Sydney Ringer would be overwhelmed today by the implications of his simple experiment performed over 120 years ago showing that the heart would not beat in the absence of Ca$^{2+}$. Fascination with the role of Ca$^{2+}$ has proliferated into all aspects of our understanding of normal cardiac function and the progression of heart disease, including induction of cardiac hypertrophy, heart failure, and sudden death. This review examines the role of Ca$^{2+}$ and the L-type voltage-dependent Ca$^{2+}$ channels in cardiac disease.
The L-type calcium channel in the heart: the beat goes on

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Sydney Ringer would be overwhelmed today by the implications of his simple experiment performed over 120 years ago showing that the heart would not beat in the absence of Ca²⁺. Fascination with the role of Ca²⁺ has proliferated into all aspects of our understanding of normal cardiac function and the progression of heart disease, including induction of cardiomyopathy, heart failure, and sudden death. This review examines the role of Ca²⁺ and the L-type voltage-dependent Ca²⁺ channels in cardiac disease.

When Sydney Ringer (1) discovered the vital role of Ca²⁺ in the heart, investigations took a leap forward and have continued unabated (2). Austrian scientist Otto Loewi, best known for his work on autonomic transmitters and discovery of “chemical vagusstoff,” recognized the connection between digitalis and Ca²⁺ in 1917–1918. Although he always believed that Ca²⁺ was the key to understanding life’s processes, the Nobel Prize in Physiology and Medicine was awarded to Loewi and Sir Henry Hallett Dale in 1936 for their studies on neurotransmitters.

Ca²⁺ is the link in excitation-contraction (EC) coupling (Figure 1), which starts during the upstroke of the action potential (AP) and causes the opening of the L-type voltage-dependent Ca²⁺ channel (L-VDCC). Interest in high-voltage–activated L-VDCCs began with biochemical and continued with molecular characterizations, culminating in the cloning of the pore-forming α₁ subunit and the auxiliary channel subunit α₃/β in rabbit skeletal muscle (3–5). Although the L-VDCC subunits are most abundant in fast skeletal transverse tubules, Ca²⁺ influx is not required for contraction in skeletal muscle, unlike cardiac muscle, which requires Ca²⁺ entry with each beat and triggers Ca²⁺ release from the sarcoplasmic reticulum (SR) via Ca²⁺-release channels, e.g., ryanodine receptor 2 (RyR2). This amplifying process, termed Ca²⁺-induced Ca²⁺ release (CICR) by A. Fabian-to, causes a rapid increase in intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) from ~100 nM to ~1 μM to a level required for optimal binding of Ca²⁺ to troponin C and induction of contraction (2).

There is a close correlation between activation of the L-type Ca²⁺ current (I₅ᵥ) and cardiac contraction. Contraction is followed by Ca²⁺ release from troponin C and its reuptake by the SR via activation of the SR Ca²⁺-ATPase 2a (SERCA2a) Ca²⁺ pump in addition to extrusion across the sarcolemma via the Na⁺/Ca²⁺ exchanger (NCX). In the human heart under resting conditions, the time required for cardiac myocyte depolarization, Ca²⁺-induced Ca²⁺ release, contraction, relaxation, and recovery is 600 ms. This process occurs approximately 70 times a minute or over 2 billion times in the average lifespan. Ca²⁺ is also required for maintenance of cell integrity and gene expression (6) relevant to the growth and development of the embryonic heart (7). L-VDCCs are regulated by the adrenergic nervous system and may interact with G protein–coupled receptors (8).

Cardiac L-VDCC structure

The L-VDCCs are heterotetrameric polypeptide complexes comprising the α₁, α₃/β, β, and, in some tissues, γ subunits (Figure 2) that allow depolarization-induced calcium influx into the cytosol. In all excitable tissues, Ca²⁺ channels invariably contain α₁, α₃/β, and β subunits. These are considered the functional minimum core for Ca²⁺ channel assembly. The accessory subunits (β, α₃/β) are tightly but not covalently bound to the α₁ subunit and modulate the biophysical properties and trafficking of the α₁ subunit to the membrane.

α₁ subunits

The Ca²⁺ channel α₁ subunit (170–240 kDa) consists of 4 homologous motifs (I–IV), each composed of 6 membrane-spanning α-helices (termed S1 to S6) linked by variable cytoplasmic loops (linkers) between the S5 and S6 segments (Figure 2). To date, 10 α₁ subunit genes have been identified and separated into 4 classes: Ca₆.1 (α₁a, 1.2 (α₁c), 1.3 (α₁d), and 1.4 (α₁e)). Only the α₁c (dihydropyridine-sensitive [DHP-sensitive]) subunit is expressed in high levels in cardiac muscle. Ca₆.2.1 (α₁s), 2.2 (α₁b), and 2.3 (α₁o) form P/Q-, N-, and possibly R-type channels, respectively, and are all found in brain. They are primarily responsible for initiation of synaptic transmission at fast synapses in the nervous system. They have a larger intracellular loop connecting domains II and III, which contains a synaptic protein interaction site that binds SNAP proteins involved in exocytosis (9). Ca₆.3.1 (α₁h), 3.2 (α₁i), and 3.3 (α₁j) form T-type channels that are localized to the brain, kidney, and heart and were originally called low-voltage-activated channels. Unlike L-type channels, they are relatively insensitive to DHPs. Ca₃ channels conduct T-type Ca²⁺ currents, which are important in a wide variety of physiological functions, including neuronal firing, hormone secretion, smooth muscle contraction, cell proliferation of some cardiac tissue, and myoblast fusion. In the

Nonstandard abbreviations used: ACE, angiotensin-converting enzyme; AF, atrial fibrillation; AID, atrioventricular node dysfunction; AP, action potential; β-AR, β-adrenergic receptor; BID, β-interaction domain; BTZ, benzothiazepine; [Ca²⁺]ᵢ, intracellular Ca²⁺ concentration; CaM, calmodulin; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; CCB, Ca²⁺ channel–blocker; CDI, Ca²⁺-dependent inactivation; CREB, cAMP-responsive element–binding protein; DHP, dihydropyridine; EC, excitation-contraction; IC₆₅, Ca²⁺ current; IC₆₇, L-type Ca²⁺ current; IQ, i-isocitrate-glutamate; L-VDCC, L-type voltage-dependent Ca²⁺ channel; NCX, Na⁺/Ca²⁺ exchanger; PAA, phenylalkylamine; PKA, protein kinase A; RyR2, ryanodine receptor 2 (cardiac); SH3-GK, Src homology 3-granule kinase; SR, sarcoplasmic reticulum; VDCC, voltage-dependent Ca²⁺ channel; VDI, voltage-dependent inactivation; VF, ventricular fibrillation.

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heart, T-type channels are abundant in sinoatrial pacemaker cells and Purkinje fibers of many species and are important for maintenance of pacemaker activity by setting the frequency of AP firing. T-type channels have been shown to be reexpressed in the ventricles of some animal models of heart failure, suggesting that T-type channels play a role in cardiac disease. The neuronal T-type channels can generate low-threshold spikes that lead to firing bursts and oscillations that are prominent in the thalamus and implicated in a variety of neurological disorders (10). In addition to these well characterized Ca²⁺ channels, the cloning of a single Ca₄-like protein in 1999 suggested the existence of a fourth subfamily (11). This novel protein contains conserved amino acids found in Ca²⁺ (EEEE) or Na⁺ channels (DEKA), including EEKE residues in the corresponding

**Figure 1**
A model illustrating the Ca²⁺ signaling pathways implicated in hypertrophy and heart failure. Stimulation of the β₁-AR activates G_s (stimulatory G proteins), which activates adenylyl cyclase (AC), causing production of cAMP. This stimulates cAMP-dependent PKA, which phosphorylates (P) and alters the function of numerous substrates important for SR Ca²⁺ regulation, including the L-VDCC, RyR2, and phospholamban (PLN). β₂-ARs couple G_s/Ras/MEK1/2/ERK1/2 pathways to hypertrophy (G_i, inhibitory G protein; MEK1/2, mitogen-activated protein kinase). Subsequently, the activated β₁-AR is desensitized when it is phosphorylated by β₁-AR kinase-1 (β₁ARK1). During hypertrophy, β₁-AR expression increases. In heart failure, while the levels of PLN protein expression remain unchanged (or decreased), the phosphorylation status at Ser16 and Thr17 is decreased, even though the levels of SR Ca²⁺-ATPase 2a (SERCA2a) are decreased. Cardiac SR-associated protein phosphatase-1 (PP-1) removes phosphate at Ser16 in PLN and is upregulated in heart failure. Calstabin2 (FKB12.6) plays a role in stabilizing RyR2 in order to help maintain the channel in a closed state during diastole. RyR2 is hyperphosphorylated in heart failure, and calstabin2 dissociates from RyR2. Elevated NCX is an adaptive change in heart failure that becomes maladaptive and may be responsible for both arrhythmogenesis and contractile dysfunction. PKC-α expression and activity are elevated in heart failure. Calcineurin (CN) is activated by sustained elevation of [Ca²⁺]. It dephosphorylates nuclear factor of activated T cells (NFAT), enabling its translocation to the nucleus, which is sufficient to induce hypertrophy. Hypertrophic stimuli, such as α₁-adrenergic agonists, Ang II, and endothelin-1 (ET-1), all elevate [Ca²⁺] and activate the CN-NFAT, Ca²⁺/CaM-CaMKII, and PKC-MAPK-NFAT signaling systems through G protein–coupled receptors (GPCRs) and PLC-DAG-IP₃-dependent Ca²⁺ release [PLC, phospholipase C; DAG, diacylglycerol, IP₃, inositol (1,4,5)-trisphosphate]. Transcription factors, such as myocyte-enhancer factor 2 (MEF2) and GATA4 (cardiac zinc finger transcription factor) are located in the nucleus and serve as endpoints for hypertrophic-signaling pathways. AT₁, type 1 angiotensin II receptors; G_i, βγ subunit of the activated Gi-binding protein; G_s, heterotrimeric GTP-binding protein, consisting of G_s and G_i2, which dissociate upon receptor activation; NHE, Na⁺/H⁺ exchanger, regulates cytosolic pH; PIP₂, phosphatidylinositol 4,5-biphosphate; T tubule, transverse tubule.

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review
The α1 subunit harbors the ion-selective pore, voltage sensor, gating machinery, and the binding sites for channel-modulating drugs (2, 5, 12) and is autoregulatory. The pore is asymmetric, with conserved glutamate residue (EEEE) (or aspartate residue, in some positions in low voltage–activated channels) comprising the ion-selectivity filter(s) (13–16), located between segments S5 and S6 of each motif (Figure 2). Cloning and analysis of the VDCC have revealed that the positively charged fourth transmembrane segment (S4) of each motif is highly conserved and is likely to form an α-helix in which every third or fourth residue is basic (Arg or Lys). It is thought that the S4 α-helices traverse the membrane electric field and, in response to a depolarizing stimulus, move outward into the extracellular space, initiating conformational changes from nonconducting to conducting states of the channel (sliding-helix model) (reviewed in ref. 17). However, the emerging models are still controversial. Most structure-function studies support a spiral or rotational motion of the S4 or S3 plus S4 α-helices through the channel protein in order to move gating charges across the membrane electric field. In contrast, Jiang et al. (18), on the basis of the crystal structure of the Aeropyrum pernix K+ channel, proposed a model involving positively charged S4 α-helices.
charged “voltage-sensor paddles” (each S4 segment forms half of a voltage-sensor paddle), which are located near the intracellular membrane surface at negative resting membrane potentials. In response to depolarization, the voltage-sensor paddles move through the lipid membrane bilayer to their external position, opening the pore by pulling on the S4–S5 linker. According to this model, the charged residues of S4 are not exposed to the intracellular solution at all, based on the finding that the S3B–S4 loop is not accessible to site-specific Fab fragments. However, this model does not explain how the voltage-sensor paddle of an ion channel operates in transporting positive charges across the lipid membrane. Starace and Bezanilla argued against this model (19), pointing out several discrepancies. They suggested a “proton pore” model in a K+ channel voltage sensor, demonstrating that replacement of the first S4 arginine by histidine in the Shaker K+ channel creates a proton pore when the cell is hyperpolarized. Proton transport occurs when depolarization of the membrane moves histidine residues coupled to the voltage sensor from the internal to external phase. Their model, in contrast to Jiang’s model, shows that a small conformational change can transfer charges across the focused transmembrane field. Compelling experimental evidence from both models put the readers in a position to decide which model is favorable.

**Accessory subunits**

### αδ subunits

The αδ subunits are closely associated with the α1 subunit by surface interaction and are intracellularly linked through a disulfide bridge to a small protein, the δ subunit. The α2 subunit is entirely extracellular, and the δ subunit has a single transmembrane region with a very short intracellular part. The α2 and δ subunits are encoded by the same gene, which is separated by proteolytic cleavage (20). Ellis et al. (3) first cloned the α2/δ subunit from rabbit skeletal muscle, thinking there was only one product from the gene. Presently, at least 4 isoforms encoded by separate genes have been identified (α2/δ1, 2, 3, 4) (20–22).

The issue of in vivo structure-function has yet to be resolved. In heterologous expression systems, coexpression of the α2/δ subunit affects α1 function by increasing channel density, charge movement, and $b_{max}$ of drug binding (e.g., the DHP isradipine) with smaller effects on $K_p$ and variable minor effects on channel kinetics (refs. 4, 23, 24, and references therein). It is probable that the α2/δ and β subunits “drive” the α1 subunit to the membrane in the correct insertion mode. The α2/δ1 subunit is ubiquitously distributed and possesses a stereo-selective high-affinity binding site for certain GABA-antagonists, such as the drug gabapentin, which is widely used to treat epilepsy, pain, sleep disorders, and many other paroxysmal neurological conditions (25–27). The α2/δ2 subunit also binds gabapentin but at low affinity while α2/δ3 and α2/δ4 do not bind this drug. Mice deficient in α2/δ2 exhibit neurological dysfunction, such as enhanced seizure susceptibility and cardiac abnormalities, namely a tendency to develop bradycardia (28). The recently cloned human α2/δ1 subunit (21) is localized to fetal liver, colon, pituitary, and adrenal gland and is associated with the α1C subunit (Ca. 1.2) and the β3 subunit. This reinforces the complexity of L-VDCCs since subunit association appears to confer biophysical properties (13, 23). This rich diversity opens avenues for exciting physiological and pathological discoveries (29).

### The γ subunits

It was originally thought that the γ subunit was the product of a single gene and only existed in skeletal muscle (22). It is interesting that characterization of a genetic defect that induces epileptic seizures in stargazer mice (30) led to the detection of a family of at least 5 novel isoforms of the γ subunit that are almost exclusively expressed in the brain. To date, 8 genes encoding a variety of γ subunit isoforms have been identified (31). Although the γ1 subunit is associated specifically with skeletal muscle Ca1.1 channels, there is evidence that the γ2 subunit interacts with α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor subunits (30) and possibly other membrane-signaling proteins. Unlike other auxiliary subunits (β and αδ), the γ subunits do not have a significant role in the membrane trafficking of the Ca2+ channels. The γ1 subunit modulates the biophysical properties of the Ca2+ channel. Clearly, this is a very important Ca2+ channel subunit, but since it does not appear to be expressed in heart, it is not discussed further here.

### β subunit structure

Four β subunit isoforms (β1–β4) have been described. All are hydrophilic, nonglycosylated, and located within the cell; only β1 has been reproducibly shown to form cardiac L-VDCCs. The β subunit does not have a membrane-spanning region. It is tightly bound to a highly conserved motif in the cytoplasmic linker between repeats I and II of all cloned high voltage–activated α1 subunit isoforms, called the α-interaction domain (AID) (32, 33), and also to a secondary site (34). Just recently, elucidation of the high-resolution 3D structure (34–36) of a β subunit has shed light on the molecular mechanism of binding of the α1 to β subunits. Previous work suggested that the β subunit interacts with α1 primarily through the β-interaction domain (BID), which binds directly to the AID. However, Van Petegem and colleagues (35) reported that the BID engages the AID through a conserved hydrophobic cleft, termed the α-binding pocket (ABP). Interference with AID-ABP binding might provide a novel way to modulate Ca2+ channel function in pathological states. The I–II loop of the α1 subunit contains an endoplasmic reticulum retention signal that restricts cell surface expression. The β subunit reverses the inhibition imposed by the retention signal (37). Recent structural modeling and X-ray crystallography revealed that the β subunits share homology with the Src homology 3-guanylate kinase (SH3-GK) module of the membrane-associated guanylate kinase family of scaffolding proteins (38). Structural and biochemical characterization of this SH3-GK module of the β subunit by McGee et al. (39) demonstrated that the functional β subunit requires intramolecular and intermolecular interaction between the SH3 and GK domains. This hypothesis was elaborated by introducing mutations in the rat β2a subunit. The interaction between the α1A and β2a subunit was disrupted and, as a consequence, the inactivation kinetics of the Ca2+ channel currents were altered. After coexpression of β2a subunits with complementary mutations in their SH3 and GK domains, these deficits were functionally rescued through intramolecular β subunit assembly. This model indicates that the BID includes elements from both the β-SH3 and β-GK domains.

### The roles of the β auxiliary subunits in influencing channel function

Though much knowledge has been accumulated about the multiple roles of the β subunit in the processing and functioning...
of the L-VDCC using heterologous recombinant coexpression of the different subunits (23, 40), the physiological significance of subunit interaction in the context of native tissue is only just beginning to be studied via transgenic approaches and adenovirus-mediated intracellular incorporation of genes encoding Ca\textsuperscript{2+} channel β subunits into single cells (5, 41, 42). In general, coexpression of β subunits modulates the biophysical properties of the L-VDCC α1 subunit, producing a leftward shift of the current-voltage relationship, which is consistent with the involvement of the S4 region of the α1 subunit voltage-sensor region. However, the precise interpretation of the data is not straightforward due to the fact that oocytes and most mammalian cells express endogenous α1, α2/3, and β subunits (40). Moreover, it has been shown that α1 subunits expressed in the absence of β subunits are not regulated by the β-adrenergic system (43) or by pH changes (44). Frequency- and prepulse-dependent facilitation of L-VDCC activity is regulated by certain classes of β subunits (45). By employing an antisense strategy to lower the numbers of endogenous β subunits in oocytes, leaving only α1 subunits intact, a loss of current occurred, implying that the β subunit functions in the assembly and expression of the α1 subunit (46). Our group provided evidence that the β subunits have a chaperone-like role in trafficking α1C subunits from the ER to the plasma membrane, and its insertion in the proper geometry (47). Recently, Kobilinsky et al. (48) identified, within the C terminal, a 153-aa sequence in the human cardiac short β2 and β2g subunits that was essential for modulating Ca\textsuperscript{2+} channel function and interaction with the α1C subunit. These 2 functional, short β2 subunit splice variants lack the protein kinase A (PKA) phosphorylation site and the BID (32) as well as part of the β-SH3-GK domain and helix α1. Viard et al. (49) demonstrated that a region of this β2a subunit is involved in PI3K-induced increases of Cav1.2 (rat brain) channel density. The PI3K-induced regulation is mediated by phosphatidylinositol 3,4,5-trisphosphate-activated protein kinase B (also called Akt) and requires phosphorylation of β2 subunits on Ser574, which is common to all splice variants of the β2 subunits. These results indicate that PI3K regulates VDCC trafficking to the plasma membrane and may be a general mechanism for the regulation of Ca\textsuperscript{2+} entry in excitable cells (49). The β1 subunit has a crucial role in EC coupling, as proven in β1-KO mice, which suffer from impaired EC coupling and early lethality (50). The exact mechanism for the lack of EC coupling is not known, but it is possible that the deficiency in the assembly process of the α1/β1 complex results in the degradation of the α1 subunit. The role of the β2 subunit in EC coupling is unclear (51). β2-null mice have no detectable abnormalities in the heart (52).

Hullin et al. (53) cloned 2 distinct β subunits, β2 and β3, from rabbit heart and showed an association with the α1 subunit of the L-VDCC. The aa homology of these subunits was similar to that of β1, originally cloned from skeletal muscle (12). Subtypes of the β subunits are revealed frequently, e.g., the 2 splicing products, β2a and β2b, which have been shown to lend more diversity to channel function. It should be noted that β2a, an important subtype of this subunit, is palmitoylated (54, 55). This modification is associated with membrane targeting of nontransmembrane proteins (such as the β subunit) to specific areas including the plasma membrane. Another subtype, the β2s, has been cloned from rabbit brain (53). The role of the β subunits in L-VDCC expression is well characterized although we still do not know how the β subunits modulate preexisting α1 subunit expression. Colecraft et al. (56) devised a novel system in which recombinant adenoviruses were used to express GFP-fused β1.4 subunits in cultured adult rat cardiomyocytes. While all 4 subunits (β1a, β2a, β3, β4) increased L-VDCC density, their effects on inactivation kinetics were nonuniform. The conclusion of this study was that overexpression of the newly cloned rat splice variant of a β2 subunit in adult rat heart cells yielded channels that were identical to those in the native unmodified rat heart cells. The authors stated that this work provided an “experimental paradigm to explore novel function of ion channel subunits in their native environments.” While this system is superior to heterologous systems, it does not provide the whole animal model necessary to explore beyond a single cell.

β3 is most abundant in brain but is also expressed in heart, aorta, trachea, lung, and skeletal muscle. In 2 studies (57, 58), β3-KO models were engineered. In 1 study, (58) a high-salt diet led to hypertension, smooth muscle hypertrophy, cardiac hypertrophy, reduced channel in aortic cells, slowed inactivation rate, and a decreased DHPP-sensitivity. In the other study (57), β3-KO mice showed altered pain processing due to a decrease in the expression of N-type Ca\textsuperscript{2+} channels via functional alterations of Ca\textsuperscript{2+} currents in neurons projecting to the spinal cord. Expression levels of various β subunit isoforms (53, 56, 59) indicate that altered single-channel behavior in human heart may be due to differential effects and changes in β subunit gene products.

A valuable approach to characterizing β subunits in normal and diseased tissues is to compare normal and abnormal tissues from human heart with a mouse model of a similar disease. Although β subunits appear rate limiting for L-VDCC expression (56, 60), our group found that IC\textsubscript{a} density in cardiomyocytes from Tg mice overexpressing the α1C subunit of L-VDCC increased by 30–40% in comparison with WT mice without significant change in IC\textsubscript{a} activation and inactivation kinetics prior to the development of heart failure. However, Western blots revealed a 2.7-fold increase in Ca\textsubscript{a}1.2 protein expression (61, 62). This discrepancy was resolved when it was shown that single-channel gating was impaired in cardiomyocytes of 4-month-old α1C-Tg mice. Immunoblot analysis also showed that auxiliary subunits β2 and α2/3 play important modulator roles, and their expression was decreased. Interestingly, in the older age groups of Tg mice with the heart failure phenotype, the β2 subunit expression pattern is reversed, and we observed a 2-fold increase of protein expression levels (A. Schwartz, unpublished data) compared with that of WT littermates. These data lend credence to the concept that in human heart failure, alterations of auxiliary subunits play a vital role in changing the kinetics of the L-VDCC pore unit (61). We hypothesize that some types of heart failure are possibly L-VDCC β subunit channelopathies.

**Role of calmodulin as a signaling molecule in Ca\textsuperscript{2+}-dependent inactivation and EC coupling**

The cardiac L-VDCC displays long-lasting openings and minor voltage-dependent inactivation (VDI) components. In the heart, Ca\textsuperscript{2+}-dependent inactivation (CDI) is compatible with the length of the Ca\textsuperscript{2+}-mediated plateau phase in the AP. Therefore, Ca\textsuperscript{2+}-induced inhibition of the cardiac VDCC plays a critical role in controlling Ca\textsuperscript{2+} entry and downstream signal transduction as well as ensuring that contraction and relaxation cycles of the heart muscle fiber are coordinated.
Several lines of evidence suggest that the Ca\(^{2+}\)-binding protein calmodulin (CaM) is a critical sensor in mediating the inactivation process. In the C terminal tail of the pore-forming \(\alpha_{1C}\) subunit of the L-VDCC, there is a Ca\(^{2+}\)-dependent CaM-binding isoleucine-glutamine (IQ) motif that has been implicated in autoregulation (Figure 2). Substitution of isoleucine (I1624) by alanine (Ala) prevents \(\alpha_{1C}\) binding to CaM and as a consequence eliminates CDI, promoting Ca\(^{2+}\)-dependent facilitation (63–65), which contributes to a force-frequency relationship of heart. Originally, an EF hand was identified as a crucial determinant of CDI. Peterson et al. (66) suggested that a 4-aa cluster (VVTL) within the EF-hand region was involved in the CDI process; however, subsequent studies could not confirm this finding. Pitt et al. (67), using Ca\(^{2+}\)-insensitive CaM mutants (with all 4 Ca\(^{2+}\) binding sites destroyed), found that the \(\alpha_{1C}\) subunit contains an apoCaM site requiring 10–100 nM Ca\(^{2+}\) for tethering. Two other sequences (labeled peptides A and C) between the EF hand and the IQ motif are also implicated in CaM binding. Upon depolarization and concomitant Ca\(^{2+}\) influx, [Ca\(^{2+}\)], elevates to a \(\mu\)M level causing Ca\(^{2+}\) binding to the prebound CaM, which
then rapidly engages the IQ motif and promotes inactivation. Recently, Kim et al. (68) identified 11654 in the CaM-binding IQ motif as a link between the Ca²⁺ sensor and the downstream inactivation machinery. The study suggested a unified model for CDI and VDI of L-VDCC utilizing the I-II linker of the channel as a blocking particle (Figure 3). However, the molecular details of the interaction between the CaM and the C terminal of the α1C need to be further elucidated. Kobrinisky et al. reported (48) that the α1C subunit N terminal tail may have a role in channel trafficking and is a target for β subunit modulation. The authors proposed hypothetical molecular arrangements of the β subunit modulation of the Ca₁.2 channel, implying that the β subunit acts as a “molecular wedge” that prevents the N terminus of the α₁C subunit from blocking the pore. The data, generated from coexpression studies in African green monkey kidney cells (COSs), provided evidence that the CDI is not mediated solely by determinants of the α₁C subunit C terminal tail.

CaM is also involved in EC coupling. Yang and coworkers (69) demonstrated that adenosine expression of a mutant CaM lacking all 4 Ca²⁺-binding sites (CaM 1–4) surprisingly markedly enhanced the amplitude of [Ca²⁺], transients in cultured rat ventricular myocytes. The underlying mechanism involves the augmentation of I_Ca, density with slowed inactivation time associated with an elevation of the Ca²⁺/CaM-dependent protein kinase II (CaM(KII)). One of the appealing working hypotheses explaining the CaM 1–4 positive inotropic effect is that the excessive CaM 1–4 displaces apoCaM from the L-VDCC α₁C subunit C terminal tail, resulting in an increase in apoCaM concentration in the cytosol. Despite the increase in apoCaM concentration, the total CaM concentration remains unchanged. This process prevents CaM activation at the C terminus of α₁C, which would concomitantly increase the Ca²⁺/CaM available to activate CaM(KII). Ca²⁺/CaM activates CaM(KII), which increases L-VDCC by inducing a gating mode characterized by long channel openings (70). CaM–CaM(KII)–L-VDCC crosstalk has a central role in contractility and Ca²⁺ homeostasis. In addition, substantial evidence supports its involvement in cardiac hypertrophy and failure (71). A growing body of evidence suggests that CaM(KII) is a proarrhythmic signaling molecule (72). Results by Tessler and colleagues (73) indicated that Ca²⁺-dependent regulation of I_Ca (transient outward K⁺ current, encoded by K₁.4) is achieved mainly via CaM(KII) activation in human atrial myocytes. Since CaM(KII) is upregulated during atrial fibrillation (AF), there is evidence that changes in [Ca²⁺], homeostasis may initiate electrical remodeling during AF. CaM(KII) structure and function data and other recent evidence together suggest that CaM(KII) inhibition may serve as a potential strategy for treating myocardial dysfunction and arrhythmias in the setting of structural heart disease (71, 74). An important signaling molecule involved in the complex pathway regulating Ca²⁺ homeostasis is the nuclear factor cAMP-responsive element–binding protein (CREB) (75), which is a major downstream target for CaM(KII). CREB normally regulates the transcription of target genes that encode contractile proteins, proteins involved in generating energy, and proteins required for cardiac myocyte growth and viability. A variety of intracellular signaling molecules may be involved in CREB phosphorylation and activation, including PKA and CaM(KII) in response to elevations in [Ca²⁺]. Studies suggest that therapies designed to increase CREB activity in the failing heart might slow the progression of heart failure.

**Ca²⁺ channel antagonists and Ca²⁺-binding domains**

The L-VDCC is an important pharmacologic target in the treatment of a number of conditions. In the late 1960s, Fleckenstein showed that Ca²⁺-antagonists, such as verapamil, protected the rat heart against structural damage associated with prolonged [Ca²⁺], overload. Among the many problems that arise from Ca²⁺ overload, ventricular ectopic rhythm, etc., ventricular fibrillation (VF), is the most prominent and can be life threatening. In pharmacological models, Ca²⁺ channel blockers (CCBs) are considered promising drugs to treat supraventricular arrhythmias, hopefully preventing lethal VF. We emphasize, however, that the effects of these drugs have not emerged as unequivocally favorable in all clinical studies to date. Verapamil and diltiazem can, in some cases, prevent episodes of acute ischemic VF in humans, but they do not have as much of a beneficial effect on overall mortality as the β-blockers and the angiotensin-converting enzyme (ACE) inhibitors. The clinical implications of this finding, reported in different clinical trials, are similar to those reported for encaidine and flecainide, class IC antiarrhythmics, in the Cardiac Arrhythmia Suppression Trial (76). Patients suffering from coronary disease may die of either heart failure or arrhythmias. Likewise, arrhythmias ascribable to disorders of conduction are also treated with CCBs that function, presumably, by inhibiting conduction disturbances in the sinoatrial or atrioventricular nodes. Although CCBs bind specifically to regions of the α₁C subunit of the L-VDCC, these drugs are not currently judged as being helpful in the setting of congestive heart failure. In fact, all clinical trials to date, with the exception of the Prospective Randomized Amlodipine Survival Evaluation (PRAISE I) study on congestive heart failure patients, were failures. However, a subgroup analysis (PRAISE II) revealed that improved clinical symptoms were seen only in patients with heart failure of a nonischemic cardiomyopathic nature. A favorable effect on survival was found only in patients without a history of angina (77). In the Third Vasodilator-Heart Failure Trial (78) (V-HeFT III), felodipine was administered to patients with congestive heart failure in a setting of stable therapy with enalapril, diuretics, and digoxin. The drug had neither a beneficial nor deteriorating effect despite the improvement in exercise performance and LV function, as reported previously in the V-HeFT II trial in patients with chronic heart failure (79). Chronic nifedipine (which has strong peripheral vasodilating effects) therapy caused a higher incidence of clinical deterioration and worsening of heart failure (80, 81). The DEFIANT-I study (Doppler Flow and Echocardiography in Functional Cardiac Insufficiency: Assessment of Nisoldipine Therapy) was a double-blind randomized study of the effects of the DHP nisoldipine on LV size and function after acute myocardial infarction. Diastolic LV function improved in patients recovering from acute myocardial infarction (82). The Danish Verapamil Infarction Trial II (DAVITT II) demonstrated that long-term treatment with verapamil significantly improved reinfarction survival after acute myocardial infarction (83). Hypertensive patients are often treated with CCBs to reduce cardiovascular disease risk, but the overall benefit compared with atenolol and hydrochlorothiazide and ACE inhibitors is both controversial and problematic. The Controlled Onset Verapamil Investigation of Cardiovascular End Points (CONVINCE) trial indicated that the effectiveness
of CCB therapy was comparable to diuretic and β-blocker treatment in reducing cardiovascular disease (84). According to the Multicenter Diltiazem Postinfarction Trial (85) analysis, diltiazem exerted no overall effect on mortality or cardiac events in a large population of patients with previous infarction, but in patients with pulmonary congestion, diltiazem was associated with an increased number of cardiac events and mortality. Interestingly, the increase in mortality was not accompanied by a worsening of heart failure. Despite these concerns, in the Studies of Left Ventricular Dysfunction Trial (SOLVD), 3–35% of the patients were treated with CCBs in addition to digitals and diuretics. Concomitant CCB use was associated with significantly increased risks of fatal and nonfatal myocardial infarction (86). CCBs have a favorable systemic vasodilator effect and should improve diastolic relaxation. Besides that, one would assume that CCBs, in inhibiting Ca2+ influx into myocardial cells, might be beneficial because theoretically they could reduce Ca2+ overload, an important trigger for activating certain down-regulated channels in cardiac muscle as well. A use-dependent pattern of the L-VDCC in cardiac dysfunction. Clinical trials to date, however, have been disappointing. In fact, it has been suggested that the effects of CCBs on mortality in patients with heart failure may be associated with increased sympathetic activity. Summing up the therapy of heart failure, the β-adrenergic receptor (β-AR) blockers, ACE inhibitors, diuretics, and aldosterone receptor(s) inhibitors (spironolactone and new derivatives) have achieved therapeutic success probably through multiple actions that culminate in reduced load on the heart.

Use dependence

Less detailed structural information is known regarding the mechanism of use-dependent block, a feature that is critical to the activity of therapeutically successful L-VDCC antagonists. Starmer et al. (93) proposed the “guarded receptor hypothesis” in the setting of the Na+ channel to explain the mechanism of use-dependent block by the Na+ channel blocker antiarrhythmic drugs although this model may be applicable for the Ca2+ channels in cardiac muscle as well. A use-dependent pattern is described where peak \( I_{Ca} \) is progressively reduced by a train of depolarizing test pulses. Verapamil and diltiazem preferentially interact with the open and inactivated states of the channel (reviewed in ref. 94). The more frequently the Ca2+ channel opens, the better is the penetration of the drug to the binding site. This explains their preferential effect on nodal tissue in paroxysmal supraventricular tachycardia. The lack of use dependence and the presence of voltage sensitivity of the DHPs in regard to their binding explains their vascular selectivity. Herin et al. (95) were among the first to establish a relationship between Ca2+ channel inactivation and use-dependent Ca2+ channel block by PAA. Since that time, single amino acids have been identified as inactivation determinants in motifs IIIS6, IVS6, and IVS5, with some of these also serving as high-affinity determinants for the DHP receptor site (94–96).

The L-VDCC in heart failure

There is overwhelming evidence that EC coupling in the heart depends on the function of the L-VDCC. Although considerable information is known regarding the role of the L-VDCC in EC coupling, the consequence of increased Ca2+-channel density in hypertrophy and cardiac failure remains speculative. In fact, most investigators report no change in the L-VDCC or downregulation in end-stage heart failure. Nevertheless, almost everyone agrees that alteration of intracellular Ca2+ handling in the myocardium is relevant to both human and animal models of heart disease (as reviewed by Benita et al. in ref. 97). Haase and coworkers (98) investigated the effect of α1 and β subunits in cardiac preparations from normal and hypertrophied human hearts. They found a significant increase in the number of DHP receptors expressed in hypertrophied hearts compared with normal hearts. Hullin et al. (99) examined the role of L-VDCC subunits expressed in allografts from hearts with diastolic heart failure. The transcript and protein expression levels of the β subunit were decreased while the expression levels of other subunits were unchanged; however, the interpretation of the results from transplanted hearts may be more complex.

On the functional level, Schroeder et al. (100) used single-channel analyses to demonstrate that single-channel current activity was markedly enhanced in the failing heart compared with nonfailing control hearts as a result of increased open probability and availability. However, extrapolating these findings to whole-cell current is difficult. Although an increased \( I_{Ca} \) would be predicted from these single-channel data, no increase was found when \( I_{Ca} \) was measured in whole-cell preparations. Such a discrepancy might be explained if the cardiomyocytes from these failing hearts expressed fewer L-VDCCs than normal although the authors reported no significant changes in either α1C subunit transcript or protein expression levels. Schroder et al. (100) proposed 2 alternative explanations: (a) the increased channel activity reflects an increase in PKA-dependent phosphorylation of the α1C (due to altered dephosphorylation) in the failing heart; and (b) auxiliary channel subunits expressed in the cardiomyocytes modulate \( I_{Ca} \).
α1C subunit mRNA levels. These data support the concept that reduced expression of β subunits is responsible for the reduction of functional L-VDCC.

In contrast with the rabbit model, the expression of the L-VDCC α1C and α2 subunits was not decreased in atrial myocardium of patients with chronic AF, suggesting no change in ICa density (102). These results are in conflict with other published results demonstrating a decrease in ICa density (103–105) and protein content of the α1C subunit of L-VDCC in clinical AF (106, 107).

However, we have to remember that whole cell ICa is determined by the equation $ICa = N \times i \times p_o \times f_{axis}$, where N is the number of functional channels in the cell, i is the single channel current amplitude, $f_{axis}$ is the fraction of available channels, and $p_o$ is the open probability of the available channels to be in the open state. Therefore, the reduction of ICa could be the result of a change in single-channel gating characteristics. Indeed, Klein et al. (107) revealed an increased channel open probability associated with reduced protein expression of the α1C subunit. Christ et al. (105), on the contrary, suggest that an increase in protein phosphatase 2A activity contributes to the impaired ICa density in AF, which may imply reduced single-channel activity. Overall, based on the results available to date, it appears there are other regulatory pathways and factors impacting the L-VDCC during AF and heart failure (108–110). It is of considerable interest that, after the many years since the cloning of the L-VDCC, a mutation has finally been demonstrated in the α1C subunit (111). The related disease, now called Timothy Syndrome, is characterized by syncpe and sudden death from cardiac arrhythmias and is attributed to a G406R as well as a G402S mutation, occurring in exon 8.

**EC coupling gain**

Ca²⁺ entry via L-VDCCs is the major trigger for SR Ca²⁺ release (Ca²⁺ sparks) by RyR2, and summation of the Ca²⁺ sparks during depolarization of the cell underlie the basis for [Ca²⁺]i homeostasis. Single cardiomyocytes from failing rat heart displayed smaller [Ca²⁺]i transients and compromised contractility despite the unchanged ICa (112). These results are in conflict with other published results from hypertrophied spontaneously hypertensive rats, found an increase in contractility, [Ca²⁺], transients, and the average Ca²⁺ spark amplitude (big sparks) without any alteration in ICa or SR Ca²⁺ load. These results suggest an increase in EC coupling gain (115), which means that the coupling between Ca²⁺ entry through L-VDCC and Ca²⁺ release from the SR would be enhanced although this could not account entirely for the big Ca²⁺ sparks. Several other possibilities include altered transverse-tubule structure and increased phosphorylation of RyR2 and L-VDCC (122).

Reduced EC coupling gain may explain how Tg animals develop heart failure. For example, Tg overexpression of NCX1 (123) resulted in a phenotype with severe hypertrophy associated with a reduction of EC coupling gain as a consequence of the increased ICa and decreased [Ca²⁺], transient. According to the authors’ interpretation, the overexpressed NCX behaves like a sponge and helps reduce the amount of Ca²⁺ entering through the L-VDCC before the trigger-induced Ca²⁺ release from SR. In contrast, Henderson et al. (124) reported a cardiac-specific NCX KO mouse model with normal cardiac function. The cardiac phenotype included decreased ICa associated with normal [Ca²⁺], transient amplitude. The authors hypothesized that the interaction between trigger Ca²⁺ and RyR2 is more efficient in the KO mice than in WT mice (EC coupling gain increase). Interestingly, at the onset of cardiac hypertrophy in the α1C Tg model, the gain function of EC coupling was uncompromised, and SR Ca²⁺ content, and unitary properties of Ca²⁺ sparks were unchanged (62).

It is common knowledge that heart failure is characterized by a disruption in the cardiac β-AR system. Data by Grandy et al. (125), for example, provide evidence that cardiac-specific overexpression of β2-AR increases EC coupling gain. Interestingly, ICa is decreased and accompanies the increased SR Ca²⁺ load and the frequency and amplitude of spontaneous Ca²⁺ sparks. It has been assumed that augmentation of β-AR signaling has therapeutic potential, and these observations suggest that increased EC coupling gain is the underlying mechanism.

Clearly, the link between reduced EC coupling gain and heart failure is important; however, more investigation is required to improve our understanding of the defects in EC coupling in pathological cases.

We have endeavored here to provide an insight into the importance of Ca²⁺ in the life of the cardiac myocyte with a special emphasis on L-VDCCs, viewing molecular data through the filter of fundamental physiology. It is our belief that molecular-based cardiac physiology is at the doorstep of a major breakthrough, at which time a more complete understanding of the biochemical and biophysical processes will drive successful therapeutic interventions and treatment of heart disease.

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