# Chapter 5
# Modulation of Calcium Handling: Calcium-Channel Modulators

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## L-Type $Ca^{2+}$ Channels: Verapamil and Diltiazem

### *Electrophysiological Properties. Differences*

Several calcium channels (L, N, T, P, Q and R) were identified in humans. Of these channels, voltage-sensitive L-type and T-type calcium channels are mainly operative in cardiovascular system. There are five classes of calcium channel blocking drugs: phenylalkylamines, dihydropyridines, benzothiazepines, diphenylpiperazines, and a diarylaminopropylamine. Clinically available calcium channel blockers are dihydropyridines, benzothiazepines and phenylalkylamines. These calcium channel

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blockers (CCB) are selective and inhibit voltage–sensitive L-type slow calcium channels mediated calcium influx in cardiac myocytes and in smooth muscle by slowing the activation of the L-type calcium channel and also delay its recovery from inactivation. To show its effect, the drugs travel through the slow channel and bind to channel from the inner side of the membrane. They bind more effectively when the channels are in open and in inactivated state and reduce opening frequency of the channels. Verapamil and diltiazem block calcium channels in use (frequency)-dependent (effect is more apparent at faster rates) and in voltage-dependent fashion (more effective blockage in depolarized fibers).

Whereas phenylalkilamines are relatively selective for myocardial cells, dihydropyridines are relatively selective for smooth muscle cells, especially in arterial beds. Benzothiazepine CCB (diltiazem) has an intermediate effect between phenylalkylamine and dihydropyridines in their selectivity for vascular and myocardial cells (Table 5.1).

As sinus node/AV node cells and working myocardial cells have different electrophysiological properties, verapamil and diltiazem (non-dihydropyridine CCB, ND-CCB) exert different effects on nodal cells, fibers and working myocardial cells. By blocking calcium current through L-Type channel ($I_{CaL}$) in nodal cells, ND-CCBs reduce maximum diastolic potential, the slope of spontaneous diastolic depolarization in phase 4, and maximal action potential amplitude of phase 0. Thus, ND-CCBs prolong conduction time and refractory periods of the AV node (prolonged AH interval and lengthened AV nodal anterograde/retrograde refractory periods). Nevertheless, ND-CCBs show no effect on atrial and ventricular activation time, i.e. P wave duration, HV interval and QRS duration (ND-CCBs do not have a significant effect on action potential amplitude, V max of phase 0, resting membrane voltage or refractoriness of atrial, myocardial working cells as well as purkinje fibers as these cells have fast-response characteristics related to $I_{Na}$) [1].

However, ND-CCBs by blocking $I_{Ca}$ in Phase 2 and 3 reduces the plateau height of the action potential in phase 2,

Table 5.1 Selectivity of calcium channel blockers

| | **Arterial vasodilation** | **Negative inotropy** | **Sinus node depression** | **AV node depression** |
|---|---|---|---|---|
| Dihydropiridine CCB (e.g. Nifedipine) | ↑↑↑↑↑ | ↑ | 0 or reflex tachycardia | Neutral |
| Diltiazem | ↑↑↑ | ↑↑ | ↑↑↑↑↑ | ↑↑↑↑↑ |
| Verapamil | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑↑ | ↑↑↑↑↑ |

Dihydropyridine CCB (nifedipine) is the most selective for arterial vasodilatation, while diltiazem and verapamil have more effects on the heart (negative inotropy, sinus node and AV node depression)

may shorten the action potential slightly in all cardiac fibers, and prolong the it slightly in Purkinje fibers. Verapamil suppresses triggered activity by reducing diastolic calcium load and hence early and late afterdepolarizations [1].

Verapamil is a racemic mixture of d- and l-isomer of verapamil. D-isomer of verapamil shows to some extent $I_{Na}$ blocking effect, therefore some anaesthetic activity. Clinical effect of verapamil is through the l-isomer of verapamil. Verapamil may also block the alfa receptors and increase the vagal effect on AV node. As verapamil does not block beta receptors, reflex sympathetic stimulation that occurs as a response to peripheral vasodilation and transient hypotension (by $I_{CaL}$ blockage of smooth muscle cells) may diminish its direct effect on sinus node (slowing the sinus rate).

Similar to working myocardium, ND-CCBs have no direct effect on refractoriness of accessory pathways. Moreover, via reflex sympathetic stimulation they may enhance the conduction over the accessory pathway in patients with Wolff-Parkinson-White syndrome (along with direct AV-Nodal blocking effect, verapamil may increase ventricular response over the accessory pathway during atrial fibrillation).

## *Pharmacokinetics*

ND-CCBs can be administered per os or by the intravenous route. Both of the drugs show high first-pass effect, high plasma protein binding, and extensive hepatic metabolism. Although enteral absorption of these drugs is almost complete, bioavailability of ND-CCBs is substantially reduced (~20–35% for verapamil and ~40% for diltiazem) by high first pass effect and extensive hepatic metabolism. The begin of the effect of these drugs by oral administration depends on formulation. Diltiazem and verapamil are available in a variety of preparations from different manufacturers, and those preparations may have different

pharmacokinetics. Therefore, dosing may vary depending on type of preparation and manufacturer. The first effect of conventional tablets is usually seen in 30–60 min, however the beginning of effect and peak effect are delayed in slow releasing, long acting forms. The peak effect of intravenous administration is within first 15 min. ND-CCBs are extensively bound to plasma proteins (70–85% for diltiazem and 90% for verapamil). They are metabolized rapidly and almost completely in liver into several active and inactive metabolites via CYP enzyme system (mainly via CYP3A4). Principal metabolite of diltiazem is desacetyldiltiazem (20% of the effect of diltiazem), and major metabolite of verapamil is norverapamil (20% of the effect of verapamil). Half-life of ND-CCBs is between 2 and 11 h and is prolonged in elderly, in patients with hepatic disease, or when they are administered repeatedly due to saturation of hepatic enzymes. Excretion is for mainly in urine as metabolites (>70%); and approximately 2–4% of a dose is excreted unchanged (Table 5.2).

## *Adverse Effects, Drug Interactions and Contraindications*

Most of the adverse effects of ND-CCBs are due to the extension of their pharmacological effects.

*Cardiac side effects:* Excessive inhibition of calcium influx in SA node, AV node and myocardial cells can cause serious bradycardia, atrioventricular block, cardiac depression, heart failure and cardiac arrest. Concomitant use of other cardiodepressant drugs like β-blocking drugs increase the susceptibility to side effects and also the severity of side effects.

*Extracardiac side effects:* Extracardiac side effects are usually rare. The most commonly reported side effects are constipation, dizziness, and headache.

Table 5.2 Pharmacokinetics of verapamil and diltiazem

| | Verapamil | Diltiazem |
|---|---|---|
| Absorption (oral, %) | >90 | >90 |
| Bioavailability (%) | 20–35 | 40 |
| Onset of action[a], oral (min) | 90–120 | <30 |
| Peak effect[a], h | 1–6 | 2–4 |
| Protein binding (%) | 90 | 70–85 |
| Plasma half-life, h[b] | 4–8 | 3–4 |
| Distribution volume (Lt/kg) | 5.0 ± 2.1 | 3.3 ± 1.2 |
| Metabolism | 80% first pass effect, active metabolites | 60% first pass effect |
| Active metabolite | Nor-verapamil | Desacetyldiltiazem, N-Desmethyldiltiazem |
| Excretion (%) | | |
| Renal | 70 (3–4% unchanged) | 35(2–4% unchanged) |
| Fecal | 16 | 65 |

[a]Onset of action and peak effect depend on the formulation of drug. Diltiazem is available in a range of formulations, including standard (onset of action 15 min, peak plasma concentrations after 2–4 h, and plasma t1/2 of 3–4 h) and longer-acting (peak plasma concentrations approximately 8–11 h after dosing, and average plasma t1/2 of 6–8 h). With verapamil, the time to maximal concentration is ~80–90 min with the immediate-release; and ~7–8 h with modified-release preparations

[b]Plasma half-life of verapamil increases during long term administration

*Verapamil*

- Very common (10% or more): Headache
- Common (1–10%): Dizziness, lethargy, constipation (particularly in the elderly up to 10%), dyspepsia, nausea, diarrhoea, flatulence
- Uncommon (0.1–1%): Abdominal discomfort/pain, oedema.
- Rare: gynecomastia, reversible hepatic impairment, paresthesia, gingival hyperplasia, erythromelalgia, hyperprolactinemia.

*Diltiazem:* generally mild side effects.

- Common (1–10%): Headache, fatigue, tiredness/malaise, accidental injury, pain, scalp irritation, rash
- Rare: gingival hyperplasia, exfoliative dermatitis, angioneurotic edema, erythema multiforme, vasculitis, transient increased liver transaminases

## Drug Interactions

ND-CCBs are frequently administered concomitantly with other medications (antihypertensive drugs, vasodilators etc.). ND-CCBs may affect absorption, distribution, metabolism, and excretion of concomitant drug, and may interact with drug transport proteins (Table 5.3).

Concomitant use of CYP3A4 inducers (e.g. carbamazepine, phenytoin, rifampicin) may reduce plasma levels and CYP3A4 inhibitors (e.g. ketoconazole, itraconazole, ritonavir, erythromycin, fluoxetine, valproic acid, cimetidine, other CCBs, HIV protease inhibitors, grapefruit juice) may increase verapamil's plasma levels. Therefore, caution is advised when administered concomitantly with such drugs.

Similarly, due to competitive inhibition of CYP3A4 with ND-CCBs may lead to an increase of plasma levels of other CYP3A4 metabolized drugs (e.g. cyclosporin, tacrolimus, ketoconazole, carbamazepine, sildenafil).

Table 5.3 Drug interactions with verapamil

| Mechanism of interaction | Tissue distribution | Substrates |
|---|---|---|
| Drugs absorption and elimination via MDR1 (ABCB1, P-gp1): *Verapamil inhibits P-gp and affects oral absorption and renal excretion of the listed drugs*[a] | Liver<br>Kidney<br>Intestine<br>BBB<br>BTB<br>BPB | Amitriptyline, Amiodarone, Amprenavir, Anticancer agents, Apixaban, Atorvastatin, Cefoperazone, Chorambucil, Chlorpromazine, Cimetidine, Ciprofloxacin, Cisplatin, Clarithromycin, Colchicine, Cyclosporine, Dabigatran, Dexamethasone, Digoxin, Diltiazem, Domperidone, Doxorubicin, Erythromycin, Edoxaban, Estradiol, Etoposide, Fentanyl, Fexofenadine, Grepafloxacin, Hydrocortisone, Imatinab mesylate, Indinavir, Itraconazole, Ketoconazole, Lansoprazole, Levofloxacin, Lidocaine, Loperamide, Losartan, Lovastatin, Methadone, Methotrexate, Methylprednisolone, Morphine, Nadolol, Nelfinavir, Norfloxacin, Nortriptyline, Ondansetron, Omeprazole, Pantoprazole, Phenytoin, Pravastatin, Propranolol, Progesterone, Quinidine, Ranitidine, Ritonavir, Rivaroxaban, Rhodamine 123, Saquinavir, Tacrolimus, Testosterone, Timolol, Trimethoprim, Verapamil, Vincristine |

Table 5.3 (continued)

| Mechanism of interaction | Tissue distribution | Substrates |
|---|---|---|
| Metabolism of drugs via CYP3A4: *Verapamil is a moderate inhibitor of CYP3A4, it decreases the metabolism of the listed drugs and thus increases the plasma level and clinical effect*[a] | Liver | Alprazolam, Amiodarone, Atorvastatin, Buspirone, Carbamazepine, Cisapride, Clarithromycin, Cyclosporine, Dapsone, Dihydropyridine calcium, channel blockers, Diltiazem, Efavirenz, Erythromycin, Ergot alkaloids, Estrogens, Fentanyl, Lovastatin, Midazolam, Nefazodone, Phosphodiesterase, inhibitors, Pioglitazone, Prednisolone, Progesterone, Protease Inhibitors, Quinidine, (R)-, Warfarin, Rifampin, Sertraline, Sirolimus, Simvastatin, Tacrolimus, Testosterone, Trazadone, Triazolam |
| Pharmacodynamical interactions: *Verapamil potentiates the effects of drugs with similar pharmacodynamics* | Heart | Beta blockers, Digoksin, Amiodarone, Clonidine, Alfa Adrenergic Blockers, Disopyramide, Neuromuscular Blocking Agents |

*P-glycoprotein 1* (permeability glycoprotein, P-gp) also known as multidrug resistance protein 1 (*MDR1*) or ATP-binding cassette sub-family B member 1 (*ABCB1*) or cluster of differentiation 243 (*CD243*). It is an important protein of the cell membrane that pumps (ATP-dependent efflux pump with broad substrate specificity) many foreign substances out of cells. *BBB* blood-brain barrier, *BPB* blood-placental barrier, *BTB* blood-testis-barrier

[a]The list does not represent all of the drugs metabolized via CYP3A4

Combination of ND-CCBs with drugs that exert similar effects may potentiate effect of these drugs. This combination (e.g. combination of ND-CCBs with beta blockers, antiarrhythmic drugs or cardiac glycosides) may pose an increased risk of deep bradycardia, heart block, hypotension and/or decreased cardiac contractility, congestive heart failure. ND-CCBs increase the depression of cardiac contractility, conduction and automaticity and the vasodilatation produced by general anesthetics, or effect of neuromuscular blocking agent.

ND-CCBs increase the plasma levels of quinidine, lovastatin, atorvastatin, simvastatin, increase the risk of lithium toxicity. Verapamil is also an inhibitor of P-glycoprotein that decreases the clearance of digoxin and increases its plasma levels (50–75%); thus, the maintenance dose of digoxin should be reduced to avoid the risk of bradycardia or AV block. Unlike verapamil, diltiazem does not interact with digoxin. New oral anticoagulants (dabigatran, rivaroxaban, apixaban, edoxaban) are substrates of P- glycoprotein, thus concomitant verapamil treatment can affect the bioavailability of these drugs (i.e. increase of plasma concentrations). Interaction between verapamil and new oral anticoagulants can be minimized if NOACs are administered 2 h prior to verapamil; or alternatively dose reduction of NOAC (dabigatran and edoxaban) should be considered.

Elevated serum calcium levels due to high doses of vitamin D and/or high intake of calcium salts may reduce the response to diltiazem.

Verapamil may increase blood alcohol concentrations and prolong its effects.

## Pregnancy

There are no well controlled studies with ND-CCBs in pregnant or in breastfeeding women. In animal studies, CCBs were shown to have some teratogenic effects. Therefore,

ND-CCBs are classified as *pregnancy category C* by FDA. Thus, during the pregnancy or breastfeeding period ND-CCBs should only be used if the potential benefits to the pregnant woman outweigh any possible risks to the foetus.

## *Clinical Uses*

### Treatment and Prophylaxis of Paroxysmal SVT and Control of Ventricular Rate in Atrial Flutter/ Atrial Fibrillation

Intravenous verapamil or diltiazem may be used in hemodynamically stable supraventricular tachycardias (SVT) either for rate control or to terminate the tachycardia (class IIa indication, level of evidence B) [2]. ND-CCBs have been shown to successfully terminate SVTS in 64–98% of patients [2]. These agents are especially helpful in patients who cannot tolerate beta blockers or have recurrence after conversion with adenosine. Diltiazem and verapamil should not be used in patients with suspected systolic heart failure.

In patients with broad complex tachycardias, it is critical to distinguish SVT with aberrant conduction (or SVT with pre-existing bundle brunch block) from ventricular tachycardia or pre-excitation. The administration of intravenous verapamil or diltiazem in patients with either VT or a pre-excited AF may lead to hemodynamic compromise or may accelerate the ventricular rate and lead to ventricular fibrillation.

#### Parenteral (in Acute Treatment)

*Diltiazem:* 0.25 mg/kg over 2 min (under monitoring of blood pressure and ECG); may be repeated after 15 min (0.25–0.35 mg/kg over 2 min) or maintenance dose: 5–15 mg/h IV.

*Verapamil:* 5–10 mg (0.075–0.15 mg/kg) over 2 min; may be repeated after 30 min as 10 mg (0.15 mg/kg) over 2 min.

Oral

Verapamil (Immediate release tablets): 240–480 mg/day in 3 or 4 divided doses.

## Treatment of Hypertension

In the contemporary guidelines for the management of high blood pressure, calcium channel blockers are referred as first line therapy along with ACE inhibitors/Angiotensin receptor blockers, diuretics (and beta blockers) [3]. However, guidelines essentially refer to long acting dyhidropyridine–CCBs (vasoselective CCBs) rather than verapamil or diltiazem. Verapamil or diltiazem is mostly used as an alternative to beta blockers in patients who have contraindications to beta blockers or cannot tolerate beta blockers.

Diltiazem

*Extended Release Capsules*

- Initial dose: 120–240 mg orally once a day
- Maintenance dose: 120–540 mg orally once a day
- Maximum dose: 540 mg/day

*Extended Release Coated Capsules*

- Initial dose: 180–240 mg orally once a day, increasing the dose as needed
- Maintenance dose: 240–360 mg orally once a day
- Maximum dose: 480 mg/day

*Extended Release Tablets*

- Initial dose: 180–240 mg orally once a day, increasing the dose as needed
- Maximum dose: 540 mg/day

Verapamil

*Immediate Release Tablets*

- Initial dose: 40–80 mg orally three times a day
- Maintenance dose: If adequate response is not obtained with the initial dose, dose may be titrated upward to max. dose of 480 mg/day

*Extended Release Capsules*

- Initial dose: 100–200 mg orally once a day (preferentially at bedtime)
- Maintenance dose: If adequate response is not obtained with the initial dose, dose may be titrated upward to max. dose of 400 mg/day.

*Extended Release Tablets*

- Initial dose: 180 mg orally once a day (preferentially at bedtime)
- Maintenance dose: If adequate response is not obtained with the initial dose, dose may be titrated upward to max. dose of 480 mg/day

*Sustained Release Capsules*

- Initial dose: 120–240 mg orally once a day (preferentially in the morning)
- Maintenance dose: If adequate response is not obtained with the initial dose, dose may be titrated upward to max. dose of 480 mg/day

*Sustained Release Tablets*

- Initial dose: 120–180 mg orally once a day (preferentially in the morning with food)
- Maintenance dose: If adequate response is not obtained with the initial dose, dose may be titrated upward to max. dose of 480 mg/day

Verapamil SR is also available as a fixed combination with trandolapril for treatment of arterial hypertension.

## Treatment of Chronic Stable and Vasospastic Angina Pectoris

ND-CCBs are effective in patients with angina acting by relieving coronary vasoconstriction, lowering heart rate, negative inotropic effect and peripheral vasodilatation [1]. Verapamil has been approved for all kind of anginas (effort, vasospastic, unstable). Compared to beta blockers, verapamil has similar anti-anginal effect in patients with chronic stable angina, however with less new diabetes, fewer angina attacks and less psychological depression. Diltiazem has similar effect like verapamil with more favorable side-effect profile. There is no study comparing the antianginal effect of verapamil with diltiazem.

Combination of ND-CCBs with beta blockers should be avoided due to increased risk of heart block, sinus bradycardia, and heart failure. ND-CCBs should not be used in patients with stable angina and left ventricular dysfunction. Beta blocker may be combined with dyhidropyridine CCBs like amlodipine, long acting nifedipine, felodipine, lacidipine or lercanidipine.

### Dosis

*Diltiazem*

*Immediate Release Tablets*

- Initial dose: 30 mg orally four times a day (before meals and bedtime), increasing gradually every 1–2 days until the optimal response is attained
- Maintenance dose: 180–360 mg orally per day in divided doses (3–4 times a day)

*Extended Release Capsules*

- Initial dose: 120–180 mg orally once a day, increase the dose every 7–14 days as needed
- Maximum dose: 540 mg/day

*Extended Release Coated Capsules*

- Initial dose: 120–180 mg orally once a day, increase the dose every 7–14 days as needed
- Maximum dose: 480 mg/day

*Extended Release Tablets*

- Initial dose: 180 mg orally once a day, increase the dose every 7–14 days as needed
- Maximum dose: 360 mg/day

*Verapamil*

*Immediate Release Tablets*

- Initial dose: 40–120 mg orally three times a day
- Maintenance dose: If adequate clinical response is not obtained with the initial dose, dose may be titrated upward to max. dose of 480 mg/day

*Extended Release Tablets*

- Initial dose: 180 mg orally once a day (preferentially at bedtime)
- Maintenance dose: If adequate response is not obtained with the initial dose, dose may be titrated upward to max. dose of 480 mg/day

Contraindications

- Cardiogenic shock, severe hypotension, HF, AMI with pulmonary congestion.
- LV Dysfunction (LVEF <40%), severe aortic stenosis
- 2nd and 3d-degree AV block, sick sinus syndrome, SA block, or severe sinus bradycardia (in patients without pacemaker)
- Atrial fibrillation or atrial flutter associated with an accessory pathway
- Hypersensitivity to drug
- Pregnancy and lactation (relative contraindication)

# Modulation of Abnormal $Ca^{2+}$ Homeostasis/Cycling

## *Abnormal $Ca^{2+}$ Homeostasis/Cycling and Arrhythmia Risk*

Calcium ions are plays important role not only in generation of action potential but also in excitation-contraction coupling and in various other cellular processes through calcium regulated enzymes or calcium sensitive ion channels. Disorders of calcium handling (either due to dysfunction of calcium channels or due to dysfunction in regulatory proteins) result in disorders in depolarization, in repolarization and also calcium overload in myocardial cells.

Calcium ions play a pivotal role in excitation-contraction coupling. Cardiac excitation-contraction coupling is initiated by opening of voltage dependent $Na^+$ channels, which mediates an influx of $Na^+$ ions and depolarizes the myocytes membrane. The action potential spreads along the membranes of transvers tubules (T-Tubules) to the interior of muscle fiber. Depolarization of cell membrane activates voltage-gated (slow) L-type $Ca^{2+}$ channels during phase 1 and especially phase 2 of action potential and allow inward $Ca^{2+}$ current which creates plateau in the action potential. These "trigger $Ca^{2+}$" ions bind to ryanodine receptor in the sarcoplasmic reticulum (storage organelle of calcium ions) membrane and induce "$Ca^{2+}$-induced $Ca^{2+}$ release" of the sarcoplasmic reticulum [4]. The T-tubules of cardiac muscle, are much more developed and have five times greater diameter than that of the skeletal muscle T-tubules, however sarcoplasmic reticulum is less well developed. Therefore, the strength of contraction depends to a great extent on the concentration of calcium ions in the T-tubules (i.e. extracellular fluid). Sudden 500-fold increase of cytosolic $Ca^{2+}$ concentration induces the

contraction of myofilaments by binding of calcium to the troponin C. In heart muscle, calcium pulse lasts longer than skeletal muscle and is about 300 ms. At the end of phase 2, L-type $Ca^{2+}$ channels are inactivated and influx of calcium ions ends abruptly. Subsequently, calcium ions rapidly pumped out of the sarcoplasm back into the sarcoplasmic reticulum (re-uptake via sarco−/−endoplasmic reticulum calcium-ATPase, SERCA) and T-tubule (via sodium-calcium exchanger and Na/K-ATPase). The removal of calcium ions from sarcoplasm is an energy dependent (active) process.

Abnormal calcium homeostasis appears in various cardiac diseases like chronic heart failure, myocardial ischemia, left ventricular hypertrophy, or channelopathies and plays crucial role in development or arrhythmias. Up- or down regulation of channels, dysfunctions (functional or structural) in any of those channels or in regulatory proteins lead to "calcium-leak" or "calcium sparks" which triggers early (EAD) and delayed afterdepolarizations (DAD). EADs and DADs represent a substantial function in triggering of arrhythmias [5, 6].

## *SERCA2a, NCX, RyR2 Channels*

### Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA2a)

In cardiac muscle, the removal of intracellular calcium from sarcoplasm is essential part of the regulation of cardiac contractility. After contraction of myofilaments, calcium ions are pumped out from sarcoplasm, and maintaining low cytosolic calcium concentration relies upon SERCA (into SR) and plasma membrane calcium ATPase and $Na^{+}/Ca^{2+}$ Exchanger-NCX (into extracellular fluid) pumps.

There are three human SERCA genes encoding up to ten isoforms of calcium channels. The SERCA1 isoform is specific for the SR in fast-twitch skeletal muscle. SERCA2 has been found in the SR of slow-twitch skeletal muscle and in other tissues. The SERCA2a isoform is expressed in the SR of cardiac muscle. SERCA2b is the major isoform expressed in smooth muscle and non-muscle tissues and also has a housekeeping function (essential for most mammalian cells). SERCA2a may play a role in the pathogenesis of cardiac hypertrophy and failure. SERCA2a activity is regulated by phospholamban.

The function of SERCA is controlled by the regulatory protein phospholamban (PLB/PLN). PLB operates as a subunit and inhibits SERCA. Increased β-adrenergic stimulation via cAMP induced protein kinase A pathway phosphorylates the PL and due to increased phosphorylation reduces the association between SERCA and PLB. Phosphorylation of PL leads to dissociation of PLB, interrupts the inhibition and hence increases the SERCA mediated calcium transport. Another regulatory protein is calcium binding protein calsequestrin. Calsequestrin is located in SR and helps the SR to store an extraordinarily high amount of calcium ions by binding 18–50 $Ca^{2+}$ ions to each molecule of calsequestrin. As Calsequestrin binds calcium ions with very low affinity, they can also easily be released back to sarcoplasm. Reduction of free calcium concentration within the SR enhances the pump function of SERCA by decreasing the high concentration gradient between sarcoplasm and SR. Phosphorylation of calsequestrin increases the calcium binding capacity of calsequestrin nearly twofold. Under pathological circumstances SERCA2 can also be regulated by microRNAs (miR-25 suppresses SERCA2 in heart failure).

Experimental inhibitor of SERCA is thapsigargin and experimental stimulator is istaroxime.

SERCA3 is expressed in many tissues. SERCA3 is associated with the relaxation of vascular smooth muscle and may also play a role in insulin secretion in beta cell through the regulation of calcium signalling.

## Natrium/Calcium Exchanger (NCX)

NCX is a dimeric transporter of ten transmembrane helices. It is an antiporter membrane protein that removes calcium from sarcoplasm. It uses the energy that is stored in the large concentration gradient of sodium (higher concentrations in extracellular fluid) by allowing sodium to flow down across the plasma membrane into sarcoplasm and in exchange counter transport calcium ions into extracellular fluid. The NCX removes a single calcium ion in exchange for the import of three sodium ions.

NCX may reverse direction of flow along with the changes in sodium gradient. Most of the time NCX is in the $Ca^{2+}$ efflux position. Nevertheless, during the upstroke of the cardiac action potential there is a large influx of Na ions and results in a large increase in intracellular sodium for a very short of time. This causes the reversal of the flow via NCX (pump sodium ions out of the cell and $Ca^{2+}$ ions into the cell). Subsequent activation of L-type calcium channel and influx of $Ca^{2+}$ results in rise of sarcoplasmic calcium concentration and NCX returns to its calcium efflux position.

Na/K-ATPase indirectly contributes to calcium homeostasis. It rectifies the sodium influx by pumping actively sodium ions out of sarcoplasm and prevents the increase of sodium concentration.

Several pathological conditions may abnormally switch the NCX to reverse the flow in $Ca^{2+}$ influx position. (1) Higher internal sodium than usual (when cardiac glycoside blocks Na/K -ATPase pump, which results in an increase of sarcoplasmic $Na^{+}$ concentration) (2) the sarcoplasmic reticulum release of calcium is inhibited (results in a decrease in sarcoplasmic calcium concentration) (3) other calcium influx channels are inhibited (results in a decrease in sarcoplasmic $Ca^{2+}$ concentration) (4) when the action potential duration is prolonged.

## RyR2 Channels

Ryanodine receptors (RyRs) are huge intracellular calcium channels [7]. RyR2 is the cardiac ryanodine receptor and consists of high molecular weight homotetramer. Each monomer

has at least six transmembrane segments to form the pore region of the channel and also a large cytoplasmic regulatory domain that serves as a docking platform for multiple regulatory proteins and enzymes (including calmodulin, the FK506-binding protein FKBP12.6/calstabin2, protein kinase A, CaM-dependent kinase II, protein phosphatases 1 and 2A, phosphodiesterase, junctin, triadin, and calsequestrin).

RyRs take part in different signalling pathways in different tissues. In cardiac muscle, cardiac isoform (RyR2) plays a crucial role in excitation contraction coupling by mediating major calcium release from the intracellular calcium store, the sarcoplasmic reticulum (other minor source of calcium ions is the extracellular fluid largely via $I_{CaL}$). The depolarization of cell membrane activates voltage-gated L-type $Ca^{2+}$ channels and leads inward calcium current ($I_{CaL}$). As calcium ions are primary physiological ligand of RyRs, calcium binding to ryanodine receptor triggers the opening of RyRs and thus induces enormous "calcium-induced calcium release".

RyR2 activity is also modulated by multiple other subunit proteins and enzymes. Caffeine, low concentration of ryanodine (<10 μM) or cyclic ADP-ribose activate the channel. On the contrary, magnesium, ruthenium red, or higher concentrations of ryanodine (≥100 μM) inhibit the channel. In muscle cells, calcium-free calmodulin (apoCalmodulin) activates the RyR channel, and calcium bound calmodulin inhibits the channel. Phosphorylation of the RyR by cAMP-dependent protein kinase and by Calcium/calmodulin-dependent protein kinase II dissociates FKBP12 or FKBP12.6 from the RyR complex. When by FKBP12 or FKBP12.6 is dissociated from the FKBP-RyR complex, the RyR2 channel is activated.

Furthermore, calcium release from RyR has been shown to regulate ATP production in heart and pancreas cells.

The mutations of RyR2 and its regulatory proteins are shown to be associated with catecolaminergic polymorphic ventricular arrhythmias and sudden cardiac death.

## *Arrhythmogenic Substrate Is Disease-Specific: AF, HF, Channelopathies*

### Atrial Fibrillation (AF)

AF is a complex, multifactorial arrhythmia. Major arrhythmogenic mechanisms are ectopic activity (as a trigger and driver of AF) and re-entry (different types of functional and/or structural re-entries, play role in maintenance of AF). Abnormal calcium handling plays critical role not only in promoting the ectopic activity, but also facilitating the maintenance of atrial fibrillation by enhancing the re-entry [8]. Rapid atrial rate during AF causes several progressive structural (increased fibrosis, cellular disarray etc.) and functional changes (further alterations in calcium handling, abbreviated AP duration and wavelength, changes in cellular physiology etc.) as well. Abnormal calcium handling is pivotal in AF pathophysiology by enhancing AF, and as a mediator which leads to functional and structural changes of AF (AF begets AF).

Focal ectopic activities in atria are mostly due to DADs (i.e. triggered activity). DADs are oscillations of the membrane potential occurring after phase 3 of action potential. DADs are results mainly from spontaneous diastolic SR calcium releases through RyR2, which increases cytoplasmic calcium levels and hence activates NCX and generates depolarizing transient-inward current. When transient-inward current sufficiently depolarizes the membrane potential, voltage-gated sodium channels are activated and a triggered action potential is generated. Initially, DADs occur as result of increased SR calcium load and higher RyR2-protein expression. Thereafter, additionally functional and structural changes occur (like increase in CaMKII-dependent RyR2 phosphorylation, reduced activity of protein phosphatase 1, reduced levels of regulatory proteins like sarcolipin and FKBP12.6, and increased calcium sensitivity and expression of NCX) which further increase the susceptibility to cellular DADs.

Re-entry is facilitated by shortening of effective refractory period and slowing in atrial conduction. At initial phase of AF, high atrial rate related cellular calcium overload reduces inward calcium current through L-type calcium channels (LTCC) to protect cell from cytotoxic calcium overload which results in action potential shortening and wavelength abbreviation. Reduction of inward calcium current may result from calcium-dependent reduction in gene expression, from posttranslational modifications like LTCC hypophosphorylation and from calpain-mediated breakdown of channel subunits. Through several calcium dependant pathways, density of the inward rectifier kalium current $IK_1$ and the acetylcholine-dependent inward-rectifier kalium current ($IK_{ACh}$) are also increased which in turn further shorten action potential duration and cause a more negative resting membrane potential, thereby increasing atrial excitability and facilitate the formation of rotors.

Moreover, re-entry is also enhanced by fibroblast-to-myofibroblast transition and myofibroblast proliferation which is triggered through several calcium dependant pathways as well.

## Heart Failure

Calcium is the pivotal intracellular mediator between sarcolemmal action potential and myofilament activation, contraction and relaxation. Apart from excitation contraction coupling, calcium is crucial for physiological stress adaptation. A higher diastolo-systolic calcium gradient increases force generation and cardiac output. In heart failure, independent from the cause of myocardial injury, several compensatory mechanisms like myocardial hypertrophy, increasing the filling pressure and activation of neuro-hormonal pathways are activated to overcome the depressed cardiac function. However, a chronic hyperactivation of neuro-hormonal pathways contributes to progressive maladaptive remodelling of the heart, progressive deterioration of pump function, and arrhythmias. These maladaptive responses are associated with abnormal intracellular calcium metabolism, and show important similarities beyond the reason of heart failure (decreased calcium transients,

enhanced diastolic SR calcium leak and diminished SR calcium sequestration, that lead to defective excitation contraction coupling i.e. impaired contractility and relaxation) [9]. Despite preserved calcium influx current density through L-type calcium channel, the ability of any given calcium current to activate SR calcium sparks (i.e. impaired calcium induced calcium release) was significantly diminished which leads to reduction in yield of excitation contraction coupling (ECC). Along with reduced SR calcium release via RyR2 and subsequent reduced calcium re-uptake and sequestration via SERCA2a, there is an increase in open probability of RyR2 and SR diastolic calcium leak. Elevated diastolic calcium level further contribute to reduced contractile force generation, impaired relaxation, and abnormal force–frequency relationship.

In addition, due to chronic hyperadrenergic state, desensitization of beta-adrenoreceptors and thus reduced global intracellular cAMP synthesis occur. Alterations of second messenger system leads to alterations in expression and functions of regulatory proteins (like calmodulin-dependent protein kinase II, Protein kinase A, protein kinase C, Calcineurin, and S100A1) [10]. The ratio of phospholamban to SERCA2 is increased and contributes to increased diastolic calcium levels and cardiac dysfunction. Furthermore, due to the changes in the expression and function of NCX and SERCA2, there is a shift toward increased outward calcium current via NCX to the extracellular space and a net decrease in SR calcium re-uptake.

Beside electrical remodelling, there is a structural remodelling (structural changes of the T-tubules, SR storage organelles, and/or the architecture of the dyads) as well which contributes to defective EC coupling by altering the geometry of the calcium release unit.

## *Channelopathies*

### Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

CPVT is an inherited arrhythmia syndrome characterized by physical or emotional stress triggered polymorphic ventricular

tachycardia in patients with structurally normal hearts. CPVT is caused by mutations in RyR2 or CASQ2 which result in defective intracellular/sarcoplasmic calcium handling [11, 12]. Two forms of CPVT have been described resulting from mutations in either the RyR2 or CASQ2. Mutations in RyR2 (CPVT type 1) are identified in about half of the patients with CPVT and are inherited in an autosomal dominant form. Mutations in CASQ2 (CPVT type 2) are inherited in autosomal recessive pattern and found only 1–2% of patients with CPVT. Some other rare mutations in other genes (such as Ank2, TRDN and CALM1) related to intracellular calcium handling have also been identified in patients with clinical features similar to CPVT.

Mutations in CPVT-1 cause a gain of function in the RyR, and mutations in CASQ2 result in a decreased calcium binding affinity leading to higher levels of free SR calcium which triggers store-overload induced calcium release. As a result, mutations in these two genes precipitate diastolic calcium leak into sarcoplasma under adrenergic stimulation. Increased amount of diastolic calcium leads to an activation of the NCX resulting in a depolarizing positive inward current that induces delayed afterdepolarizations (DAD), triggered activity, premature ventricular beats and bidirectional/polymorphic VT. Corresponding to the intensity of exercise, the complexity of arrhythmias will increase from single ventricular extrasystoles (mostly polymorphic) to bidirectional VT or to polymorphic VT.

## Brugada Syndrome/Short QT Syndrome

Brugada syndrome is a rare genetic disease, characterized by coved type ST-segment elevations in the right precordial leads, increased risk of fatal ventricular arrhythmias and sudden cardiac death. Short QT syndrome is also rare genetic disease characterized by very short QT intervals on ECG, increased risk of fatal ventricular arrhythmias and sudden cardiac death. In 2007, two missense mutations in the CACNA1C (A39V and G490R) and one in the CACNB2

(S481L) which are encoding the alpha1- and beta2b-subunits of the L-type calcium channel were reported in patients with Brugada pattern ECG and short QT interval (QTc < 360 s) [13]. Both mutations result in a loss of function of Cav1.2 that leads to diminished depolarizing inward calcium in plateau phase, shortening of the action potential (short QT phenotype) and loss of the action potential dome (Brugada syndrome phenotype).

In 2011, a loss-of-function mutation in the CACNA2D1 (nucleotide c.2264G > C; amino acid p. Ser755Thr), the gene encoding Cavα2δ-1 subunit of calcium channel was found in a patient with short QT interval and documented VF.

## Long QT Syndrome (LQTS 8, Timothy Syndrome)

Long QT syndrome is rare genetic disease which causes abnormal, prolonged repolarization and thus increased risk of fatal arrhythmias. The Timothy Syndrome (TS) is a rare, autosomal dominant inherited form of the LQTS and is classified as LQTS type 8. In TS, gain of function mutations of the gene CACNA1c (chromosome 12p13.33) which encodes the calcium channel Cav1.2 (voltage-dependent L-type calcium channel subunit alpha-1C) is responsible for prolongation of action potential [14]. The mutations result in reduced Cav1.2 channel inactivation, which leads to sustained depolarizing inward calcium currents during the plateau phase and thus prolonged plateau phase and action potential (prolonged QT interval). This prolongation in turn leads to increased risk of spontaneous, abnormal secondary depolarizations (after-depolarizations), lethal arrhythmias, and sudden death. Since the calcium channel Cav1.2 is highly expressed in many tissues like brain, patients with TS have many cardiac (QT prolongation, congenital heart diseases like patent ductus arteriosus, patent foramen ovale, hypertrophic or dilated cardiomyopathy, bradycardia, atrioventricular block) and also extracardiac manifestations (syndactyly, craniofacial findings like low-set ears, depressed nasal bridge, premaxillary underdevelopment, baldness at

birth, as well as intermittent hypoglycemia, immune deficiency, autism spectrum disorders and language, motor, and generalized developmental delays).

## *Modulation of Abnormal $Ca^{2+}$ Homeostasis*

– $Ca^{2+}$ channel blockers (diltiazem, verapamil): mechanism of action, clinical uses

Mechanism of action and clinical uses of NDCCB are discussed in details in previous chapter.

– RyR2 blockers/stabilizers, dantrolene, rycals, CaMKII inhibitors, SERCA2a, Flecainide

### RyR2 Blockers/Stabilizers

Dysfunctions or dysregulations of ryanodine receptors in heart (RyR2) cause fatal arrhythmias and is involved in heart failure, whereas dysfunctions in ryanodine receptors in skeletal muscle (RyR1) result in malignant hyperthermia and myopathies like central core or minicore myopathy.

Mutations, defective regulation and distorted subunit composition of ryanodine channels cause diastolic calcium leakage from the sarcoplasmic reticulum, which in turn triggers arrhythmias and weaken cardiac contractility. Hence, modulation of RyR2 mediated abnormal calcium homestasis have emerged as a potential therapeutic target for arrhythmias. Pharmacological agents known to modulate RyR2 are listed in Table 5.4 [15].

Ryanodine is a plant alkaloid, which binds with high affinity and specificity to its receptor in the SR (ryanodine receptor has been named after this alkaloid). RyR2 has both a high- and low-affinity binding site for ryanodine, which are responsible for the concentration dependent effects of ryanodine on the activity of RyR. At nanomolar concentrations ryanodine increases the open probability without affecting

Table 5.4 RyR2 modulating agents

| Agonists | Antagonists |
|---|---|
| Purine derivatives (caffeine)[a] | Ruthenium red[b] |
| Digitalis glycosides (digoxin)[a] | Dantrolene[a] |
| Suramin[a] | Ryanoids (ryanodine)[a,b] |
| Volatile anesthetics (halothane)[a] | Local anesthetics (tetracaine)[b] |
| 4-Chloro-m-cresol (4-CMC)[a] | 1,4-Benzothiazepines (JTV519/K201)[c] |
| Peptide toxins (IpTx)[a] | |
| Macrocyclic compounds (FK506)[c] | |

[a]Modulators of channel gating
[b]Modulators of ion translocation
[c]Allosteric modulator subunit interactions

rates of ion movement, at submicromolar concentrations locks the RyR, and increases open probability to almost 100%, leads to a long-lasting subconductance (~50% of the normal conductance), however at micromolar concentration fully closes them and inhibits SR calcium release.

Ruthenium red, is a water-soluble dye that inhibit SR calcium release from both the cytosolic and luminal sides of the channel. As ruthenium red is neurotoxic, it is not an ideal candidate for drug development.

Charged local anaesthetics (tertiary amines like procaine, tetracaine, and lidocaine or quaternary amines like QX572, QX314) also inhibit RyR2 channels. Procaine appears to be more selective for RyR2 compared to RyR1. They show their effects by stabilizing RyR2 in a closed transformational state and decrease the open probability of the channels (tetracaine and procaine), or by voltage dependent blockade of channels and reduce the conductance without an effect on open probality of channel (lidocaine and quaternary amines).

## Dantrolene

Dantrolene is a hydantoin derivative used for the treatment of malignant hyperthermia and is the only currently approved drug for clinical use [16]. In skeletal muscle, 10–100 micromolar dantrolene inhibits abnormal calcium release from the SR. The sensitivity to dantrolene to RyR2 is lower than RyR1. Dantrolene prevents aberrant calcium release by stabilizing interdomain interactions within the RyR2.

## Stabilizers of Calsitabin-RyR2 Complex (1,4-Benzothiazepines Derivatives/Rycals)

Rycals are JTV519, S44121/Arm036 and S107. They stabilize the interaction between inhibitory subunit calstabin2 (FK506 binding protein 12.6, FKBP12.6) and hyperphosphorylated RyR2 (closed conformational state), reduce the channel-opening probability, and hence prevent arrhythmgenetic diastolic calcium leak, contractile dysfunction and calcium overload [17]. JTV519 prevents triggered ectopic activity triggered by a SR calcium leak through FKBP12.6-depleted RyR2 in ventricular and in pulmonary vein cardiomyocytes. Nonetheless, JTV519 also blocks voltage-gated sodium, voltage gated L-type calcium, and kalium channels (Ikr, Ik1, IAch) and its effectiveness in treatment of clinical arrhythmias is yet uncertain.

## CaMKII Inhibitors

KN-93 is a potent and specific inhibitor of calcium/calmodulin-dependent protein kinase II (CaMKII). Dysregulation of CaMKII pathway is considered to be associated not only with arrhythmias but also with Alzheimer's disease and Angelman syndrome. In hearts, CaMKIIδ enhances calcium current via L-type channels by augmentation of peak calcium influx and slowed inactivation of the calcium current, thus it may act pro-arrhythmic by triggering early afterdepolarizations (EADs).

### SERCA2a Activator (N106)

In heart failure, activity and expression of the cardiac sarcoplasmic reticulum calcium ATPase (SERCA2a) is characteristically decreased. The small ubiquitin-like modifier type 1 (SUMO-1) is a regulator of SERCA2a and SUMOylation of SERCA2a improves its function. N106 is a small molecule that directly activates the SUMO-activating enzyme and consequently increases SUMOylation of SERCA2a.

### Flecainide/Propafenone

Flecainide and propafenone are class Ic antiarrhythmics and blocks sodium channels. However, both of these drugs also block the open state of RyR2, prevent diastolic calcium leak, and hence supress DADs and triggered arrhythmias [18]. Furthermore, their sodium channel blocking properties also contribute to RyR2 blocking effect by preventing DADs reaching to threshold potential and trigger premature beats.

## Medical Treatment of Inherited Cardiac Arrhythmias Syndromes Associated to Mutations in Calcium Handling

### *Idiopathic VT*

Ventricular tachycardias in structurally normal hearts are mostly focal in origin and underlying mechanisms are triggered activity, abnormal automaticity, or rarely re-entry within the Purkinje fibers. Idiopathic VTs most commonly originate from the RV outflow tract. Other locations of focal idiopathic VTs include the LV outflow tract, aortic cusps, mitral and tricuspid annuli, papillary muscles. Treatment options for idiopathic VTs are catheter ablation and medical therapy. Medical therapy may be indicated in patients with

mild to moderate symptoms. As the underlying mechanism mostly is cAMP-mediated EADs or DADs, medical treatment include beta-blockers, verapamil, diltiazem (rate of efficacy of 20 to 50%) that supress cAMP mediated pathways and triggered activity. Alternative therapy includes class IA, IC and III agents.

## *CPVT*

CPVT is resulted from mutations in RyR2 or CASQ2 which result in stress induced diastolic calcium leak into sarcoplasma and DADs mediated triggered activity. Medical treatment includes β-blockers, verapamil, or a combination of both to reduce diastolic calcium and to prevent DADs. In addition, ryanodine channel blocker flecainide and sympathetic denervation may be added to therapy in some patients who are refractory to β-blocker/verapamil therapy.

## *Brugada/Short QT Overlap Syndrome*

Quinidine is the only drug used in long term medical treatment of Brugada/Short QT Syndrome. Although quinidine is class I antiarrhythmic agent (blockers of the fast inward sodium current), it also displays inhibitory effect on the slowly inactivating, tetrodotoxin-sensitive Na current, slow inward calcium current (ICa), rapid (IKr) and slow ($I_{Ks}$) components of the delayed potassium rectifier current, inward potassium rectifier current ($I_{KI}$), the ATP-sensitive potassium channel ($I_{KATP}$) and Ito.

## *Long QT Syndrome Type 8*

In long QT syndrome 8, there is a progressive prolongation of action potential duration and QT interval, induction of DADs, and both DAD and EADs-mediated triggered activities due to gain of function mutations of the calcium channel

Cav1.2. Antiarrhythmic treatment includes β-blocker, verapamil, mexilatine, and ranolazine to maintain repolarization stability and to prevent DADs and EADs.

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