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L-Type Calcium Channels: Structure and Functions

Tianhua Feng, Subha Kalyaanamoorthy and
Khaled Barakat

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Abstract

Voltage-gated calcium channels (VGCCs) manage the electrical signaling of cells by allowing the selective-diffusion of calcium ions in response to the changes in the cellular membrane potential. Among the different VGCCs, the long-lasting or the L-type calcium channels (LTCCs) are prevalently expressed in a variety of cells, such as skeletal muscle, ventricular myocytes, smooth muscles and dendritic cells and forms the largest family of the VGCCs. Their wide expression pattern and significant role in diverse cellular events, including neurotransmission, cell cycle, muscular contraction, cardiac action potential and gene expression, has made these channels the major targets for drug development. In this book chapter, we aim to provide a comprehensive overview of the different VGCCs and focus on the sequence-structure-function properties of the LTCCs. Our chapter will summarize and review the various experimental and computational analyses performed on the structures of the LTCCs and their implications in drug discovery applications.

Keywords: CaV1.2, L-type calcium channel, ion channel blocker, high-voltage activation, low-voltage activation

1. Introduction

1.1. L-type calcium channel introduction

The voltage-gated calcium channels (VGCCs/CaVs), are transmembrane ion channel proteins that selectively conduct calcium ions through the cell membrane in response to the membrane potential during depolarization. In 1953, Paul Fatt and Bernard Katz discovered the existence of calcium-conducting ion channels in the crustacean muscle [1]. Following the initial discovery of the presence of calcium conducting ion channels in the crustacean muscle cells, several

reports confirmed the presence of these channels in various mammalian cell types including, skeletal, cardiac muscles and all excitable cells. These calcium channels were firstly classified into two types based on their activation voltage and conductance, the high-voltage-activated (HVA) and the low-voltage-activated (LVA) calcium channels [2]. The HVA and LVA channels were reported to have distinct gating properties and pharmacological profiles [2, 3]. Hess et al. [4] found that the HVA channels are sensitive to 1,4-dihydropyridine (DHPs) antagonists and DHPs agonists help in stabilizing the HVA channels in the open-conducting state for a prolonged time. Interestingly, some of the identified HVA calcium channels exhibited preferences to different tissues and different sensitivity to DHP and other toxin antagonists, which led to the identification and classification of diverse HVA channels.

DHP-sensitive channels were found to be present in various cells and exhibited a long-lasting activation length and hence are called the DHP channel or the L-type calcium channel (LTCC) [5, 6]. ω -CTX-sensitive calcium channels were pronounced for their roles in the nervous system and are thus classified as N-type (non-L or neuronal) channels. ω -AGA-sensitive channels were initially found in the Purkinje cells of the cerebellum and are, therefore, named as P-type channels. Another close homolog of the P-type channel produced by alternative splicing of the CACNA1A gene was found and is referred to as the Q-type calcium channel. In addition to these three types of HVA, some calcium-conducting channels were found to be insensitive to any of these antagonists and have been classified as R-type (resistant) channels (Table 1).

Only one type of calcium channel has been reported among the LVA channels, namely, the transient-opening calcium channel (also called T-type channel). The T-type channels are similar to L-type channels in their diverse expression and antagonist resistant properties. However, small single-channel conductance and ability to be activated at lower membrane potentials make them distinct from the L-type channels.

The prevalence of N-, P/Q-, and R-channels in neurons, and L- and T-channels in broad cellular types, shows the distinctive functional roles of the calcium channels. Besides their role in the characterization of the homologous channels, the calcium channel antagonists remain promising for their ability to specifically-modulate the different types of channels [10]. The varied sensitivities of the HVA and LVA channels to different antagonists show the potential for engineering these antagonists to selectively-alter the calcium conduction in different cells for various functions.

Type	Antagonists sensitivity					Ref
	1,4-DHP	Phenylalkylamine	Benzothiazepine	ω -CTX	ω -AGA	
CaV1 L-type	Blocks	Blocks	Blocks	Resistant	Resistant	[7]
CaV2.1P/Q-type	Resistant	Resistant	Resistant	Resistant	Blocks	[8]
CaV2.2 N-type	Resistant	Resistant	Resistant	Blocks	Resistant	[9]
CaV2.3 R-type	Resistant	Resistant	Resistant	Resistant	Resistant	[9]

Table 1. Blocker sensitivities of different HVA channels.

Ten mammalian VGCCs have been identified, of which the L-type calcium channel includes four members, CaV1.1-CaV1.4, the P-/Q-type includes CaV2.1, the N-type includes CaV2.2, the R-type includes CaV2.3, and the T-type includes three members, CaV3.1–3.3. Their sequence similarity and evolutionary relationship are shown in **Figure 1**. The following sections of the chapter will focus on comprehending the structure, and function of the LTCCs and their implications in drug discovery applications.

1.2. Distribution of LTCCs

The distribution of LTCCs varies widely across its' members as their functions vary in different excitable cells [9]. Transcripts for all L-type channel isoforms have been detected in lymphocytes for endocrine functions [11]. Among the four LTCCs types, CaV1.1 is mainly distributed in skeletal muscle and plays a role in muscle contraction. It is co-expressed with ryanodine receptors (RYRs) in GABAergic neurons, which produces gamma-aminobutyric acid (GABA) [12]. CaV1.2 and CaV1.3 show a highly overlapping expression pattern in many tissues and are mostly present in same cell types, such as in adrenal chromaffin cells, cardiac and neuronal cells [13]. CaV1.2 and CaV1.3 are predominantly located post-synaptically on the cell soma and in the spine and shaft of dendrites in the neurons [14]. CaV1.2 and CaV1.3 are also expressed in the sinoatrial node (SAN) and atrial cardiomyocytes and play a role in cardiac pacemaker activity [15]. In cardiomyocytes, CaV1.2 is mainly involved in the excitation-contraction coupling. CaV1.3 are found in the pancreas and kidney, where it correlates with endocrine secretion, and in the cochlea to regulate the auditory transduction (**Figure 1**). CaV1.4 is primarily expressed in the retinal cells and helps in normal visual functions [16].

When the LTCCs detect the electrical signal on the cell membrane, they transform these signals into other physiological activities, such as contraction of the muscle, secretion of hormones, and regulation of genes [18, 19]. These processes can generally be summarized as excitation-contraction [18], excitation-secretion [19], and excitation-transcription coupling [12], respectively.

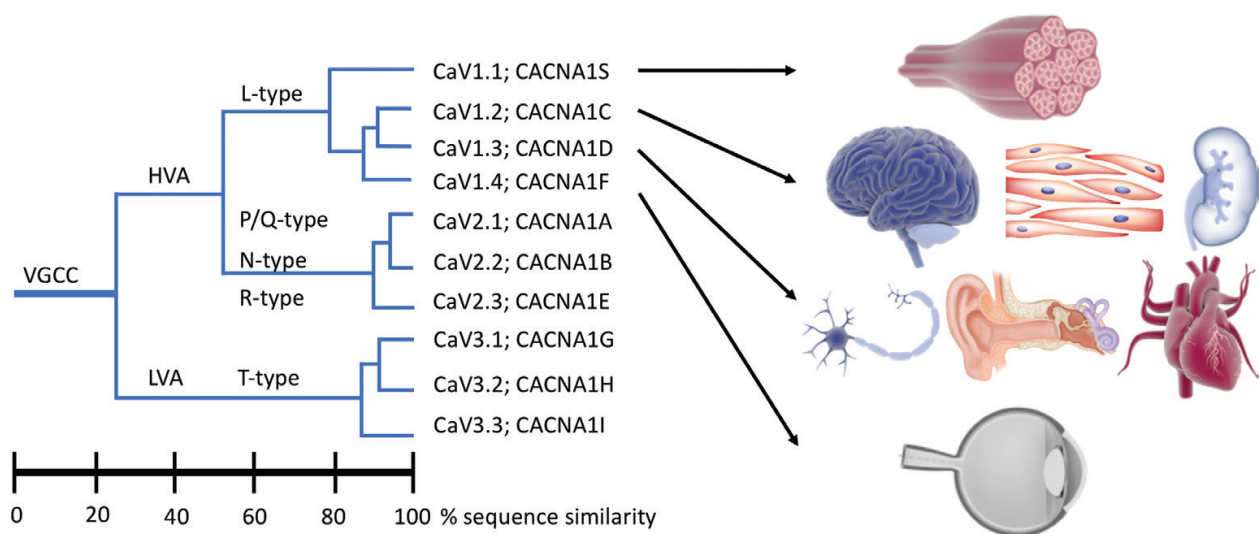


Figure 1. Phylogenetic tree showing the evolutionary relationship among the members of the VGCCs [2, 17]. And, the major distribution of the four LTCC isoforms in human tissues [12, 13, 18]. The tree was constructed using the Clustal omega. The scale in the figure shows the percentage of sequence identity in the CaV α_1 subunit of different channels.

2. Sequence-structure organization of L-type calcium channels

2.1. LTCC: domain organization

The purified LTCCs contains five subunits, the principal or pore-forming subunit, α_1 (170 kDa) and different auxiliary subunits, α_2 (150 kDa), β (50–78 kDa), δ (17–25 kDa), and γ (32 kDa). The auxiliary subunits are non-covalently linked to the α_1 subunit for modulating the biophysical properties and trafficking of the α_1 subunit onto the membrane [20]. The α_1 subunit corresponds to the pore-forming segment of LTCC to allow the passage of Ca^{2+} ions and is composed of approximately 2000 amino acids (AAs). The other components serve as auxiliary subunits and modify the function of the channel. For example, the β subunit and $\alpha_2\delta$ -subunit accelerate the activation and deactivation kinetics of the channel and significantly increases the maximal-conductance of ionic current [21]. The β subunit, which lacks the membrane-spanning region, is localized on the intracellular region of the channel. The α_2 and δ subunit, although expressed by a single gene, are cleaved into two separate proteins during post-translational modification resulting in a glycosylated extracellular α_2 and a smaller membrane-spanning δ subunit that are held together ($\alpha_2\delta$ -subunit) by a disulfide bond. The transmembrane γ -subunit, another component of LTCCs has not been found in CaV1.2 and CaV1.3 of the cardiac cells [13]. The γ -subunit has not been extensively studied because of their relatively limited distribution and trivial functional roles. **Figure 2** shows the arrangement of CaV subunits.

2.2. LTCC: sequence and splice variants

The pore-forming α_1 -subunits are expressed by 10 genes, the CACNA1S (CaV1.1 α_1), CACNA1C (CaV1.2 α_1), the CACNA1D (CaV1.3 α_1), the CACNA1F (CaV1.4 α_1), the CACNA1A (CaV2.1 α_1), the

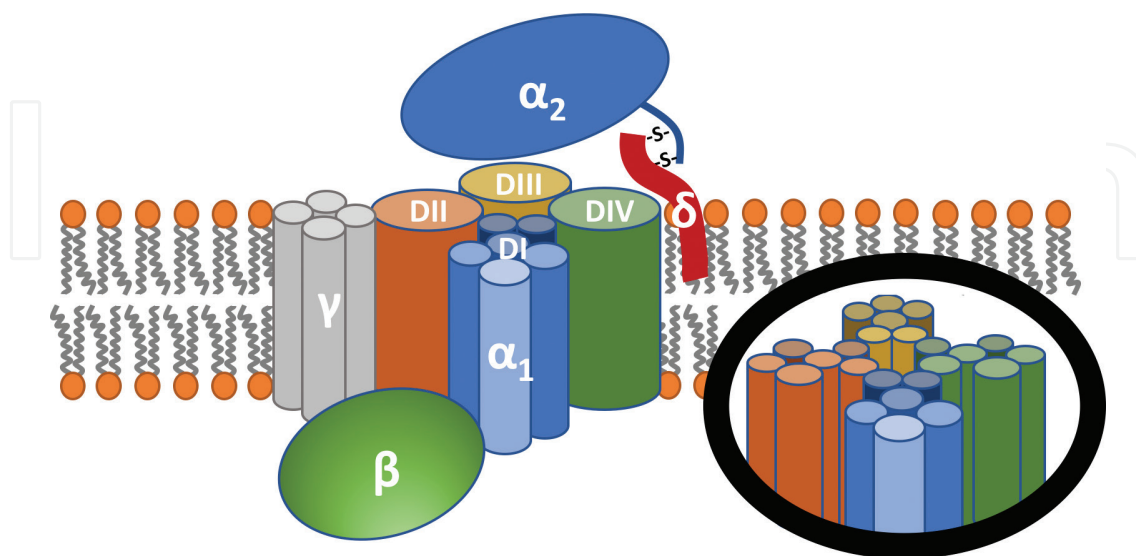


Figure 2. The LTCC complex. The pore-forming transmembrane α_1 -subunit, the intracellular β -subunit, the extracellular α_2 -subunit co-linked with the transmembrane δ -subunit, and the transmembrane γ -subunit are shown [3, 13].

CACNA1B (CaV2.2 α_1), the CACNA1E (CaV2.3 α_1), the CACNA1G (CaV3.1 α_1), the CACNA1H (CaV3.2 α_1), and the CACNA1I (CaV3.3 α_1). The members of the three families (CaV1, CaV2, and CaV3) share high sequence similarity (above 80%). In particular, the CaV1 and CaV2 families have relatively high sequence similarity, when compared with that of the LVA CaV3 family. All these channels have large numbers of potential splice variants expressed in different tissues [12]. The splicing sites are mainly distributed in the structurally flexible regions, such as N-terminal, C-terminal, and linkers between the transmembrane domains. They contribute to regulation of genes, gaining diversity in proteins, and in fine-tuning the physiological functions of the channel.

2.3. Domain organization

The LTCC polypeptide forms a heterotetramer and includes the pore-forming transmembrane α_1 -subunit, the intracellular β subunit, and an extracellular $\alpha_2\delta$ subunit. Most of the pharmacological and gating properties of LTCCs are accomplished by their α_1 -subunits. The structural topology of the α_1 -subunits is highly conserved among the members of the LTCCs and is made up of the cytoplasmic N- and C-terminal domains and four intervening transmembrane domains (DI-DIV). Each transmembrane domain is composed of six transmembrane α -helices (S1–S6), where S1–S4 helices are known as the voltage sensing domain (VSD), and S5–S6 forms the pore domain [22]. VSD detects the changes in the membrane potential and PD helps in the selective passage of calcium ions through the channel pore. The S4 helix of the VSD encompasses several conserved positively charged residues, whereas, the S1–S3 helices are dominated by negatively charged amino acids. When the membrane is depolarized, the movement of the S4 helices is transmitted to the cytoplasmic ends of the S5 and S6 helices, through the S4–S5 linkers, resulting in the opening of the activation gate formed by the S6 helices on the inner side of the channel [3, 13].

The membrane-associated P-loop in each domain between the two helices, S5 and S6, form the selective filter of the channel. The selectivity of calcium channels relies on the P-loops domains and their calcium ion binding sites. The selectivity filter of VGCC includes conserved glutamate residues (E–E–E–E) in the P-loop region [5]. Their side chains can restrain Ca²⁺ at the right coordination and let Ca²⁺ enter into the pore region. The recent research identified three aspartic acid residues along the selectivity filter from extracellular to intracellular. Amino acid substitution and crystallization, has helped in locating the three binding sites for the Ca²⁺ ions [5]. Although the bacterial calcium channel is different from the mammalian LTCCs in their amino acid sequence and structural features, the structure of CavAb has provided valuable insights into calcium ion selectivity conferred by the selectivity filter.

The N-terminus and C-terminus region of LTCC are both located in the cytosolic space. Although the major sequence of the N-terminus is composed of random loops, it also includes a calmodulin interaction domain, known as N-terminal spatial Ca²⁺ transforming elements (NSCaTE) [23]. The length of the C-terminus is much longer than N-terminus and contains several binding sites for various proteins that modulate the LTCCs activity (shown in **Figure 3**). Proteolytic cleavage of the C-terminal domain generates two fragments, the proximal C-terminal regulatory domain (PCRD) and the distal C-terminal regulatory domain (DCRD). The upstream sequence of the cleaved site contains the PCRD, IQ domain, pre-IQ domain, and the EF-hand motif. This region is important for Ca²⁺/CaM binding and regulation.

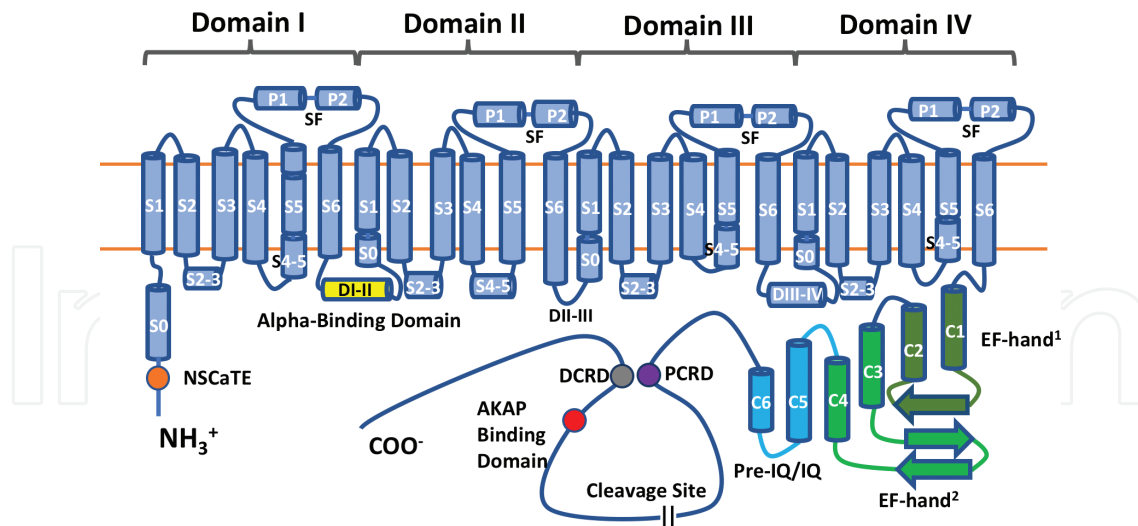


Figure 3. The secondary structure topology of the α_1 -subunit of LTCCs. The N-terminal domain is followed by four homologous transmembrane domains and the C-terminal domain. Each of the transmembrane domains is made of six helices and a membrane associated P-loop. The orange, purple, red, and gray dots indicate the location of NSCaTE, PCRD, AKAP binding domain, and DCRD, respectively [3, 22]. The linker of DI and DII, colored in yellow, is the alpha-binding domain (ABD). The sequence from C1 to C2 and from C3 to C4 shows the two EF-hand motifs. Sequence from the end of C4 to the end of C6 colored in light blue is pre-IQ and IQ domain. The cleavage site is located in the sequence between DCRD and PCRD. The secondary structure is based on the PDBsum database.

The downstream sequence from the cleavage site includes the A-kinase-anchoring-protein (AKAP) binding domain (ABD) and DCRD [3]. When the DCRD is proteolytically cleaved, the cleaved fragment can remain non-covalently bound to the PCRD, thus allowing the two regions of the C-terminal domain to interact with each other and perform the auto-inhibitory function for the LTCCs [24]. The DCRD serves as an effective auto-inhibitory domain for the LTCCs or as a transcriptional modular when it enters the nucleus [24]. The ABD of the distal C-terminus plays a vital role in PKA-induced phosphorylation of the DCRD. The AKAP binds with the ABD and helps PKA identify the phosphorylation sites in the cleaved fragment. The phosphorylation shuts down the auto-inhibition of LTCC and facilitates the Ca^{2+} influx [13].

Coexpression and co-assembly of $\text{CaV}\beta$ and $\text{CaV}\alpha_2\delta$ subunits with $\text{CaV}\alpha_1$ have a significant role in LTCCs trafficking [25]. The $\text{CaV}\beta$ subunit, which belongs to the membrane-associated guanylate kinase (MAGUK) protein family is composed of three domains similar to that of the MAGUK family, except for the missing PDZ in the N-terminus. The two conserved structural domains of the $\text{CaV}\beta$, the SH3 and the guanylate-kinase (GuK) like domain are linked together by a HOOK domain. The HOOK domain of the $\text{CaV}\beta$ isoforms has variable lengths and share a relatively low overall amino acid identity and plays an important role in the $\text{CaV}\beta$ interaction with other proteins [26]. Similar to the DCTD, the HOOK domain possesses sites for phosphorylation and alters the conduction state of LTCCs. The $\text{CaV}\beta$ subunit interacts with the 18-residue long DI-II linker (or the alpha interaction domain (AID)) of the α_1 subunit. The α -binding pocket (ABP), a hydrophobic groove formed by the surrounding α -helices, in the GuK domain of the $\text{CaV}\beta$ subunit interacts with the AID [27]. The high-affinity association between AID and ABP markedly influences the cell surface expression of functional channels [26].

Another important co-expressed protein component of the LTCC complex is the $\text{CaV}\alpha_2\delta$ subunit. The $\alpha_2\delta$ subunit remains to be a promising target for the treatment of neuropathic pain and mutations that affect the function of $\text{CaV}\alpha_2\delta$ -1 were found to cause cardiac dysfunctions [25]. The $\text{CaV}\alpha_2\delta$ subunit is a disulfide-linked polypeptide that interacts with the α_1 subunit on the extracellular space through its' α_2 segment, while the δ segment serves as an anchor fixing the subunit to the membrane. The $\text{CaV}\alpha_2\delta$ contains a similar domain arrangement to various plasma proteins, which includes Von Willebrand factor type-A (VWFA) and the calcium channel and chemotaxis (CACHE) domain [28]. The VWFA domain found in $\text{CaV}\alpha_2$ promotes the trafficking of the α_1 subunit to the membrane and acts as a receptor for the extracellular ligands, such as thrombospondins. This VWFA domain also contains a metal ion-dependent adhesion site (MIDAS), which allows precise coordination of the VWFA domain with bound protein ligand [29]. Mutation of this site can result in the loss of $\text{CaV}\alpha_2\delta$ subunits' regulatory function to the $\text{CaV}1.2$, $\text{CaV}2.1$, and $\text{CaV}2.2$. Nevertheless, the $\text{CaV}\alpha_2\delta$ subunit can still help in trafficking the $\text{CaV}\alpha_1$ subunit to the cytoplasmic membrane. The CACHE domain is located at the downstream sequence of VWFA domain in the extracellular side. This domain is known to have a possible role in small-molecule recognition [21, 28].

3. Three-dimensional structures of LTCCs

Elucidating the three-dimensional (3D) structure of membrane proteins is challenging due to their intricate environmental conditions. Until now, there are no complete 3D structures available for the human voltage-gated calcium channels. The 3D structures of two specific regions of VGCCs in complex with their auxiliary subunits have been resolved, the AID- $\text{CaV}\beta$ complex and the IQ domain-calmodulin ($\text{Ca}^{2+}/\text{CaM}$) complex. Recently, the structure of *Arcobacter butzleri* calcium channel (CaVAb) and the mammalian $\text{CaV}1.1$ were determined using crystallography and cryo-electron microscopy (EM) techniques, respectively. These structures have provided significant insights on the ion selectivity and drug-binding sites in the calcium channels.

3.1. The AID- $\text{CaV}\beta$ complex

The crystal structures of three isoforms of $\text{CaV}\beta$ have been resolved in complex with the AID (i.e., short polypeptides from the DI-II linker of $\text{CaV}\alpha_1$) from different species [30]. The 2.2 Å resolution structure of rabbit $\text{CaV}\beta_2$ isoform was crystallized in complex with an 18-residue long polypeptide, corresponding to the AID of $\text{CaV}1.1\alpha_1$ (PDB ID: 1T3L). The core region of rat $\text{CaV}1.2\beta_3$ isoform was crystallized (PDB ID: 1VYV) with a polypeptide (49 AAs) at 2.6 Å resolution. Chen et al. crystallized the single structure of $\text{CaV}\beta_4$ isoform at 3 Å resolution (PDB ID: 1VYU) [30]. Their core structures, which includes the SH3 and GuK domains, exhibit high similarity. A chimeric complex of rat $\text{CaV}\beta_2$ isoform and first 16 residues of human $\text{CaV}1.2$ AID region was crystallized at 1.97 Å resolution (PDB ID: 1T0J) [26]. Mutation analysis showed that three $\text{CaV}1.2$ AID residues, Tyr447, Trp440, and Ile441 are important for the interaction between the $\text{CaV}\beta$ subunit and the AID [26, 30].

In 2012, a 2.0 Å resolution crystal complex of rabbit CaV1.2 DI-DII linker and CaV β_2 isoform was determined [27]. Not until recently, the 3D structure of the last isoform of CaV β subunit, the CaV β_1 , has been identified in a complex with the complete cryo-EM model of rabbit CaV1.1. The mechanism that CaV β regulates CaV α_1 is achieved through the transmitted motions of DI-S6. Before association with CaV β , AID is in a coil-type structure. The CaV β acts as a chaperone and helps AID undergo a coil to helix transition during the binding [31]. The α -helix of AID propagates the upstream sequence of DI-S6. They form a rigid connection between the GuK domain of the CaV β and the channel pore, and mechanically transduce their binding to channel gating states [30]. The N-terminus of the CaV β is anchored to the membrane, which restricts the motion and orientation of the CaV β binding to the AID and connecting the DI-S6 segment. These coupled motions help CaV β effectively regulate the gating properties of calcium channel.

3.2. The IQ domain-calmodulin (CaM) complex

Calmodulin (CaM) is a small and conserved calcium-binding messenger protein that plays an essential role in all the HVA channels. In the case of LTCCs, binding with Ca²⁺/CaM is known to pronounce calcium-dependent inhibition of the channel current. Calmodulin, being localized in the cytosolic region, detects the changes in the levels of intracellular Ca²⁺ and modulates the interaction of LTCCs with other proteins. Four EF-hand motifs distributed equally on the N- and C-terminus of the CaM works as the calcium ion sensor. Each of EF-hand motifs is composed of two alpha helices and is connected by a flexible loop with the Ca²⁺ binding site located in the middle. The Ca²⁺/CaM has a higher binding affinity to LTCC and therefore associates with the LTCC complex even at low cytoplasmic Ca²⁺ concentrations. The IQ domain and the pre-IQ domain, upstream sequence of the IQ domain, serve as the binding site for the calmodulin. CaM is known to play a regulatory role in the calcium-dependent inactivation of LTCCs. However, the trafficking function of Ca²⁺/CaM remains controversial, due to inconsistent results in different expression systems [32]. In hippocampal neurons, CaV1.2 trafficking to the distal dendrites is accelerated by the presence of Ca²⁺/CaM, and not by the apo-CaM [33].

From 2005 to 2012, several structures containing a short polypeptide from CaV1.1 or CaV1.2 and calcium-bound calmodulin (Ca²⁺/CaM) were determined. In 2005, three structures of the CaV1.2 IQ domain bound to the hydrophobic pocket of the Ca²⁺/CaM protein were resolved [34, 35]. In those complexes, Ca²⁺/CaM exists in a 2:1 ratio with the IQ domain [36]. IQ domain engages itself in the hydrophobic pockets, present in the N-terminal and C-terminal Ca²⁺/CaM lobes, through sets of conserved 'aromatic anchors'. In the CaV1.2, three residues (Tyr1627, Phe1628, and Phe1631) downstream of IQ domain bind the hydrophobic Ca²⁺/C lobe pockets. The three upstream residues (Phe1618, Tyr1619, and Phe1622) bind the Ca²⁺/N lobe pockets [34]. The lengths of CaV1.2 α_1 IQ domains vary among the resolved structures. For example, the 3D structures of human IQ domain have been resolved with 37 residues (PDB ID: 2BE6), and 21 residues (PDB ID: 2F3Z, PDB ID: 2VAY) [37], and 21 residues from *Cavia porcellus* (PDB ID: 2F3Y). In 2009, Fallon et al. resolved the extended structure of IQ domain to include the pre-IQ domain, which comprised of 77 residues from human CaV1.2 C-terminus (PDB ID: 3G43) [38]. In 2010, the structure of PreIQ and IQ domain from human CaV1.2 containing 78 residues (PDB ID: 3OXQ) was crystallized in complex with Ca²⁺/CaM at 2.55 Å resolution [36]. In 2012, Liu and Vogel reported a novel-binding motif (NSCaTE) from N-terminus of CaV1.2

and CaV1.3 to have a higher affinity for binding Ca²⁺/CaM when compared to that of the binding region in C-terminus [23]. Using NMR, they reported the 3D structure of a 24-residue long NSCaTE motif in complex with the Ca²⁺/CaM (PDB ID: 2LQC). Until now, the Ca²⁺/CaM complex structure has only been resolved with CaV1.1 and CaV1.2 of the LTCCs. Although CaV1.4 binds to Ca²⁺/CaM, their interaction has not been reported to have any functional regulation.

3.3. The structure of bacterial CaV channel

In 2014, the first structure of a bacterial calcium channel (CaVAb) was resolved by performing specific mutations on the *Arcobacter butzleri* sodium channel (NaVAb) [5]. The quaternary structure of CaVAb is a symmetrical homo-tetramer, which is similar to its NaVAb prototype. Four identical domains assemble to form the main structure of the channel, with each of the domain (containing 237 residues) encompassing six transmembrane helices. The topological features of the transmembrane domain of the CaVAb are similar to that of the LTCC. Tang et al. performed several mutations to elucidate the structural basis of Ca²⁺ selectivity and reported the crystal structures of 13 variants that conferred different mutations in NaVAb.

Each monomer is composed of a voltage-sensing domain (S1–S4) and a pore-forming domain (S5–S6). Four positively charged arginines in the voltage-sensing domain detect the changes in the membrane potential. The voltage-sensor movements are transmitted to the pore-forming domain through a cytoplasmic linker that connects the S4 and S5 helices. Three negatively charged aspartate residues at the selectivity filter (Asp177, Asp178, and Asp181) were found to be essential for binding the Ca²⁺ ion and render selectivity to the channel. The paper revealed that the ion-selective mechanism is based on three Ca²⁺ binding sites, site-1 (Asp178), site-2 (Asp177, Leu176), and site-3 (Thr175). A single substitution at site-177, from Glu to Asp, enhanced the calcium selectivity by 1000 times over sodium, which was sufficient to convert the sodium channel to calcium channel. Although 181D is not directly involved in Ca²⁺ coordination and lies outside of the ion-conducting pore, it generates an electronegative environment to attract the extracellular cations. Binding of one Ca²⁺ blocks the pore and prevents the entry of the monovalent cations. The entry of second Ca²⁺ induces electrostatic repulsion on the first Ca²⁺, thereby forcing it to flux into the cytoplasm. Thus, the extracellular calcium ions fluently permeate into the intracellular side in response to the concentration gradient [5].

3.4. Structure of CaV1.1

In 2015, Wu et al. reported the complete structure of the mammalian CaV1.1 complex at 4.2 Å resolution using the cryo-EM technique [39]. Three auxiliary subunits were isolated from the rabbit skeletal muscle, the pore-forming α_1 -subunit, the extracellular $\alpha_2\delta$ -subunit, and the transmembrane γ -subunit. The fourth auxiliary subunit was included in the complex by docking the crystal structure of rat CaV β_2 (PDB ID: 1T0J) on the AID of CaV1.1 α_1 subunit. Following this complex, two rabbit CaV1.1 complexes at resolution 3.9 Å (PDB ID: 5GJW) and 3.6 Å (PDB ID: 5GJV) were reported [40]. This CaV1.1 construct included 1873 amino acid residues. While the 3D coordinates of most parts of the CaV1.1 α_1 -subunit were resolved, some of the cytoplasmic (N-terminus: 1–31, DI–DII linker: 377–416, DII–DIII linker: 670–787, and C-terminus 1516–1873) and extracellular segments (DI S3–S4: 140–160, DIII S3–S4: 886–891, and DIV S3–S4: 1206–1228) were found to be missing (Figure 4).

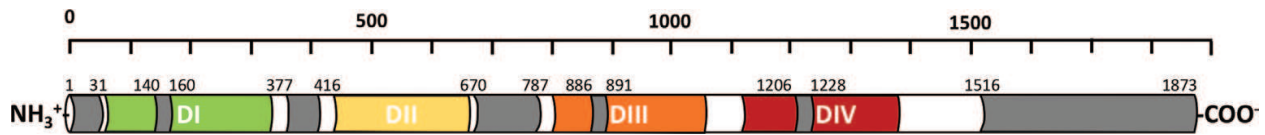


Figure 4. The missing residues in the rabbit CaV1.1- α_1 structure (PDB ID: 5GJV) shaded in gray.

The rabbit CaV1.1 is composed of four inter-connected homologous domains, each of which includes the voltage-sensing and pore-forming domain. The S4 helix of the VSD is composed of six charged residues when compared to four residues in human CaVs [41]. Remarkably, the asymmetric pore-region of CaV1.1 is formed by the four S5, and S6 bundles and the tightly packed inner gate showcased a closed conformation and inactivated conduction-state of the CaV1.1 channel. The auxiliary CaV α_2 subunit included four tandem cache domains and one VWA domain. The cysteine residues, Cys1074 in CaV δ and Cys406 in CaV α_2 formed a disulfide bond at the binding region between VWA domain and CaV δ . In the VWA domain, the MIDAS residues (Ser263, Ser265, Asp261, Thr333, and Asp365) and CaV α_1 DI S1–S2 residue (Asp78) are bound to a calcium ion. Both the previous and latest 3D structures identified for the CaV γ subunit included four transmembrane α -helices, however, in this CaV1.1 structure, additional extracellular β -sheets have been resolved together with the regions of the two termini. The second and third transmembrane-helices in CaV γ and DIV S3–S4 in CaV α_1 are directly involved in interactions through hydrophobic forces. The Cryo-EM structure of the rabbit CaV1.1, have thus brought novel insights on the multi-domain structure of VGCC, especially the association of CaV with the auxiliary proteins.

3.5. Computational modeling

Computational modeling and simulation remain to be a promising technique to reveal fundamental biological mechanisms, biomolecular interactions and predicting the effects of modulators. In CaV, modeling-based studies were previously performed to understand how LTCC blockers bind the calcium channel [42–44]. Tikhonov and Zhorov generated homology models of the open- and closed-state conformation of the pore-forming domains of CaV1.2 using the crystal structure of the KvAP and KCSA channels as the template [45]. The generated models were used to dock three types of LTCC blockers, benzothiazepine, phenylalkylamine, and dihydropyridine. The docking analysis showed that the all the three ligands bind near the S5–S6 helices of domain III and IV and the CaV residues, tyrosine in S6-DIII, tyrosine in S6-DIV, and glutamine in S5-DIII, are important for binding these ligands close to the pore domain of the channel. Since no experimentally-resolved structure of small molecule-CaV was available, *in silico* the docking analysis performed in this study provided useful insights for understanding ligand-binding in CaV.

Adiban et al. used the structure of the CaVAb (PDB ID: 4MVQ) to model the selectivity filter of the CaV with defined charges. They performed molecular dynamics (MD) simulation to calculate the potential of mean force and showed that the affinity for Ca²⁺ in site-2 (Asp177, Leu176) is higher than that within the two other sites, site-1 (Asp178) and site-3 (Thr175). Their study also showed that, in the absence of calcium ions, the CaVAb could allow the passage of Na⁺ ions, but not Cl⁻ ions [46]. This study using the structure of CaVAb, was helpful in understanding the structure–function relationships of the calcium channel.

All of the computational models built for CaV until now were based on templates with low sequence identity (<30%). Studying molecular systems built with low-identity templates is quite challenging since the accuracy of the model is highly dependent on the similarity between the template and the target protein [47]. While the oligomeric structures of bacterial homologs are useful for modeling the transmembrane domains (TMDs), building the large intracellular domains that connect the TMDs using these structures is not feasible. With the availability of the CaV1.1 complex and other structural data, obtaining better quality homology-based models for the human CaV channels, especially the LTCCs, is now possible. Nevertheless, sophisticated methods and high-performance computing would be needed for modeling the multi-domain architecture of the human CaVs. Building these models can be helpful in understanding the structure-function-dynamic properties, the Ca²⁺ influx mechanisms and effects of small molecules on these channels [48, 49].

4. Activation mechanisms of LTCCs

LTCC, being a voltage-gated ion channel, remains mostly sensitive to the changes in the membrane potential and the VSD (S1–S4 helices) of the LTCCs play a crucial role in sensing the voltage changes across the membrane. In addition to the voltage-dependent gating mechanisms, the channel's conductivity also depends on the intracellular Ca²⁺ concentration, and thus is modulated by both self-regulatory and extrinsic mechanisms.

4.1. Voltage-dependent activation/inactivation mechanism

The cardiac action potential is a classic example of the voltage-dependent mechanism. During the action potential, the ions channels undergo several conformational transitions and regulate the exchange of ions across the membrane. When the intracellular gates of the channel are open, the channel is referred to be conducting. The smooth passage of ions through the open-pore of the channel generates the electrical current across the membrane. When the membrane depolarizes, the inactivation gates of the channel are closed, while the intracellular gates are still open to allow the decay of current levels. Inactivation is a way to decrease the availability of the open-state of the channel at more depolarized membrane potentials [50]. The closed state of the channel corresponds to the closed intracellular gates, which hinders the ion passage, and results in a non-conducting state.

The action potential can be divided into five phases (Phase 0–4, shown in **Figure 5**) and the concerted activities of various ion channels in the heart help in maintaining the cardiac rhythm. In simple terms, at phase 0, the membrane is initially at the resting potential (–90 mV), where the LTCCs are in a closed-state (no Ca²⁺ ion passage). When depolarization occurs, and the membrane potential reaches a threshold voltage of –70 mV, the inward sodium ion (Na⁺) channels are activated allowing the flow of the I_{Na} current. This current flow further increases the membrane potential to a more positive value and reaches a peak, when the activation of LTCCs are initiated, and the sodium channels are inactivated. During phase 1, early and rapid repolarization occurs by the brief activation of K⁺ channels and the LTCCs remain in a pre-open state. After phase 1, the opening of the LTCCs (open/activated state) slows the

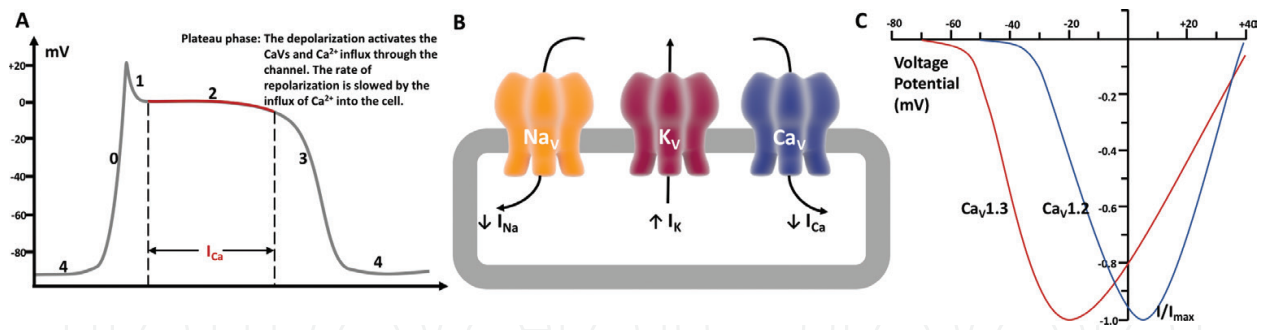


Figure 5. (A) Cardiac action potential (left) [54]. (B) Activities of three types cation channels during phase 2. The inward sodium and calcium current decays with time; the outward potassium current is activated allowing the cell to move to resting state [55]. (C) $Ca_V1.2$ and $Ca_V1.3$ voltage potential amplitude. Plot re-generated from Xu and Lipscombe data [56].

repolarization down. This phase of the cardiac action potential, the phase 2, is called a plateau phase and is maintained by the balancing act of the Ca^{2+} and K^+ ions (shown in **Figure 5**). This Ca^{2+} influx that occur during phase 2 initiates the contractile function of the cardiac cells. Close to the end of phase 2, when more Ca^{2+} ions are released an auto-inhibitory signal is triggered resulting in a non-conducting state or closed state of the calcium channel.

During phase 3, the cell tries to return to the resting state by the gradual inactivation of the calcium channels (inactivated state) and continued efflux of K^+ ions [51]. At the end of phase 3 inward rectifying K^+ channels are activated to reset the membrane potential to the resting state. The last phase of the action potential, in phase 4, the membrane returns to resting potential (-90 mV), which allows the LTCCs to transit from the inactivated state to the closed state for the next cycle of events. This resting potential of the membrane is maintained by the continued leak of the K^+ ions.

Given the tissue-specific localization of the members of LTCCs and their different functional roles relevant to the region of expression, these channels also have different activation threshold and kinetics. For example, the $Ca_V1.1$ and $Ca_V1.2$ require a depolarized threshold of $+7$ and -30 mV, for their activation [2, 52]. The $Ca_V1.3$ and $Ca_V1.4$ do not require a depolarized threshold as strong as $Ca_V1.1$ and $Ca_V1.2$, to be activated and have relatively low activation thresholds of about -55 and -40 mV (**Figure 5**), respectively. Similarly, $Ca_V1.3$ channels are known to activate with fast kinetics when compared to that of the $Ca_V1.1$ [53]. Although $Ca_V1.3$ is closely related to $Ca_V1.2$, it seems to share more similar properties with $Ca_V1.4$, including rapid activation kinetics, low activation threshold, and lower sensitivity to 1,4-DHPs [53].

4.2. Calcium-dependent activation/inactivation mechanism

The calcium-dependent channel regulation process requires the participation of multiple segments, such as the β -subunit [57], Ca^{2+}/CaM [58], CaMKII [59], N-terminal and C-terminal regions of the LTCC $Ca_V\alpha_1$. Calcium-dependent inactivation serves as the autoinhibitory control for the LTCCs to control the levels of intracellular calcium. It is mediated by the interactions between the Ca^{2+}/CaM and Ca_V pre-IQ/IQ domains. Disruption to this interaction has

been known to attenuate the calcium-dependent inactivation process. At the resting potential, apo-CaM associates with the alpha-subunit of the channel. When Ca^{2+} ions bind the CaM, the fully charged CaM with four Ca^{2+} ions allow the two polarized lobes of the CaM, the Ca^{2+}/N lobe, and the Ca^{2+}/C lobe, to envelope the alpha-binding helix. Specific interactions of two lobes with the IQ domain initiate different calcium-dependent regulations. For example, in CaV1.2 and CaV1.3, CDI is caused by the interaction between the IQ domain and the Ca^{2+}/C -lobe, while CDF is facilitated by the interaction of the Ca^{2+}/N -lobe and the IQ domain [24, 35]. In contrary, the IQ domain interactions with the Ca^{2+}/N -lobe and the Ca^{2+}/C -lobe in the CaV2.1 channel, lead to CDF and CDI, respectively [60, 61].

Ferreira et al. [62] reported that calcium-dependent mechanisms could speed up the inactivation process. They used barium in the place of calcium ion and found that the channels undergo rapid activation and slow inactivation due to the lack of intracellular calcium. Their result showed that voltage-dependent mechanisms alone in the absence of calcium-dependent mechanisms would lead to slower inactivation [24, 53]. While CaM promotes calcium-dependent inactivation, the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) counteracts the above process and helps in the re-activation of the channel in a calcium-dependent way. CaMKII phosphorylates the $\text{CaV}\beta$ subunit and the C-terminus of $\text{CaV}\alpha_1$ at their specific phosphorylation sites, resulting in the disruption of CaM-CaV channel interactions. Increase in the intracellular calcium ion level activates the CaMKII and reduces the effects of CDI [12, 59]. CaMKII enables the channel gates to be frequently left in the open state for a long time thus, prolonging the plateau phase of the action potential at high frequency.

5. L-type calcium channels modulators

The LTCCs are considered as an important target for the treatment of various diseases [9, 63–66]. CaV1.1, the major isoform of the skeletal LTCCs is reported to correlate with hypokalemic periodic paralysis, which is characterized by muscular weakness or paralysis [63]. CaV1.2 and CaV1.3, being more expressed in the heart and the brain, their dysfunction results in severe disease states, such as Timothy's syndrome, cardiac arrhythmia [6], bipolar disorder, and autism [64, 65]. Any abnormality in the cardiac LTCCs leads to long-QT syndrome (LQTS), where the QT interval of the cardiac action potential is prolonged, a condition that causes heart arrhythmias or sudden cardiac death (SCD) [67]. The Timothy Syndrome (TS), is an extremely rare multisystem LQTS subtype, that is mainly caused by the dysfunctions of LTCC and Ca^{2+} handling proteins. As the only LTCC subtype in the retinal cells, mutations in the CaV1.4 gene are known to weaken the normal visual functions and cause night blindness. Modulating LTCCs, therefore, remains to be an important avenue for the treatment of several diseases.

5.1. Small molecule modulators

Fleckenstein showed that small organic molecules, like verapamil, specifically inhibited the Ca^{2+} current from LTCCs [66]. Since then, several small-molecule modulators of the LTCCs

have been identified. Most LTCC drugs can be grouped into one of these three groups, phenylalkylamines, benzothiazepines, and dihydropyridines [9]. All LTCCs exhibit a similar pharmacological profile upon treatment with the drug, including the high-affinity frequency-dependent block [9]. These three classes of drugs bind to three allosterically linked receptor sites on the LTCCs and block the inward calcium current [68]. Previous studies showed that these three classes of drugs bind at three distinct sites, however with overlapped binding domains [43, 69]. All three binding sites are close to the pore and are located within the S5 and S6 helices of DIII and S6 helix of DIV [68]. Tikhonov and Zhorov choose the KvAP and KcsA crystal structure as the template to model the open state and the close state of CaV1.2, respectively. Based on this template, they investigated the binding mode of dihydropyridine [43] and benzothiazepine [42] and confirmed their binding in this region. However, the co-crystallized structure of CaVAb-verapamil complex suggests that the verapamil-like phenylalkylamines can bind within the pore of the channel [6].

5.1.1. DHPs and related drugs

The 1,4-dihydropyridine (1,4-DHP) is an effective and specific LTCCs blocker that is commonly used for the treatment of cardiovascular diseases, such as vasodilation, angina, and hypotension [45, 68]. DHP-derived drugs, such as amlodipine, clevidipine, and felodipine are also used for the treatment of cardiac diseases [7]. Nimodipine, another DHP-based drug, regulates the LTCCs distributed in neurons and helps in improving the outcomes of neurological treatments [7, 65]. Most DHP-based antagonists prefer binding to the inactivated states of the channel and stabilize them to block the Ca²⁺ influx. Since most 1,4-DHPs are lipophilic, they also tend to bind on the outer surface of the channel facing the lipid molecules and form interactions with the S6 of DIII and DIV [53]. Four DIII residues (Tyr1152, Ile1153, Ile1156, Met1161) and one DIV residue (Asn1472) were found to be important for binding DHP-based drugs. Amino acid substitutions on the residues mentioned above were found to cause more than a five-fold decrease in the binding affinity of these compounds. Also, substitutions in the DIII and DIV residues of CaV1.2 (Phe1158, Phe1159, Met1160, Tyr1463, Met1464, Ile1471) are known to cause the about two- to fivefold decreases in the binding affinity [69].

5.1.2. Phenylalkylamine and related drugs

Verapamil is the prototype phenylalkylamine and is the only drug currently available from this class for clinical use [9, 68]. It is widely used for the treatment of hypertension. The significant differences in the binding affinities of phenylalkylamine towards the different conduction states of the channel show that these drugs, similar to DHPs, most likely bind to the inactivated state of LTCCs [7, 66, 68]. It has been shown that phenylalkylamine binding causes the channel to hardly recover from the repolarization [68, 69]. It is known from the co-crystallized structure of CaVAb that phenylalkylamine binds in the central cavity of the pore on the intracellular side of the selectivity filter [6]. Upon binding, the drug results in the physical blockade of the channel and thus, preventing the passage of calcium ions. The Br-verapamil interacts with the surrounding residues, including Met174, Leu176, and two Thr206, from the S6 helices of each domain.

5.1.3. Benzothiazepine and related drugs

Diltiazem, a clinically approved LTCC antagonist from the benzothiazepine class, is used for the treatment of arrhythmias. The diltiazem exhibits modest selectivity for LTCCs in vascular smooth muscle over cardiac muscle. Similar to the other two types, benzothiazepine tends to bind the inactivated state of LTCCs and share similar binding site as that of the 1,4-DHPs. Based on the photoaffinity labeling experiments, the binding sites of diltiazem was located within the S6 of DIII and DIV. Specific amino acid residues, Tyr1463, Ala1467, and Ile1470 that are present in the S6 helix of the DIV have been identified to be important for benzothiazepine blocking [68].

In addition to the α_1 -subunit, the $\alpha_2\delta$ -subunit of LTCCs are also considered as a promising target for regulating the channel functions [70]. Pregabalin and gabapentin are $\alpha_2\delta$ -subunit targeting small molecules that are being used for the treatment of chronic neuropathic pain [9, 29]. It has been shown that these two molecules are sensitive only to $\text{CaV}\alpha_2\delta_1$ and $\text{CaV}\alpha_2\delta_2$ and not to other isoforms of $\text{CaV}\alpha_2\delta$ [21]. These drugs work through the association of $\text{CaV}\alpha_2\delta$ and $\text{CaV}\alpha_1$ and inhibit the calcium channel activity in two ways. First, the association of $\text{CaV}\alpha_2\delta$ and $\text{CaV}\alpha_1$ increases the current amplitude. Binding with ligands inhibits this effect. Second, these drugs modify the channel activation by affecting the channel surface trafficking [71]. Interactions with ligands decrease the $\alpha_2\delta$ -subunit surface expression and LTCC trafficking, which in turn reduces the overall calcium inward currents [70]. Using alanine-scanning mutagenesis these drugs have been identified to bind to an arginine residue (Arg217) on the extracellular side of the channel. Due to their extracellular binding characteristics, these drugs are not required to enter the cell to bind their target, which makes them an attractive alternative therapy for the treatment of neurological diseases [7]. **Table 2** summarizes the binding region, targeted disease, and chemical structure of LTCC targeting drugs.

5.2. Peptide modulators

Apart from the small molecular modulators, few natural toxin proteins have been known to specifically block the LTCCs. Two groups of toxins that selectively block the $\text{CaV}2$ subfamily have been identified: the ω -conotoxin family of pore blockers and the functionally heterogeneous ω -agatoxin family of pore blockers [8]. Recently, Findeisen et al. reported a peptide-based inhibitor for the CaV . The $\text{CaV}\beta$ subunit exhibits a chaperone-like function and induces a helical conformation on the AID from its' coiled structure, upon binding. The helix-conformation of AID has a higher binding affinity towards the $\text{CaV}\beta$ subunit [27, 72]. Based on this mechanism, Findeisen et al. [57] developed a stapled meta-xylyl (m-xylyl) AID, that is compatible to bind the $\text{CaV}\beta$ subunit. The stapled peptide, having a higher helix propensity had a higher chance to bind the $\text{CaV}\beta$ when compared to that of the native AID. This peptide inhibited the interaction between the AID and $\text{CaV}\beta$ (K_d $\text{CaV}1.2$ -AID: 6.6 ± 2.0 nM, K_d AID-CEN: 5.2 ± 1.5 nM) and served as an antagonist for the calcium channel. The crystal structure of the stapled peptide in complex with the AID region of the human $\text{CaV}1.2$, and the rat $\text{CaV}\beta_2$ was resolved at 1.7 and 2.0 Å resolution, respectively (PDB ID: 5V2Q, 5V2P). Thus, peptide-based inhibitors are also of interest in modulating the CaV channel.

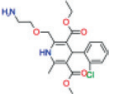
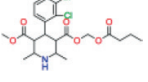
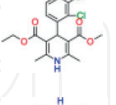
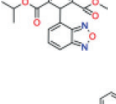
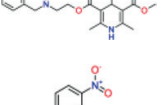
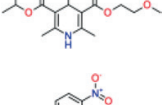
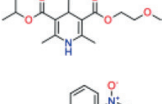
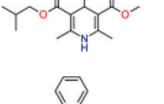
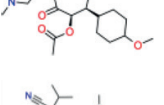
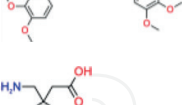
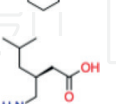
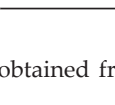
Drug	Drug class	Binding region	Targeted disease	2D-structure
Amlodipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension, chronic stable angina	
Clevidipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension	
Felodipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension	
Isradipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension	
Nicardipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension, chronic stable angina	
Nifedipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension, vasospastic angina, chronic stable angina	
Nimodipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Improve neurologic outcome	
Nisoldipine	1,4-Dihydropyridine	CaV α 1 IIIS5-S6, IIIS6, IVS6	Hypertension	
Diltiazem	Benzothiazepine	CaV α 1 IIIS6, IVS6	Hypertension	
Verapamil	Phenylalkylamine	CaV α 1 IS6, IIS6, IIIS6, IVS6	Hypertension, chronic stable angina, cluster headache	
Pregabalin	Gamma-aminobutyric acid (GABA)	CaV α 2 δ	Neuropathic pain, epilepsy, generalized anxiety disorder	
Gabapentin	Gamma-aminobutyric acid (GABA)	CaV α 2 δ	Epilepsy, neuropathic pain	

Table 2. Common L-type calcium channel inhibitors. The list of drugs and the 2D structures are obtained from the Drugbank database.

6. Discussion

Voltage-gated calcium channels (VGCCs), which are responsible for the calcium flux in cells, play a key role in many physiological processes, including neurotransmission, cell cycle, muscular contraction, cardiac action potential, gene expression, and protein modulation.

Their abnormal functions result in increased intracellular calcium levels and trigger serious pathological effects from cardiovascular and neuronal diseases to cancer. VGCCs are therefore considered as a significant target and development of isoform-specific modulators for VGCCs remain promising in pharmaceutical research.

The diverse expression patterns of the L-type and T-type channels show that these channels are pharmacologically important in several cancers, Parkinson's disease, sensory diseases and cardiac diseases. The N-type channels, although present in different organs, is known for their activity in the nervous system and are considered as a target for pain and nervous disorders. On the other hand, the P/Q-type, which is preferably expressed in the neuronal cells, is attributed to neurological diseases, such as migraine, Alzheimer's and ataxia. For several decades, non-selective calcium channel blockers have been used for targeting the calcium channels for various treatments. The FDA approval of ziconotide (N-type channel blocker) and the specificity of ω -agatoxin to P-/Q-type channels have attracted the development of more isoform-specific blockers [9]. Given the diversity in the roles and expression of calcium channels, specific targeting of these channels seems to be a promising strategy for therapeutic innovations.

7. Conclusion

In this chapter, we provide a comprehensive overview of the different types of VGCCs, especially the LTCCs, their sequence, structure, distribution, functional, and biophysical/biochemical variations. Numerous studies have reported these key features, including the structural and functional properties of different CaV channels. For instance, the recent structures of CaVAb and the rabbit CaV1.1 paved the way for understanding the structure–function connections of ion-selectivity mechanism, self-regulation, and small-molecule interactions. However, till date, the complete structures of the human CaV isoforms have not been resolved, which remains to be a hurdle for understanding the intrinsic disease-related mechanisms of the channel. With the advent and application of various technologies, such as cryo-EM, NMR, crystallography, molecular modeling, and molecular dynamics, it could be possible to resolve the complete structure of the human isoforms. Having this structural information in-hand would help in understanding the structure–function relationships of these channels, and thereby in the development of isoform-specific calcium channel modulators.

Author details

Tianhua Feng¹, Subha Kalyanamoorthy¹ and Khaled Barakat^{1,2,3*}

*Address all correspondence to: kbarakat@ualberta.ca

1 Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

2 Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada

3 Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, Alberta, Canada

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