lone AF patients for mutations in the *GJA1* gene, encoding connexin 43. They found a frameshift mutation in one patient (c.932delC) that led to truncation of the C-terminal domain of connexin 43. However, the mutation was absent in the lymphocyte DNA of the same patient, suggesting a somatic mosaicism. Additional, protein trafficking studies demonstrated intracellular retention of the mutant protein and negatively influenced gap junction formation of wild-type connexin 43 and connexin 40. Electrophysiological studies revealed only reductions in coupling when coexpressed with wild-type connexins.

Renin-Angiotensin-Aldosterone System Genetic Variants

The *ACE* gene, encoding the angiotensin-converting enzyme, contains a common polymorphism based on the insertion (I) or deletion (D) of a 287-bp intronic DNA fragment. Carriers of the D allele are found to have higher ACE activity and, subsequently, higher angiotensin II levels. Angiotensin II in turn stimulates cardiac fibrosis and conduction heterogeneity. Ultimately this may set to stage for AF.

Bedi et al. [88] studied a cohort of 340 patients with heart failure, of whom 51 developed AF. They screened for single nucleotide polymorphisms in genes that control production of angiotensin-converting enzyme (ACE) and eNOS. eNOS is present in endothelial cells, with increased expression in response to wall stress, and is considered to have protective effects on the atrial myocardium directly or via alterations in ACE activity. They found an association of the *ACE* D/D (odds ratio 1.5, 95 % confidence interval 0.8–2.4) and *eNOS* 894T/T genotypes (odds ratio 3.2, 95 % confidence interval 1.7–6.2) and AF. In a case control study Yamashita et al. [89] could not demonstrate a significant association between *ACE* gene insertion/deletion polymorphisms and AF. Tsai et al. [90] investigated several gene polymorphisms of the

Table 35–2. Single nucleotide polymorphisms associated with common AF

Gene	SNP	Subjects	Type AF	Age	Ethnicity	HR	(A)symp	UHD	Mechanism
KCNE1 (minK) [76]	S38G	108	CAF	63	Chin	na	na	Yes	$I_{Ks}\downarrow$
KCNE1 (minK) [78]	S38G	331	CAF	73	Cauc	na	na	Yes	$I_{Ks}\downarrow$
KCNE5 [79]	C97T	158	PAF, CAF	66	Cauc	na	na	na	Possible (I_{Ks})
KCNH2 (HERG) [80]	K897T	1,207	PAF, CAF	69/60[a]	Cauc	na	symp	Yes	Possible
KCNH2 (HERG) [81]	rs1805120	297	PAF, CAF	66	Chin	na	na	Yes	Possible
SCN5A [82]	H558R	157	PAF	52	Cauc	72	na	No	Excitability/cond vel ↓
SCN5A [83]	M138I	375	PAF, CAF	56	Multi	na	na	Yes	Excitability/cond vel ↓
	E428K								
	H445D								
	N470K								
	E655K								
	T1131I								
	R1826C								
	V1951M								
eNOS [78]	T-786C	331	CAF	73	Cauc	na	na	Yes	Stimulate ACE?
eNOS [88]	G894T	51	PAF, CAF	63	Cauc	na	na	Heart failure	Stimulate ACE?
GJA5 [84]	−44A *and* +71G	30	PAF	33	Cauc	na	symp	WPW	Dispersed conduction?
GJA5 [85]	−44A *and* +71G	173	PAF, CAF	70	Chin	na	symp	Yes	Dispersed conduction?
GJA5 [86]	rs10465885	1,090	PAF, CAF	≤60	Cauc	na	na	No	Dispersed conduction?
GJA1[87]	c.932delC	10	PAF	<55	Cauc	na	na	No	Dispersed conduction?
AGT [90]	M235T	250	CAF	68	Chin	na	symp	Yes	Atrial remodeling?
	G-6A								
	G-217A								
AGT [91]	A-20C C/C	968	PAF, CAF	68	Cauc	na	na	70 % hypertension	Atrial remodeling?
	A-20C C/C *and* ACE D/D								
ACE [88]	D/D	51	PAF, CAF	63	Cauc	na	na	Heart failure	Atrial remodeling?
ACE [92]	D/D	404	PAF, CAF	71	Cauc	na	na	60 % hypertension	Atrial remodeling?
ACE [93]	D/D	97	PAF, CAF	73	Chin	na	na	Hypertension + LVH	Atrial remodeling?
GNB3 [98]	C825T	227	PAF, CAF	58	?	na	na	59 % hypertension	Possible ($I_{K,Ach}$)
IL6 [105]	G-174C	110	Post op AF	61	?	na	na	Yes	Inflammation?
IL10 [106]	C-1306T	196	PAF, CAF	68	Jap	na	na	40 % hypertension	Inflammation?
MMP2 [106]	A-592C	196	PAF, CAF	68	Jap	na	na	40 % hypertension	Atrial remodeling?
MMP9 [108]	C-1562T	128	PAF, CAF	73	Chin	na	na	Hypertension + LVH	Atrial remodeling?
TIMP2 [107]	G-418C	128	PAF, CAF	73	Chin	na	na	Hypertension + LVH	Atrial remodeling?
NPPA [109]	rs5063	384	PAF, CAF	59	Chin	na	na	Yes	Possible

ACE angiotensin-converting enzyme, *AF* atrial fibrillation, *(A)* symp (a) symptomatic, *CAF* chronic/permanent AF, *Cauc* Caucasian, *Chin* Chinese, *cond vel* conduction velocity, *na* not available, *HR* heart rate, *Jap* Japanese, *LVH* left ventricular hypertrophy, *PAF* paroxysmal AF, *Post op AF* post operative AF, *SNP* single nucleotide polymorphism, *UHD* underlying heart disease, *WPW* Wolf Parkinson White syndrome, *?* unknown

[a]Mean age of the discovery cohort/mean age of the replication cohort

renin angiotensin system in 250 Chinese patients with AF in the setting of underlying heart diseases and 250 matched controls. They observed, comparable to the data of Yamashita, no association between the *ACE* gene insertion/deletion polymorphism and AF. The same was found for the investigated polymorphism of the angiotensin II receptor gene. However, the angiotensinogen (*AGT)* gene M235T, G-217A and G-6A polymorphisms were associated with AF. The odds ratios for AF were 2.5 (95 % confidence interval 1.7–3.3) with M235/M235 plus M235/T235 genotype, 3.3 (95 % confidence interval 1.3–10.0) with G-6/G-6 genotype, and 2.0 (95 % confidence interval 1.3–2.5) with G-217/G-217 genotype, respectively.

Ravn et al. [91] genotyped 9,235 individuals from the Danish general population for the A-20C, G-6A, T174M, and M235T polymorphisms in the *AGT* gene and the insertion/deletion (I/D) polymorphism in the *ACE* gene. During long-term follow-up, 968 participants developed AF. The hazard ratio of the A-20C homozygous C/C carriers was 1.5 (95 % confidence interval 1.1–2.1, P = 0.01), and for

double homozygotes (A-20C C/C *and* ACE D/D) the hazard ratio for AF was 2.4 (95 % confidence interval 1.4–4.1, P = 0.001). The absolute 10-year risk for AF was 61 % for A-20C C/C carriers.

Fatini et al. [92] studied an Italian cohort of AF patients (106 with lone AF, 404 with AF and concomitant cardiovascular conditions) and compared the allele frequency of the *ACE* I/D polymorphism to controls (n = 520). The *ACE* D allele was associated with lone AF, and AF in the setting of other cardiovascular conditions.

Similar results were found by Huang et al. [93] in a Chinese cohort of patients with hypertensive heart disease (n = 626), including 97 AF patients. The *ACE* DD genotype was associated with AF in that particular cohort. In the same cohort, a polymorphism (C-344T) of the *CYP11B2* gene, encoding aldosteron synthase, was screened, but no relation with AF was found. Watanabe et al. [94] found that within individuals of European ancestry with lone AF, the D allele was associated with longer PR interval (12.0 ms increase per D allele). P-wave duration showed a similar trend. So, ACE activity, modified by the I/D polymorphism may play a role in cardiac remodeling in individuals with AF. Furthermore, these data might explain why drugs affecting the renin angiotensin system may have beneficial effects on the prevention of AF [95–97].

G-Protein Genetic Variant

G-protein coupled receptors are transmembrane receptors and are important for signal transduction. G-proteins regulate ion channel function and intracellular enzyme function, and may set the stage for AF.

Schreieck et al. [98] determined the genotype of the C825T polymorphism in the G-protein β(beta)3 subunit (*GNB3*) gene in 291 patients with AF and 292 controls. This polymorphism is a risk factor for hypertension [99]. In this study, 59 % of AF patients and 62 % of controls suffered from hypertension. Patients with coronary artery disease, valvular heart disease or cardiomyopathy were excluded. The TT genotype was significantly less present in the AF group when compared to the control group (5.8 % vs. 12.0 %) and associated with a 51 % decrease in the unadjusted risk for the occurrence of AF (OR, 0.49; 95 % CI, 0.28–0.85; p = 0.01). The presence of CC and CT genotypes were not statistically different between both groups.

Inflammatory Genetic Variants

Inflammatory biomarkers like C-reactive protein (CRP) and interleukin-6 (IL6) have been associated with AF [100–102]. Also, focal lesions of inflammation have been found in atrial myocardium [103, 104], however, the pathophysiological mechanism by which inflammation increases AF susceptibility is not well understood.

Gaudino et al. [105] assessed the role of the -174G/C *IL6* (*IL6*) gene polymorphism in 110 patients undergoing coronary artery bypass surgery. This polymorphism has been previously associated with postoperative interleukin 6 levels. In the postoperative period, 26 patients developed AF. Analysis of the polymorphism demonstrated a significant prevalence of the GG genotype in patients with AF (34 % vs. 10 %). This resulted in an OR of 3.25 (95 % CI 1.23–8.62).

Kato et al. [106] performed an association study for 40 polymorphisms of 32 candidate genes, selection based on the available literature, and lone AF in 1,069 Japanese individuals. They found that the A-592C polymorphism of the *IL10* gene, encoding interleukin 10, decreases the risk for AF. Common polymorphisms in *IL6* and *IL10* genes show a possible role of inflammation and AF.

Matrix Metalloproteinases and Tissue Inhibitors Genetic Variants

Matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are suggested to play an important role in extracellular matrix metabolism and may contribute to atrial tissue remodeling.

Kato et al. [106] (see description previous section) demonstrated that the C-1306T polymorphism of the *MMP2* gene, encoding for matrix metalloproteinase 2, increases the risk for AF.

Gai et al. [107] included 881 Chinese hypertensive patients with left ventricular hypertrophy, of which 128 with AF. The genotypes of the

MMP2 C-1306T and C-735T polymorphisms and *TIMP2* G-418C polymorphisms were determined. The homozygous and heterozygous C allele carriers had more often AF (odds ratio 1.77, 95 % confidence interval 1.21–2.92, P = 0.009) after adjusting for age, left atrial size, left ventricular mass index, and antihypertensive drugs. The C allele led to lower plasma TIMP-2 concentrations. In this study no association between *MMP2* polymorphisms and AF was found.

In the same Chinese hypertensive heart disease cohort (n = 881), Gai et al. [108] screened two common polymorphisms (C-1562T and R279Q) of the *MMP9* gene, encoding matrix metalloproteinases-9. They found that the T allele carriers of the C-1562T polymorphism had an increased risk of AF with an odds ratio of 1.94, 95 % confidence interval 1.20–3.14, P = 0.006. These findings suggest a possible role of MMPs and TIMPs in atrial remodeling.

Atrial Natriuretic Peptide Genetic Variants

Ren et al. [109] performed a case control association study and mutational analysis of the *NPPA* gene in 384 AF patients and 844 controls from a Chinese GeneID population. An association between single nucleotide polymorphism rs5063 and lone AF was found (1.63 95 % confidence interval 1.09–2.43, P = 0.003), however no association between rs5063 and AF in the whole cohort was present.

In addition, Roberts et al. [110] examined two single nucleotide polymorphisms, rs5063 and rs5065, in two separate North American cohorts of AF patients. Both single nucleotide polymorphisms in the *NPPA* gene region, result in amino acid changes of atrial natriuretic peptide. In total, 620 AF patients and 2,446 controls were genotyped. However, neither of both polymorphisms was associated with AF in these North American cohorts.

Candidate Gene Association Replication

Most of the above mentioned findings, however, lack replication in additional cohorts. Sinner et al. [9] aimed to replicate the reported associations between single nucleotide polymorphisms and AF in the general population as found by candidate gene association studies published in the literature. They used two independent case-control samples from Germany (2,145 cases with AF from the German Competence Network for Atrial Fibrillation and 4,073 controls from the KORA S4 study) and the United States (790 cases and 1,330 controls from the Massachusetts General Hospital). They were unable to replicate any of the association between single nucleotide polymorphisms and more common AF. This lack of replication is largely caused by the complexity of more common AF, and the used approach. Common AF is influenced by both environmental influences and multiple genetic variants. The genetic variants underlying more common AF exert individually only minor effects. There are millions of single nucleotide polymorphisms, making it extremely difficult to find a genetic variant causative for AF in the community by screening only potentially interesting genes in relatively small candidate gene association studies. However, some other factors merit consideration when interpreting the various candidate gene association studies. In addition to the genetic heterogeneity there is wide variety in phenotypes of AF. There may be specific forms of AF that have different genetic backgrounds [9]. The phenotypic information for both cases and controls at the above mentioned association studies varies widely. Candidate gene association studies assume a direct relation between the genotype and phenotype, however, gene-gene interactions, gene-environment interactions, genetic mosaicism, and other genetic, epigenetic and transcriptional mechanisms may impact the association between genetic variants and common AF.

Finally, a degree of publication bias needs to be considered when interpreting results of individual association studies. Samani et al. demonstrated in a meta-analysis of 14 studies (>8,500 subjects), for the I/D polymorphism of the *ACE* gene locus, that the estimate of most of the larger studies lie at or below the pooled estimate of the odds ratio, whereas the smaller studies are inevitably positive. This may have led to an overestimation of the true effect of the D allele [111].

Genome Wide Association Studies

With the advances in genotyping technologies, GWAS have become a novel method to find these common genetic variants with low effects by screening all single nucleotide polymorphisms instead of limiting the search to interesting candidate genes. GWAS relies on the fact that genetic variance at one locus can predict with high probability genetic variance at an adjacent locus, typically over distances of 30,000 base pairs of DNA in the human genome (called "haplotypes"). So, only 500,000 markers are genotyped and represent the information of all other base pairs in the whole genome (approximately 3×10^9). As a consequence, GWAS is able to detect genetic variants present in >5 % of the general population ("common variants") [112]. To evaluate each of the 500,000 single nucleotide polymorphisms for a potential relation with AF multiple statistical testing has to be performed. To overcome false positive results by correcting for multiple testing, large patient cohorts are required. To date, three susceptibility loci associated with common AF have been uncovered.

4q25 Locus Associated with AF

Investigators of DeCODE genetics were the first to discover AF susceptibility loci [10]. They studied over 300,000 single nucleotide polymorphisms in 3,580 subjects with AF and 19,256 controls in Iceland, all of European ancestry. They identified two polymorphisms (rs2200733 and rs10033464), on the long arm of chromosome 4 (4q25) that were highly associated with AF. These results were replicated in other populations in Iceland, Sweden, United States, and Hong Kong, and improved both the validity and generalizability. Neither variant was correlated with obesity, hypertension, or myocardial infarction suggesting that the genetic variants are not associated with AF by affecting those risk factors. The variant on chromosome 4q25 lies in a region with no known genes, the closest gene is *PITX2* over 150,000 base pairs away [10, 113]. The most significantly associated SNP at this locus, rs2200733, had a risk allele frequency of approximately 20 % in individuals of European ancestry and 60 % in individuals of Asian ancestry. Many others have confirmed these findings. The CHARGE-AF investigators have replicated the association between the chromosome 4q25 locus, rs2200733, and AF in individuals of European ancestry [113]. The GWAS of the CHARGE-AF consortium have confirmed this region as an AF susceptibility locus [12]. In accordance with these findings, a small Italian study found also the 4q25 locus associated with prevalent AF and atrial flutter [114]. Others found rs2200733 associated with incident AF after coronary artery bypass grafting [115], and catheter ablation for AF [116]. In addition to all the replication efforts in cohorts of European ancestry, replication of the association between rs2200733 and AF in a Chinese population was also demonstrated [117]. Each copy of the minor allele of SNP rs2200733 carries a 1.68-fold increased risk of AF (95 % CI, 1.50–1.87). Subjects heterozygous for the risk allele have 1.68-fold relative risk for AF, comparable to conventional AF-risk factors, like increasing age (OR ~ 2 per decade), male sex (OR 1.5), hypertension (OR 1.5) and diabetes mellitus (OR 1.5) [3]. Subjects homozygous for the risk allele have an estimated 2.8-fold relative risk of AF.

16q22 Locus Associated with AF

Genome-wide analyses from two independent consortia identified another susceptibility locus for AF on chromosome 16q22 [12, 118]. The most significantly associated variants were located within or around the zinc finger homeobox 3 (*ZFHX3*) gene. In both studies, the most significantly associated variants at this locus were located in intron 1, in close proximity of each other and had risk allele frequencies of approximately 20 %. These consortia studied 8,222 individuals with AF and 58,439 control subjects, all of European ancestry.

1q21 Locus Associated with AF

Another GWAS included subjects with lone AF (without overt cardiovascular diseases), and identified a novel genetic locus for lone AF on chromosome 1q21 [11]. This study

comprised of 1,335 subjects with lone AF and 12,844 controls. The strongest association with lone AF was at the chromosome 4q25 or *PITX2* locus. A second locus was identified at chromosome 1q21 near a calcium-activated potassium channel, *KCNN3* (*KCa2.3* or *SK3*). This single nucleotide polymorphism, rs13376333, is located between the first and second exon of *KCNN3*, and each minor allele conferred an approximately 50 % increase in the odds of lone AF.

Functional Implications of AF GWAS Loci

The unbiased GWAS results have uncovered genetic variants underlying common AF, however the exact mechanisms how each single nucleotide polymorphism increases the susceptibility of AF is still uncertain. *PITX2* encodes for the paired-like homeodomain transcription factor 2 that seems an interesting candidate gene for AF. Mommersteeg et al. [119, 120] demonstrated in an experimental mouse model that PITX2 is important in embryonic development of pulmonary myocardial sleeves and in the formation of sinus node in the left atrium. However, it remains unclear how these early developmental effects of PITX2 cause AF years later. Wang et al. [121] was the first to demonstrate that *PITX2c* is expressed in post-natal mouse hearts, and that *PITX2c* deficient mice were prone to develop atrial arrhythmias. Kirchhof et al. [122] demonstrated that *PITX2* expression levels were approximately two orders of magnitude higher in the left atrium compared to the right atrium or the ventricles in human hearts. An interesting small study determined a relation between two AF risk alleles on chromosome 4q25 (rs2200733, rs10033464) and AF recurrence after catheter ablation of AF [116]. Whether this genotype can be used for risk stratification and patient selection for certain therapies warrants more research. Data regarding potential mechanisms explaining the association between the locus at the transcription factor *ZFHX3* gene and AF is lacking. The polymorphism associated with lone AF lies at an intronic region of the calcium-activated potassium channel *KCNN3* on chromosome 1q21 [11]. This gene encodes KCNN3 (or SK3), a member of a family of Ca^{2+}-activated potassium channels gated by changes in intracellular Ca^{2+} concentration [123]. KCNN3 channels have a prominent role during the late phase of the cardiac action potential in both mice and human [124–126].

Atrial Standstill

Atrial standstill is a rare arrhythmia, characterized by the total absence of electrical and mechanical activity in the atria. On the electrocardiogram, P waves are absent and usually a junctional escape rhythm is present. Also intra-atrial electrograms are absent and the atria are inexcitable by electrical stimulation. On the echocardiogram, the atrial wall does not contract and the mitral A-wave is missing. Atrial standstill may be complete or partial. In the latter, electrical silence exists in one part of the atria together with a rapid atrial rate in another part of the atria at the same time [127, 128]. Atrial standstill may be transient as well as permanent, depending on the underlying condition. The treatment consists of ventricular pacemaker implantation in combination with anticoagulation since atrial standstill is associated with an increased risk of thrombo-embolic complications. Atrial standstill may be due to different non-heritable causes including digitalis or quinidine intoxication, hyperkalemia, myocardial ischemia or infarction, cardiac surgery, myocarditis, diabetes and hypothermia [129] Atrial standstill is associated with a number of inheritable disorders. Familial forms in the absence of underlying (cardiac) disease (isolated familial atrial standstill) have also been described.

Atrial Standstill in Heritable (Cardiac) Disorders

Atrial standstill occurs in up to 45 % of patients with Emery-Dreifuss muscular dystrophy [130]. This disease can be inherited as a X-linked recessive, autosomal dominant, or autosomal recessive disorder. The X-linked form is caused by mutations in the gene encoding emerin, whereas the autosomal dominant forms are caused by mutations in the gene encoding

Table 35–3. Mutations associated with familial atrial standstill

Gene (mutation)	No. of families (subjects)	Age	Ethnicity	UHD
Homozygous GJA5 (−44A/+71G) *and* SCN5A (D1275N) [37]	1(4)	22–33	Cauc	No
Heterozygous GJA5 (−44A/+71G) *and* SCN5A (L212P) [139]	1(1)	3	Jap	No
SCN5A (R367H) [133]	1(2)	37	Jap	Brugada

Cauc Caucasian, *Jap* Japanese, *UHD* underlying heart disease

lamin A/C. The disease is characterized by progressive skeletal muscle wasting with contractures. Cardiac involvement may include the development of cardiomyopathy, and AV conduction abnormalities. AF and atrial flutter, often anticipating atrial standstill, may occur. Atrial standstill has also been described in patients suffering from autosomal recessive spinal muscular atrophy type 3 (Kugelberg-Welander syndrome) [131].

Fazelifar et al. [132] described two Iranian siblings, one male aged 34 years and one female aged 44, who were evaluated because of frequent syncope. Both had atrial standstill on the 12-lead ECG with a broad QRS escape rhythm, mildly enlarged ventricles and atria on echocardiography and a left ventricular ejection fraction between 30 and 40 %. There were no signs of amyloidosis or muscular disease. The male developed syncope due to self-terminating polymorphic ventricular tachycardia. The family history disclosed two sudden deaths in a brother and a nephew at the ages of 34 and 21 years, respectively. The nephew previously had a pacemaker implanted because of symptomatic bradycardia. Although no genetic analysis was performed, an autosomal inheritance of this form of dilated cardiomyopathy in combination with atrial standstill is likely. A case of father and son with Ebstein's anomaly and atrial standstill has been reported [133] as well as a single case with this combination [134]. Takehara et al. [135] reported a case of atrial standstill in a patient with Brugada syndrome (Table 35.3). This 37-year old Japanese woman suffered from recurrent syncope. Her ECG demonstrated coved-type ST elevation compatible with Brugada syndrome. During admission she suffered from episodes of ventricular fibrillation that were preceded by a long pause due to atrial standstill. Atrial standstill could also be provoked by administration of procainamide. A mutation in exon 9 of the *SCN5A* gene leading to amino acid substitution R367H was found in the patient as well as in her son. Her son's ECG, however, demonstrated a saddle back ST-elevation. The family history was negative for sudden cardiac death.

Isolated Familial Atrial Standstill

Familial forms of atrial standstill are extremely rare. A number of families have been described in the last decades [136–140]. In 2003 a Dutch family with progressive form of atrial standstill has been reported [37]. In this family atrial standstill was present in four individuals (Table 35.3). Symptoms of dizziness and syncope started in their early twenties, progressing in the years thereafter and prompting medical evaluation by 30–40 years of age. On invasive electrophysiologic study, the atria could be either partially or not be stimulated. Genetic analysis revealed a mutation in the cardiac sodium channel gene *SCN5A* (p.D1275N) in all three living affected individuals and five unaffected family members. Additionally, two gap junction protein connexin 40-related polymorphisms (−44G > A and +71A > G) were found in all affected patients and five other healthy relatives. Eight relatives were *homozygous* for both polymorphisms of which three were affected with atrial standstill. Therefore, only the combination of the *SNCA5* mutation and the rare *GJA5* genotype lead to atrial standstill in this family. Remarkably, AF was only present in one individual in this family, aged 68 years, after myocardial infarction, although AF has been described in D1275N carriers [36]. Functional measurements of D1275N sodium channels revealed a shift of the voltage dependence of activation towards more depolarized voltages, resulting in decreased excitability. The combination of the rare *GJA5* genotypes was demonstrated to cause a reduction in connexin

40 expression in vitro. Two years later, Makita et al. [141] reported a Japanese boy presenting with sick sinus syndrome in combination with paroxysmal AF at the age of 3 years (Table 35.3). When he was 11 years old, total atrial standstill was present. No underlying (cardiac) disease was present. Genetic screening revealed a mutation in exon 6 of the *SCN5A* gene leading to substitution of proline for leucine at position 212 (L212P). His father, although asymptomatic, was also found to be the carrier of this mutation. Additionally, a *heterozygous GJA5* polymorphism (−44GA/+71AG) was found in the boy and in his asymptomatic mother and maternal grandmother. In conclusion, a genetic defect in *SCN5A* seems to underlie familial atrial standstill but in itself is not leading to the severe phenotype. Coinheritance of *GJA5* polymorphisms modifies the clinical expression of the arrhythmia.

Conclusion

Our understanding of the genetics underlying AF is still limited, still much of the heritability of AF is uncovered, but large advances have been made. The basis was set by observations of families with AF, with and without other cardiovascular conditions. The mutations found in these families are rare but have large effects. Although we learned that these genetic variants do not play a major role in more common AF, the identification of monogenic genetic variants in families with AF has increased our understanding of mechanisms underlying AF [142]. More common AF has also a heritable component, but is a complex trait; caused by the interaction of environmental factors and multiple genetics variants with small effects. Recently, large advances have been made with the introduction of GWAS. The observations in the general population with AF suggest some form of genetic control in the pathogenesis of this more common form of AF, however exact mechanisms are still uncertain. Atrial standstill is an extremely rare arrhythmia and is usually caused by a reversible condition. Furthermore it may be associated with a variety of heritable conditions with distinct phenotypes. Cases of isolated familial atrial standstill have been described. Data on genetics of AF are promising and may help to understand why some people develop AF while others will never do, may help to increase the understanding of the pathogenesis of AF, increase the predictability of AF, and eventually, provide novel targets for AF therapies. Ultimately, this research may contribute to a decrease in AF burden for the individual patient and for public health in general.

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36
Idiopathic Ventricular Fibrillation

Sami Viskin, Arnon Adler, and Bernard Belhassen

Abstract

Idiopathic ventricular fibrillation (VF) is an uncommon disease that manifests as syncope or cardiac arrest caused by rapid polymorphic ventricular tachycardia (VT) or VF in the absence of organic heart disease. Because the term "idiopathic" means "absence of identifiable etiology", idiopathic VF is essentially a diagnosis by exclusion. However, typical clinical and electrophysiological characteristics present in some patients often allows for a straight-forward positive diagnosis. Moreover, it is now clear that many patients with idiopathic VF have, in fact, a genetic channelopathy, presenting in the form of "idiopathic VF with early repolarization" or "congenital short QT syndrome (SQTS)". This chapter summarizes the history of this disease as we know it since its first description in 1929 to the most recent developments in our understanding of its pathophysiology. Idiopathic VF leads to syncope or cardiac arrest typically during early adulthood and involves a relatively high incidence of arrhythmic storms (with clusters of VF episodes) that fail to respond to conventional antiarrhythmic therapy (including amiodarone) but respond exquisitely to intravenous isoproterenol and oral quinidine. The mode of onset of spontaneous arrhythmias in idiopathic VF, namely, the triggering of rapid polymorphic VT/VF by single ventricular extrasystoles with very short (R-on-T) coupling intervals. The extrasystoles triggering VF have been mapped mainly to the His-Purkinje fibers in the left ventricle and may be ablated. The clinical data linking idiopathic VF to the congenital SQTS and the malignant early repolarization syndrome are presented and the genetic mutations so far described are presented.

Keywords

Ventricular fibrillation • Sudden death • Short QT syndrome • Early repolarization

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Idiopathic ventricular fibrillation (VF) is an uncommon disease that manifests as syncope or cardiac arrest caused by rapid polymorphic ventricular tachycardia (VT) or VF in the absence of organic heart disease. In the first edition of this book we wrote that idiopathic VF is a disease of unknown etiology. Although this remains true for the majority of cases it is now clear that many patients with idiopathic VF have in fact a genetic channelopathy presenting in

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the form of "idiopathic VF with early repolarization" (discussed in detail in a previous chapter) or "congenital short QT syndrome (SQTS) with not so short QT interval." Because the term "idiopathic" means "absence of identifiable etiology" idiopathic VF is essentially a diagnosis by exclusion. However typical clinical and electrophysiological characteristics present in some patients often allows for a straight-forward positive diagnosis.

History

In 1929, Dock published what probably represents the first description of idiopathic VF [1]. This case-report describes a 36-year-old male with clusters of syncope caused by documented VF that terminated spontaneously. Organic heart disease was appropriately excluded with the technologies then available. Similar case reports followed and in 1987 Belhassen published the first series of idiopathic VF [2], emphasizing the importance of electrophysiological evaluation with programmed ventricular stimulation and the high efficacy of quinidine for preventing inducible and spontaneous VF [2].

In 1990, we published the first systematic review on idiopathic VF [3], including data for 54 cases published by then. The typical characteristics of idiopathic VF, including the onset of symptoms during early adulthood in both genders, the relatively high incidence of arrhythmic storms (with clusters of VF episodes), the high inducibility rate of VF with programmed ventricular stimulation and the excellent response to quinidine therapy, were first summarized in that review.

The mode of onset of spontaneous arrhythmias in idiopathic VF, namely, the triggering of rapid polymorphic VT/VF by single ventricular extrasystoles with very short (R-on-T) coupling intervals, already evident from the initial reports, was described by Leenhardt and Coumel [4] as "short-coupled variant of torsade de pointes" and was finally described in detail by us in 1997 [5]. Six years later, Haissaguerre demonstrated that the short-coupled extrasystoles triggering VF in this disease are very-early ectopic beats originating from Purkinje fibers [6].

The differential diagnosis of idiopathic VF also evolved in recent years. When we wrote the first review on this topic in 1990 [3], the differential diagnosis included (in addition to subtle forms of organic heart disease) the following arrhythmia-disorders: the long QT syndrome (described in 1957) [7–9] the catecholamine sensitive polymorphic VT (CPVT) (described in 1995) [10], and the syndrome of nocturnal sudden death of South East Asia (known since 1960) [11]. However, in 1992, the Brugada brothers described patients with otherwise idiopathic VF who had a peculiar electrocardiogram showing right bundle branch block with persistent ST-segment elevation in the right precordial leads [12]. It soon became evident that >20 % of patients thought until then to have idiopathic VF had what we now call "Brugada syndrome" [13]. Moreover, in 1997 it became clear that the "syndrome of unexplained nocturnal sudden death in South East Asia" was in fact, an "endemic" manifestation of Brugada syndrome in Asia [14]. Then, in the year 2000, the congenital short QT syndrome was described [15, 16], and since such patients have inducible [16] and spontaneous [17] ventricular arrhythmias that are indistinguishable from those of idiopathic VF patients, we proposed in 2004 that idiopathic VF may very well be *a* "*short QT syndrome with not very short QT intervals*" (QTc intervals in the range of 340–360 ms) [18]. Finally, in 2008, Haissaguerre [19], Nam [20] and our group [21] show that the "early repolarization pattern" (the combination of J-waves and ST-segment elevation), long believed to represent a completely benign electrocardiographic pattern, is strongly associated with a history of idiopathic VF, further promoting the concept of "J-wave syndromes" [22]. The congenital short QT and early repolarization syndromes are described in detail in Chaps. 27 and 30.

Etiology

As often happens with diseases initially termed "idiopathic," it is likely that "idiopathic VF" does not represent a single entity but rather represents different diseases with similar electrocardiographic characteristics, including different channelopathies. For example, some cases of

"idiopathic VF" may have in fact a congenital SQTS. Although the SQTS was originally defined as an arrhythmic syndrome with baseline QTc <300 ms [15, 16, 23], it is now clear that no single QTc value distinguishes all healthy individuals from all patients with SQTS [24]. In fact, carriers of SQTS mutations with QTc intervals as long as 362 ms (that would be considered within the low-normal range) [24], have been clearly described [25]. At the same time, we have shown that patients with idiopathic VF (particularly males) have shorter QT intervals than age-matched controls (often in the 360–370 ms range) and others have shown that idiopathic VF patients have apparently normal heart rate at baseline but have insufficient QT prolongation during slow heart rate [26, 27], suggesting that idiopathic VF could represent a continuum [24]. Interestingly, in a very large kindred (involving three distinct families with an apparent founder mutation) of idiopathic VF in the Netherlands, carriers of the risk-haplotype had electrocardiograms that were defined as "strictly normal," and the carriers had QT intervals that were not different from those of the non-carriers [28]. Still, the mean QTc of the risk-haplotype carriers (395 ms) would fall shorter than the 10th percentile of the QTc of the healthy population [29]. Also, the presumed underlying genetic disorder (over-expression of DPP6) would be expected to increase transient outward (I_{TO}) current, shortening the action potential in some areas more than others, again pointing towards "the short QT hypothesis". On the other hand, other cases of idiopathic VF appear to have marked J-waves rather than short QT intervals [19, 21]. Experimental models [22] and anecdotal reports of idiopathic VF patients with J-waves studied with novel non-invasive imaging modalities [30] suggest that steep repolarization gradients – caused by shorter than normal action potentials in some ventricular areas, rather than in the whole ventricle – underlie many cases of idiopathic VF [31]. Interestingly, some studies of "early repolarization syndrome" also report that patients with idiopathic VF with J-waves also have *shorter QT intervals* than age- and gender-matched controls [19, 32]. On the other hand, the fact that only a minority of patients with idiopathic VF report a familial history of sudden death [3, 19, 33] is a strong argument against the role of genetic channelopathies in all cases.

Ventricular arrhythmias in idiopathic VF are invariably triggered by ventricular extrasystoles with very short coupling intervals [3–5], and Haissaguerre has elegantly shown that these short-coupled extrasystoles originate from Purkinje fibers [34, 35]. Purkinje-fibers ectopic beats have also been linked to VF-initiation in patients with organic heart disease [36], particularly during or shortly after myocardial infarction [37–39]. One can propose the following arrhythmic mechanisms could explain the very short-coupled extrasystoles that originate from Purkinje fibers and trigger idiopathic VF: (1) micro-reentry at the Purkinje-muscle junctions [40]; (2) late phase-3 early-depolarization induced trigger activity [41] due to unprovoked calcium sparks in Purkinje fibers and (3) parasystole originating from Purkinje fibers [42] fortuitously firing on the T-wave of the normal beat. Regardless of the mechanism underlying the firing of Purkinje extrasystoles, it is the short refractory period of the surrounding myocardium, either at the entire ventricular level in patients with SQTS, or at a regional level in patients with early-repolarization syndrome, that allows the closed-coupled extrasystoles to propagate and trigger VF.

Clinical Manifestations

Patients with idiopathic VF present with either syncope or cardiac arrest in early adulthood. The mean age at presentation in several series has been 35–45 years and the vast majority are older than 20 years and younger than 65 years old at the time of presentation [3, 19]. Two thirds of the patients are males [3, 19].

The arrhythmias provoking syncope (Fig. 36.1) and those causing cardiac arrest are similar in terms of mode of onset, ventricular rate and polymorphic morphology. It is not clear why some events of polymorphic VT terminate spontaneously (causing syncope) while others deteriorate to fine ventricular fibrillation (causing cardiac arrest) (Fig. 36.1b, c). However, the proportion of patients presenting with cardiac arrest

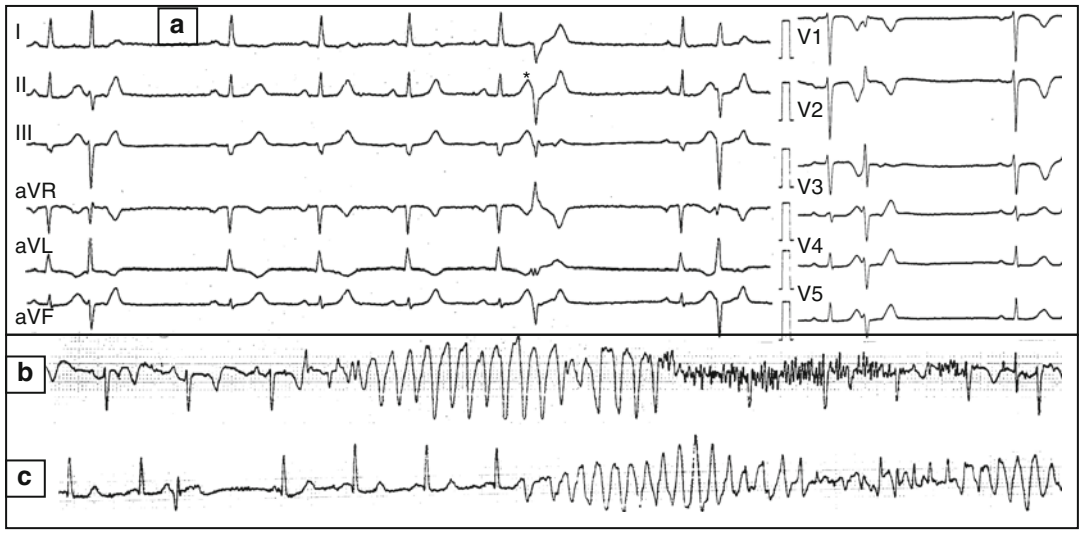

Figure 36–1. Typical example of idiopathic VF. This 54 year old female was referred for neurological consultation because of "recurrent seizures". Her baseline electrocardiogram shows sinus rhythm with normal PR, QRS and QT intervals (*panel* **a**). However there are several ventricular extrasystoles with varying coupling intervals, including short-coupled extrasystoles falling on the peak of the preceding T wave (*). Because of the short-coupled extrasystoles the patient was admitted to the Cardiology Department (instead of the Neurology Department). Soon thereafter a self terminating episode of rapid polymorphic ventricular tachycardia was recorded during one of her "seizures" (*panel* **b**). Ventricular fibrillation requiring defibrillation was also recorded shortly thereafter (*panel* **c**). The patient was diagnosed as "idiopathic ventricular fibrillation" and has been free of ventricular arrhythmias during treatment with quinidine for more than 9 years

(as opposed to syncope) is much higher in idiopathic VF than in other channelopathies causing polymorphic ventricular arrhythmias like the long QT syndrome or CPVT [43]. In other words, arrhythmias in idiopathic VF occur rarely, but once they occur they are generally sustained and frequently fatal [28]. As a rule, syncope and cardiac arrest in idiopathic VF are *not* related to effort or emotional stress [3, 19, 33]. Sleep-related arrhythmias, which are common in sodium channelopathies (Brugada syndrome and long QT syndrome of the LQT3 type), are rare in idiopathic VF [3, 19, 33]. Finally, about 25 % of patients with idiopathic VF present with arrhythmic storms, that is, with clusters of VF episodes recurring within 24–48 h [3, 33, 44]. Some of these VF clusters have been triggered by fever [45].

Electrocardiogram

The electrocardiogram (ECG) during sinus rhythm has been conventionally defined as normal. It should be noted, however, that when the entity "idiopathic VF" was first defined, the ECG was considered "normal" when pathologies known at the time were excluded [3]. Specifically, the QT was considered normal because it was not prolonged [3]. However, as discussed above, a considerable proportion of patients with idiopathic VF have "relatively short" QT intervals at baseline [18], QT intervals that appear normal but are shorter than those of comparable healthy controls [19, 32], or QT intervals that are normal at baseline but fail to prolong adequately during braydcardia [26, 27]. The T-peak to T-end interval (the interval from the summit to the end of the T wave), which is a marker of the dispersion of repolarization and arrhythmic risk in the long QT syndrome [46] and Brugada syndrome [47], is normal in idiopathic VF [18].

The early repolarization pattern is also observed in idiopathic VF patients far more frequently than among comparable healthy controls [19, 21, 48, 49] and some use the term "early repolarization syndrome" to describe patients with otherwise idiopathic VF who have this ECG pattern [19, 49]. In its most characteristic form, early

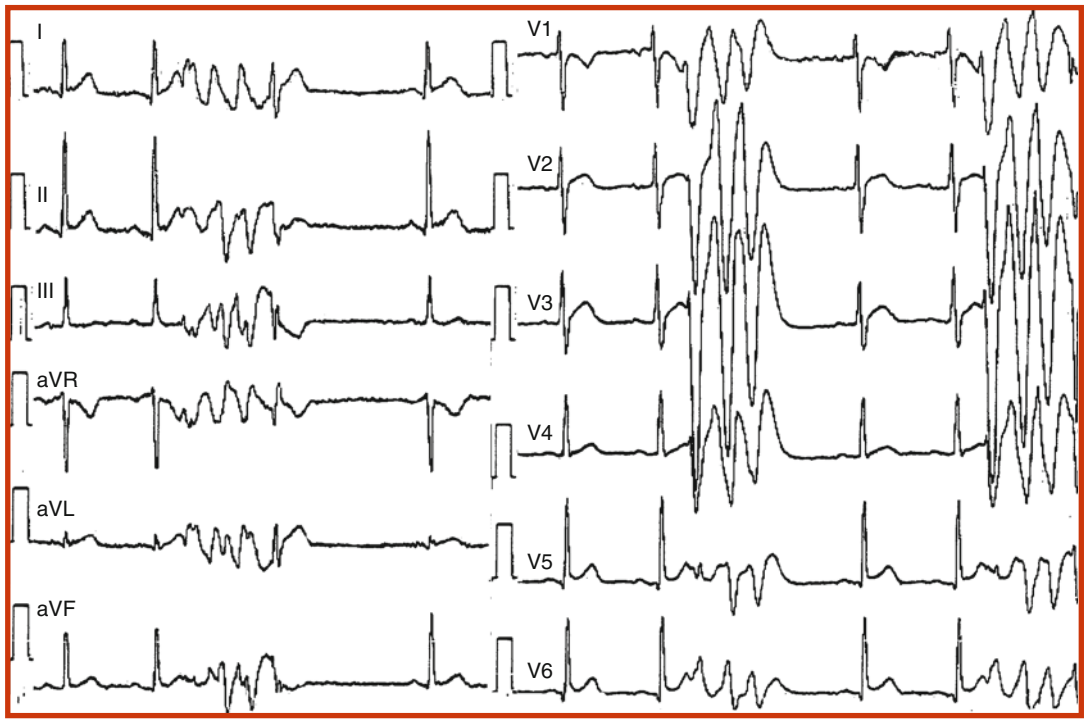

Figure 36–2. Typical mode of onset of idiopathic VF. Note the very short coupling interval of the extrasystoles initiating the polymorphic ventricular tachycardia. Also, note that despite the polymorphic morphology of the arrhythmia, when more than one VT episode is recorded (like in the precordial leads), the first, second and third complexes of the tachycardias are remarkably similar

repolarization in idiopathic VF patients takes the form of a distinct J-wave followed by an horizontal ST-segment [50]. Based on our case control series [21] and using conditional-probability formulas [21] we estimate that (1) the estimated odds for developing idiopathic VF for an individual in the 35–45 years age-range are 3.4 in 100,000; (2) the risk increases to 11 in 100,000 once J-waves are found in the ECG and (3) the risk increases to 30 in 100,000 if the J-waves are followed by horizontal ST segment [50].

Ventricular extrasystoles only rarely occur in patients with idiopathic VF, but when they do, they have varying coupling intervals with some extrasystoles closely coupled to the preceding complex (mean coupling interval = 302 ± 52 ms in our series [5], 297 ± 41 ms in the series of Haissaguerre [35], 300 ± 35 ms in the series of Champagne [51] and ≤340 ms in the series by Nam [49]). Because of the short coupling interval, the extrasystoles fall on the summit or the descending limb of the T wave (Figs. 36.1 and 36.2). In our series [5], all VF episodes started by ventricular extrasystoles falling within 40 ms (with the vast majority falling within 20 ms) of the peak of the T wave. There appears to be an inverse relationship between the coupling interval of the extrasystoles and the risk for malignant arrhythmias with longer bursts of polymorphic VT triggered by extrasystoles with shorter coupling intervals [35]. Nam reported that the arrhythmias in idiopathic VF with early repolarization are preceded by long-short cycles more often than VF in Brugada syndrome [49]. However, in our experience and that of others, arrhythmias in idiopathic VF are (as a rule) *not* pause dependent [3, 19, 33, 44, 51].

Morphology of Extrasystoles

In early reports showing the onset of idiopathic VF [3, 33, 52], a similar ECG pattern of the first short-coupled ventricular extrasystole was observed, namely left bundle branch block

pattern and left axis (Fig. 36.2). However, later reports [34, 35], showed that extrasystoles with other patterns do exist, suggesting various possible origin sites. Interestingly, when multiple episodes of polymorphic VT are recorded with 12-lead recordings, the morphology of the initiating beats of all these episodes is similar. This applies not only to the first complex, but to the second and third complexes of the polymorphic arrhythmias as well (Fig. 36.2). The last observation supports the notion that idiopathic VF has a focal origin (see below) [34, 35].

Electrophysiologic Data

Patients with idiopathic VF have normal A-H and H-V intervals, and their ventricular refractory periods are within normal limits [2, 53]. This is in contrast to patients with Brugada syndrome, who often have prolonged H-V interval [12] and patients with short QT syndrome, who have short refractory periods in the atrium and the ventricle [23, 54].

The ventricular arrhythmias induced by programmed ventricular stimulation are invariably of polymorphic morphology, namely polymorphic VT or VF (Fig. 36.3). Induction of monomorphic VT excludes the diagnosis of idiopathic VF. This is at variance with patients with Brugada syndrome who also have primarily VF [55], but rarely have monomorphic VT [56–60].

The inducibility rate is a function of the protocol used during programmed ventricular stimulation. Many electrophysiologists are reluctant to shorten the coupling interval of the delivered ventricular extrastimuli beyond a "nominal" value of 200 ms. This is because the risk of "accidentally" inducing VF in healthy individuals also increases as the coupling interval of the second and third extrastimuli are shortened below 200 ms [61–63]. Indeed, in small studies performed 20 years ago, 9 % of healthy individuals – *without* documented or suspected spontaneous ventricular arrhythmias – had inducible VF when the coupling intervals were limited only by ventricular refractoriness [61–63]. Moreover, an additional 40 % of the last group of healthy controls had inducible non-sustained polymorphic VT and this lead to premature discontinuation of the pacing protocol [61–63]. Therefore, one must recognize that *at least* 9 % of healthy individuals will have inducible VF if aggressive protocols of extrastimulation (with double and triple extrastimulation with coupling intervals shorter than 200 ms) are used [64]. On the other hand, in our experience, as many as *80 % of patients with idiopathic VF* have inducible VF with aggressive protocols of extrastimulation consisting of double and triple ventricular extrastimulation at two right ventricular pacing sites and using repetition of extrastimulation at the shortest coupling interval that captures the ventricle [2, 52, 53]. This very high-inducibility rate suggests that the induction of VF, with aggressive protocols of extrastimulation, is a valid endpoint of programmed ventricular stimulation that then may be used for guiding antiarrhythmic therapy with quinidine in patients with idiopathic VF (Fig. 36.3) (see section "Prognosis and Therapy of Idiopathic VF").

Recently, Haissaguerre et al. performed endocardial recordings in patients with idiopathic VF at a time when they had frequent spontaneous ventricular extrasystoles and/or bursts of polymorphic VT [34, 35]. The investigators were able to locate the site of origin of these ventricular arrhythmias in 27 patients. Successful localization of the site of origin of the ventricular arrhythmias was guided by recording of very early endocardial recordings and confirmed by abolition of ventricular arrhythmias following radiofrequency ablation of the firing focus. Purkinje potentials were recorded at the site of origin of ventricular arrhythmias in 23 (85 %) out these 27 patients (in the left ventricular septum in ten patients, the anterior right ventricle in nine patients and in both locations in four). The Purkinje potentials preceded the local myocardial activation by 11 ± 5 s during sinus rhythm and by 10 ± 150 ms during spontaneous ventricular ectopy [34, 35]. Based on these endocardial recordings, it seems that the arrhythmias in idiopathic VF have a focal origin and that the triggering focus is within the Purkinje fibers in the majority of patients. Of note, the firing focus was not within the Purkinje network in only four (15 %) patients and in all these patients the arrhythmias originated in the right ventricular outflow tract (RVOT). It is

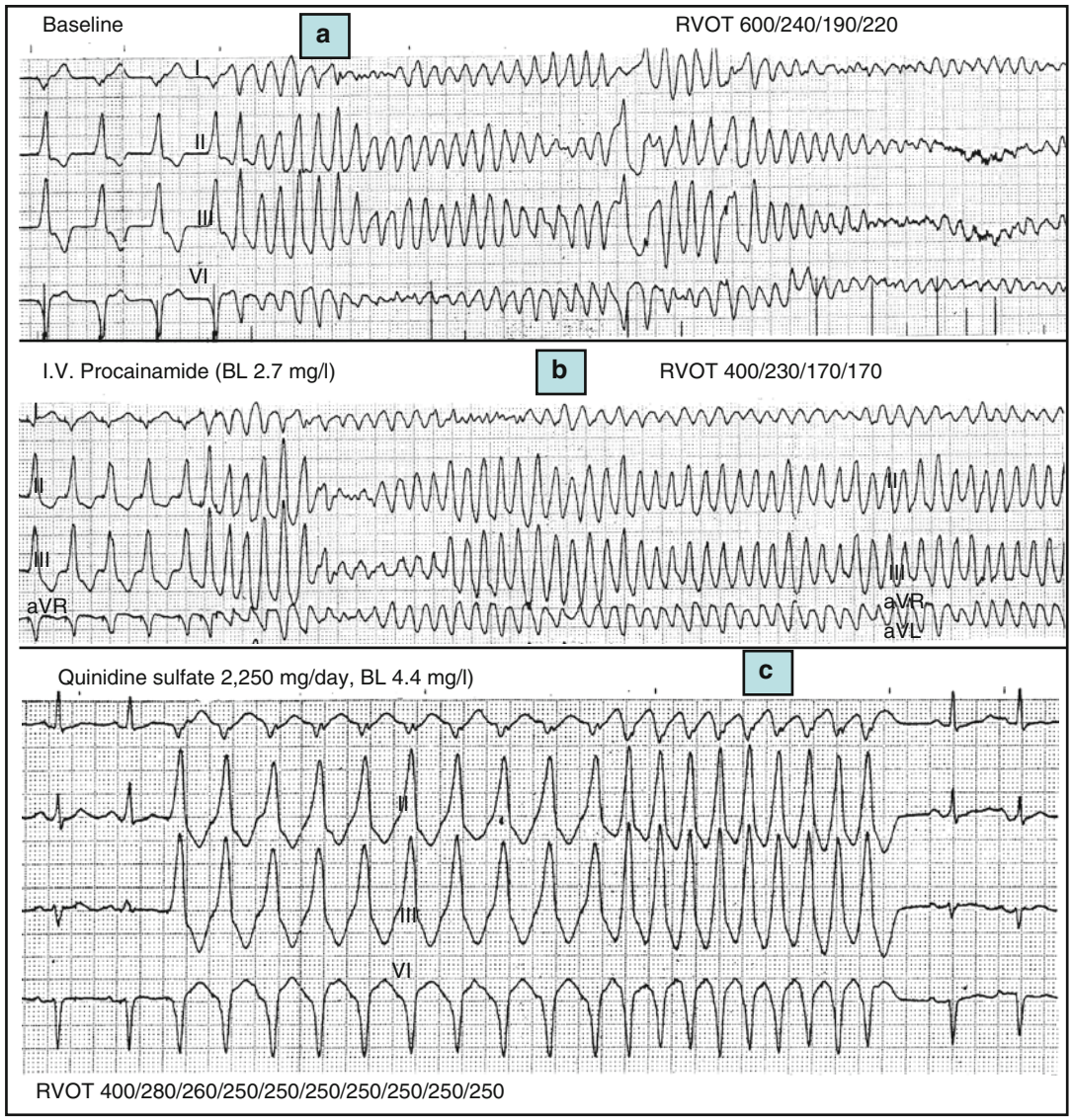

Figure 36–3. Typical results of electrophysiologic study in idiopathic VF. *Panel* **a**: At the baseline study, VF is induced by triple ventricular extrastimulation with short coupling intervals from the right ventricular outflow tract. Basic ventricular pacing at 100 beats/min (cycle length 600 ms) is followed by three extrastimuli 240, 190 and 220 ms apart and this initiates VF that required DC shock for termination. *Panel* **b**: After intravenous administration of 1,000 mg procainamide the protocol of extrastimulation is repeated and VF is induced again. Note that despite of therapeutic levels of procainamide the effective refractory period is sufficiently short to allow ventricular capture of the ventricle with short coupling intervals (230, 170 and 170 ms for the first, second and third extrastimuli, respectively). *Panel* **c**. After 5 days of oral therapy with quinidine it is no longer possible to capture the ventricle with short coupling intervals. No ventricular arrhythmias could be induced despite a maximally aggressive protocol of extrastimulation, including nine extrastimuli. This cardiac arrest survivor has been free of arrhythmias for >5 years on quinidine therapy

possible that idiopathic VF originating from the RVOT and the "short-coupled variant of RVOT" described by our group [65], and the "malignant idiopathic RVOT-VT" described by Noda [66] represent the same entity.

Diagnosis

Diagnosing idiopathic VF in a cardiac arrest survivor is straightforward when the onset of spontaneous polymorphic VT/VF is recorded

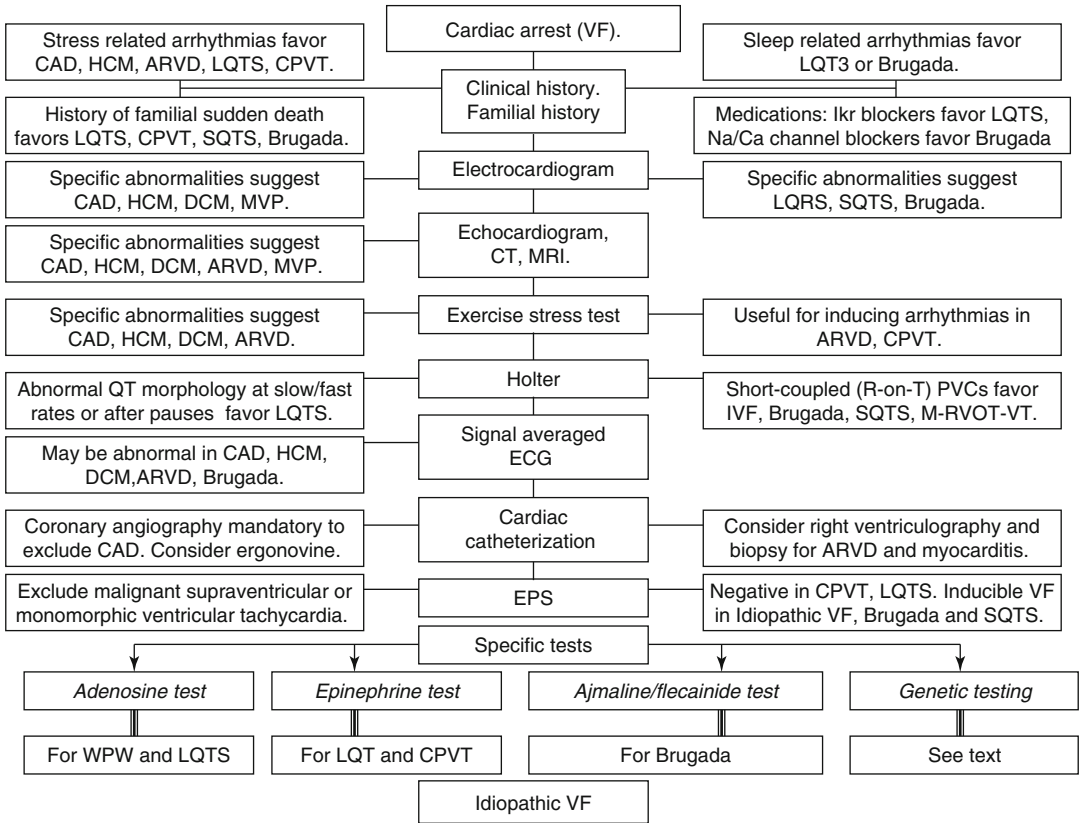

Figure 36–4. Proposed basic algorithm to diagnose idiopathic VF. Abbreviations in the algorithm are as follows: *CAD* coronary artery disease, *HCM* and *DCM* hypertrophic and dilated cardiomyopathy, respectively, *ARVD* arrhythmogenic right ventricular dysplasia/cardiomyopathy, *LQTS* long QT syndrome, *CPVT* catecholaminergic polymorphic ventricular tachycardia, *LQT3* long QT syndrome of LQT3 genotype, *SQTS* short QT syndrome, *MVP* mitral valve prolapse, *CT* computerized tomography, *MRI* magnetic resonance imaging, *M-RVOT-VT* malignant form of idiopathic ventricular tachycardia originating from the right ventricular outflow tract, *WPW* Wolff-Parkinson-White syndrome

(usually during an arrhythmic storm) and this shows initiation of polymorphic VT/VF by very short coupled ventricular extrasystoles (Figs. 36.1 and 36.2) [3, 5]. This is because the only three other conditions that lead to such characteristic mode of VF initiation (myocardial ischemia [67, 68], Brugada syndrome [69] and short QT syndrome [117]) can be identified with appropriate testing.

More often, however, patients are admitted after resuscitation from cardiac arrest and have documented ventricular fibrillation, but recordings of the arrhythmia onset are not available. In such cases, the diagnosis of idiopathic VF is established by excluding all identifiable causes and further supported by the inducibility of VF with programmed ventricular stimulation. A discussion of all potential causes of sudden death is beyond the scope of this chapter, but a practical approach is presented in Fig. 36.4.

The diagnosis of idiopathic VF should be considered in patients presenting with syncope without documented arrhythmias. Having said that, it must be emphasized that in the overwhelming majority of patients presenting with syncope in the absence of heart disease, a diagnosis of vasovagal syncope (rather than arrhythmic syncope) will be evident already from the clinical history. Also, the vast majority of patients with syncope that does *not* appear to be of vasovagal origin

also have electrocardiographic or echocardiographic abnormalities that will suggest an underlying diagnosis. Therefore, only very rarely one is confronted by a patient presenting with syncope in whom the history is sufficiently worrisome to suggest an arrhythmic origin, yet all non-invasive studies, including exercise [70–72] and standing tests [73] to exclude exercise-induced arrhythmias and long QT syndrome, as well as drug challenges to exclude Brugada syndrome [74, 75] long QT syndrome [76, 77] and Wolff Parkinson White [78], are all negative. In such cases, electrophysiologic evaluation can be performed to exclude intra-His block as the cause of syncope. However, recommending the performance of programmed ventricular stimulation to a patient with syncope who has no evidence of heart disease and no documented arrhythmias (particularly closed-coupled ventricular extrasystoles) is problematic. This is because, in the absence of organic heart disease, inducible ventricular arrhythmias (if any), are likely to be of polymorphic morphology. Understanding the significance of inducible VF in the absence of documented spontaneous arrhythmias is difficult because such arrhythmias may be induced in at least 9 % of healthy individuals [64]. Therefore, performance of programmed ventricular stimulation should only be performed when both physician and patient are prepared to accept the induction of VF as a "positive" response.

Differential Diagnosis

Subtle Forms of Organic Heart Disease

Excluding all forms of organic heart disease is essential before the diagnosis of idiopathic VF is considered. However, it should be noted that some forms of organic heart disease may cause malignant ventricular arrhythmias at a time when the anatomic abnormalities are minimal and difficult to detect by imaging modalities. For example, patients with hypertrophic cardiomyopathy due to Troponin-T mutations may be at risk for arrhythmic death at a time when left ventricular hypertrophy is still mild [79, 80]. Similarly, right ventricular dysplasia is sometimes identified as the underlying cause of sudden death only during forensic examination and despite negative extensive diagnostic workup [81]. Of note, subtle anatomic abnormalities, like mitral valve prolapse without hemodynamic significance, should not necessarily be accepted as the cause of cardiac arrest. On the other hand, signs of severe left ventricular dysfunction after resuscitation should not necessarily be used to exclude the diagnosis of idiopathic VF because prolonged resuscitation may result in transient electrocardiographic and echocardiographic abnormalities that are indistinguishable from those seen in patients with dilated cardiomyopathy [82, 83]. If such abnormalities resolve, the diagnosis of idiopathic VF should obviously be considered.

Wolff-Parkinson-White Syndrome

Patients with atrioventricular accessory pathways may have minimal or no ventricular preexcitation (i.e., may have narrow QRS complexes) if they also have fast conduction along the AV node or if their accessory pathways is located on the left lateral wall (far away from the sinus node). Yet, these pathways may have short refractory periods. Such patients may develop atrial fibrillation with rapid ventricular rates that may deteriorate to VF. If the preexcited atrial fibrillation is not recorded and the patient is found in VF, the near-normal electrocardiogram during sinus rhythm may lead to a wrong diagnosis of "idiopathic VF" because all imaging tests will be normal. The wrong diagnosis of idiopathic VF may gain further support from electrophysiologic studies if *atrial* stimulation is not performed prior to ventricular pacing because programmed ventricular stimulation is likely to induce VF in patients with WPW [84]. Therefore, excluding accessory pathways – either with adenosine injection as a bedside test or with atrial pacing during electrophysiologic studies – is a mandatory step in the work-up of VF survivors even when the electrocardiogram is judged to be "normal". Of note, rare cases of cardiac arrest caused by very rapid supraventricular tachyarrhythmias in patients without Wolff-Parkinson-White have also been described [85].

Catecholamine Sensitive Polymorphic VT

Physicians may opt to skip performance of exercise testing in cardiac arrest survivors reasoning that coronary angiography will eventually reveal any significant coronary lesion. We have encountered patients with CPVT who were erroneously diagnosed as "idiopathic VF" only because exercise stress testing was not performed. Since all other tests, including electrophysiologic studies, are invariably normal in this disease, exercise stress test is a mandatory test in cardiac arrest survivors. Although the majority of patients with CPVT have a pathognomonic response to exercise (exercise-induced atrial fibrillation followed my multifocal ventricular extrasystoles, bidirectional VT and polymorphic VT), it was recently recognized that some patients with genetically proven CPVT have only single ventricular extrasystoles that look like typical "benign right ventricular outflow tract (RVOT) extrasystoles" during maximal exercise [86, 87]. More recently, a family with CPVT in which affected individuals had negative exercise stress tests but were identifiable by marked post-pause QT prolongation, was described [88]. Such patients could easily be misdiagnosed as "idiopathic VF."

Long QT Syndrome (LQTS)

The QTc intervals of the healthy population, as well as the QTc of patients with LQTS have a normal distribution and there is considerable overlapping between the QTc of both populations. Importantly, 12 % of patients with genetically proven LQTS have "normal" QT when the latter is defined as QTc <440 ms [89]. Identifying patients with LQTS who have borderline QT is especially challenging in the LQT1 genotype because the T-wave morphology, which is frequently abnormal in LQT2 and LQT3, is most often normal in LQT1. Fortunately, the epinephrine challenge test is especially effective for unraveling abnormal QT responses in LQT1 [76, 77]. We recently described that the sudden tachycardia induced by standing unmasks LQTS in borderline cases and use that as a bedside test [73].

Short QT Syndrome (SQTS)

The newly described SQTS [15, 16, 23] is caused by genetic mutations involving the same potassium channels that cause the LQTS but with an opposite effect [90–93]. In other words, in the SQTS there is excessive outflow of potassium currents, shortening the action potential duration and the effective ventricular refractory period. Distinguishing idiopathic VF from SQTS is not easy. Although the original cases of SQTS had extremely short QT intervals (QTc shorter than 300 ms [15, 16, 23] more recently described cases of genetically proven SQTS have QTc intervals of 360 ms [25]. Also, we recently showed that "relatively short" QT intervals (QTc <360 ms) are frequently observed in healthy males but are statistically more common in males with idiopathic VF [18]. Moreover, patients with idiopathic VF have normal QT intervals at normal heart rates, but their QT fails to lengthen as their heart rate slows down, leading to abnormally short QTc values during bradycardia [26, 27]. Finally, patients with SQTS and patients with idiopathic VF share the following clinically characteristics: (1) Both patient groups have similar spontaneous [5, 17] and inducible [3, 23] ventricular arrhythmias; (2) both patient groups appear to respond especially well to quinidine therapy [33, 52, 54, 94, 95]; (3) both patient groups are at risk for inappropriate ICD-shocks because of intracardiac T-wave oversensing [96, 97]. Patients with SQTS may be misdiagnosed as "idiopathic VF" if the QT interval is measured only during relatively rapid heart rates. This is because the main problem in the SQTS is failure of appropriate QT lengthening during bradycardia [23].

Brugada Syndrome

We estimated that one out five patients originally diagnosed as "idiopathic VF" have in fact Brugada syndrome [13] and very similar numbers were reported by others [98]. Moreover, if all patients with idiopathic VF undergo systematic testing with repeated electrocardiograms [99] and Holter recordings [100] with the right precordial electrodes placed at higher positions,

as well as pharmacological challenge tests using sodium-channel blockers, as many as 40 % with idiopathic VF could be diagnosed as Brugada syndrome [101].

Short-Coupled Variant of Right Ventricular Outflow Tachycardia

The right ventricular outflow tract (RVOT) is the site of origin of the most common type of ventricular tachycardia (VT) occurring in patients *without* organic heart disease [33]. This RVOT-VT has a distinctive morphology (QRS complexes with left bundle branch block pattern and tall R waves in the inferior leads) and, in general, does not lead to hemodynamic decompensation. Therefore, RVOT-VT is considered a benign arrhythmia [33]. However, our group [65] and the group of Noda and Shimizu [66] recently described patients with otherwise typical "benign RVOT ectopy" who went on to develop spontaneous VF or polymorphic VT. It is not clear if patients with idiopathic VF and patients with this newly described form of "polymorphic VT from the RVOT" represent different aspects of one disease or two distinct disorders [102]. However, several characteristics differ among both groups: (1) otherwise typical *monomorphic* RVOT-VT is *also* seen in patients with malignant polymorphic RVOT-VT [65, 66] but is never seen in idiopathic VF. (2) Only 5 % of patients with "malignant polymorphic VT" have inducible VF [65, 66] by programmed ventricular stimulation, whereas the majority of patients with idiopathic VF have inducible VF [33, 52]. (3) the coupling interval of the ventricular extrasystoles initiating the malignant ventricular arrhythmias is invariably very short in idiopathic VF [3, 5] but is longer, varying from "relatively short" [65] to "normal," [66] in the "polymorphic RVOT-VT" [103]. The last observation is consistent with the results of intracardiac mapping performed by Haissaguerre [35]: In that series, idiopathic VF originated from Purkinje fibers in 86 % of the patients and from the RVOT in the remaining 14 %. Again, the coupling interval of the extrasystoles initiating VF was *longer* for arrhythmias originating in the RVOT than for arrhythmias triggered by Purkinje fibers (355 ± 30 ms vs. 280 ± 26 ms, $p < 0.01$) [35].

Prognosis and Therapy of Idiopathic VF

The rate of recurrence of malignant ventricular arrhythmias in idiopathic VF, in the absence of therapy, is unacceptably high. At a mean follow-up of 6 years, more than 40 % of patients have recurrent VF and the risk is higher for those with normal electrocardiograms (that is, after excluding those with possible Brugada syndrome) [104]. In a recent series of patients with "truly idiopathic VF" (that is, excluding not only those with Brugada-type electrocardiogram at baseline but also those who developed ST-segment elevation when challenged with sodium channel blockers), the risk for recurrent VF was 39 % at 3.4 ± 2.3 years [51]. Therefore, once a diagnosis of idiopathic VF is made, some form of therapy is mandatory. Therapy may include ICD implantation, drug therapy with quinidine, radiofrequency ablation of the triggering focus or combinations of the above.

Drug Therapy with Quinidine

The very first patients with idiopathic VF described in the literature, back in 1929 [1] and 1949 [105], were treated with quinidine after multiple episodes of spontaneous polymorphic VT and VF were clearly documented. Both patients had an excellent response [1, 105]. In fact, a second publication reporting the long term follow-up of the patient initially reported in 1949, established that this patient eventually died of cancer at old age, without ever experiencing arrhythmia recurrence while on quinidine therapy for 40 years [106]. In 1987, Belhassen pioneered the therapy of idiopathic VF with EPS-guided quinidine after observing that VF is easily inducible at the baseline state but no longer inducible after quinidine therapy [2]. Of note, one of the patients included in that original report [52], has completed >25 uneventful years of electrophysiologic-guided therapy

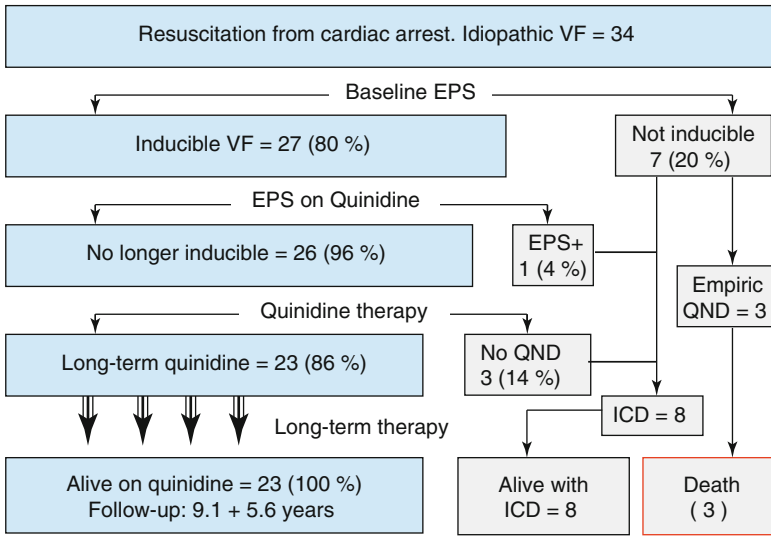

Figure 36–5. Our experience with quinidine therapy as published in 1999 [53]. *VF* ventricular fibrillation, *EPS* electrophysiologic study with programmed ventricular stimulation *EPS+* positive electrophysiologic study, i.e., inducible VF with programmed ventricular stimulation, *QND* quinidine, *ICD* implantable cardioverter defibrillator

with amiodarone and quinidine after experiencing arrhythmic storms of VF in the absence of therapy and recurrent arrhythmic syncope on amiodarone alone [94].

In 1990, when we first reviewed the topic of idiopathic VF [3], we found that the recurrence rate of cardiac arrest was high during therapy with other antiarrhythmic drugs (including amiodarone, beta-blockers or verapamil) [3]. The high-rate of arrhythmia recurrence with verapamil is worth noting because that drug was empirically proposed by Leenhardt and Coumel to treat the "short-coupled variant of torsade de pointes" [4], an entity that probably represents idiopathic VF. In contrast, we found that the recurrence rate with quinidine was nil [3]. By the time the ICD became commercially available, *we continued to recommend quinidine as the sole therapy for appropriately selected patients with idiopathic VF, including patients resuscitated from spontaneous cardiac arrest* [107]. Our criteria for quinidine therapy in VF survivors includes *all the following*: (1) diagnosis of idiopathic VF with or without Brugada syndrome; (2) inducible VF in the absence of drugs with programmed ventricular stimulation (Fig. 36.3); (3) no-inducible arrhythmias during oral quinidine therapy despite a very aggressive protocol of ventricular stimulation (Fig. 36.3) [2, 108, 109] (4) informed consent by a patient who is well informed of the risk and benefits of ICD and quinidine therapy for this disease [107]; (5) repeated assertion of drug compliance during long-term follow-up (compliance is assessed with quinidine serum levels and quinidine-effect on the QT interval). Our results using such approach were published in 1999 [53]. These results are shown in Fig. 36.5 and may be summarized as follows: Of 34 patients with idiopathic VF (all after resuscitation from cardiac arrest), 26 (80 %) had inducible VF at baseline electrophysiologic study and all but one of them were rendered non-inducible with quinidine. Side effects from quinidine led to discontinuation of quinidine therapy in 14 % of our patients. Nevertheless, 23 patients (2 out of 3 patients from the original cohort of cardiac arrest survivors) remained on quinidine therapy (without ICD back-up) and all are alive and completely free of arrhythmic symptoms that now exceeds 10 years. The long-term effectiveness of quinidine for preventing VF induction has been confirmed [110]. Relatively early in our experience, three patients who had negative electrophysiologic studies in the absence of drugs received empiric quinidine. All these patients died 4–8 years after the original VF episode. These 3 patients discontinued follow-up long before they died. Therefore, we do not know if the fatalities were due to poor compliance or due to drug failure and weather such failure was related to the fact that quinidine therapy for these particular patients was empiric and not

guided by electrophysiologic studies (since these 3 patients were non-inducible at baseline). Nevertheless, we no longer recommend empiric use of quinidine for non-inducible patients after spontaneous cardiac arrest, a subgroup of patients for whom ICD implantation is mandatory. However, we have successfully used quinidine to control arrhythmic storms of ventricular fibrillations in patients who originally received an ICD either because of non-inducibility in the baseline EPS or because of (the extremely rare) persistence of inducibility while on quinidine therapy. The excellent response of VF storms in idiopathic VF with Brugada syndrome has also been repeatedly reported [60, 111–113]. Of note, the fatalities that occurred 4–8 years after the first VF event clearly demonstrate that patients with idiopathic VF remain at risk for fatal arrhythmia-recurrence even after long asymptomatic periods. Thus, long asymptomatic periods after the first VF episode should not be interpreted as resolution of an unidentified "myocarditis" and cannot be taken as a "good prognostic sign."

Radiofrequency Ablation

Catheter-based radiofrequency ablation of the triggering focus is now an accepted mode of therapy for *atrial* fibrillation. Haissaguerre [35, 44] and others [66, 114, 115] have used the same concept to treat idiopathic VF. This form of therapy has been used primarily to treat patients with implanted ICDs who are receiving multiple ICD shocks because of arrhythmic storms. The first successful ablation was reported by Aizawa in 1992 [114] whereas relatively large series have been reported by Haissaguerre [35] and by Noda and Shimizu [66]. The series of Haissaguerre [35] and Noda [66] differ in the site of origin of the targeted arrhythmias: Noda targeted polymorphic VT originating from the right ventricular outflow tract [66]. In contrast, 85 % of the polymorphic ventricular arrhythmias ablated by Haissaguerre were mapped to the Purkinje system in the right or left ventricle while the site of origin of the VF was in the right ventricular outflow tract in only 4 (15 %) patients [35]. An acute successful abolition was achieved in all cases while 24 patients (89 %) had no recurrence of VF without drug during follow-up. However, the risk for recurrent spontaneous VF during long-term follow-up was 18 % [116], demonstrating that radiofrequency ablation should not be seen as "curative" or as an alternative to ICD implantation.

ICD Implantation

No doubt that ICD offers the most effective therapy for preventing arrhythmic death in idiopathic VF. Indeed, ICD implantation is considered "the only" effective therapy for idiopathic VF by most authors. However, when comparing ICD implantation to quinidine therapy for idiopathic VF, one should also take into consideration the potential adverse events of all these interventions.

In AVID, a large multicenter study of ICD implantation for malignant ventricular arrhythmias in patients with organic heart disease [117], the risk of adverse events, serious enough to warrant re-intervention, was 12 % [118]. Since only experienced electrophysiologists from prestigious centers participated in AVID, this 12 % complication rate is likely to be a moderate estimate. Moreover, the rate of complications from ICD implantation in idiopathic VF could likely be higher than the 12 % reported in AVID. This is because patients in AVID were relatively old (mean age 65 ± 11 years) [117] and had a 3-year mortality rate of 25 % despite the ICD related to their underlying organic heart disease [117]. In contrast, patients with idiopathic VF are significantly younger and have an extremely low risk for non-arrhythmic cardiac death. The 28 % risk of long-term complications after ICD implantation for Brugada syndrome [119] is more representative of the risk for idiopathic VF patients.

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