Regulation of Ca\textsubscript{v}1.2 Current: Interaction With Intracellular Molecules

Takeshi Kobayashi\textsuperscript{1,}\textsuperscript{*}, Yoichi Yamada\textsuperscript{1}, Mitsuhiro Fukao\textsuperscript{1}, Masaaki Tsutsuura\textsuperscript{1}, and Noritsugu Tohse\textsuperscript{1}

\textsuperscript{1}Department of Cellular Physiology and Signal Transduction, Sapporo Medical University School of Medicine, South 1 West 17, Chuo-ku, Sapporo 060-8556, Japan

Received January 10, 2007

Abstract. Ca\textsubscript{v}1.2 (\(\alpha_{1c}\)) is a pore-forming subunit of the voltage-dependent L-type calcium channel and is expressed in many tissues. The \(\beta\) and \(\alpha_2/\delta\) subunits are auxiliary subunits that affect the kinetics and the expression of Ca\textsubscript{v}1.2. In addition to the \(\beta\) and \(\alpha_2/\delta\) subunits, several molecules have been reported to be involved in the regulation of Ca\textsubscript{v}1.2 current. Calmodulin, CaBP1 (calcium-binding protein-1), CaMKII (calcium/calmodulin-dependent protein kinase II), AKAPs (A-kinase anchoring proteins), phosphatases, Caveolin-3, \(\beta_2\)-adrenergic receptor, PDZ domain proteins, sorcin, SNARE proteins, synaptotagmin, CSN5, RGK family, and AHNAK1 have all been reported to interact with Ca\textsubscript{v}1.2 and the \(\beta\) subunit. This review focuses on the effect of these molecules on Ca\textsubscript{v}1.2 current.

Keywords: Ca\textsubscript{v}1.2, calcium, channel, current

Introduction

Previous investigators have shown that the voltage-dependent calcium channel is a multi-subunit complex comprising a pore-forming subunit (\(\alpha\) subunit) and regulatory/auxiliary subunits (\(\beta\), \(\alpha_2/\delta\), and \(\gamma\)) \cite{1}. Ca\textsubscript{v}1.2 is one of the \(\alpha\) subunits (\(\alpha_{1c}\)) of the voltage-dependent L-type calcium channel and is expressed in many tissues including heart, ovary, pancreas, brain, and vascular smooth muscle (2). In addition to the \(\beta\) and \(\alpha_2/\delta\) subunits, which affect the kinetics and expression of Ca\textsubscript{v}1.2, many molecules have been reported to be involved in the regulation of Ca\textsubscript{v}1.2. Previous binding studies revealed that some of these molecules could bind multiple regions of Ca\textsubscript{v}1.2 (e.g., CaM can bind to the N terminal tail, the I-II linker, and the C terminus) \cite{2}. Several different molecules bind the same region of Ca\textsubscript{v}1.2 (e.g., CaM, CaBP1, and CaMKII all bind to the N terminal tail of Ca\textsubscript{v}1.2), indicating that these molecules may compete with each other to regulate the kinetics of Ca\textsubscript{v}1.2 current. Therefore, we must consider all of these molecules to gain a better understanding of the function of Ca\textsubscript{v}1.2 current. In this review, we focus on the molecules that interact with Ca\textsubscript{v}1.2 and the \(\beta\) subunit.

Calmodulin

Calmodulin (CaM) is a ubiquitously expressed calcium-binding protein. CaM is thought to be involved in calcium-dependent inactivation (CDI), calcium-dependent facilitation (CDF), and the rundown phenomenon of Ca\textsubscript{v}1.2 current \cite{2,3}. Although several models have been proposed, the exact mechanism of the regulation of Ca\textsubscript{v}1.2 current by CaM remains unclear \cite{2}. The A, C or CB, and IQ regions within the C terminal tail of Ca\textsubscript{v}1.2 have been reported to bind CaM \cite{2,4} (see Fig. 1). Synthetic peptides or YFP-tagged proteins containing these regions bind CaM with different affinities and with varying degrees of dependence upon calcium \cite{4,5}. Using chimeric molecules comprising Ca\textsubscript{v}1.2 and CaM, Mori et al. \cite{6} showed that a single molecule of CaM binds to Ca\textsubscript{v}1.2. High-resolution crystal structure analysis revealed that the calcium/CaM-IQ region complex forms at least two conformations \cite{7}. CaM can form several different structures depending on the particular binding partner, e.g., myosin light chain, calcium/CaM-dependent kinase kinase peptide, adenylyl cyclase exotoxin, and so on \cite{2}. CaM also can bind to the N terminal tail and I-II linker of Ca\textsubscript{v}1.2 \cite{4,8}. These reports led us to speculate that CaM may form a variety

*Corresponding author. tkobaya@sapmed.ac.jp
Published online in J-STAGE: April 4, 2007
doi: 10.1254/jphs.CR0070012

Invited article
Fig. 1. Alignment of the deduced amino-acid sequence of human CaV1.2 (UniProt accession number Q13936). The bars show the putative binding regions or fragments of CaV1.2 that can interact with CaM, CaBP1, AKAP15/18, PP2A, NIL-16, sorcin, Sx1A, SNAP-25, Syt1, and CSN5. Some confusion over the numbering of the amino acid sequence of these molecules has arisen because different investigators have described channels from different species and/or different splice variants. To avoid this confusion, we have used the amino acid residue numbering of the binding region/fragments based on the amino-acid sequence of human CaV1.2 (Q13936). Black shading indicates putative domain I (amino acid residues 125 – 405), domain II (525 – 753), domain III (901 – 1186), and domain IV (1240 – 1524).
of structures in the regulation of Ca\(_{v}\)1.2 current. The rundown phenomenon is usually observed within 1–3 min of patch excision in the inside-out patch-clamp mode. This rundown phenomenon is prevented by the application of CaM and ATP to the intracellular solution within 1 min of patch excision. Application of either CaM or ATP alone could not restore channel activity (3). This report indicated that ATP plays an important role in the regulation of Ca\(_{v}\)1.2 channel activity by CaM. Future experiments will be needed to reveal the exact role of ATP in the relationship between Ca\(_{v}\)1.2 and CaM.

**CaBP1**

Calcium-binding protein-1 (CaBP1) is a member of the EF-hand superfamily of proteins and is closely related to CaM (9). CaBP1 is expressed in the brain, retina, and gastrointestinal smooth muscles, but not in cardiomyocytes (9, 10). Zhou et al. (8, 11) showed that CaBP1 can bind to the N terminus, the III-IV linker region, and the C-IQ region at the C terminus of Ca\(_{v}\)1.2. CaBP1 can compete with CaM for interaction with the IQ region. In cells transfected with Ca\(_{v}\)1.2, CaBP1 prevented CDI and caused CDF of the Ca\(_{v}\)1.2 current, although CaM promotes strong CDI of the Ca\(_{v}\)1.2 current. Deletion of the N terminus of Ca\(_{v}\)1.2 abolished the effects of CaBP1 on CDI, indicating that the interaction of CaBP1 with the N terminus of Ca\(_{v}\)1.2 is essential for Ca\(_{v}\)1.2 modulation by CaBP1. The significance of the interaction of CaBP1 with the III-IV linker region of Ca\(_{v}\)1.2 remains unclear.

**CaMKII**

Calcium/CaM-dependent protein kinase II (CaMKII), a multifunctional Ser/Thr protein kinase, has been reported to be involved in CDF and voltage-dependent facilitation (VDF) of Ca\(_{v}\)1.2 current (12–14). Hudmon et al. (12) reported that CaMKII binds to the N terminus and III-IV linker region of Ca\(_{v}\)1.2 in the presence of calcium/CaM. CaMKII can bind to the N terminus, the I-II, II-III, and III-IV linker regions and the C terminus of Ca\(_{v}\)1.2 in the presence of calcium/CaM and ATP. They concluded that the interaction of CaMKII with the C terminus of Ca\(_{v}\)1.2 is essential for CDF. In contrast, Grueter et al. (13) reported that CaMKII binds to the C terminus of the \(\beta_{2a}\) subunit and that phosphorylation of Thr498 in the \(\beta_{2a}\) subunit is essential for modulation of CDF by CaMKII. Recently, Lee et al. (14) reported that VDF of the Ca\(_{v}\)1.2 current requires the phosphorylation of Ser1512/Ser1570 of Ca\(_{v}\)1.2 (Ser1535/Ser1593 of human Ca\(_{v}\)1.2) by CaMKII. Mutation of Thr498 in the \(\beta_{2a}\) subunit to Ala did not prevent VDF. These reports led us to speculate that the mechanism of signaling through CaMKII, which mediates CDF and VDF, may involve different parts of the channel sequence or a more complicated mechanism.

**AKAPs**

A-kinase anchoring proteins (AKAPs) contain a common structural motif that tethers PKA through interaction with a dimer of kinase regulatory subunits. AKAPs bind several PKA substrates and regulate PKA-dependent phosphorylation of these substrates (15). Although more than 70 different AKAPs have been discovered, we focus here on AKAP15/18 and MAP2B, which have been reported to associate with Ca\(_{v}\)1.2.

AKAP15 and AKAP18 were independently identified as 15- and 18-kDa AKAP by Gray et al. (16) and Fraser et al. (17), respectively. However, amino acid alignment has revealed that these two proteins are identical. Thus, this AKAP will be referred to as AKAP15/18 in this review. AKAP15/18 is expressed in many tissues, including heart, brain, skeletal muscle, and lung (16, 17). Previous studies reported that AKAP15/18 contributes to cAMP- and PKA-dependent augmentation of the Ca\(_{v}\)1.2 current (17, 18). Hulme et al. (18) reported that AKAP15/18 interacts directly with the distal C terminus of Ca\(_{v}\)1.2 via a leucine zipper (LZ)-like motif. Disruption of this interaction using competing peptides inhibits regulation of the Ca\(_{v}\)1.2 current by the \(\beta\)-adrenergic receptor in rat ventricular myocytes. AKAP15/18 also has a myristoylation site at the N terminal glycine residue and two palmitoylation sites at Cys4 and Cys5, which are required for localization to the plasma membrane (17). AKAP15/18 mutated at these three residues was unable to localize to the periphery of the cell and had no effect on Ca\(_{v}\)1.2 current in response to the application of a cAMP analog. These reports led us to speculate that AKAP15/18 interacts reversibly with Ca\(_{v}\)1.2 at the plasma membrane.

Microtubule-associated protein 2 (MAP2) is a neuron-specific AKAP. Davare et al. (19) reported that MAP2b, a splice variant of MAP2, binds directly to Ca\(_{v}\)1.2. The relative contribution of AKAP15/18 and MAP2 to Ca\(_{v}\)1.2 phosphorylation in neurons remains unclear.

**Phosphatases**

As mentioned above, the anchoring of PKA by Ca\(_{v}\)1.2 is essential for efficient phosphorylation of the latter. Since phosphorylated proteins are dephosphorylated by phosphatases, it is reasonable to hypothesize that a phosphatase is localized at or near Ca\(_{v}\)1.2. Davare et al. (20) reported that protein phosphatase 2A (PP2A) binds
directly to the C terminus of Cav1.2. A co-immunoprecipitation analysis has shown that neither protein phosphatase 1 (PP1) nor protein phosphatase 2B (PP2B or calcineurin) binds to Cav1.2. Hall et al. (21) reported that a stretch of amino acid residues from 1958 to about 2000 of rabbit Cav1.2 (2011-2053 of the human sequence) might be crucial for PP2A binding. However, the results of electrophysiological studies are not always consistent with those of binding studies. Dialysis with the catalytic subunits of PP2A, but not PP1, resulted in a decrease in the L-type calcium current in native cardiomyocytes (22). In contrast, inhibitor-2, a specific inhibitor of PP1, increased the L-type calcium current, whereas fostriecin, a specific inhibitor of PP2A, did not (23). PP2B reduced Cav1.2 current in smooth muscle (24), but not in neurons (25). Phosphatases can also bind to AKAPs (15).

**Caveolin-3 and β2-adrenergic receptor**

Caveolae are flask-shaped invaginations of the plasma membrane that participate in vesicular trafficking and signal transduction in various cell types (26). Caveolins are the major structural protein of caveolar membranes, and Caveolin-3 is expressed predominantly in muscle (27). Recently, an immunoprecipitation analysis revealed that Caveolin-3, Cav1.2, β2-adrenergic receptor (not β1-adrenergic receptor), G-protein-αs, adenylyl cyclase, PKA, and PP2A form a macromolecular signal complex in cardiomyocytes (28). Silencing of Caveolin-3 mRNA using siRNA eliminated the response to β2-adrenergic stimulation of the L-type calcium current in neonatal mouse cardiomyocytes. This report led us to speculate that Caveolin-3 is necessary for the regulation of the Cav1.2 channel by the β2-adrenergic receptor. However, studies in the brain, which lacks Caveolin-3 expression (27), revealed regulation of the Cav1.2 channel by β2-adrenergic stimulation (29). Therefore, future experiments are necessary to reveal the detailed mechanism of β2-adrenergic receptor regulation of the Cav1.2 channel.

**PDZ domain proteins**

It has been shown that calcium influx via postsynaptic L-type calcium channels activates the transcription factor cAMP response element-binding protein (CREB) and CRE-dependent transcription (30, 31). Cav1.2 contains a class 1 PDZ domain-binding carboxy-terminal motif, VSX1L, that has been shown to interact with the PDZ domain (31). Weick et al. (30) reported that interactions between the Cav1.2 subunit and PDZ domain proteins are required to activate CREB efficiently in hippocampal neurons. NIL-16 (neuronal interleukin-16 precursor protein) (32) is the only PDZ domain protein that is known to interact with Cav1.2. Because NIL-16 is expressed within the hippocampus (32), the interaction between Cav1.2 and NIL-16 is proposed to be important for efficient signaling to CREB in hippocampal neurons (30). However, co-immunoprecipitation experiments examining potential interactions between Cav1.2 and NIL-16 in neurons have thus far been unsuccessful, although a GST pull-down assay provided evidence for direct interaction of these two proteins (32).

**Sorcin**

Sorcin is a 21.6-kDa calcium-binding protein that is a member of the penta EF-hand protein family and is widely distributed among mammalian tissues (33). Biochemical studies have demonstrated that sorcin binds directly to both cardiac RyRs (33) and Cav1.2 (34). Although the current-voltage relationship was virtually identical in sorcin-overexpressing TG and WT myocytes, the rapid component of L-type calcium current inactivation (tau-fast) was significantly accelerated in sorcin-overexpressing TG myocytes compared to WT controls (35). Meyers et al. (34) reported that sorcin interacted with the C terminus (amino acid residues 1622 – 1748) of rabbit Cav1.2 (1640 – 1766 in human Cav1.2). This site largely overlaps the CaM-binding domain (see Fig. 1). It is not known whether there is synergistic activity between sorcin and CaM.

**SNARE proteins and synaptotagmin**

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins are characterized by the presence of a SNARE motif, which is a coiled-coil domain of 60 – 70 amino acids critical for SNARE complex formation (36). In neurons, SNARE proteins mediate secretion of neurotransmitters from the nerve terminal (37). X-ray crystallography has revealed that the target membrane SNARE protein (t-SNARE or Q-SNARE), syntaxin 1A(Sx1A) and SNAP-25, and the vesicle membrane SNARE protein (v-SNARE or R-SNARE), synaptobrevin, can form a SNARE complex (36). Synaptotagmin I (Syt1) is localized on synaptic vesicles and is thought to be a vesicular calcium sensor (38). Wiser et al. (39) reported that Sx1A, SNAP-25, and Syt1 can bind to the II-III linker region of Cav1.2 (amino acid residues 753 – 893 in rat Cav1.2, 753 – 893 in human Cav1.2) based on a GST pull-down assay. Whole-cell Ba2+ currents mediated by Cav1.2/β2ζ/α2/δ subunits co-expressed with Sx1A and Syt1 in Xenopus oocytes are greater than those mediated by Cav1.2
The calcium current in this area is increased due to interaction of CaV1.2 with Syt1. Subsequently, Syt1 senses the increase in the calcium concentration, resulting in fusion of the synaptic vesicle with the target membrane. Recently, Cho et al. (41) demonstrated the tight association of CaV1.2 with syntaxin 2 and SNAP-23. Vendel et al. (42) reported that β1 interacts with Syt1.

**CSN5**

COP9 signalosome subunit 5 (CSN5), also known as Jun activation domain-binding protein 1 (Jab1), is thought to be a group of coactivators that stabilizes complexes of the transcription factors cJun or JunD, so as to increase the specificity of target gene activation by AP-1 proteins (43). Kameda et al. (44) reported that CSN5 interacts specifically with the II-III linker region of CaV1.2 (amino acid residues 775 – 994 in rat CaV1.2, 745 – 984 in human CaV1.2). Silencing of CSN5 mRNA using siRNA decreased the endogenous protein level of CSN5 and activated CaV1.2 current in COS7 cells. However, the physiological role of CSN5 in native cells remains unclear.

**RGK family**

Rem, Rem2, Rad, and Gem/Kir are members of a Ras-related GTPase protein subfamily (RGK family) that exhibit distinct patterns of tissue-specific expression [Rem (45), Rem2 (46), Rad (47), and Gem/Kir (48)]. They have all been shown to interact directly with the β subunit, resulting in the down-regulation of calcium channel function [Rem (45, 49, 50), Rem2 (46), Rad (45), and Gem/Kir (48)]. The AID peptide of CaV1.2 inhibited the binding of Gem to the β3 subunit. Immunofluorescence microscopy showed that co-expression of CaV1.2 and the β3 subunit with Gem/Kir or Rem2 blocked cell-surface expression of CaV1.2 (48, 51). These reports suggest that all members of the RGK family may interfere with the interaction between the β subunit and CaV1.2 and with the trafficking of CaV1.2 to the plasma membrane, resulting in a reduction of the CaV1.2 current. However, surface biotinylation studies by Finlin et al. (46, 50) demonstrated that Rem and Rem2 did not reduce the membrane expression of CaV1.2. Co-expression of CaV1.2 and the β2 subunit with Rem did not block expression of CaV1.2 on the cell surface (49). Therefore, the nature of the mechanisms regulating calcium channel activity by RGK family members remains unclear. In addition, Rad and Rem are robustly expressed in the heart, but the physiological role of Rad and Rem in the heart remains unclear (45, 47).

**AHNAK1**

Ahnak (meaning “giant” in Hebrew) is a 700kDa, 5643aa protein also known as desmoyokin (52). Hohaus et al. (53) revealed that ahnak1 is expressed in cardiomyocytes and showed that the C terminus of ahnak1 interacts with the β2 subunit. Recently, the Ile5236Thr mutation of Ahnak1 was identified in patients with hypertrophic cardiomyopathy. Haase et al. (54) showed that the application of a C terminal ahnak1 fragment (amino acid residues 5215 – 5288) containing the Ile5236Thr mutation to cardiomyocytes resulted in an increase in the calcium current as well as a slight leftward shift in its voltage dependence. These effects are similar to those of isoproterenol in normal cardiomyocytes. The Ile5236Thr mutated ahnak1 peptide induced partial dissociation of the wild-type ahnak1-fragment and the β2 subunit. PKA-mediated phosphorylation of both ahnak1 and the β2 subunit also led to the partial dissociation of the wild-type ahnak1-fragment/β2 subunit complex. Therefore, they proposed that β-adrenergic stimulation leads to partial dissociation of the ahnak1/β2 subunit complex, followed by an increase in the calcium current though CaV1.2. Stimulation of the β-adrenergic receptor stimulation in vivo by intramyocardial infusion of isoproterenol resulted in substantial phosphorylation of membrane-localized ahnak1, but not the β2 subunit (52). These reports led us to speculate that ahnak1 may be an important target of PKA-mediated phosphorylation in the enhancement of L-type calcium current by the β-adrenergic receptor.

**Summary and perspectives**

In this review, we described the molecules that can bind and regulate the CaV1.2 channel. CaM can bind to two different elements of the IQ-region of CaV1.2, indicating that binding of CaM to the IQ region may produce differential effects on the kinetics of CaV1.2 current. This model led us to speculate that other molecules in addition to CaM may have differential effects on channel kinetics following interaction with the same region of CaV1.2. CaM, CaBP1, and CaMKII can bind multiple regions of CaV1.2. This suggests that other molecules shown to bind a single region of CaV1.2 in previous reports, may also bind multiple regions of CaV1.2. Furthermore, several molecules can bind the same or closely associated regions of CaV1.2. Therefore, the CaV1.2 channel may form part of a multi-molecule
complex that is regulated by multiple signaling pathways.

Calcium is an intracellular messenger in excitation-contraction (E-C) coupling and excitation-transcription (E-T) coupling (55). In E-C coupling, a rise in intracellular calcium activates myofilaments to produce contraction. In E-T coupling, calcium regulates gene transcription and expression via the calcium/CaM-CaMK-HDAC (histone deacetylase) pathway and the calcium/CaM-PP2B-NFAT (nuclear factor of T-cell activation) pathway. The Cav1.2 channel plays a pivotal role in E-C and E-T coupling because calcium entry through the Cav1.2 channel triggers these couplings. As shown in Fig. 1, some molecules can bind the same region of Cav1.2, indicating that the binding of one molecule to Cav1.2 may result in the dissociation of another molecule from Cav1.2. Therefore, this dissociated molecule may become a second messenger that regulates the function of other molecules (excitation-regulation (E-R) coupling).

References

23. duBell WH, Rogers TB. Protein phosphatase 1 and an opposing protein kinase regulate steady-state L-type Ca2+ current in mouse cardiac myocytes. J Physiol. 2004;556:79–93.
26. Razani B, Woodman SE, Lisanti MP. Caveolae: from cell


28 Balejepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ. Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation. Proc Natl Acad Sci U S A. 2006;103:7500–7505.


40 Cohen R, Elferink LA, Atlas D. The C2A domain of synaptotagmin alters the kinetics of voltage-gated Ca2+ channels Ca(v)1.2 (L-type) and Ca(v)2.3 (R-type). J Biol Chem. 2003;278:9258–9266.


