Voltage-Gated Calcium Channels and Their Roles in Cardiac Electrophysiology

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Abstract

In cardiomyocytes voltage-gated Ca^{2+} channels are major players in cardiac cellular electrophysiology and cellular excitation-contraction coupling. Accordingly, Ca^{2+} channel dysfunction contributes to the development of cardiac arrhythmias and impaired cardiac contractile function. In addition, Ca^{2+} entry through voltage-gated Ca^{2+} channels is an important regulator of gene transcription and cardiac cellular metabolism. In order to fulfil these tasks reliably, Ca^{2+} channels are highly regulated by specific subunit compositions and various signaling pathways. This chapter provides an overview of the role of voltage-gated Ca^{2+} channels in cardiac cellular electrophysiology and summarizes their molecular composition, biophysical properties, and regulatory mechanisms, with a special focus on L-type Ca^{2+} channels.

4.1 Introduction

Sarcolemmal Ca^{2+} influx into cardiomyocytes through Ca^{2+}-permeable ion channels plays a critical role in cellular physiology (Bers 2008). On the one hand, electrogenic Ca^{2+} influx results in depolarization of the cellular membrane potential, thereby

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D. Thomas, C. A. Remme (eds.), *Channelopathies in Heart Disease*, Cardiac and Vascular Biology 6, https://doi.org/10.1007/978-3-319-77812-9_4

contributing to the action potential (AP) plateau in atrial and ventricular cardiomyocytes and to cellular automaticity in the sinoatrial and atrioventricular nodes (Heijman et al. 2016). On the other hand, Ca^{2+} ions are important intracellular second-messenger molecules, involved in excitation-contraction (EC) coupling, regulation of cellular function by phosphorylation, and regulation of gene expression (Fearnley et al. 2011; Harada et al. 2012a; Yue et al. 2011; Kreusser and Backs 2014; Rose et al. 2012; Du et al. 2010).

Voltage-activated Ca^{2+} channels represent the major route of Ca^{2+} entry into cardiomyocytes in response to depolarizations of the cellular membrane potential (Rose and Backx 2014). So far, 10 members of the family of voltage-gated Ca^{2+} channels have been identified in mammals. Based on their biophysical and pharmacological properties, they are further grouped into L-, P/Q-, R-, and T-type Ca^{2+} channels. However, only L-type and T-type Ca^{2+} channels (LTCC and TTCC, respectively) are expressed in cardiomyocytes, whereas the others are most abundant in neurons (Bers 2008; Catterall 2011; Rose and Backx 2014). LTCC are expressed in all cardiomyocytes, whereas TTCC are restricted to myocytes in the sinoatrial (SAN) and atrioventricular (AVN) nodes (Vassort et al. 2006). In addition, TTCC are expressed in atrial myocytes in a species-dependent manner. Smaller species (mouse, guinea pig) show higher TTCC current amplitudes compared to larger species (rabbit, pig), and TTCC appear to be absent in human atrial cardiomyocytes (Catterall 2011; Ono and Iijima 2010).

In this chapter, we provide an overview of the role of Ca^{2+} channels in cellular electrophysiology and intracellular Ca^{2+} handling. We introduce their molecular composition followed by their characteristic biophysical properties and an overview of the most important regulatory mechanisms. We will focus predominantly on the LTCC given its central role in cardiac pathophysiology but will highlight the major differences between LTCC and TTCC.

4.2 The Role of L-Type and T-Type Ca^{2+} Currents in Cardiac Cellular Electrophysiology

Depending on the cell type, Ca^{2+} channels are involved in several fundamental electrophysiological processes in the heart (Bers 2008). In sinoatrial and atrioventricular nodes, depolarizing Ca^{2+} currents through TTCC and $Ca_v1.3$ LTCC have been suggested to contribute to diastolic depolarization and may thereby play an important role in pacemaker activity and regulation of heart rate (Capel and Terrar 2015; Bers 2008; Vassort et al. 2006). On the other hand, $Ca_v1.2$-mediated L-type Ca^{2+} current ($I_{Ca,L}$) is responsible for the AP upstroke in SAN and AVN myocytes. Inhibition of $I_{Ca,L}$ is therefore a commonly used approach to inhibit AV-nodal conduction to reduce ventricular rate in patients with atrial fibrillation (rate control) (Kirchhof et al. 2016).

In the working myocardium, LTCC are a major contributor to the AP "plateau" at which the membrane potential remains at a relatively depolarized level for a few hundred milliseconds prior to being repolarized (Fig. 4.1) (Heijman et al. 2016; Bers

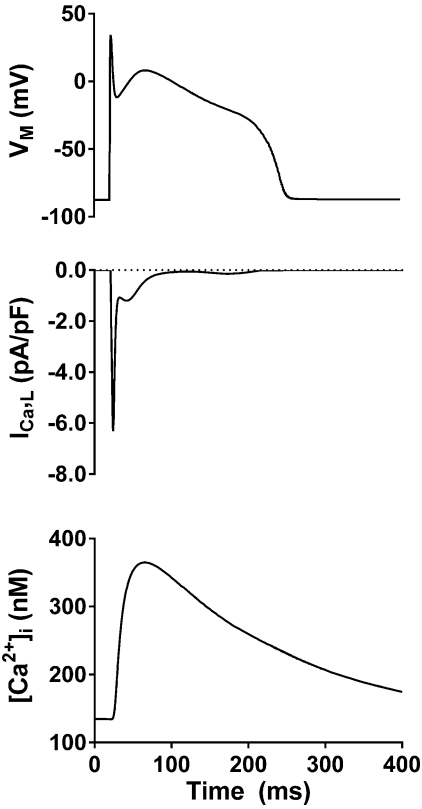

Fig. 4.1 L-type Ca^{2+} current ($I_{Ca,L}$) activity during a ventricular action potential. Membrane potential (V_M), Ca^{2+} transient, and $I_{Ca,L}$ during 1 Hz pacing in the Heijman et al. canine ventricular cardiomyocyte model (Heijman et al. 2011)

2008). This long plateau phase is a hallmark of cardiac cellular electrophysiology. The Ca^{2+} entering the cardiomyocyte through LTCC also rapidly triggers a much greater Ca^{2+} release from the sarcoplasmic reticulum (SR) through Ca^{2+}-release channels known as "ryanodine receptor channels" (RyRs, RyR2=cardiac form), a process termed Ca^{2+}-induced Ca^{2+} release (Fig. 4.2) (Voigt et al. 2012a; Grandi et al. 2011; Bers 2008). During the resulting intracellular Ca^{2+} transient (Voigt et al. 2014; Voigt et al. 2012b), Ca^{2+} binds to troponin-C in the myofilaments and initiates cardiomyocyte contraction, a process referred to as excitation-contraction coupling (EC coupling) (Bers 2002). During diastole Ca^{2+} is removed from the cytosol by Ca^{2+} reuptake into the SR, mediated via the SR Ca^{2+} ATPase ("SERCA," SERCA2a=predominant cardiac form) and by Ca^{2+} extrusion into the extracellular space via forward-mode Na^+/Ca^{2+} exchanger (NCX, NCX1=cardiac form) (Bers 2008; Voigt et al. 2012b; Hohendanner et al. 2013).

In ventricular cardiomyocytes LTCC are located mainly within membrane invaginations, so called t-tubules (Fig. 4.2). This organization allows their close interaction with RyR2 on the SR within dyadic junctions throughout the whole volume of the myocyte. Thus, t-tubules conduct membrane depolarizations to LTCC deep in the cell, activating RyR2 throughout the cellular volume (Song et al. 2005) and ensuring simultaneous uniform SR Ca^{2+} release. In contrast, atrial cardiomyocytes do not have

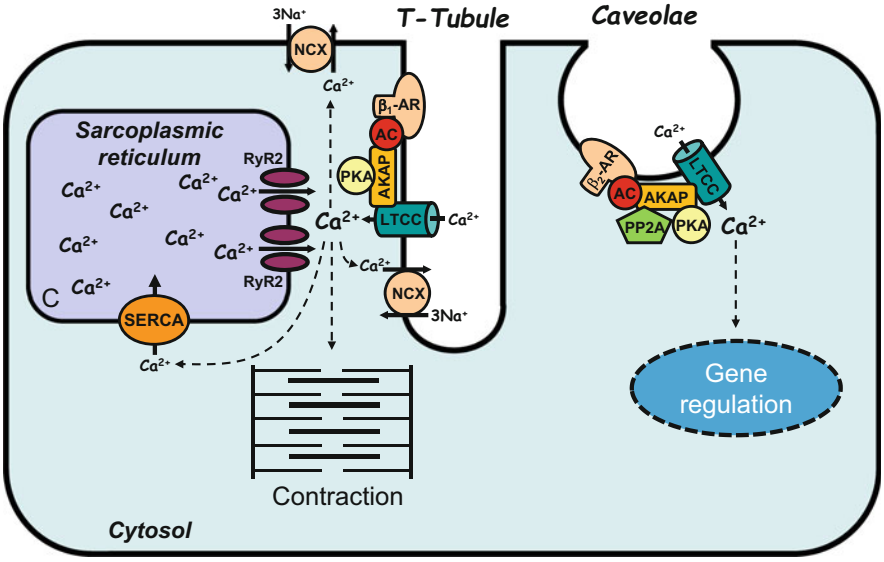

Fig. 4.2 Distinct L-type Ca^{2+} channel (LTCC) macromolecular complexes within t-tubules and caveolae. Ca^{2+} enters ventricular cardiomyocytes through t-tubular LTCC and triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) through Ca^{2+}-release channels known as "ryanodine receptor channels" (RyRs, RyR2=cardiac form). The released Ca^{2+} binds to troponin-C in the myofilaments and initiates cardiomyocyte contraction. During diastole Ca^{2+} is removed from the cytosol by Ca^{2+} reuptake into the SR, mediated via the SR Ca^{2+} ATPase ("SERCA," SERCA2a=predominant cardiac form) and by Ca^{2+} extrusion into the extracellular space via forward-mode Na^+/Ca^{2+} exchanger (NCX, NCX1=cardiac form). LTCC complexes within t-tubules include β_1-adrenergic receptor (β_1-AR), adenylyl cyclase (AC), and protein kinase A (PKA). Additional LTCC subpopulations in caveolae are implicated in signaling to the nucleus to regulate the transcription of genes. Caveolar LTCC complexes are composed of β_2-AR, AC, PKA, and protein phosphatase 2A (PP2A)

an extensive t-tubule network or may have only rudimentary t-tubular structures. As such, LTCC are expressed mainly around the periphery of atrial cardiomyocytes (Bootman et al. 2011; Brandenburg et al. 2016; Richards et al. 2011). Therefore, the close interaction of RyRs and LTCC occurs only in the immediate subsarcolemmal space, and the SR Ca^{2+} release occurs as a centripetal Ca^{2+} wave: RyR2s close to the sarcolemma get activated first and sequentially activate neighboring RyR2s toward the cell-center (Greiser et al. 2009; Wakili et al. 2010). Recently, Brandenburg et al. have provided evidence for voluminous axial tubules that harbor LTCC with extensive junctions to RyR2 in the SR in atrial cardiomyocytes. Although less extensive than the t-tubular system in ventricular cardiomyocytes, these axial tubules may be connected to the cellular surface membrane by the sparse t-tubule network, thereby partially synchronizing intracellular Ca^{2+} release from the SR (Brandenburg et al. 2016). Given the critical role of LTCC in EC coupling, their inhibition can have profound negative inotropic effects.

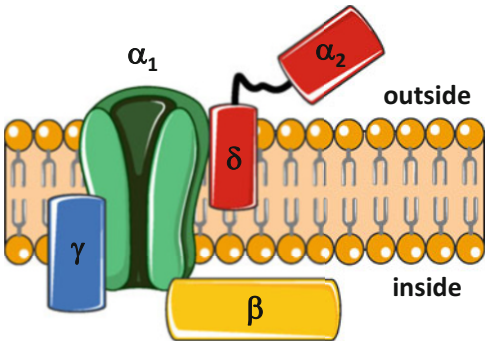

Fig. 4.3 Molecular composition of the L-type Ca^{2+} channel. See text for further details

4.3 Molecular Composition of LTCC and TTCC

4.3.1 L-Type Ca^{2+} Channels

The LTCC consist of a pore forming α_1-subunit and the accessory β-, α_2-δ-, and γ-subunits (Catterall 2000, 2011) (Fig. 4.3). Of the four currently known α_1-subunits ($Ca_v1.1$, *CACNA1S*; $Ca_v1.2$, *CACNA1C*; $Ca_v1.3$, *CACNA1D;* and $Ca_v1.4$, *CACNA1F*), only two are expressed in the heart (Schram et al. 2002). Similar to cardiac voltage-gated Na^+ channels, $Ca_v1.2$ and $Ca_v1.3$ consist of four domains with six transmembrane segments each, containing pore-forming loops and the voltage sensor as well as drug-binding sites. Whereas the $Ca_v1.2$-mediated L-type Ca^{2+} current ($I_{Ca,L}$) represents a major route of Ca^{2+} entry in all cardiomyocytes, $Ca_v1.3$-mediated Ca^{2+} currents are predominantly found in the SAN, conduction system, and atrial cardiomyocytes.

The β-subunits bind to a single site on a loop between domains I and II of the α-subunit (AID, α interaction domain) (Chen et al. 2004). The β_2-isoform (*CACNB2*) is the major cardiac isoform. Co-expression of this β-subunit with the Cav1.2 pore-forming subunit enhances activation and inactivation kinetics, shifts the inactivation curve to more negative potentials (see below), and enhances affinity to dihydropyridines (Mitterdorfer et al. 1994). In addition, Cavβ acts as a chaperone that antagonizes an endoplasmatic reticulum retention signal in the α_1-subunit, thereby supporting the correct folding and membrane targeting of the channel (Arikkath and Campbell 2003). In agreement, recent work has shown that a Cavβ_2-mimetic peptide can increase LTCC trafficking, overcoming dysregulation of LTCC density in obesity (Rusconi et al. 2016).

The α_2-δ-subunits are encoded by the same genes (*CACNAD1–4*) (Jay et al. 1991). They are cleaved after transcription and re-linked with disulfide bonds. Of the four known isoforms, α_2-δ_1 and α_2-δ_3 are expressed in the heart. α_2-δ-subunits have been suggested to facilitate the formation of functional sarcolemmal Ca^{2+} channels but may also alter channel gating, shifting voltage dependence of activation and inactivation to more hyperpolarized potentials (see below) (Davies et al. 2007).

The Ca^{2+} channel γ-subunit is encoded by eight genes with γ_4-, γ_6-, γ_7-, and γ_8-subunits expressed in the heart (*CACNG4*, *CACNG6–8*). The γ-subunits alter activation and inactivation properties of the channel in expression systems (Yang et al. 2011). However, their role in cardiac cellular electrophysiology remains unclear.

In addition to these core subunits, the LTCC contain numerous other associated proteins within its large macromolecular complex. These proteins can regulate channel function (e.g., through phosphorylation, as discussed below) or channel trafficking and targeting [e.g., the protein myoscape, which has recently been shown to interact with LTCC and to influence its surface expression (Eden et al. 2016)].

4.3.2 T-Type Ca^{2+} Channels

TTCC are mediated by the Ca_v3 family of Ca^{2+} channels, consisting of $Ca_v3.1$–$Ca_v3.3$ (*CACNA1G*, *CACNA1H*, and *CACNA1I*, respectively) (Catterall 2011). Interestingly, these α_1 subunits share only <25% amino acid sequence identity with Ca_v1 channels (Catterall 2011), suggesting an early divergence during evolution and potentially providing a structural basis for the differences in electrophysiological properties (discussed below). In the heart, TTCC consist predominantly of Cav3.1 and Cav3.2, whereby the exact balance is species and condition dependent (Perez-Reyes 2003). In contrast to LTCC, the α_2-δ and β subunits do not appear to modulate TTCC gating, although they may affect trafficking of the α_1 subunits (Dubel et al. 2004).

4.4 Voltage-Gated Ca^{2+} Channels: Biophysical Properties

$I_{Ca,L}$ was first described in Purkinje fibers and described as "slow inward current" in order to distinguish this current from the fast sodium current (Reuter 1967, 1979). Shortly afterward the presence of $I_{Ca,L}$ in all types of cardiomyocytes including atrial, SAN, and AVN was proven (Vassort et al. 2006). $I_{Ca,L}$ is named after its characteristic slow voltage-dependent inactivation resulting in "long-lasting" activity of the current, as well as large single-channel conductance and open time. Furthermore, $Ca_v1.2$-carried $I_{Ca,L}$ is characterized by high voltage of activation [$V_{1/2}$ for activation between −10 mV and −15 mV (Mangoni et al. 2006)], marked upregulation in response to the activation of cAMP-dependent phosphorylation pathways, and sensitivity to Ca^{2+} channel antagonists (1,4-dihydropyridines, phenylalkylamines, benzothiazepines) (Bers 2002; Catterall 2011; Rose and Backx 2014; Tang et al. 2016). $Ca_v1.3$-mediated currents activate at more negative membrane potentials ($V_{1/2}$ of activation between −40 mV and −30 mV), and their inactivation curve is also shifted to the left (Mangoni et al. 2006). These biophysical properties are consistent with the notion that $Ca_v1.3$ contributes to the diastolic depolarization in the SAN and thus is an important determinant of the pacemaker activity in the heart.

The second family of Ca^{2+} currents was initially described in dorsal root ganglion neuron in starfish eggs (Hagiwara et al. 1975). In contrast to $I_{Ca,L}$, these Ca^{2+}

currents showed rapid voltage-dependent inactivation and were therefore termed T-type Ca^{2+} currents ($I_{Ca,T}$) for their "transient" openings and small ("tiny") single-channel conductance. In addition, T-type Ca^{2+} currents are activated at much more negative membrane potentials and were insensitive to conventional Ca^{2+} channel inhibitors (Vassort et al. 2006; Catterall 2011). Because of their relatively negative activation potential ($V_{1/2}$ of activation between −60 mV and −50 mV), which is within the diastolic range, TTCC have been suggested to contribute to diastolic depolarization in the SAN and AVN (Mangoni et al. 2006; Capel and Terrar 2015; Vassort et al. 2006). In contrast, because of their small amplitude and their rapid inactivation, the contribution of TTCC to Ca^{2+} influx in ventricular cardiomyocytes is negligible (Catterall 2011; Ono and Iijima 2010).

4.4.1 Activation of Ca^{2+} Channels

In patch-clamp experiments, $I_{Ca,L}$ is activated by a depolarizing step-pulse protocol. Following depolarization from the resting membrane potential, the current amplitude reaches a peak value within 5–7 ms followed by inactivation of the current despite sustained depolarization (Fig. 4.4) (Bers and Perez-Reyes 1999; Voigt et al. 2012b, 2014; Bers 2001; Hadley and Hume 1987). The current reaches its maximal amplitude in response to depolarizations between 0 and +10 mV. Despite maximum activation of the channel, further depolarizations result in smaller $I_{Ca,L}$ amplitudes as the voltage approaches the reversal potential and the driving force (E_m–E_{rev}) for Ca^{2+} currents diminishes (Fig. 4.4). The balance between channel activation and reduced driving force results in the typical bell-shaped $I_{Ca,L}$ current-voltage relationship. In order to illustrate the channel activation independent of the driving force, the conductance of the channel, i.e., the LTCC-mediated current divided by the driving force, is plotted versus the membrane potential. This results in a typical sigmoidal activation curve with $V_{1/2}$ of activation typically seen between −10 and −15 mV (Fig. 4.5) (Mangoni et al. 2006).

4.4.2 Inactivation of Ca^{2+} Channels

Following sustained depolarization, $I_{Ca,L}$ undergoes a typical decay which depends on time, voltage, and intracellular Ca^{2+} (Bers and Perez-Reyes 1999). The contribution of voltage-dependent inactivation and intracellular Ca^{2+}-dependent inactivation (VDI and CDI, respectively) can be determined experimentally by replacing Ca^{2+} as a charge carrier with Ba^{2+} or monovalent ions. In absence of divalent cations, the LTCC-mediated current shows very slow inactivation, which under these conditions is largely due to voltage-dependent inactivation and from which the name of the channel (L-type) was derived. Using Ba^{2+} as a charge carrier results in a faster inactivation of the current, although the Ba^{2+}-mediated current inactivation is still much slower than in the presence of Ca^{2+} as a charge carrier (Bers and Perez-Reyes 1999; Bers 2008; Catterall 2011).

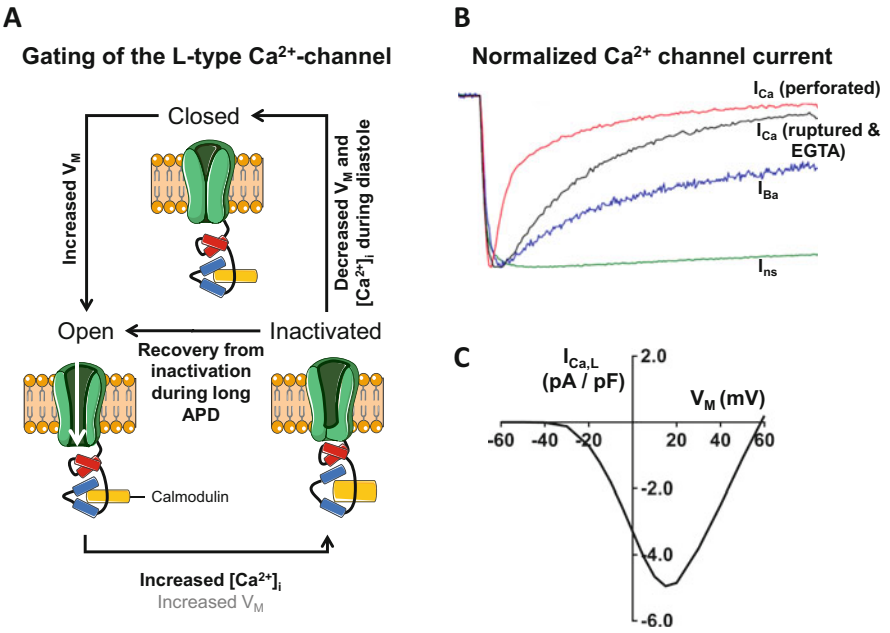

Fig. 4.4 L-type Ca^{2+} current activation and inactivation. (**a**) Schematic illustration of three gating states of the L-type Ca^{2+} channel (LTCC). Following depolarization from the resting membrane potential, the channel is activated within 5–7 ms followed by inactivation of the current despite sustained depolarization. (**b**) LTCC inactivation with Ca^{2+}, Ba^{2+}, or monovalent cations (ns) as the charge carrier. Currents were measured at 0 mV except for I_{ns} at −30 mV to obtain comparable activation, and peak currents were normalized. I_{Ca} with sarcoplasmic reticulum (SR) Ca^{2+} release was recorded using the perforated patch-clamp technique and 2 mM external Ca^{2+}. I_{Ca} with no SR Ca^{2+} release was recorded in the whole-cell configuration with 10 mM EGTA in the pipette. I_{Ba} was recorded in the whole-cell configuration with 2 mM external Ba^{2+} and 10 mM EGTA in the pipette. I_{ns} was measured in divalent cation-free conditions. Reprinted with kind permission from Bers (2001). (**c**) Current-voltage relationship of canine ventricular L-type Ca^{2+} current modeled according to Heijman et al. (2011)

CDI of LTCC represents a feedback control mechanism to prevent extensive Ca^{2+} overload by increasing LTCC inactivation, thereby decreasing Ca^{2+} influx, in the presence of high intracellular Ca^{2+}. Interestingly, buffering intracellular Ca^{2+} transients with ethylene glycol tetra-acetic acid (EGTA) slowed CDI of LTCC-mediated current but did not completely abolish it. These data indicate that both Ca^{2+} entering the cell through LTCC, which is not buffered by the relatively slow buffering properties of EGTA, and the Ca^{2+} released from the SR during systolic Ca^{2+} transients contribute to the LTCC inactivation (Richard et al. 2006). Calmodulin (CaM) attached to the C-terminus of the LTCC α-subunit has been identified as the Ca^{2+} sensor for CDI (Qin et al. 1999; Sanchez-Alonso et al. 2016). In particular, Ca^{2+} binding to CaM leads to a conformational change allowing an intracellular loop of the α-subunit to interact with the channel pore and induce inactivation.

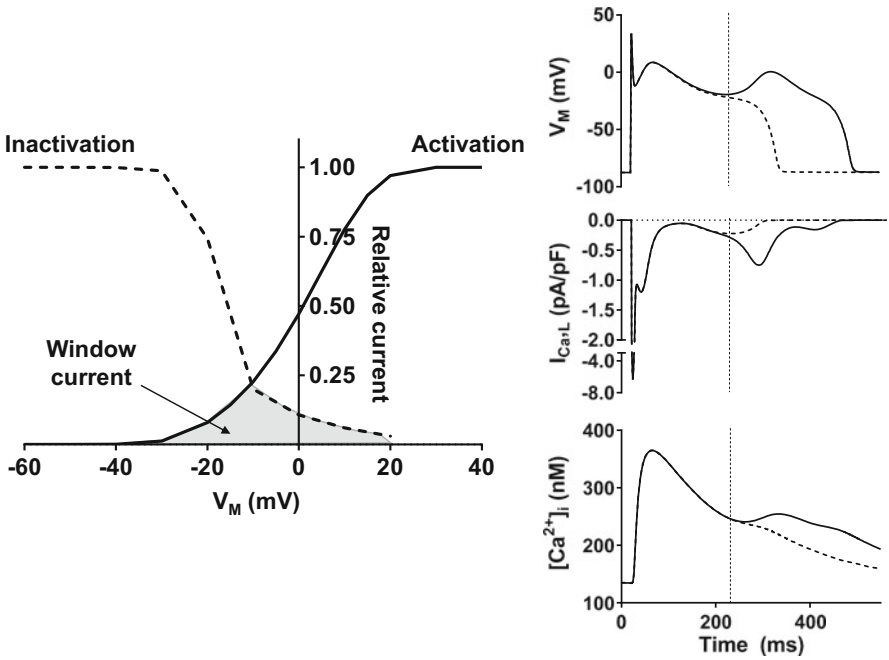

Fig. 4.5 L-type Ca^{2+} current may contribute to the development of early afterdepolarizations. (**a**) Steady-state activation and inactivation curves of L-type Ca^{2+} current modeled according to Heijman et al. (2011). Overlap of both curves (indicated in gray) illustrates the presence of a window current, which may contribute to the generation of early afterdepolarizations (**b**, EADs)

The channel availability during a depolarizing pulse depends on the preceding holding potential and follows a sigmoidal relationship with $V_{1/2}$ of inactivation between −35 mV and −45 mV, depending on the molecular channel composition, which varies from species to species (Fig. 4.5) (Yuan et al. 1996; Benitah et al. 2010). Upon repolarization from a depolarizing voltage pulse, the LTCC recover from voltage-dependent inactivation, thereby increasing the availability of the channel. The time course of recovery from inactivation is generally assessed using a paired pulse protocol with variable inter-pulse duration and in large mammals has a time constant between 20 and 70 ms (Fulop et al. 2004; Heijman et al. 2011; Li et al. 2000). If there is insufficient time for complete recovery between consecutive depolarizing pulses during fast rates, LTCC can accumulate in the inactivated state, resulting in a rate-dependent reduction of $I_{Ca,L}$ (Li et al. 2000).

4.5 Phosphorylation-Dependent Regulation of LTCC

The marked upregulation in response to cAMP-dependent protein phosphorylation pathways is a major characteristic of $I_{Ca,L}$ that contributes significantly to the positive inotropic effect of β-adrenergic receptor (β-AR) stimulation (Catterall

2011; Rose and Backx 2014; Heijman et al. 2011). Moreover, given the central role of Ca^{2+} influx through LTCC in cardiomyocyte function, it is not surprising that LTCC are regulated by multiple local signal transduction cascades. Most of them involve phosphorylation of the LTCC complex by protein kinases and dephosphorylation by protein phosphatases (Hofmann et al. 2014; Treinys and Jurevicius 2008).

4.5.1 β-Adrenergic Signaling and cAMP-Activated Protein Kinase A

It is well known that stimulation of the sympathetic nervous system leads to an increase in heart rate and cardiac contractility as part of the so-called "fight or flight" response (Ripplinger et al. 2016; Reuter 1974). This is mainly mediated by the release of catecholamines, which stimulate G_s-protein-coupled β-AR, leading to increased cAMP levels and activation of cAMP-dependent protein kinase A (PKA). Cardiac LTCC are a major target for PKA-mediated phosphorylation, and it was shown already in the 1970s that PKA-mediated phosphorylation of LTCC results in increased $I_{Ca,L}$ amplitude, prolonging the AP plateau and increasing contractility (Reuter 1974). Within the following years, several possible phosphorylation sites at the Cav1.2 α-subunit (Ser1627, Ser1700, Thr1704, Ser1928) or β-subunit (Ser143, Ser459, Ser165, Ser478, Ser479) have been proposed, mainly based on data obtained in expression systems (Hofmann et al. 2014). However, in vivo experiments with mice in which these sites were muted or truncated revealed mostly unaltered fight-or-flight reactions (Brandmayr et al. 2012). In agreement, recent work has shown that Cav1.2 activity in the brain, but not in the heart, requires phosphorylation of Ser1928, suggesting that there are tissue-specific differences in Cav1.2 regulation (Qian et al. 2017). The exact PKA-phosphorylation sites responsible for the PKA-mediated $I_{Ca,L}$ regulation in the heart are still under debate (Hofmann et al. 2014).

The PP2A catalytic subunit can bind directly to the LTCC α_{1C} subunit, and channel-bound PP2A plays an important role in the inhibition of $I_{Ca,L}$, although other mechanisms of PP2A/LTCC interaction have also been described (Heijman et al. 2017). PKA is targeted to LTCCs by A-kinase anchoring proteins (AKAPs) (Wong and Scott 2004). AKAP15 interacts with the C-terminus of $Ca_v1.2$ and targets PKA near the phosphorylation site Ser1928 (Gray et al. 1997; Hulme et al. 2002). AKAPs are involved in the organization of LTCC in distinct subcellular compartments with specific molecular compositions. Examples of such LTCC subpopulations include those found in t-tubules and those in caveolar plasma membrane domains outside of t-tubules (Fig. 4.2) (Balijepalli et al. 2006; Best and Kamp 2012). This spatial organization allows precise local regulation of $I_{Ca,L}$ (Sanchez-Alonso et al. 2016). LTCC complexes within t-tubules include β_1-AR, adenylyl cyclase, and PKA, whereas caveolar LTCC complexes are composed of β_2-AR, adenylyl cyclases, PKA, PP2A, and caveolin 3 (Balijepalli et al. 2006; Best and Kamp 2012; Rose and Backx 2014). The differential compartmentalization of $Ca_v1.2$ channels results in different regulation, involving stimulation by β_1-AR in the t-tubule or β_2-AR in the caveolae (Nikolaev et al. 2010).

To ensure this compartment-specific regulation of $I_{Ca,L}$ by β_1-AR or β_2-AR, local cAMP compartmentation is required. An important mechanism that limits cAMP diffusion between cellular compartments is the cAMP degradation by PDEs (Mika et al. 2012). There are 11 families of PDEs, 4 of which are classically responsible for cAMP degradation in the heart: PDE1, PDE2, PDE3, and PDE4 (Osadchii et al. 2005). PDE2–4 contribute to the regulation of $I_{Ca,L}$ in atria and ventricle (Mehel et al. 2013; Molina et al. 2012; Rivet-Bastide et al. 1997; Vandecasteele et al. 2001).

4.5.2 Ca^{2+}/Calmodulin-Dependent Protein Kinase (CaMKII)

CaMKII-dependent phosphorylation of the LTCC has been linked to an increase of LTCC activity in response to increased stimulation frequencies, often referred to as "facilitation" (Richard et al. 2006). "Facilitation" reflects a Ca^{2+}-dependent increase of $I_{Ca,L}$ amplitude and slowing of $I_{Ca,L}$ inactivation, which does not occur with other divalent cations such as Ba^{2+} as charge carrier. Facilitation occurs likely as a result of CaMKII-mediated phosphorylation (Lee 1987; Lee et al. 2006). However, the physiological role of this process is not entirely clear. It may counterbalance the direct Ca^{2+}-dependent inactivation described above.

4.5.3 G_q-Protein-Coupled Receptors and Activation of Protein Kinase C

Protein kinase C (PKC) activation is a major step in the signaling cascade activated by G_q-coupled receptors such as α_1- and α_2-AR, endothelin, angiotensin II, and muscarinic receptors. Although there is evidence for PKC-dependent phosphorylation of $Ca_v1.2$ and Cavβ2, the consequences of PKC activation on $I_{Ca,L}$ remain controversial (Kamp and Hell 2000; Shistik et al. 1998). PKC-dependent activation can increase (Alden et al. 2002) or decrease (Yue et al. 2004) $I_{Ca,L}$ or can even have biphasic effects (Weiss et al. 2004). For a detailed discussion, we refer the interested reader to Benitah et al. (2010).

4.5.4 Nitric-Oxide Dependent Signaling and Activation of Protein Kinase G

Results about the effects of protein kinase G (PKG)-mediated LTCC phosphorylation on $I_{Ca,L}$ are discrepant and may depend on cell type and experimental conditions (in vivo vs. in vitro). PKG activation occurs in response to NO-dependent stimulation of guanylate cyclase and the resulting increase of cyclic guanosine monophosphate (cGMP) levels (Tamargo et al. 2010; Hare 2003). The rise in cGMP may influence $I_{Ca,L}$ not only by direct PKG-mediated phosphorylation but also by cGMP-dependent activation (PDE2) or inhibition (PDE3) of PDEs that

control cAMP (Vandecasteele et al. 2001). cGMP-dependent signaling may thereby modulate the LTCC response to β-AR stimulation (Abi-Gerges et al. 2001, 2002). Whether cGMP-dependent signaling facilitates or inhibits cAMP-signaling seems to depend on tissue and species (Han et al. 1994, 1996; Wang et al. 2000; Kirstein et al. 1995; Martynyuk et al. 1997).

Furthermore, NO may regulate $I_{Ca,L}$ activity in a cGMP-independent manner. The α_{1C}-subunit of the LTCC (Cav1.2) contains more than 10 cysteine residues that may be nitrosylated and involved in LTCC regulation. Nitrosylation of LTCC has been suggested to inhibit $I_{Ca,L}$ in atrial and ventricular myocytes (Sun et al. 2006; Carnes et al. 2007; Rozmaritsa et al. 2014). Interestingly, S-nitrosylation increases during ischemia reperfusion and AF, thereby contributing to a reduction in $I_{Ca,L}$, SR-Ca^{2+} load, and Ca^{2+}-induced cardiac injury, which represents an important cardioprotective mechanism (Tamargo et al. 2010).

4.5.5 Adenosine Monophosphate-Activated Protein Kinase

Adenosine monophosphate-activated protein kinase (AMPK), a serine/threonine kinase, is activated in response to increased AMP/ATP ratios and represents a sensor of cellular energy status. AMPK has been recently shown to physically associate with $Ca_v1.2$ and regulate $I_{Ca,L}$. This may contribute to adaptation of cellular activity to the energy metabolism (Harada et al. 2012b, 2015).

4.6 The Role of L-Type Ca^{2+} Currents in Cardiac Pathophysiology

Dysregulation of LTCC and TTCC at the transcriptional, translational, and post-translational level plays an important role in a wide variety of diseases, as recently summarized in a number of reviews (Venetucci et al. 2012; Heijman et al. 2014; Adams and Snutch 2007; Bartos et al. 2015). Here, we summarize LTCC dysregulation [since TTCC are absent from working myocardium in humans (Catterall 2011; Ono and Iijima 2010)] in two major cardiovascular pathologies: atrial fibrillation and heart failure.

In patients with long-standing persistent (chronic) atrial fibrillation, $I_{Ca,L}$ is significantly reduced, contributing to the proarrhythmic AP shortening and the reduction in Ca^{2+}-transient amplitude, which plays an important role in the stroke-promoting atrial contractile dysfunction (Voigt et al. 2012b, 2014). The molecular mechanisms underlying the reduced $I_{Ca,L}$ are diverse and can involve reduced expression of Cav1.2, increased microRNA-328-mediated reduction in Cav1.2 protein levels, or altered S-nitrosylation, phosphorylation, or calpain-mediated degradation of LTCC (Heijman et al. 2014). In addition, dysregulation of $Cav\beta_2$ expression (Ling et al. 2017), reducing trafficking of LTCC to the plasma membrane, may contribute. In contrast to chronic atrial fibrillation, $I_{Ca,L}$ is not reduced in patients with paroxysmal atrial fibrillation that were in normal sinus rhythm when

the tissue was obtained (Voigt et al. 2014), suggesting that the reduction in $I_{Ca,L}$ is a protective mechanism to reduce Ca^{2+} entry in response to a persistent high activation rate. Consistent with this notion, rapid pacing of atrial cardiomyocytes results in reduction of $I_{Ca,L}$ within hours (Qi et al. 2008).

Data on $I_{Ca,L}$ in heart failure are variable, with some studies reporting a decrease in $I_{Ca,L}$, whereas most others found no differences in $I_{Ca,L}$ between human ventricular cardiomyocytes from failing and non-failing hearts (Richard et al. 1998; Bartos et al. 2015; Nattel et al. 2007). Similarly, the mRNA level of Cav1.2 was either unchanged (Ambrosi et al. 2013) or increased (Soltysinska et al. 2009) in end-stage heart failure patients compared to non-failing controls. Some studies suggest a blunted response of $I_{Ca,L}$ to β-adrenergic stimulation in heart failure, partly due to increased basal phosphorylation (Bartos et al. 2015; Nattel et al. 2007). However, little is known about alterations in the composition of the LTCC macromolecular complex in heart failure.

Activation and inactivation curves of $I_{Ca,L}$ are overlapping resulting in a "window current," a voltage range, which enables a persistent $I_{Ca,L}$ through LTCC that are activated but do not completely inactivate. If AP duration is prolonged, for example, in heart failure, LTCC will spend more time in this voltage range, allowing $I_{Ca,L}$ to recover from inactivation and become conducting again (Fig. 4.5). Under these conditions, the increase in $I_{Ca,L}$ may induce an abnormal membrane depolarization. These so-called early afterdepolarizations may trigger Torsades de Pointes (TdP) arrhythmias at the tissue scale, which are an important cause for sudden cardiac death in patients with heart failure and long QT syndromes (Weiss et al. 2010).

Long QT syndrome type-8 (also known as Timothy syndrome, TS) has been attributed to gain-of-function mutations such as G406R, G402R, and A1473G that lead to the disruption of the LTCC inactivation (Betzenhauser et al. 2015; Napolitano and Antzelevitch 2011). In addition to marked QT prolongation at birth, intrauterine bradycardia and AV conduction block are characteristic hallmarks of TS. The characteristic extra-cardiac phenotype including dysmorphic facial features, syndactyly, and autism often leads to the diagnosis. The occurrence of ventricular tachycardia is the major cause for the limited life expectancy with average survival of 2–3 years. On the other hand, loss-of-function mutations in the α_1, β_2, and $\alpha_2\delta$ subunits of the LTCC have been found to be associated with early repolarization syndrome and Brugada syndrome. The loss of function may thereby result from changes in gating, permeation, or trafficking. A more detailed overview on Ca^{2+} channel mutations and their role in cardiac arrhythmia syndromes is beyond the scope of this chapter and therefore discussed elsewhere in this book and in excellent recent reviews (Betzenhauser et al. 2015; Napolitano and Antzelevitch 2011).

4.7 Conclusions

In cardiomyocytes LTCC-mediated Ca^{2+} currents are the major route for Ca^{2+} entry. Most importantly, $I_{Ca,L}$ is a major player in cardiac cellular electrophysiology and in cellular EC coupling (Bers 2002, 2008). In addition, Ca^{2+} entry through LTCC is an

important regulator of gene transcription and cardiac cellular metabolism (Makary et al. 2011; Wakili et al. 2011). In order to fulfil these tasks reliably, LTCC are highly regulated by specific subunit compositions and various signaling pathways (Benitah et al. 2010; Catterall 2000, 2011; Kohlhaas et al. 2017). According to their diverse role in several important physiological mechanisms, it is not surprising that abnormalities in LTCC activity contribute to the pathophysiology of many common heart diseases such as atrial fibrillation, ischemia reperfusion injury, and heart failure (as discussed elsewhere in this book). Furthermore, in channelopathies affecting LTCC, both loss-of-function and gain-of-function mutations lead to rare arrhythmia syndromes, which are discussed more extensively elsewhere in this book.

Compliance with Ethical Standards

Funding N.V. is supported by the DZHK, by the German Research Foundation (DFG VO 1568/3-1, IRTG1816 RP12, SFB1002 TPA13) and by the Else-Kröner-Fresenius Stiftung (EKFS 2016_A20). J.H. is supported by the Netherlands Organization for Scientific Research (NWO/ZonMw Veni 91616057).

Conflict of Interest Jordi Heijman declares that he has no conflict of interest. Cristina E. Molina declares that she has no conflict of interest. Niels Voigt declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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