L-type Ca^{2+} channels in the heart: structure and regulation

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Summary. *This review analyzes the structure and regulation mechanisms of voltage-dependent L-type Ca^{2+} channel in the heart. L-type Ca^{2+} channels in the heart are composed of four different polypeptide subunits, and the pore-forming subunit α_1 is the most important part of the channel. In cardiac myocytes, Ca^{2+} enter cell cytoplasm from extracellular space mainly through L-type Ca^{2+} channels; these channels are very important system in heart Ca^{2+} uptake regulation. L-type Ca^{2+} channels are responsible for the activation of sarcoplasmic reticulum Ca^{2+} channels (RyR2) and force of muscle contraction generation in heart; hence, activity of the heart depends on L-type Ca^{2+} channels. Phosphorylation of channel-forming subunits by different kinases is one of the most important ways to change the activity of L-type Ca^{2+} channel. Additionally, the activity of L-type Ca^{2+} channels depends on Ca^{2+} concentration in cytoplasm. Ca^{2+} current in cardiac cells can facilitate, and this process is regulated by phosphorylation of L-type Ca^{2+} channels and intracellular Ca^{2+} concentration. Disturbances in cellular Ca^{2+} transport and regulation of L-type Ca^{2+} channels are directly related to heart diseases, life quality, and life span.*

Voltage-dependent Ca^{2+} channels

Voltage-dependent calcium channels (VDCCs) were first identified by Fatt and Katz in 1953 (1). Hagiwara et al. subsequently studied these channels extensively and suggested to divide them into distinct types (2). According to the electrophysiological properties, VDCCs were divided into two major classes: low voltage-activated and high voltage-activated Ca^{2+} channels. According to the pharmacological and biophysical properties of VDCCs, they were subdivided into several functional channel types and named T-type, L-type, N-type, P/Q-type, and R-type Ca^{2+}channels. Low voltage-activated channel class consists of only T-type Ca^{2+} channels that open at low membrane potentials and inactivate very rapidly. R-type is sometimes called intermediate voltage-activated Ca^{2+} channels. Voltage-dependent Ca^{2+} channels have been divided into different types considering their activation threshold, conductance, the time and voltage dependence of inactivation, selectivity to divalent cations, channel gating, open- and close-time duration, pharmacological properties (3, 4).

L-type Ca^{2+} channels ("L"ong lasting) are often called dihydropyridine receptors (DHPR). They are sensitive to various 1,4-dihydropyridines, some of them blocking (nifedipine, nicardipine) and some increasing (Bay K 8644) Ca^{2+} current through L-type Ca^{2+} channels (4). These high voltage-activated channels are distributed in various tissues: skeletal muscle, heart, brain, endocrine cells, neurons, and other. They have quite high conductance (between 11 and 25 pS) and slow time- or voltage-dependent inactivation (~500 ms). There are a big variety of L-type Ca^{2+} channels, and the same cell can express several types of L-type Ca^{2+} channel. The functional properties of L-type Ca^{2+} channels in heart, secretory cells, and neurons are quite similar but in skeletal muscle, conductance is smaller (~11 pS) and properties significantly differ (3).

Structure of L-type Ca^{2+} channel

Cardiac L-type Ca^{2+} channels are composed of four polypeptide subunits (α_1, β, α_2/δ) and form hetero-tetrameric complex with molecular mass of about 400 kDa (Fig.). In skeletal muscle and brain, Ca^{2+} channels have the fifth subunit (γ), but in heart, γ is not expressed (4, 5, 6). The hydrophobic α_1 polypeptide is entrenched in cell membrane, while β subunit locates in cytoplasm. The δ subunit is anchored in cell membrane and has a single transmembrane segment with a short intracellular part and a long glycosylated extracellular part. The α_2 peptide is extracellular subunit of the Ca^{2+} channel. The α_2 and δ subunits of L-

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type Ca^{2+} channel are tightly bound together through a disulfide bridge (6, 7) (Fig.).

It is elucidated that the α_1 subunit is the most important polypeptide of the Ca^{2+} channel-forming proteins; it forms the channel pore for ion flow. The α_1 subunit consists of four homologous motifs (I-IV), each composed of six transmembrane segments (S1-S6) (Fig.). The α_1 subunit is responsible for voltage-dependent Ca^{2+} channel opening (positively charged arginine and lysine in the S4 segment serves as voltage sensor) and channel selectivity for calcium ions. The α_1 subunit has specific binding sites for Ca^{2+} channel blockers (1,4-dihydropyridines, phenylalkylamines, benzothiazepines) (4, 6). Intracellular part of the α_1 subunit has two sequences called domains L and K that are involved in Ca^{2+}-induced channel inactivation: both like calmodulin (CaM)-binding site and like Ca^{2+} sensor (6, 8). To date, at least 10 different α_1 subunit genes have been identified, but only α_{1C} ($Ca_v1.2$) isoform is expressed at high levels in cardiac muscle (6).

Four different β subunit isoforms (β_1-β_4) have been described by now, and the β_2 isoform is expressed in the heart (6). The β subunit functions in the expression of the α_1 subunit. The β subunits have a chaperone-like role in trafficking α_1 subunits from the endoplasmic/sarcoplasmic reticulum to the plasma membrane and its insertion in the proper conformation (4, 6). The β subunit has a prominent role in β-adrenergic regulation of the Ca^{2+} channel and in response to the changes of the pH of the cell; also, β subunit influences Ca^{2+} channel facilitation (6). The β subunits are

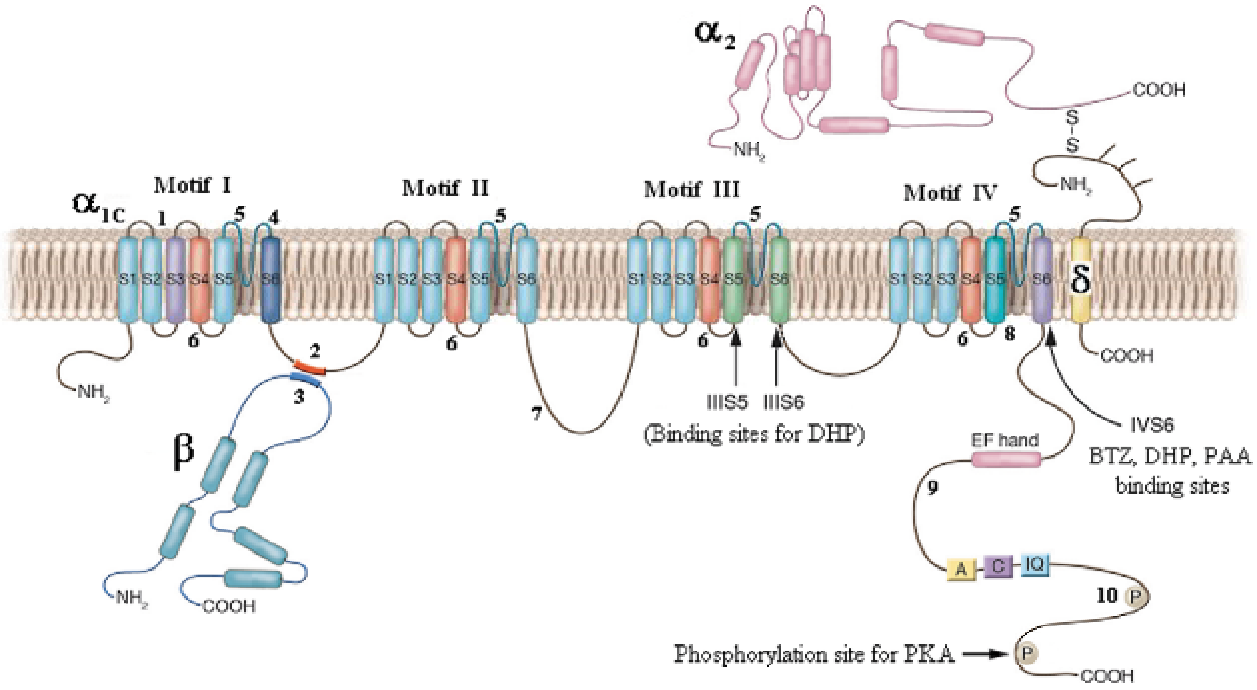

Fig. **Structure of L-type Ca^{2+} channel**

The pore forming α_{1C} subunit is composed of four homologous motifs (I–IV), each of which consists of six putative transmembrane segments (S1–S6). The α_2 subunit is extracellular, the δ subunit contains transmembrane segment, intracellular part and extracellular sequence related to α_2 through a disulfide bond. The β subunit of the Ca^{2+} channel is located intracellulary. The γ subunit in not expressed in the heart and is not shown in Fig.

1) Voltage-dependent activation kinetics site; 2) α interaction with β subunit – AID (α interaction domain); 3) β interaction with α subunit – BID (β interaction domain); 4) voltage-dependent inactivation site (IS6 and neighboring regions); 5) ion selectivity site (pore forming S5-S6 linker regions); 6) voltage sensor (positively charged segments) – IS4, IIS4, IIIS4, IVS4; 7) excitation-contraction coupling; 8) role of use-dependent block; 9) Ca^{2+}-dependent inactivation area; 10) phosphorylation site for CaMKII (determines phosphorylation-dependent I_{Ca} facilitation). DHP – 1,4-dihydropyridine, BTZ – benzothiazepine, PAA – phenylalkylamine.
(Modified from Bodi et al. The L-type calcium channel in the heart: the beat goes on. J Clin Invest 2005;115:3306-3317).

involved in channel regulation by phosphorylation of channel-forming proteins. Sites possible for phosphorylation by various protein kinases (PKG, PKA, PKC) have been identified in these subunits (7, 9). In addition, the β subunit increases Ca^{2+} current amplitude, accelerates the kinetics of Ca^{2+} channel activation, and alters pharmacological properties of the channel (6, 7).

The α_2 and δ subunits are encoded by the same gene, and presently, at least four isoforms ($\alpha_2/\delta_{1,2,3,4}$) of this protein, encoded by separate genes are known (6). The α_2/δ subunits have less influence for the action of L-type Ca^{2+} channel than the β subunit. The α_2/δ subunits slightly increase Ca^{2+} current amplitude; they fractionally quicken inactivation of the channel and can change the properties of Ca^{2+} channel activation. The α_2/δ subunits can affect channel density and probably participate in "driving" the α_1 subunit to the plasma membrane (6).

The all Ca^{2+} channel subunits – the main pore-forming subunit α_1 and auxiliary subunits β, α_2/δ – have many protein isoforms. Such a plenty of expressed subunits and the variety of possible combinations assure proper work of the channel and functional differences in separate tissues. Recently, there are attempts to identify the spatial organization of L-type Ca^{2+} channel. Serysheva and colleagues have revealed three-dimensional structure of L-type Ca^{2+} channel of rabbit skeletal muscle; they propose an asymmetric structure consisting of two major regions called a "heart-shaped" and a "handle-shaped" region (10). The spatial organization of cardiac Ca^{2+} channel is similar, but cardiac Ca^{2+} channels are more compact (they lack the γ subunit), and functional protein in membrane has a tapering structure compared to the channel form of the skeletal muscle (5).

In cardiac myocytes, Ca^{2+} current through L-type Ca^{2+} channels (L-type Ca^{2+} current or I_{Ca}) is the main way for Ca^{2+} influx from extracellular space into cytoplasm; I_{Ca} triggers the contraction of heart muscle and regulates the force of contraction (11–13).

Regulation of L-type Ca^{2+} channel by protein kinase A

Phosphorylation of Ca^{2+} channel-forming proteins by protein kinase A (PKA) is the main and mostly explored way for Ca^{2+} channel activation. PKA itself is activated by second messenger cAMP. Phosphorylation of L-type Ca^{2+} channels by PKA increases the probability and duration of the open state of the channels; because of that, I_{Ca} of the cell is increased. I_{Ca} is stimulated by PKA in further intracellular signalization way: 1) agonist binds its receptor; 2) receptor stimulation activates GTP-binding protein (G_s), which stimulates adenylyl cyclase (AC); 3) stimulated AC intensively produces cAMP from ATP; 4) increased concentration of cAMP activates PKA; 5) PKA phosphorylates several proteins related to excitation-contraction coupling, including L-type Ca^{2+} channels (11, 14–16). cAMP is degraded by cAMP phosphodiesterases (PDEs), and the signaling cascade is then suppressed limiting cAMP-dependent phosphorylation; also signaling cascade is terminated by serine/threonine phosphatases that remove a phosphate group from kinase-phosphorylated proteins (14, 17, 18). Several different agonists (catecholamines, glucagon, histamine, serotonin) can activate AC if are bound to their receptors and induce cAMP-dependent I_{Ca} stimulation.

The stimulation of β-adrenergic receptors and subsequent activation of PKA, which results in modulation of L-type Ca^{2+} channel, is the best-investigated pathway of Ca^{2+} channel regulation. To date, three types of β-adrenergic receptors (β-ARs) from different tissues – β_1, β_2 and β_3-ARs – have been identified and cloned. β receptor subtype and number varies significantly depending on the object of investigation. β_1-ARs dominate in mammalian heart, although the stimulation of β_2-ARs can have a significant effect here also (19, 20). The effect of catecholamines in human heart is generally attributed to β_1- and β_2-ARs, with a respective contribution of each receptor subtype that varies significantly depending on the cardiac tissue, the pathophysiological state, the age, or the developmental stage (21). The stimulation of β-ARs increases the activity of single Ca^{2+} channels, and then I_{Ca} of whole cell is increased. However, expression of a β_3-AR in human myocardium was also demonstrated (22, 23), but its influence on L-type Ca^{2+} channel regulation is still under discussion.

PKA-dependent phosphorylation of the channel-forming protein α_{1C} can occur at Ser-1928 (24). It is also found that PKA phosphorylates the auxiliary β subunit of the Ca^{2+} channel at three sites (Ser-459, Ser-478, and Ser-479) *in vitro* (25). The phosphorylation of L-type Ca^{2+} channel subunit α_{1C} by PKA is critically dependent on close localization of both components – PKA and L-type Ca^{2+} channel, whereas phosphorylation of the β subunit by PKA does not require such a close PKA dislocation (26). PKA is anchored near its targets through association with an A-kinase-anchoring protein called AKAP. If the association of an AKAP with PKA is interrupted, PKA-dependent phosphorylation of skeletal and cardiac L-

type Ca^{2+} channels is not observed. It is thought that PKA and cardiac Ca^{2+} channels are associated through mAKAP or AKAP15; they bind PKA in a close proximity of cardiac Ca^{2+} channel (14, 26).

The suppression of AC activity is one of the most common pathways to interrupt PKA-dependent channel stimulation. AC is usually suppressed by activation of G_i proteins. It is showed that stimulation of various G_i-coupled receptors does not change basal I_{Ca} in most cases, but reduces $I_{Ca,}$ increased via stimulation of β-adrenergic receptors (27). Stimulation of muscarinic receptors suppresses PKA-dependent stimulation of the Ca^{2+} channel, because muscarinic receptors activate G_i and suppress AC. It is showed that M_2 muscarinic receptors can suppress I_{Ca} of cardiac cells by activation of phosphatases or by changing cGMP-dependent phosphodiesterase activity in participation of NO (28). G_i proteins can be activated (and cAMP synthesis can be blocked) not only by muscarinic receptors, but by other cardiac receptor types too: adenosine (A_1), opiates, and atrium natriuretic peptides (14).

Activation of phosphodiesterases is another way to reduce PKA-dependent channel phosphorylation. Phosphodiesterases (PDEs) hydrolyze cAMP and cGMP and decrease their intracellular concentration. It assures local signaling of cyclic nucleotides (compartmentation), prevents cAMP diffusion to the whole cytosol and inadequate target activation (15, 18). Cardiac PDEs belong to five families: 1) PDE1, which hydrolyzes both cAMP and cGMP, is activated by Ca^{2+}-calmodulin and is mainly expressed in nonmyocytes of cardiac tissue; 2) PDE2, which also can hydrolyze both cAMP and cGMP and is stimulated by cGMP; 3) PDE3, which is a cGMP-inhibited cAMP-PDE; 4) PDE4, which hydrolyzes cAMP; 5) PDE5, which hydrolyzes cGMP (18). PDEs from families PDE1, PDE3, PDE4, and PDE5 can be phosphorylated by various kinases (PKA, PKG), and therefore their activity can be altered (17, 18). Within PDE families, multiple isoforms exist, and until now, at least a dozen of different PDE isoforms have been found in heart (18).

Regulation of L-type Ca^{2+} channel by protein kinase C

A prominent role in L-type Ca^{2+} channel regulation plays protein kinase C (PKC). Stimulation of various types of $G_{q/11}$ protein-coupled receptors (endothelin, α_1-adrenergic, angiotensin II and muscarinic receptors) activates PKC. Receptor-activated $G_{q/11}$ protein stimulates phospholipase C. Phospholipid PIP_2 (phosphatidylinositol 4,5-bisphosphate) is a substrate plasma membrane-located substrate, which cleaves PIP_2, generating inositol triphosphate (IP_3) and diacylglycerol (DAG). DAG is a neutral lipid and transmits signals within plasma membrane. Together, DAG, phosphatidylserine, and sometimes Ca^{2+} activate PKC. At least 10 PKC isoforms were defined by now, and these are divided into three subfamilies: the classical PKCs, the novel PKCs, and atypical PKCs. The number of PKC isoforms and levels of their expression vary in different tissues and depend on the species and developmental stage of the animal (9, 29, 30). Several PKC isoforms are present in the heart: the isoforms α, β, and εPKC are detected in both neonatal and adult ventricular myocytes, ηPKC is present in cultured neonatal ventricular myocytes. It is thought that the isoforms λ and ζPKC also can be expressed in the heart. The most inconsistently detected cardiac isoform is βPKC (29).

The effect of PKC on I_{Ca} can be highly diverse. PKC can either increase (31) or decrease I_{Ca} (32). In addition, it is known the biphasic effect of PKC on cardiac I_{Ca}, i.e. I_{Ca} was increased and decreased during the same experiment (33).

The mechanism of the effect of PKC on the activity of cardiac L-type Ca^{2+} channels is not exactly known. It is established that the α_{1C} and β_2 subunits of cardiac L-type Ca^{2+} channel can be phosphorylated by PKC *in vitro* (14). PKC phosphorylates the N' terminus of the α_{1C} subunit of L-type Ca^{2+} channel, and the effect on the channel can be either stimulating (33) or suppressive (34).

Because agonists that activate PKC often produce inositol 1,4,5-trisphosphate-induced release of Ca^{2+} from the sarcoplasmic reticulum through IP_3 channels, the PKC-induced channel stimulation can be masked by Ca^{2+}-induced inactivation of channels (9). PKC-induced effects can be dependent on isoform of PKC that is activated. The expression of PKC isoforms in the heart can vary during development and depend on object and pathology. The activation of PKC induces translocation of the enzyme towards its targets; therefore, various isoforms of PKC can spread unequally in intracellular space and phosphorylate different protein targets. For instance, εPKC translocates to cross-striated regions in ventricular myocytes, which places near sarcolemmal T-tubules, where L-type Ca^{2+} channels are located. In the phosphorylation of membrane targets, specific anchoring proteins referred to as RACKs (receptors for activated C kinases) are playing an important role; PKC interacts with RACKs in an isoform-specific manner (14, 29). Data suggest that

the βPKC isoform stimulates I_{Ca} (31) and εPKC suppresses I_{Ca} (32).

Regulation of L-type Ca^{2+} channel by protein kinase G

The effect of PKG (protein kinase G) on the activity of cardiac L-type Ca^{2+} channels is not exactly clear, and the results of research on cGMP/PKG activation cascade are quite diverse. There are data showing that cGMP/PKG pathway suppresses Ca^{2+} channels (35); however, other data suggest stimulation of I_{Ca} (36). The effect of cGMP/PKG signalization pathway on I_{Ca} can be related not only to the direct action of PKG on L-type Ca^{2+} channel but also to the NO- and cGMP-related regulation of PDEs, because PDEs hydrolyze cAMP and cGMP and alter the activity of cyclic nucleotide-dependent enzymes (18). The effect of NO on cardiac I_{Ca} can have a cGMP-independent manner (37). In rabbit atrial myocytes, NO enhances I_{Ca} in a cAMP-independent manner through the activation of PKG (37).

The rise of cGMP can reduce I_{Ca}, and there are several possible pathways of such I_{Ca} regulation: 1) direct phosphorylation of Ca^{2+} channel by PKG; 2) activation of phosphatases (that dephosphorylate Ca^{2+} channels) by PKG; 3) cGMP-dependent stimulation of PDE2 that hydrolyzes cAMP. Therefore, the rise of cGMP level can influence I_{Ca} not only by phosphorylation of the channel, but also by modulation of PKA-dependent channel regulation (9, 35, 37). Because of multiple effects of cGMP, the findings of studies vary, and the influence of PKG on L-type Ca^{2+} channel activity is hard to identify.

The effect of tyrosine kinases on the activity of L-type Ca^{2+} channels

Protein tyrosine kinases (PTKs) regulate myocardium response to the β-adrenergic stimulation of L-type Ca^{2+} channels; however, the role of PTK in L-type Ca^{2+} channel regulation is not well known. It is thought that PTK-evoked effects on I_{Ca} depend on tissue, species, and age, because some of membrane-associated PTKs are receptors of various growth and differentiation factors. Investigation of PTK effect on cardiac L-type Ca^{2+} channel gives mixed results, but most of data show channel activation by PTK (38, 39).

The use of PTK blockers (genistein, T23) revealed that PTKs are involved in cardiac L-type Ca^{2+} channel regulation and commonly increase $I_{Ca.}$ (39). There are data showing the suppression of I_{Ca} by PTK in human atrial myocytes, but our laboratory has demonstrated a biphasic effect of PTK suppression (by genistein) on I_{Ca} in human atrial myocytes – a brief suppression of I_{Ca} is rapidly replaced by stimulation (40). This demonstrates that PTKs can both stimulate and suppress I_{Ca} in human atrial myocytes. It has been hypothesized that inhibition (by genistein) of membrane-associated PTKs decreases I_{Ca}, whereas inhibition of cytosolic PTKs increases I_{Ca} (41). No clear overall picture about PTK effect on the activity of L-type Ca^{2+} channel has emerged at this moment, but it is thought that PTKs can alter I_{Ca} sensitivity to stimulatory effect of β-ARs by affecting them.

Inactivation of L-type Ca^{2+} channels

Cytosolic Ca^{2+} concentration decreases during diastole: Ca^{2+} ions are pumped back into intracellular stores and removed through sarcolemma. For maintenance of intracellular Ca^{2+} homeostasis and balanced cardiac activity, Ca^{2+} influx into cytoplasm via L-type Ca^{2+} channels has to be terminated. Inactivation of L-type Ca^{2+} channels depends on two mechanisms: voltage-dependent inactivation and Ca^{2+}-dependent inactivation. These two mechanisms control Ca^{2+} influx into cardiomyocytes; together they regulate signal transduction to sarcoplasmic reticulum (SR) Ca^{2+} channels (RyR2) and assure normal contraction and relaxation of the heart. The Ca^{2+}-dependent inactivation (CDI) mechanism predominates versus voltage-dependent inactivation in cardiac cells (42, 43), and there is general agreement to conclude that in mammalian cells, CDI depends primarily on Ca^{2+} released from the SR (44, 45). Therefore, I_{Ca} amplitude and kinetics depends highly upon SR content and RyR2 activity (11, 12). Fast Ca^{2+}-dependent inactivation serves as a negative feedback for Ca^{2+} to limit rapidly its own entry via L-type Ca^{2+} channels. Slow voltage-dependent inactivation prevents a premature rise in I_{Ca} when intracellular Ca^{2+} concentration decreases and CDI terminates during maintained depolarization (12).

The Ca^{2+} binding-protein calmodulin (CaM) plays a critical role in Ca^{2+}-induced inactivation of L-type Ca^{2+} channel. It was found that L-type Ca^{2+} channel α_{1C} mutant subunits with changed amino acid sequence in C terminus conducted Ba^{2+} and Ca^{2+} currents with almost identical kinetics of inactivation (8). It was found that CDI is determined by two α_{1C} amino acid sequences that are called domains L and K. Both domains L and K were found to bind CaM involved in Ca^{2+}-dependent inactivation of L-type Ca^{2+} channel. In addition, domain L contains a sequence specific for Ca^{2+} ions – a highly specific Ca^{2+} sensor. Ca^{2+} loading of this Ca^{2+} sensor modulates the CaM binding to

CaM-binding site in α_{1C} domain L, whereas the IQ (isoleucine-glutamine) motif in domain K appears to have a role in the binding of Ca^{2+}-loaded CaM (8). It is known that α_{1C}-binded CaM can change its conformation, when saturated with Ca^{2+}, and convert from a dumbbell shape to a globular shape wrapped around the target peptide α_{1C}. It is thought that CaM molecule can bind four Ca^{2+} ions, and consequently changed conformation of both CaM and α_{1C} C terminus leads to blockage of α_{1C} pore; however, there is no clear picture of the inactivation mechanism (6, 8). There are data showing that not only α_{1C} C terminus is involved in Ca^{2+}-dependent inactivation of the channel.

Facilitation of L-type Ca^{2+} current

CaM is involved not only in inactivation of L-type Ca^{2+} channels, but also can be important in I_{Ca} stimulation mechanism. Noble and Shimoni were the first to report that an increase in the rate of stimulation produces slow increase of I_{Ca} in frog atrial fibers (46). Later, this process was described in enzymatically isolated mammalian (guinea-pig, dog, rabbit, rat, human) cardiomyocytes and was called facilitation (12, 47). The increase of I_{Ca} described by Noble and Shimoni is conditionally slow, i.e. I_{Ca} increases in minutes, and it was shown that this process is dependent on L-type Ca^{2+} channel phosphorylation by kinase CaMKII (48, 49). Protein kinase CaMKII is a Ca^{2+}/calmodulin-dependent serine/threonine kinase and has an important role in L-type Ca^{2+} channel regulation. The CaMKII isoforms γ and δ dominate in the heart and can increase their own activity by autophosphorylation (49). These CaMKII kinases are activated by low intracellular Ca^{2+} concentration; it is thought that their activation is related to Ca^{2+} influx through L-type Ca^{2+} channels but not to the SR Ca^{2+} release (12).

I_{Ca} facilitation can also have different background from CaMKII-dependent phosphorylation. In mammalian cells, fast I_{Ca} facilitation was determined; it takes place during high-frequency (0.5–5 Hz) depolarizing pulses applied to the cell after rest. The increase of I_{Ca} in this case results mainly from the slowing of I_{Ca} decay kinetics and is recorded in seconds after the first depolarizing pulse. Fast facilitation mechanism is different from earlier mentioned slow I_{Ca} facilitation and is dependent on Ca^{2+} release from SR via RyR2 at high stimulation frequencies in mammalian cardiomyocytes (12). As already mentioned, SR-Ca^{2+} release-dependent inactivation is the main mechanism of L-type Ca^{2+} channel inactivation in mammalian cardiomyocytes. Thus, reduced SR-Ca^{2+} release weakens inactivation process, and L-type Ca^{2+} channels inactivate more slowly (42, 44, 50). This inactivation slowing resulted in a significant increase of Ca^{2+} influx and I_{Ca} amplitude (50). Delgado and colleagues have showed that high-frequency (1 Hz) stimulation decreased SR-Ca^{2+} release (measured using Fluo-3 Ca^{2+} indicator) in rat cardiomyocytes and related this process to fast frequency-dependent facilitation of I_{Ca} (44). They have also found that after blocking SR-Ca^{2+} release by thapsigargin, the fast I_{Ca} inactivation phase was reduced and facilitation was eliminated (44). Thapsigargin blocks the ability of the cell to pump calcium into the SR and depletes the store; consequently, Ca^{2+} release from the SR becomes weak. Whereas, the fast frequency-dependent facilitation is not observed in frog cells because of weak and poorly developed SR; however, slow CaMKII-dependent facilitation of I_{Ca} is present in frog cells, supporting evidence for distinct types of facilitation (12).

L-type Ca^{2+} channels, I_{Ca}, and heart failure

Cardiac L-type Ca^{2+} channels are the main entrance for Ca^{2+} influx into cardiac cell and determine the activity of whole heart (11–13); therefore, the changes in channel expression and regulation may alter the heart activity and badly influence functions of whole body. Many researchers report the reduction or no change in L-type Ca^{2+} channel density (and I_{Ca}) in heart failure (6, 13), but there are data showing the increase in L-type Ca^{2+} channel density in hypertrophied and failing hearts (6). In any case, it is clear that changes in Ca^{2+} flux balance in the cardiac cell are directly related to human and animal cardiac diseases (6). In failing heart not only cardiac remodeling (the changeover of cardiac function and structure) happens (51), but also the regulation systems are altered. In ischemic and failing heart, the sympathetic system is activated, and the level of catecholamines is raised (52). Consequently, the β-adrenergic receptors are activated, and L-type Ca^{2+} channel stimulation through cAMP occurs. Such pronounced activation of the sympathetic system in patients with heart failure is inversely correlated with survival (52). The level of cardiac β-ARs (mostly β_1-ARs) in failing heart is reduced, and the β-AR-G_s coupling of remaining receptors is altered (52). β-AR blockade is a widely used treatment in heart failure (53), when cardiac activity is regulated by cAMP-dependent cascade suppression.

Metabolic inhibition is a prominent feature of the ischemic and failing heart. Despite a prolonged research of this process, its effect on L-type Ca^{2+} channels in the cell is not completely understood. Gener-

ally, it is said that inhibition of oxidative phosphorylation results in a shortage of ATP, but by what mechanism it affects L-type Ca^{2+} channel activity is not exactly clear. The findings of our laboratory showed that the suppression of L-type Ca^{2+} channels during metabolic inhibition was dependent on an intensive hydrolysis of ATP, but independent of reduced protein phosphorylation (54). Such a suppression of Ca^{2+} channels seems to be related to an increased intracellular acidosis during metabolic inhibition. Our recent experiments have shown (unpublished data) that I_{Ca} facilitation in cardiac myocytes in human was weaker than in rat, and metabolic inhibition in human cardiomyocytes had lower effect on I_{Ca} than in rat cardiac cells. This points that L-type Ca^{2+} channel regulation in failing myocardium is altered, because human cardiomyocytes were isolated from heart explants and cardiac biopsies obtained during open heart surgery, i.e. human cardiac cells were isolated from more or less impaired and pharmacologically treated cardiac tissues. Our results consist with other researchers' data: I_{Ca} facilitation in single cardiac cells is weak or absent in late-stage heart failure, and inactivation of I_{Ca} is much slower than in normal cardiac cells (50). Such alterations of I_{Ca} are related to perturbations of RyR2 and Ca^{2+} concentration in heart failure (13), whereas RyR2 dysfunction is related to cardiac arrhythmias and cardiomyopathy (12).

Širdies L-tipo Ca^{2+} kanalai: struktūra ir reguliacija

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Raktažodžiai: Ca^{2+} kanalai, kinazės, fosforilinimas, inaktyvacija, fasilitacija.

Santrauka. Šiame apžvalginiame straipsnyje analizuojama įtampos valdomų širdies L-tipo Ca^{2+} kanalų struktūra bei jų reguliacijos mechanizmai. Širdies L-tipo Ca^{2+} kanalai sudaryti iš keturių skirtingų baltyminių subvienetų, tarp kurių svarbiausias yra kanalo porą suformuojantis subvienetas α_1. Širdies ląstelių L-tipo Ca^{2+} kanalai yra pagrindinis Ca^{2+} jonų patekimo kelias iš ląstelės išorės į citoplazmą ir yra labai svarbi širdies Ca^{2+} jonų apykaitą reguliuojanti sistema. Nuo šių kanalų aktyvumo priklauso sarkoplazminio tinklo RyR2 kanalų aktyvumas ir širdies raumens susitraukimo jėga, kartu ir širdies darbas. Vienas svarbiausių L-tipo Ca^{2+} kanalų aktyvumo reguliavimo būdų yra kanalą sudarančių baltymų fosforilinimas įvairiomis kinazėmis. L-tipo Ca^{2+} kanalų aktyvumas taip pat priklauso nuo Ca^{2+} jonų koncentracijos citoplazmoje. Širdies ląstelėms būdinga Ca^{2+} srovės fasilitacija ir šis procesas priklauso nuo L-tipo Ca^{2+} kanalų fosforilinimo bei Ca^{2+} jonų koncentracijos citoplazmoje. L-tipo Ca^{2+} kanalų reguliacijos sutrikimai ir ląstelinės Ca^{2+} jonų apykaitos pokyčiai yra tiesiogiai susiję su širdies ligomis, lemia gyvenimo kokybę bei trukmę.

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