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Disrupting Calcium Channel Expression To Lower Blood Pressure: New Targeting of a Well-Known Channel

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Voltage-gated calcium channels in vascular smooth muscle cells (VSMCs) mediate many crucial Ca^{2+} -dependent processes inherent to vascular biology including arterial contraction and gene expression. As such, they are intricately involved in the regulation of blood flow to critical organs and the determination of blood pressure levels. The VSMCs lack action potentials that form the basis for excitability in neuronal and cardiac cells. Instead, under physiological conditions, VSMCs show steady-state resting membrane potentials that fluctuate between -60 mV and -40 mV in response to vasoactive substances. Within this voltage range, membrane depolarization and the open-state probability of Ca^{2+} channels are positively and tightly coupled to regulate Ca^{2+} influx and vascular tone (1). Mathematical modeling suggests that at least several thousand pore-forming Ca^{2+} channel subunits are expressed in the plasma membrane of a single VSMC (2). The channels open but only slowly inactivate during sustained depolarization, so that Ca^{2+} influx mediated by only a fraction of the channel population appears sufficient for tonic vasoconstriction.

Although the voltage-dependent Ca^{2+} channels in VSMCs play a central role in excitation-contraction coupling in small arteries, the identity of their molecular composition is largely unknown. Similarly, there is little knowledge about the physiological factors that regulate their expression under physiological conditions and during disease states. This knowledge gap may impair our ability to formulate new therapeutic interventions for the treatment of common vascular diseases that involve Ca^{2+} channel abnormalities. For example, recent findings suggest that abnormalities of vascular Ca^{2+} channels may contribute to the development of hypertension, a condition that currently affects more than 40 million Americans (3). Thus, healthcare concerns and costs dictate the importance of identifying the structural proteins that compose the Ca^{2+} channels in VSMCs, and suggest the need to define the factors that influence their expression and function.

In this regard, studies in other cell types have firmly established that voltage-dependent Ca^{2+} channels are multi-protein complexes composed of a pore-forming α_1 subunit and accessory β and $\alpha_2\delta$ subunits (Figure 1) (4–9). The α_1 subunit confers signature properties to the channel including voltage sensing, Ca^{2+} permeability, and inhibition by Ca^{2+} channel blockers. The structure of the α_1 subunit includes four repeat domains (I, II, III, and IV) each composed of six transmembrane segments (10–11). Signaling molecules, such as protein kinase A (PKA) and protein kinase C (PKC), which are important regulators of

vascular tone, can bind to intracellular domains to modify the gating of the Ca^{2+} channel. Additionally, the N- and C-terminal regions of channel subunits can modulate channel behavior. For example, the C terminus constitutively binds calmodulin to permit Ca^{2+} -dependent inactivation of the channel (12–17).

Importantly, although ten genes are available to encode different α_1 subunits, a single $\text{Ca}_v1.2$ gene appears primarily to encode the voltage-gated Ca^{2+} channels in VSMCs that show high-threshold, long-lasting (i.e., “L-type”) Ca^{2+} currents and that are sensitive to inhibition by 1,4-dihydropyridine antagonists. Indeed, the vascular $\text{Ca}_v1.2$ (α_{1C}) gene represents a smooth muscle splice variant (α_{1C-b}) that differs in four regions from the splice variant (α_{1C-a}) expressed by cardiac myocytes (18–19). Alternative splicing of exon 8 results in amino acid differences in transmembrane segment IS6 that confer an increased sensitivity for 1,4-dihydropyridine drugs (18–19). Further alternative splicing of exon 1 results in a shortened N terminus that may permit a separate promoter to regulate selectively the expression of $\text{Ca}_v1.2$ channels in VSMCs without altering channel availability in other cell types (20–21). Not surprisingly, the α_{1C} subunit plays a key role in mediating vascular reactivity. For example, depolarization-induced contraction and myogenic tone are abolished in the arteries of mice showing smooth muscle-specific inactivation of the $\text{Ca}_v1.2$ (α_{1C}) gene. In these animals, the average systemic blood pressure was ~30 mm Hg lower than in control mice, reflecting the key role of $\text{Ca}_v1.2$ channels in mediating resting vascular tone and normal levels of blood pressure in vivo (22).

Although the α_1 subunit represents the central protein that forms the pore of voltage-gated Ca^{2+} channels, other subunits are necessary for full physiological function. As studied in most detail in neurons and cardiac myocytes, four different genes encode populations of $\text{Ca}_v\beta$ subunits (β_1 , β_2 , β_3 , and β_4) that are expressed in a tissue-specific manner (10–23–25). Although heterogeneous populations of β subunits appear to be expressed in distinct cell types, a single β subunit may critically determine the availability of functional $\text{Ca}_v1.2$ channels. For example, conventional β_1 -null mice die from asphyxia at birth, revealing the requisite contribution of the β_1 subunit to Ca^{2+} channel expression in skeletal muscle cells (26). In contrast, β_2 -null mice die of a cardiac defect in utero (27) and β_4 -deficient mice show primarily neural deficits that include a “lethargic” and “stargazing” phenotype (28). Importantly, all of the β subunits belong to the membrane-associated guanylate kinase (MAGUK) family of scaffolding proteins (29), which possess a guanylate kinase (GK) and a Src homology 3 (SH3) domain (Figure 1) (30–31). The GK region of the β subunit contains a beta interactive domain (BID) that is required for high affinity interaction with the alpha interactive domain (AID) on the intracellular I–II linker of the α_1 subunit (32–35). Other lower affinity interactive domains also have been reported (10–35–36). Importantly, all four β subunits modulate the availability of α_1 subunits at the surface membrane and influence channel properties in an isoform specific manner. As scaffolding proteins, the β subunits are thought to localize and integrate intracellular signals to influence channel behavior and drug sensitivity (30–37–39). In addition to the β subunits, four distinct $\alpha_2\delta$ subunits that arise from alternative splicing of a single gene also may interact with the α_1 subunit to influence channel expression and properties (40–41).

The identities of the β subunits and $\alpha_2\delta$ subunits that contribute to the formation of functional $\text{Ca}_v1.2$ channels in VSMCs are largely unknown. Based on the detection of many of the corresponding genes, however, a diverse composition of $\text{Ca}_v1.2$ vascular channels is anticipated [for review, see (42)]. For example, transcripts encoding all four β subunits (β_1 , β_2 , β_3 , and β_4) have been detected in VSMCs from different arteries, although only expression levels of the β_2 and β_3 proteins have been confirmed. Similarly, at least two $\alpha_2\delta$ genes have been detected. Notably, the impact of β gene deletions on the expression of vascular $\text{Ca}_v1.2$ channels has only been examined in β_3 null mice (43). In these animals, the density of voltage-gated Ca^{2+} current in aortic VSMCs was reduced by 30% compared to cells from control animals. However, the β_3 null mice showed normal levels of blood pressure, suggesting that the loss of Ca^{2+} current in the aortic myocytes did not extend to the VSMCs of the resistance vessels involved in blood pressure regulation. Alternatively, the activation of compensatory mechanisms may have restored blood pressure to normal.

Although the precise composition of $\text{Ca}_v1.2$ channels in VSMCs awaits definition, an upregulation of these channels is regarded as part of the extensive biological and morphological adaptations that characterize the vasculature during the pathogenesis of hypertension (42, 44). The initial rise in blood pressure that signals the onset of genetic or “essential” hypertension likely originates from the interaction between multiple predisposing genes and environmental factors. However, if blood pressure is not restored to normal levels by compensatory mechanisms, the ion channels in the VSMCs of the arterial circulation appear to “electrically remodel” to sustain arterial contraction (44). A central component of this remodeling includes the rapid and persistent upregulation of functional $\text{Ca}_v1.2$ channels in the affected VSMCs (45–50). Importantly, increased expression of vascular $\text{Ca}_v1.2$ channels has been reported in rats with genetic and renal forms of hypertension (47, 50), suggesting that this event is not limited to single neural or endocrine profiles of the disease. For example, the mesenteric arteries of spontaneously hypertensive rats (SHR) with a genetic form of hypertension show an overabundance of $\text{Ca}_v1.2$ channels compared to similar arteries from the control Wistar Kyoto (WKY) rat strain (50). This abnormality has been shown to extend to the renal and skeletal muscle circulations of the SHR and corresponds to a higher density of Ca^{2+} current in the affected VSMCs and an accentuated Ca^{2+} -dependent tone in arteries isolated from these vascular beds (45, 47, 50–53). Interestingly, a recent report suggests that even mild and short-term increases in blood pressure are sufficient to trigger the upregulation of $\text{Ca}_v1.2$ channels in small arteries in vivo, inferring that this abnormality may occur early in the pathogenesis of hypertension (47).

Notably, an increased availability of functional $\text{Ca}_v1.2$ channel protein, rather than altered properties of the channel, appears to fully account for the elevated voltage-gated Ca^{2+} current in the VSMCs of hypertensive animals. Even early in the development of genetic hypertension, the elevated Ca^{2+} current in VSMCs does not relate to an altered voltage-sensitivity of the channel (45, 52), and normal properties of the channel persist during the chronic stage of hypertension (47). Also, single-channel recordings detect an increased number of Ca^{2+} channel openings without evidence of altered single-channel conductance or open-time distribution (53). All of these findings are consistent with the hypothesis that an

increased availability of characteristic $\text{Ca}_v1.2$ channels is the fundamental abnormality in the VSMCs of hypertensive animals.

The stimuli that link the development of high blood pressure and $\text{Ca}_v1.2$ channel expression remain unclear. At least two reports have suggested that membrane depolarization, a characteristic feature of VSMCs exposed to high blood pressure, may dynamically increase the expression of vascular $\text{Ca}_v1.2$ channels (47–54). Small increases in $\text{Ca}_v1.2$ mRNA have been detected in vessels from hypertensive rats, but these do not appear to fully account for the striking over-abundance of the $\text{Ca}_v1.2$ protein (50–55). For this reason, it is likely that events that enhance $\text{Ca}_v1.2$ channel translation, translocation, or stability in the plasma membrane represent the primary abnormal processes.

Despite the recognition that vascular $\text{Ca}_v1.2$ channels upregulate during hypertension, antihypertensive therapy directed at $\text{Ca}_v1.2$ channels is still limited to blocking Ca^{2+} influx through these channels using traditional calcium channel blockers (CCBs) (56–57). In the United States, three classes of CCBs are prescribed, including the phenylalkylamines, the benzothiazepines and the dihydropyridines. These drug classes bind to distinct but closely related sites on the $\text{Ca}_v1.2$ channel to reduce the open-state probability of the $\text{Ca}_v1.2$ channel and thereby attenuate the voltage-gated Ca^{2+} influx that is required for vascular activation. Interestingly, the increased abundance of the vascular $\text{Ca}_v1.2$ channels during hypertension may provide more binding sites for the CCBs, possibly explaining why their antihypertensive effects are more pronounced in hypertensive compared to normotensive animals and humans (56–58–60). Regardless, the antihypertensive effect of the CCBs and the risk of cardiovascular events varies between patient populations (61), suggesting that in addition to improved drug profiles, novel interventions for reducing Ca^{2+} -dependent tone may improve patient outcomes. In this respect, using molecular interventions to reverse the abnormal expression of $\text{Ca}_v1.2$ channels rather than attempting to block Ca^{2+} influx, may represent a helpful approach.

Two strategies for blunting Ca^{2+} channel expression have been achieved experimentally. Both rely on altering the assembly and release of $\text{Ca}_v1.2$ channels from the endoplasmic reticulum (ER). Notably, normal assembly and trafficking of $\text{Ca}_v1.2$ channels relies on the co-assembly of α - β complexes in the ER, which is mediated by a high affinity interaction between the AID and BID of the α and β subunits, respectively. This α - β interaction masks ER retention sequences on the intracellular I–II linker of α_{1C} , thus enabling translocation of the α - β channel complex from the ER–Golgi complex to the plasma membrane (Figure 2A) (62–63). One tactic for reducing the number of functional $\text{Ca}_v1.2$ channels involves the introduction of mutated β subunits that co-assemble in the ER with the α_{1C} subunit, but prevent its translocation to the plasma membrane (64–65). These β subunit “decoys” retain an intact BID and bind with high affinity to the AID of α_{1C} but lack the ability to mask the ER retention sites on the α_{1C} subunit to enable transit from the ER–Golgi complex. As a result, the decoys form α_{1C} - β decoy complexes that remain sequestered in the ER. Concurrently, the number of functional $\text{Ca}_v1.2$ channels at the plasma membrane is reduced (Figure 2B). Proof of principle has been demonstrated in cardiac myocytes in which overexpression of BID with truncated or missing N- or C-terminal regions decreased the plasma membrane localization of α_{1C} and also reduced voltage-gated Ca^{2+} current and

contractile function (66, 67). A similar loss of functional $\text{Ca}_v1.2$ channels was achieved by the adenoviral introduction of β subunits with truncated N- and C-terminal regions (67). Notably, the β decoys reduced the excitation-contraction coupling that relies on Ca^{2+} entry through $\text{Ca}_v1.2$ channels, but did not disrupt the kinetics of contraction or relaxation of the cardiac cells (66).

A second molecular strategy for disrupting the expression of $\text{Ca}_v1.2$ channels involves overexpressing “lure sequences” that include the AID of the α_{1C} subunit (34, 68). Using lure sequences as baits for BID by overexpressing the AID-containing I–II linker of the α subunit, the availability of endogenous β subunits to bind to the AID of α subunits and permit the trafficking of α – β complexes to the plasma membrane is reduced (Figure 2C) (68). Proof of principle for this approach has been demonstrated for $\text{Ca}_v2.1$ channels in neurons but, presumably, could be used to ameliorate $\text{Ca}_v1.2$ channel expression in VSMCs. Notably, the use of both of these molecular strategies to disrupt $\text{Ca}_v1.2$ channel assembly and trafficking would require VSMC-specific tissue targeting. To accomplish this goal, a SM22 α promoter that regulates smooth muscle cell-specific gene expression has been described that drives the functional expression of genes in small arteries in vivo (69, 70). Viral vectors are being designed that incorporate these promoter elements with specificity for smooth muscle cells, thereby limiting the overexpression of therapeutic molecules designed to downregulate $\text{Ca}_v1.2$ channels to the vasculature. Additionally, alternative promoters for the vascular and cardiac forms of the α_{1C} subunit were recently identified, and analysis of the 5' flanking sequence for these genes has revealed positive and negative regulatory elements (64, 71–73). Based on these findings, an emerging strategy for selectively decreasing $\text{Ca}_v1.2$ channel expression in VSMCs may include diminishing the expression of the vascular isoform of $\text{Ca}_v1.2$ channels by enhancing the level of negative regulatory transcription factors.

It is possible that molecular interventions to reverse the overexpression of vascular $\text{Ca}_v1.2$ channels in hypertension may have some advantages over traditional CCB therapies. First, even the long-acting CCBs require daily administration, whereas new therapeutics that use viral vectors to target the vascular-specific expression of $\text{Ca}_v1.2$ channels may have more enduring effects. Second, although newer CCBs show improved clinical profiles including higher degrees of vascular selectivity and fewer cardiac and renal side effects (74–77), genetic-based approaches may offer even higher vascular selectivity. For example, the presence of a vascular splice variant of the α_{1C} subunit (18, 19) affords a tantalizing opportunity to differentially downregulate the $\text{Ca}_v1.2$ channels in the vasculature to reduce blood pressure while maintaining expression in the heart, which relies on $\text{Ca}_v1.2$ channels for cardiac contraction.

Overall, the vision underlying these novel approaches to treat hypertension predicts that a direct attack on the pathophysiology of the disease by reducing the over-abundance of functional $\text{Ca}_v1.2$ channels has potential therapeutic advantages as new or adjunct therapy to lower blood pressure. Although simple in concept, the optimization and translation of these molecular therapies will require a detailed knowledge of the mechanisms that regulate $\text{Ca}_v1.2$ channel assembly and trafficking in VSMCs, and the events that trigger their pathogenic upregulation during the development of hypertension.

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References

1. Nelson MT, Worley JF. Dihydropyridine inhibition of single calcium channels and contraction in rabbit mesenteric artery depends on voltage. *J Physiol.* 1989; 412:22–25.
2. Rubart M, Patlak JB, Nelson MT. Ca^{2+} currents in cerebral artery smooth muscle cells of rat at physiological Ca^{2+} concentrations. *J Gen Physiol.* 1996; 107:459–472. [PubMed: 8722560]
3. Heart Disease and Stroke Statistics-2005 Update. American Heart Association; Dallas: 2005.
4. Curtis BM, Catterall WA. Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. *Biochemistry.* 1984; 23:2113–2118. [PubMed: 6329263]
5. Curtis BM, Catterall WA. Phosphorylation of the calcium antagonist receptor of the voltage-sensitive calcium channel by cAMP-dependent protein kinase. *Proc Natl Acad Sci USA.* 1985; 82:2528–2532. [PubMed: 2581248]
6. Hosey MM, Barhanin J, Schmid A, Vandaele S, Ptasiński J, O'Callahan C, Cooper C, Lazdunski M. Photoaffinity labelling and phosphorylation of a 165 kilodalton peptide associated with dihydropyridine and phenylalkylamine-sensitive calcium channels. *Biochem Biophys Res Commun.* 1987; 147:1137–1145. [PubMed: 2444223]
7. Ice KS, Takahashi M, Curtis BM, Catterall WA. Comparison of molecular properties of the voltage-sensitive Ca^{2+} channel from longitudinal smooth muscle of rabbit ileum with the channel purified from transverse tubules of rabbit skeletal muscle. *Circ Res.* 1987; 61:I24–I29. [PubMed: 2443272]
8. Leung AT, Imagawa T, Campbell KP. Structural characterization of the 1,4-dihydropyridine receptor of the voltage-dependent Ca^{2+} channel from rabbit skeletal muscle. Evidence for two distinct high molecular weight subunits. *J Biol Chem.* 1987; 262:7943–7946. [PubMed: 2439496]
9. Vaghy PL, Striessnig J, Miwa K, Knaus HG, Itagaki K, McKenna E, Glossmann H, Schwartz A. Identification of a novel 1,4-dihydropyridine- and phenylalkylamine-binding polypeptide in calcium channel preparations. *J Biol Chem.* 1987; 262:14337–14342. [PubMed: 2443504]
10. Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. *Annu Rev Cell Dev Biol.* 2000; 16:521–555. This review, although not recent, comprehensively describes the classification, subunit structure and regulation of the voltage-gated Ca^{2+} channels. [PubMed: 11031246]
11. Jurkat-Rott K, Lehmann-Horn F. The impact of splice isoforms on voltage-gated calcium channel α_1 subunits. *J Physiol.* 2004; 554:609–619. [PubMed: 14645450]
12. Kobrinsky E, Tiwari S, Maltsev VA, Harry JB, Lakatta E, Abernethy DR, Soldatov NM. Differential role of the α_{1C} subunit tails in regulation of the $\text{Ca}_v1.2$ channel by membrane potential, β subunits, and Ca^{2+} ions. *J Biol Chem.* 2005; 280:12474–12485. [PubMed: 15671035]
13. Lee A, Wong ST, Gallagher D, Li B, Storm DR, Scheuer T, Catterall WA. Ca^{2+} /calmodulin binds to and modulates P/Q-type calcium channels. *Nature.* 1999; 399:155–159. [PubMed: 10335845]
14. Liang H, DeMaria CD, Erickson MG, Mori MX, Alseikhan BA, Yue DT. Unified mechanisms of Ca^{2+} regulation across the Ca^{2+} channel family. *Neuron.* 2003; 39:951–960. [PubMed: 12971895]

15. Peterson BZ, DeMaria CD, Adelman JP, Yue DT. Calmodulin is the Ca^{2+} sensor for Ca^{2+} -dependent inactivation of L-type calcium channels. *Neuron*. 1999; 22:549–558. [PubMed: 10197534]
16. Qin N, Olcese R, Bransby M, Lin T, Birnbaumer L. Ca^{2+} -induced inhibition of the cardiac Ca^{2+} channel depends on calmodulin. *Proc Natl Acad Sci USA*. 1999; 96:2435–2438. [PubMed: 10051660]
17. Zuhlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature*. 1999; 399:159–162. [PubMed: 10335846]
18. Liao P, Yu D, Lu S, Tang Z, Liang MC, Zeng S, Lin W, Soong TW. Smooth muscle-selective alternatively spliced exon generates functional variation in $\text{Ca}_v1.2$ calcium channels. *J Biol Chem*. 2004; 279:50329–50335. [PubMed: 15381693]
19. Welling A, Ludwig A, Zimmer S, Klugbauer N, Flockerzi V, Hofmann F. Alternatively spliced IS6 segments of the α_{1C} gene determine the tissue-specific dihydropyridine sensitivity of cardiac and vascular smooth muscle L-type Ca^{2+} channels. *Circ Res*. 1997; 81:526–532. This study provided the first evidence that alternative splicing of α_{1C} confers different dihydropyridine sensitivities to $\text{Ca}_v1.2$ channels in cardiac and vascular muscle. [PubMed: 9314833]
20. Pang L, Koren G, Wang Z, Nattel S. Tissue-specific expression of two human $\text{Ca}_v1.2$ isoforms under the control of distinct 5' flanking regulatory elements. *FEBS Lett*. 2003; 546:349–354. [PubMed: 12832067]
21. Saada N, Dai B, Echeteu C, Sarna SK, Palade P. Smooth muscle uses another promoter to express primarily a form of human $\text{Ca}_v1.2$ L-type calcium channel different from the principal heart form. *Biochem Biophys Res Commun*. 2003; 302:23–28. [PubMed: 12593842]
22. Moosmang S, Schulla V, Welling A, Feil R, Feil S, Wegener JW, Hofmann F, Klugbauer N. Dominant role of smooth muscle L-type calcium channel $\text{Ca}_v1.2$ for blood pressure regulation. *EMBO J*. 2003; 22:6027–6034. This study used smooth muscle -specific inactivation of $\text{Ca}_v1.2$ to single out the $\text{Ca}_v1.2$ channels as key contributors to myogenic tone and blood pressure. [PubMed: 14609949]
23. Foell JD, Balijepalli RC, Delisle BP, et al. Molecular heterogeneity of calcium channel β -subunits in canine and human heart: Evidence for differential subcellular localization. *Physiol Genomics*. 2004; 17:183–200. [PubMed: 14762176]
24. Harry JB, Kobrinsky E, Abernethy DR, Soldatov NM. New short splice variants of the human cardiac $\text{Ca}_v\beta_2$ subunit: Redefining the major functional motifs implemented in modulation of the $\text{Ca}_v1.2$ channel. *J Biol Chem*. 2004; 279:46367–46372. [PubMed: 15339916]
25. Kamada Y, Yamada Y, Yamakage M, Nagashima M, Tsutsuura M, Kobayashi T, Seki S, Namiki A, Tohse N. Single-channel activity of L-type Ca^{2+} channels reconstituted with the β_{2c} subunit cloned from the rat heart. *Eur J Pharmacol*. 2004; 487:37–45. [PubMed: 15033374]
26. Gregg RG, Messing A, Strube C, et al. Absence of the β subunit (cchb1) of the skeletal muscle dihydropyridine receptor alters expression of the α_1 subunit and eliminates excitation-contraction coupling. *Proc Natl Acad Sci USA*. 1996; 93:13961–13966. [PubMed: 8943043]
27. Ball SL, Powers PA, Shin HS, Morgans CW, Peachey NS, Gregg RG. Role of the β_2 subunit of voltage-dependent calcium channels in the retinal outer plexiform layer. *Invest Ophthalmol Vis Sci*. 2002; 43:1595–1603. [PubMed: 11980879]
28. Dickie MM. Lethargic (lh). *Mouse News Letters*. 1964; 30:31.
29. Cohen RM, Foell JD, Balijepalli RC, Shah V, Hell JW, Kamp TJ. Unique modulation of L-type Ca^{2+} channels by short auxiliary β_{1d} subunit present in cardiac muscle. *Am J Physiol Heart Circ Physiol*. 2005; 288:H2363–H2374. [PubMed: 15615847]
30. Hanlon MR, Berrow NS, Dolphin AC, Wallace BA. Modelling of a voltage-dependent Ca^{2+} channel β subunit as a basis for understanding its functional properties. *FEBS Lett*. 1999; 445:366–370. [PubMed: 10094491]
31. Richards MW, Butcher AJ, Dolphin AC. Ca^{2+} channel β -subunits: Structural insights AID our understanding. *Trends Pharmacol Sci*. 2004; 25:626–632. [PubMed: 15530640]
32. Neuhuber B, Gerster U, Mitterdorfer J, Glossmann H, Flucher BE. Differential effects of Ca^{2+} channel β_{1a} and β_{2a} subunits on complex formation with α_{1S} and on current expression in tsA201 cells. *J Biol Chem*. 1998; 273:9110–9118. [PubMed: 9535900]

33. Canti C, Davies A, Berrow NS, Butcher AJ, Page KM, Dolphin AC. Evidence for two concentration-dependent processes for β -subunit effects on α_{1B} calcium channels. *Biophys J*. 2001; 81:1439–1451. [PubMed: 11509358]
34. Bichet D, Lecomte C, Sabatier JM, Felix R, De Waard M. Reversibility of the Ca^{2+} channel α_1 - β subunit interaction. *Biochem Biophys Res Commun*. 2000; 277:729–735. This study successfully used AID-containing peptides as lures to disrupt α - β interactions. [PubMed: 11062021]
35. Butcher AJ, Leroy J, Richards MW, Pratt WS, Dolphin AC. The importance of occupancy rather than affinity of $\text{Ca}_v(\beta)$ subunits for the calcium channel I–II linker in relation to calcium channel function. *J Physiol*. 2006; 574:387–398. [PubMed: 16627564]
36. Tareilus E, Roux M, Qin N, Olcese R, Zhou J, Stefani E, Birnbaumer L. A *Xenopus* oocyte β subunit: Evidence for a role in the assembly/expression of voltage-gated calcium channels that is separate from its role as a regulatory subunit. *Proc Natl Acad Sci USA*. 1997; 94:1703–1708. [PubMed: 9050842]
37. Berrow NS, Campbell V, Fitzgerald EM, Brickley K, Dolphin AC. Antisense depletion of β -subunits modulates the biophysical and pharmacological properties of neuronal calcium channels. *J Physiol*. 1995; 482:481–491. [PubMed: 7537818]
38. Dolphin AC. β subunits of voltage-gated calcium channels. *J Bioenerg Biomembr*. 2003; 35:599–620. This review provides detailed information on all aspects of the structure, modifications, kinetics and trafficking of $\text{Ca}_v\beta$ subunits. [PubMed: 15000522]
39. Wei X, Pan S, Lang W, Kim H, Schneider T, Perez-Reyes E, Birnbaumer L. Molecular determinants of cardiac Ca^{2+} channel pharmacology. Subunit requirement for the high affinity and allosteric regulation of dihydropyridine binding. *J Biol Chem*. 1995; 270:27106–27111. [PubMed: 7592963]
40. Klugbauer N, Marais E, Hofmann F. Calcium channel $\alpha_{2\delta}$ subunits: Differential expression, function, and drug binding. *J Bioenerg Biomembr*. 2003; 35:639–647. [PubMed: 15000524]
41. Marais E, Klugbauer N, Hofmann F. Calcium channel $\alpha_{2\delta}$ subunits-structure and Gabapentin binding. *Mol Pharmacol*. 2001; 59:1243–1248. [PubMed: 11306709]
42. Sonkusare S, Palade PT, Marsh JD, Telemaque S, Pesic A, Rusch NJ. Vascular calcium channels and high blood pressure: Pathophysiology and therapeutic implications. *Vascul Pharmacol*. 2006; 44:131–142. This review focuses on the structure and function of $\text{Ca}_v1.2$ channels in VSMCs, and their role in the pathogenesis of hypertension. [PubMed: 16427812]
43. Murakami M, Yamamura H, Suzuki T, et al. Modified cardiovascular L-type channels in mice lacking the voltage-dependent Ca^{2+} channel β_3 subunit. *J Biol Chem*. 2003; 278:43261–43267. These authors were the first to characterize the effect of β_3 gene deletion on Ca^{2+} current in VSMCs and on the regulation of blood pressure. [PubMed: 12920136]
44. Cox RH, Rusch NJ. New expression profiles of voltage-gated ion channels in arteries exposed to high blood pressure. *Microcirculation*. 2002; 9:243–257. [PubMed: 12152102]
45. Lozinskaya IM, Cox RH. Effects of age on Ca^{2+} currents in small mesenteric artery myocytes from Wistar-Kyoto and spontaneously hypertensive rats. *Hypertension*. 1997; 29:1329–1336. [PubMed: 9180637]
46. Martens JR, Gelband CH. Alterations in rat interlobar artery membrane potential and K^+ channels in genetic and nongenetic hypertension. *Circ Res*. 1996; 79:295–301. [PubMed: 8756007]
47. Pesic A, Madden JA, Pesic M, Rusch NJ. High blood pressure upregulates arterial L-type Ca^{2+} channels: Is membrane depolarization the signal? *Circ Res*. 2004; 94:e97–e104. This article provided direct evidence that high blood pressure rapidly increases the expression of $\text{Ca}_v1.2$ channels in small arteries, and suggested that pressure-induced depolarization of VSMCs may be a contributing factor. [PubMed: 15131006]
48. Simard JM, Li X, Tewari K. Increase in functional Ca^{2+} channels in cerebral smooth muscle with renal hypertension. *Circ Res*. 1998; 82:1330–1337. [PubMed: 9648730]
49. Wilde DW, Furspan PB, Szocik JF. Calcium current in smooth muscle cells from normotensive and genetically hypertensive rats. *Hypertension*. 1994; 24:739–746. [PubMed: 7527795]
50. Pratt PF, Bonnet S, Ludwig LM, Bonnet P, Rusch NJ. Upregulation of L-type Ca^{2+} channels in mesenteric and skeletal arteries of SHR. *Hypertension*. 2002; 40:214–219. This report showed that the enhanced levels of Ca^{2+} -dependent tone observed in small arteries of genetically hypertensive

rats was associated with an increased expression of the α_{1C} subunit of the $\text{Ca}_v1.2$ channel. [PubMed: 12154116]

51. Cox RH, Lozinskaya IM. Augmented calcium currents in mesenteric artery branches of the spontaneously hypertensive rat. *Hypertension*. 1995; 26:1060–1064. [PubMed: 7498968]
52. Ohya Y, Abe I, Fujii K, Takata Y, Fujishima M. Voltage-dependent Ca^{2+} channels in resistance arteries from spontaneously hypertensive rats. *Circ Res*. 1993; 73:1090–1099. [PubMed: 8222080]
53. Ohya Y, Tsuchihashi T, Kagiya S, Abe I, Fujishima M. Single L-type calcium channels in smooth muscle cells from resistance arteries of spontaneously hypertensive rats. *Hypertension*. 1998; 31:1125–1129. These authors provided the only comparison of single-channel Ca^{2+} currents between VSMCs of normotensive and spontaneously hypertensive rats. [PubMed: 9576124]
54. Ruiz-Velasco V, Mayer MB, Inscho EW, Hymel LJ. Modulation of dihydropyridine receptors in vascular smooth muscle cells by membrane potential and cell proliferation. *Eur J Pharmacol*. 1994; 268:311–318. [PubMed: 7805757]
55. Wang WZ, Saada N, Dai B, Pang L, Palade P. Vascular-specific increase in exon 1B-encoded $\text{Ca}_v1.2$ channels in spontaneously hypertensive rats. *Am J Hypertens*. 2006; 19:823–831. These authors recently demonstrated that the increase in expression of vascular $\text{Ca}_v1.2$ channels in hypertension can be attributed to a single splice variant of the channel. [PubMed: 16876682]
56. Godfraind T, Kazda S, Wibo M. Effects of a chronic treatment by nisoldipine, a calcium antagonistic dihydropyridine, on arteries of spontaneously hypertensive rats. *Circ Res*. 1991; 68:674–682. [PubMed: 1660358]
57. Spedding M, Paoletti R. Classification of calcium channels and the sites of action of drugs modifying channel function. *Pharmacol Rev*. 1992; 44:363–376. [PubMed: 1332082]
58. Miyamori I, Yasuhara S, Matsubara T, Takasaki H, Takeda R. Effects of a calcium entry blocker on cerebral circulation in essential hypertension. *J Clin Hypertens*. 1987; 3:528–535. [PubMed: 3134516]
59. Narita H, Nagao T, Yabana H, Yamaguchi I. Hypotensive and diuretic actions of diltiazem in spontaneously hypertensive and Wistar Kyoto rats. *J Pharmacol Exp Ther*. 1983; 227:472–477. [PubMed: 6631726]
60. Takata Y, Hutchinson JS. Exaggerated hypotensive responses to calcium antagonists in spontaneously hypertensive rats. *Clin Exp Hypertens A*. 1983; 5:827–847. [PubMed: 6883745]
61. Staessen JA, Fagard R, Thijs L, et al. Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (Syst-Eur) Trial Investigators. *Lancet*. 1997; 350:757–764. [PubMed: 9297994]
62. Cornet V, Bichet D, Sandoz G, Marty I, Brocard J, Bourinet E, Mori Y, Villaz M, De Waard M. Multiple determinants in voltage-dependent P/Q calcium channels control their retention in the endoplasmic reticulum. *Eur J Neurosci*. 2002; 16:883–895. [PubMed: 12372025]
63. Bichet D, Cornet V, Geib S, Carlier E, Volsen S, Hoshi T, Mori Y, De Waard M. The I–II loop of the Ca^{2+} channel α_1 subunit contains an endoplasmic reticulum retention signal antagonized by the β subunit. *Neuron*. 2000; 25:177–190. This article described the modulation of Ca^{2+} channel trafficking enabled by the masking of an ER retention signal on the α subunit by the β subunit. [PubMed: 10707982]
64. Fan IQ, Chen B, Marsh JD. Transcriptional regulation of L-type calcium channel expression in cardiac myocytes. *J Mol Cell Cardiol*. 2000; 32:1841–1849. [PubMed: 11013128]
65. Chen X, Zhang X, Kubo H, Harris DM, Mills GD, Moyer J, Berretta R, Potts ST, Marsh JD, Houser SR. Ca^{2+} influx-induced sarcoplasmic reticulum Ca^{2+} overload causes mitochondrial-dependent apoptosis in ventricular myocytes. *Circ Res*. 2005; 97:1009–1017. [PubMed: 16210547]
66. Fan QI, Vanderpool KM, O'Connor J, Marsh JD. Decoy calcium channel β subunits modulate contractile function in myocytes. *Mol Cell Biochem*. 2003; 242:3–10. This paper showed that mutant β subunits can be used as “decoys” to reduce the expression of functional $\text{Ca}_v1.2$ channels in the plasma membrane of cardiac myocytes. [PubMed: 12619859]
67. Sonkusare S, Stimers JR, Grain T, Marsh JD, Telemaque S. Expression of a β_2 subunit mutant alters Ca currents in HL-1 cells. *FASEB J*. 2006; 20:A1114.
68. Cuchillo-Ibanez I, Aldea M, Brocard J, Albillos A, Weiss N, Garcia AG, De Waard M. Inhibition of voltage-gated calcium channels by sequestration of β subunits. *Biochem Biophys Res Commun*.

2003; 311:1000–1007. This study demonstrated the feasibility of using AID-containing peptides to reduce the availability of β subunits that are needed for normal $\text{Ca}_v2.1$ trafficking. [PubMed: 14623281]

69. Jiang M, Mezentsev A, Kemp R, Byun K, Falck JR, Miano JM, Nasjletti A, Abraham NG, Laniado-Schwartzman M. Smooth muscle–specific expression of CYP4A1 induces endothelial sprouting in renal arterial microvessels. *Circ Res.* 2004; 94:167–174. These authors demonstrated the use of a smooth muscle -specific promoter, SM22 α , to express a functional enzyme in the VSMCs of a resistance vessel. [PubMed: 14670847]
70. Solway J, Seltzer J, Samaha FF, Kim S, Alger LE, Niu Q, Morrissey EE, Ip HS, Parmacek MS. Structure and expression of a smooth muscle cell-specific gene, SM22 α . *J Biol Chem.* 1995; 270:13460–13469. This paper represents an initial report of a promoter enabling smooth muscle -specific expression of a gene. [PubMed: 7768949]
71. Fan QI, Vanderpool K, Marsh JD. A 27 bp *cis*-acting sequence is essential for L-type calcium channel α_{1C} subunit expression in vascular smooth muscle cells. *Biochim Biophys Acta.* 2002; 1577:401–411. [PubMed: 12359330]
72. Fan QI, Vanderpool KM, Chung HS, Marsh JD. The L-type calcium channel α_{1C} subunit gene undergoes extensive, uncoordinated alternative splicing. *Mol Cell Biochem.* 2005; 269:153–163. [PubMed: 15786728]
73. Saada NI, Carrillo ED, Dai B, Wang WZ, Dettbarn C, Sanchez J, Palade P. Expression of multiple $\text{Ca}_v1.2$ transcripts in rat tissues mediated by different promoters. *Cell Calcium.* 2005; 37:301–309. [PubMed: 15755491]
74. Triggie DJ. 1,4-Dihydropyridines as calcium channel ligands and privileged structures. *Cell Mol Neurobiol.* 2003; 23:293–303. [PubMed: 12825828]
75. Hayashi K, Ozawa Y, Fujiwara K, Wakino S, Kumagai H, Saruta T. Role of actions of calcium antagonists on efferent arterioles—with special references to glomerular hypertension. *Am J Nephrol.* 2003; 23:229–244. [PubMed: 12840599]
76. Tanaka H, Shigenobu K. Pathophysiological significance of T-type Ca^{2+} channels: T-type Ca^{2+} channels and drug development. *J Pharmacol Sci.* 2005; 99:214–220. [PubMed: 16293935]
77. Mason RP, Marche P, Hintze TH. Novel vascular biology of third-generation L-type calcium channel antagonists: Ancillary actions of amlodipine. *Arterioscler Thromb Vasc Biol.* 2003; 23:2155–2163. [PubMed: 14512371]

Figure 1. Proposed topology of the Ca_v1.2 channel

The formation of the pore by the α_{1C} subunit is shown (lower right). Genes for four β subunits (β_1 , β_2 , β_3 , and β_4) and four splice variants of $\alpha_2\delta$ subunits have been identified. The β subunit contains a beta interactive domain (BID), which is required for high affinity interaction with the alpha interactive domain (AID) of the α subunit. The subunits also have phosphorylation sites and binding sites for signaling molecules. The formation of the pore by the α_{1C} subunit is shown (lower right). CaM, calmodulin; PKA, protein kinase A; PKC, protein kinase C.

Figure 2. Molecular interventions to reduce the expression of functional Ca_v1.2 channels

A. The co-assembly of α - β complexes in the endoplasmic reticulum (ER) permits translocation of Ca_v1.2 channels to the plasma membrane to form functional channels. **B.** Overexpressed mutant β subunits co-assemble in the ER with the α_{1C} subunit but prevent its translocation to the plasma membrane. These “decoy” β subunits retain the BID that permits interaction with the AID of the α subunit, but lack the ability to mask the ER retention signal to enable exit of the α - β complex from the ER. **C.** Overexpression of the AID of the α_{1C} subunit acts as bait for the BID of endogenous β subunits, thereby reducing their availability to bind to endogenous α_{1C} to permit its exit from the ER.