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Vascular calcium channels and high blood pressure: Pathophysiology and therapeutic implications

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Abstract

Long-lasting Ca^{2+} (Ca_L) channels of the $\text{Ca}_v1.2$ gene family are heteromultimeric structures that are minimally composed of a pore-forming α_{1C} subunit and regulatory β and $\alpha_2\delta$ subunits in vascular smooth muscle cells. The Ca_L channels are the primary pathways for voltage-gated Ca^{2+} influx that trigger excitation–contraction coupling in small resistance vessels. Notably, vascular smooth muscle cells of hypertensive rats show an increased expression of Ca_L channel α_{1C} subunits, which is associated with elevated Ca^{2+} influx and the development of abnormal arterial tone. Indeed, blood pressure per se appears to promote Ca_L channel expression in small arteries, and even short-term rises in pressure may alter channel expression. Membrane depolarization has been shown to be one stimulus associated with elevated blood pressure that promotes Ca_L channel expression at the plasma membrane. Future studies to define the molecular processes that regulate Ca_L channel expression in vascular smooth muscle cells will provide a rational basis for designing antihypertensive therapies to normalize Ca_L channel expression and the development of anomalous vascular tone in hypertensive pathologies.

Keywords

Calcium channels; Hypertension; Blood pressure; Ion channel expression; Vascular smooth muscle; Microcirculation

1. Introduction

Chronic hypertension is one of the deadliest diseases in the United States, predisposing 45 million individuals to ventricular hypertrophy, heart failure, stroke and end-stage renal damage. In all but rare cases, the etiology is unknown and the disease is thought to evolve from the interaction of multiple genes with environmental factors. Indeed, numerous pathways involving endocrine factors, neural reflexes and vascular abnormalities are thought to contribute to essential hypertension. However, regardless of these diverse origins, one

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hallmark finding in all forms of hypertension is an anomalous vascular tone that is mediated by Ca^{2+} influx through voltage-gated, “L-type” Ca^{2+} (Ca_L) channels. Under optimal conditions, the low open-state probability of Ca_L channels in the vascular smooth muscle cells (VSMCs) is designed to tightly regulate Ca^{2+} influx, vascular tone, and blood pressure. However, this process may go awry during the pathogenesis of hypertension in which an increased Ca^{2+} influx may fuel the development of an abnormal vascular tone and elevated peripheral vascular resistance.

Notably, our knowledge of cardiovascular Ca_L channels has been revolutionized in the past two decades by the availability of molecular biology, protein crystallographic and patch-clamp techniques. This knowledge has laid the foundation for understanding the relationship between the structure and function of Ca_L channels in the vasculature and the regulation of blood pressure. In this regard, studies from a number of laboratories have revealed that hypertension is associated with an upregulation of Ca_L channels in vascular beds involved in regulating blood pressure and adjusting blood flow to critical organs. The fact that this Ca_L channel abnormality extends to several animal models of hypertension infers that it may represent a common event in the pathogenesis of different forms of the disease. To provide a synopsis of the recent evidence linking Ca_L channel abnormalities to hypertension, this brief review will initially discuss the structure, distribution and function of Ca_L channels in the vasculature. Subsequently, it will summarize the recent findings indicating that Ca_L channels may be involved in the pathogenesis of hypertension. A final section will identify emerging opportunities to design new classes of antihypertensive drugs that target specific promoters or subunits of the vascular Ca_L channels.

2. Structure and classification

Voltage-dependent Ca^{2+} channels are required for normal excitation–contraction coupling in the heart, skeletal muscle and various types of smooth muscle including vascular smooth muscle cells (VSMCs). Initial purification studies using skeletal muscle revealed that voltage-gated Ca^{2+} channels were multi-subunit complexes, composed of a central pore-forming α_1 subunit and additional β , $\alpha_2\delta$ and γ subunits (Fig. 1A) (Curtis and Catterall, 1984, 1985; Hosey et al., 1987; Ice et al., 1987; Leung et al., 1987; Vaghy et al., 1987). It is now known that the large (≈ 190 – 240 kDa) α_1 subunit confers most functional properties to the Ca_L channel, including voltage sensing, Ca^{2+} permeability, Ca^{2+} -dependent inactivation, and inhibition by Ca^{2+} channel blockers. The structure of the α_1 subunit includes four repeat domains (I, II, III, IV) each composed of 6 transmembrane segments (Catterall, 2000; Jurkat-Rott and Lehmann-Horn, 2004). Modulation of channel behavior by phosphorylation is enabled by intracellular binding domains for signaling molecules including protein kinase A (PKA) and protein kinase C (PKC). In addition, the amino (N)- and carboxy (C)-termini play specific roles in modulating channel phenotype and expression. For example, the C-terminus has been implicated in Ca^{2+} -dependent signal transduction including the constitutive binding of calmodulin to permit Ca^{2+} -dependent inactivation of the channel (Kobrin et al., 2005; Lee et al., 1999; Liang et al., 2003; Peterson et al., 1999; Qin et al., 1999; Zuhlke et al., 1999).

Although the α_1 subunit determines the signature properties of the Ca_L channel, the intracellular β subunits modulate the biophysical and physiological responses. The β subunits belong to the membrane-associated guanylate kinase (MAGUK) family of scaffolding proteins, which possess a guanylate kinase (GK) and a Src homology 3 (SH3) domain (Fig. 1B) (Cohen et al., 2005). The GK region of the β subunit contains a beta interactive domain (BID), which along with other subunit sites, is required for high affinity interaction with the alpha interactive domain (AID) of the α_1 subunit (Berjukow et al., 2001; Birnbaumer et al., 1998; Catterall, 2000; Chen et al., 2004; Dolphin, 2003; Geib et al., 2002). Mutations that disrupt the SH3–GK interactions within the β subunits inhibit their binding to the α_1 subunit and prevent normal channel gating (Harry et al., 2004; McGee et al., 2004). As scaffolding proteins, the β subunits may localize and integrate intracellular signals to influence Ca_L channel gating (Balijepalli et al., 2003). For example, phosphorylation of the β subunit by PKA is considered to be responsible for PKA-induced stimulation of cloned Ca^{2+} channels in HEK cells co-transfected with α_{1C} and β_{2a} subunits (Bunemann et al., 1999).

Notably, the heterogeneous nature of the β subunits has hindered efforts to define their function. Four different genes (β_1 , β_2 , β_3 , β_4) and extensive splice variants of each gene exist (Foell et al., 2004; Harry et al., 2004; Kamada et al., 2004) that distinctly modify activation and inactivation kinetics, (Berjukow et al., 2001; Berrou et al., 2001; Berrow et al., 1995; Chen et al., 2004; Geib et al., 2002), voltage gating (Berrow et al., 1995; Birnbaumer et al., 1998; Catterall, 2000), and drug sensitivity (Berrow et al., 1995; Dolphin, 2003; Wei et al., 1995). For example, although the α_1 subunit contains inherent determinants of voltage-dependent inactivation (Cens et al., 1999; Herlitze et al., 1997; Spaetgens and Zamponi, 1999; Zhang et al., 1994), association with different β subunit isoforms may either increase or decrease its overall inactivation rate (Meir and Dolphin, 2002; Olcese et al., 1994). Importantly, all four β subunits generate a molecular signal for correct plasma membrane targeting of functional Ca^{2+} channel complexes, and thereby can profoundly affect the level of Ca^{2+} influx during cell depolarization (Birnbaumer et al., 1998; Bogdanov et al., 2000; Brice et al., 1997; Canti et al., 2000; Chien et al., 1996, 1998; Dolphin, 2003; Kobrinsky et al., 2004).

The expression and function of the α_1 subunit also depend on association with the $\alpha_2\delta$ subunits. Four distinct $\alpha_2\delta$ subunits ($\alpha_2\delta_1$, $\alpha_2\delta_2$, $\alpha_2\delta_3$, $\alpha_2\delta_4$) arise from alternative splicing of a single gene (Klugbauer et al., 2003; Marais et al., 2001), and a post-translational disulfide linkage results in a membrane-delineated dimer (De Jongh et al., 1990; Jay et al., 1991). Although the δ subunit is the portion of the protein that is anchored in the membrane, the extracellular α_2 subunit also interacts with the α_1 subunit (Gurnett et al., 1997). Co-expression of the $\alpha_2\delta$ subunits with cloned α_1 subunits leads to accelerated channel gating, a hyperpolarizing shift in voltage activation, and enhanced channel expression (Klugbauer et al., 2003). The latter effect may rely, at least in part, on the presence of the β subunits, although there is no direct evidence for interactions between these ancillary subunits (Gerster et al., 1999; Klugbauer et al., 2003; Sipos et al., 2000).

The α_1 subunits expressed in skeletal muscle and neurons also co-purify with γ subunits, which are membrane-delineated glycoproteins with 4 transmembrane segments (Arikath

and Campbell, 2003; Dolphin, 2003). Although the γ subunits show a wide tissue distribution, whether they co-assemble with α_1 subunits in other cell types is still controversial (Kang et al., 2001; Klugbauer et al., 2000; Moss et al., 2002). To date, no association between the α_1 and γ subunits has been described in cardiovascular tissues.

3. Ca_L channels in vascular smooth muscle

Initial studies indicate that the subunit composition of vascular Ca_L channels is a determinant of arterial tone and blood pressure levels. Functionally, these Ca_L channels show a unitary conductance between 20 and 30 pS, and are highly susceptible to block by nifedipine and other 1,4-dihydropyridine antagonists. Modeling studies of Ca_L currents in VSMCs suggest that at least several thousand pore-forming Ca^{2+} channel subunits are expressed in a single cell (Nelson et al., 1990). These channels inactivate slowly during sustained depolarization, so that the voltage-dependent Ca^{2+} influx permitted by a small fraction of these channels is sufficient to mediate pressure-induced constriction in small arteries and contribute to dynamic autoregulation in the coronary, cerebral and renal vascular beds (Gauthier-Rein and Rusch, 1998; Goligorsky et al., 1995; Inscho et al., 1998; Narayanan et al., 1994; Nelson et al., 1990). Thus, sustained voltage-dependent Ca^{2+} influx through the Ca_L channels maintains a tonic level of vasoconstriction and also provides an excitatory template upon which endogenous vasoactive substances may act to further modulate arterial diameter.

Surprisingly, despite their critical role in regulating excitation–contraction coupling in VSMCs, the exact subunit composition of vascular Ca_L channel complexes remains relatively obscure. In this regard, it appears that the Ca_L channels in VSMCs are minimally composed of α_1 , β and $\alpha_2\delta$ subunits. Some of the corresponding transcripts and proteins have been detected in a variety of arterial tissues (Table 1). In addition, gene deletion studies in mice have provided initial insight into the contributions of the α_{1C} and β subunits to arterial tone and blood pressure levels (Table 2). 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 α_{1C} ($\text{Ca}_V1.2$) gene. In these animals, the blood pressure level is reduced by ~30 mm Hg (Moosmang et al., 2003). Importantly, the α_{1C} subunit in VSMCs 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 (Liao et al., 2004; Welling et al., 1997). Most notably, alternative splicing of exon 8 results in amino acid differences in transmembrane segment IS6 that confer an increased sensitivity for 1,4-dihydropyridine drugs to the vascular variant (Liao et al., 2004; Welling et al., 1997). Furthermore, alternative splicing of exon 1 results in a shorter N-terminus of the α_{1C} subunits in VSMCs compared to cardiac myocytes (Saada et al., 2003). This arrangement may permit a separate promoter to selectively regulate the availability of vascular Ca_L channels without altering expression levels in other tissues affected by the same stimuli (Pang et al., 2003; Saada et al., 2003).

Based on the initial detection of multiple ancillary subunits of the Ca_L channel in arterial tissues, a diverse population of heteromultimeric Ca^{2+} channels is anticipated. Transcripts encoding all four β subunits (β_1 , β_2 , β_3 , β_4) have been detected in VSMCs, although only the

corresponding proteins for the β_2 and β_3 subunits have been reported (Table 1). Indeed, the β_1 and β_4 subunits appear to be primarily expressed in tissues outside the vasculature, including skeletal muscle and neurons, respectively (Gregg et al., 1996; Pichler et al., 1997). Thus, mice lacking the β_1 subunit die at birth from asphyxia (Gregg et al., 1996), reflecting the requisite role of the β_1 subunit in excitation–contraction coupling in skeletal muscle (Table 2). In contrast, conventional knockout of the gene encoding the β_2 subunit in mice results in embryonic lethality presumably from a cardiac defect since a transgenic and cardiac-specific rescue of the β_2 subunit permits survival (Ball et al., 2002). In the latter animals, future studies on the functional impact of β_2 subunit deletion may provide initial insights into the influence of the β_2 subunit on vascular Ca_L channel expression and the generation of arterial tone. Notably, gene deletion studies in β_3 knockout mice in which the expression of the β_2 subunit is maintained have inferred dual functional roles for the vascular β_2 and β_3 subunits (Murakami et al., 2003; Muth et al., 2001). In these animals, aortic VSMCs lacking the β_3 subunit show a 30% reduction in voltage-gated Ca^{2+} current, indicating that β_3 subunits contribute to the expression of functional α_{1C} subunits in the plasma membrane. In the same animals, the presence of residual Ca_L current in the VSMCs after deletion of the β_3 gene is interpreted to infer a redundant role for the β_2 subunit in α_{1C} trafficking and the maintenance of Ca_L channel expression (Murakami et al., 2003).

4. Role of Ca_L channels in the pathogenesis of hypertension

Abnormalities of Ca^{2+} channels are regarded as part of the extensive biological and morphological adaptations that characterize the vasculature during the pathogenesis of hypertension. Initially, during acute increases in blood pressure, small arteries and arterioles show an immediate “stretch-dependent” depolarization and constriction commonly referred to as the “myogenic” response. The myogenic response relies on the opening of Ca_L channels in the VSMCs for contraction (Nelson et al., 1990; Wang et al., 1999). In fact, the requisite role of Ca_L channels in mediating pressure-induced contraction is evident by the absence of myogenic tone in the arteries of mice in which the Ca_L channel ($\text{Ca}_V1.2$) gene is inactivated (Moosmang et al., 2003). Importantly, the myogenic response may be viewed as either a contributing or protective influence in hypertension (Cox and Rusch, 2002). On the one hand, the myogenic response of the small arteries and arterioles may amplify the initial rise in blood pressure and further elevate peripheral vascular resistance and blood pressure levels. Alternatively, in the cerebral, coronary and renal circulations that are primarily involved in the autoregulation of local blood flow, the myogenic response is postulated to dampen the transmission of the high systemic pressure to the microvasculature of the brain, heart and kidneys, respectively, thereby preventing pressure-induced damage to the capillary beds. Indeed, the loss of myogenic tone in the renal circulation has been linked to renal injury in animals and in humans with hypertension (Dworkin and Feiner, 1986; Feld et al., 1977; Gebremedhin et al., 1990; Hayashi et al., 1992, 1996; Olson et al., 1986), and also appears to precede the occurrence of hemorrhagic stroke in rats that are genetically predisposed to develop hypertension (Smeda and King, 2003).

The myogenic response of the different vascular beds continues to play a central role in regulating blood pressure levels and organ blood flow during the progression of hypertension. However, if blood pressure is not restored to normal levels by compensatory

neural and renal mechanisms, the VSMCs of the arterial circulation appear to “electrically remodel” as an adaptive response to sustain the higher levels of voltage-gated Ca^{2+} influx required for persistent arterial contraction. This adaptation is postulated to rely, at least in part, on the development of a “disease-specific” profile of vascular ion channels that promotes arterial contraction and Ca^{2+} influx (Cox and Rusch, 2002). To date, two fundamental events have been implicated as the cellular mechanisms that evolve to sustain an elevated level of voltage-gated Ca^{2+} influx and contractile activation in VSMCs exposed to chronic increases in intravascular pressure. First, a loss of resting membrane K^{+} conductance resulting in depolarization-induced opening of Ca_L channels has been observed in VSMCs from several rat models of hypertension (Harder et al., 1983; Harder et al., 1985; Stekiel et al., 1986; Stekiel et al., 1993). Second, even short-term exposure to high intravascular pressures appears to upregulate the α_{1C} subunit and the number of functional Ca_L channels in VSMCs (Lozinskaya and Cox, 1997; Martens and Gelband, 1996; Pesic et al., 2004; Simard et al., 1998; Wilde et al., 1994). Interestingly, new evidence suggests that depolarization per se promotes α_{1C} subunit expression in the VSMCs of small arteries, suggesting that this “myogenic stimulus” may both activate existing Ca_L channels and increase the number of Ca_L channels in VSMCs during the evolution of hypertension (Pesic et al., 2004). Thus, poorly controlled hypertension in humans may beget further hypertension, a clinical syndrome that is unfortunately common.

5. Membrane depolarization activates vascular Ca^{2+} channels

Harder et al. (1983, 1985) initially reported that VSMCs in small cerebral arteries of spontaneously hypertensive rats (SHR) were depolarized and generated Ca^{2+} -dependent action potentials indicative of an abnormally low permeability of the plasma membrane to K^{+} ions. Subsequently, a loss of resting K^{+} efflux resulting in membrane depolarization was found to be a common feature of VSMCs in a variety of vascular beds exposed to high blood pressure in vivo. For example, microelectrode studies in situ indicate that the resting membrane potential of mesenteric arteries is -38 mV in spontaneously hypertensive rats (SHR) compared to -43 mV in similar arteries of normotensive Wistar-Kyoto (WKY) rats (Stekiel et al., 1986). Similarly, cremaster arteries of renal hypertensive rats show a resting membrane potential of -40 mV compared to -44 mV in similar arteries of control rats (Stekiel et al., 1993). In view of the steep and positive relationship between resting membrane potential and vascular diameter at these voltages, even a loss of a few millivolts in membrane potential is predicted to activate Ca_L channels and accentuate voltage-gated Ca^{2+} influx into VSMCs to cause graded contraction.

The ionic changes that reduce the resting membrane potential of VSMCs during hypertension appear to involve a downregulation of voltage-gated K^{+} (K_V) channels in the arterial plasma membranes exposed to high blood pressure (Cox et al., 2001; Martens and Gelband, 1996; Wang et al., 1997; Yuan et al., 1998a,b). Specifically, a loss of *Shaker*-type K_V channels that contribute to the resting K^{+} efflux has been detected by patch-clamp studies in the VSMCs of SHR (Tobin et al., 2004). Additionally, the higher intraluminal pressure and enhanced sympathetic neural output that is characteristic of hypertensive animals may promote arterial depolarization and activation of voltage-gated Ca_L channels. The final consequence may be a Ca^{2+} -dependent increase in peripheral vascular resistance

that contributes to the progression of blood pressure elevation. However, a reduced membrane potential cannot explain why Ca_L current measured by patch-clamp methods is abnormally elevated in VSMCs from hypertensive animals. Since membrane potential is tightly controlled by this method, the persistence of an elevated Ca^{2+} current in the VSMCs of hypertensive animals suggests an inherent increase in Ca_L channel expression or activity (Cox and Lozinskaya, 1995; Lozinskaya and Cox, 1997; Ohya et al., 1993, 1998; Pesic et al., 2004; Simard et al., 1998).

6. Upregulation of Ca_L channels in response to high blood pressure

An enhanced voltage-gated Ca^{2+} current attributable to an increased number of Ca_L channels has been reported in the cerebral, mesenteric and renal VSMCs of SHR that exhibit a genetic form of hypertension. The elevated Ca_L current has been detected early in the development of hypertension, and does not appear to correspond to an altered voltage-sensitivity of the channel (Lozinskaya and Cox, 1997; Ohya et al., 1993). Rather, single-channel recordings have detected an increased number of Ca_L channel openings without evidence of altered single-channel conductance or open-time distribution (Ohya et al., 1998). All of these findings are consistent with the hypothesis that an increased number of functional Ca_L channel proteins rather than altered channel properties may account for the elevated Ca_L current in the VSMCs of the SHR.

In this regard, Western blots have confirmed an increased expression of the pore-forming α_{1C} subunit of the Ca_L channel in arteries of adult SHR compared to age-matched WKY rats with normal blood pressure levels (Pratt et al., 2002). Indeed, a panel of arteries from the two rat strains indicates an overabundance of α_{1C} subunits in the mesenteric, femoral and renal circulations of the SHR, suggesting that this abnormality extends to at least several vascular beds (Fig. 2). Notably, in the renal, mesenteric and skeletal muscle circulations, the increased expression of α_{1C} subunits corresponds to a higher density of Ca_L current and the development of anomalous vascular tone (Cox and Lozinskaya, 1995; Lozinskaya and Cox, 1997; Ohya et al., 1993, 1998; Pesic et al., 2004; Pratt et al., 2002). For example, in the renal arteries of adult SHR, an increased expression of α_{1C} subunits is associated with an enhanced Ca^{2+} current and an accentuated Ca^{2+} -dependent spontaneous tone that is reversed by treatment with nifedipine, a Ca_L channel blocker (Fig. 3A,B,C).

Interestingly, the membrane density of Ca_L current in VSMCs from either the SHR or WKY rat strain appears to be proportional to blood pressure levels, suggesting that the genetic background of the SHR is not required for Ca_L channel overexpression. For example, a close linear correlation exists between systolic blood pressure and Ca^{2+} current density in VSMCs isolated from small mesenteric arteries of either WKY rats or SHR during natural development (Lozinskaya and Cox, 1997). Also, the treatment of SHR with the angiotensin converting enzyme inhibitor, ramipril, decreased both systolic blood pressure and Ca^{2+} current density concurrently in mesenteric VSMCs (Cox et al., 2002). These data suggest a strong, positive relationship between blood pressure and the number of functional Ca_L channels in the vasculature in vivo and raise the intriguing possibility that high blood pressure per se may promote the expression of the α_{1C} subunit in the arterial circulation.

Recently, the hypothesis that high blood pressure induces vascular Ca_L channel expression was directly tested in the renal circulation of the aortic-banded rat. In this model of hypertension, the aorta is surgically banded between the right renal (RR) and left renal (RR) arteries resulting in the selective elevation of blood pressure in the right renal circulation located proximal to the banded site (Fig. 4 [inset]). Although the right and left renal circulations of these animals were exposed to the same genetic and neuroendocrine influences, the expression of the α_{1C} subunit was profoundly higher in the vasculature of the right kidney exposed to an elevated level of blood pressure for 4 weeks (Fig. 4A). In the same animals, patch-clamp recordings verified that the appearance of the α_{1C} subunit was associated with an increased Ca_L current in the VSMCs of the right renal circulation and the corresponding small arteries spontaneously developed vascular tone that was reversed by nifedipine (Fig. 4B,C). Interestingly, exposure of the right renal circulation to higher blood pressure levels of ~150 mm Hg for as little as 2 days was observed to cause an increased expression of the α_{1C} subunit in the affected arteries (Pesic et al., 2004). These recent findings have provided strong evidence that the upregulation of the α_{1C} subunit by high blood pressure in vivo increases the availability of functional Ca_L channels in renal VSMCs, and even short-term increases in blood pressure may rapidly enhance Ca_L channel expression.

7. How does high blood pressure induce Ca_L channel expression?

Although the stimuli that link the development of high blood pressure to Ca_L channel expression remain unclear, at least two reports have indicated that a reduced membrane potential may be a key factor that promotes the expression of the α_{1C} subunit in the plasma membrane of VSMCs (Ruiz-Velasco et al., 1994; Pesic et al., 2004). In the latter study, renal arteries from normal rats were incubated for 48 h in culture media containing either a physiological level of K^+ (4 mmol/L) to maintain normal membrane potential, or media containing a higher K^+ concentration (30 mmol/L) to depolarize the VSMCs. Under these conditions, the α_{1C} subunit was profoundly upregulated in the arteries exposed to the depolarizing media for 48 h, and the affected VSMCs showed an increased density of Ca^{2+} current indicative of an enhanced abundance of functional Ca_L channels (Fig. 5A,B). Thus, membrane depolarization, which has long been recognized as a characteristic feature of VSMCs exposed to high blood pressure, appears to dynamically upregulate the number of vascular Ca_L channels (Fig. 5C). Although there are few clues regarding other endogenous signals that may regulate Ca_L channel expression in the vasculature, a number of physiological influences including electrical activity, catecholamines, glucocorticoids, testosterone, and intracellular calcium modulate the expression of the cardiac Ca_L channel isoform (Avila et al., 2001; Davidoff et al., 1997; Maki et al., 1996; Nattel and Li, 2000; Takimoto et al., 1997). Thus, it seems plausible that Ca_L channel expression in the vasculature is dynamically determined by complex interactions between blood pressure and the multiple genetic and neuroendocrine influences that impinge on small arteries and arterioles in vivo.

In this regard, the expression of the α_{1C} subunit appears to be regulated at multiple steps that ultimately culminate in Ca_L channel formation. Small increases in transcript levels encoding the α_{1C} subunit have been detected in the mesenteric and femoral arteries of SHR that

exhibit an accentuated expression of functional Ca_L channels (Pratt et al., 2003). However, the relatively small increases in α_{1C} transcript do not appear to fully account for the striking abundance of the α_{1C} protein in the arteries of the SHR, or the pronounced increases in Ca_L current in the corresponding VSMCs (Cox and Lozinskaya, 1995; Lozinskaya and Cox, 1997; Ohya et al., 1993, 1998; Pesic et al., 2004; Pratt et al., 2002; Simard et al., 1998). For this reason, it is likely that events that enhance Ca_L channel translation, translocation or stability in the plasma membrane also are operational in SHR arteries. Unfortunately, there is little information regarding the translational processes that regulate the formation of Ca_L channel subunits in VSMCs. The sole observation that the α_{1C} transcript is affected by cAMP may indicate a possible influence of endogenous substances on transcriptional processes (Xie et al., 1999). Similarly, little is known regarding the mechanisms that regulate Ca_L channel stability or degradation, although one report indicates that the α_{1C} subunit has a short, 3-h half life in HEK cells (Chien et al., 1995). Additionally, proteolytic carboxy terminal truncation of α_{1C} in myometrial smooth muscle may be affected by estrogen and progesterone (Helguera et al., 2002).

One process that has drawn close attention is the interaction between α and β subunits in the endoplasmic reticulum (ER) that enables transport of the α/β channel complex to the plasma membrane to form more functional Ca_L channels (Herlitze et al., 2003; Hullin et al., 2003). A disruption of this process is thought to reduce Ca_L channel formation in some forms of heart failure, resulting in a decline in Ca^{2+} entry and cell contractility (Fan et al., 2003). Trafficking of α_{1C} also can be affected by PI3K and Akt/PKB in a β_2 subunit-dependent fashion (Viard et al., 2004), and by the interaction of β subunits with Rem and Rad GTPases (Beguín et al., 2001; Finlin et al., 2003). However, whether these β subunit-mediated pathways for regulating α_{1C} trafficking are functional in VSMCs remains unknown.

8. Implications for antihypertensive therapies

Recent evidence suggests that the development of anomalous arterial tone during hypertension is associated with an increased expression of vascular Ca_L channels that are minimally composed of α_{1C} , β and $\alpha_{2\delta}$ subunits. Importantly, the α_{1C} subunit is the binding site for all three of the classes (dihydropyridines, phenylalkylamines and benzothiazepines) of organic Ca^{2+} channel blockers (CCBs) that are used to treat essential hypertension in humans. The interaction of these drugs with the α_{1C} subunit reduces the open-state probability of the Ca_L channel and thereby attenuates the voltage-gated Ca^{2+} influx that is required for vascular activation. Fortuitously, the increased abundance of the vascular α_{1C} subunits during hypertension may provide more binding sites for CCBs, thereby amplifying their vasodilator action proportional to blood pressure elevation. This may help to explain why CCBs effectively lower blood pressure in hypertensive animals and humans, but only mildly reduce blood pressure levels in normotensive individuals (Godfraind et al., 1991; Miyamori et al., 1987; Narita et al., 1983; Takata and Hutchinson, 1983).

However, it is estimated that blood pressure is fully normalized in only 20% to 30% of hypertensive patients because of poor compliance due to cost, side effects, drug interactions, and practical difficulties inherent in monitoring by physicians (Dustan, 1996; Mancia and Grassi, 2002; Waeber, 2002). Thus, there is an unmet need for longer-term antihypertensive

treatment to avoid poor compliance and minimize the impact of chronic hypertension on the patient's well being. In this regard, new therapeutics targeting vascular-specific Ca_L channel expression may have more enduring effects. The presence of multiple transcripts (Pang et al., 2003; Saada et al., 2003) and a vascular splice variant of the α_{1C} subunit (Liao et al., 2004; Welling et al., 1997) may afford tantalizing opportunities to differentially downregulate the Ca_L channel in the vasculature to reduce blood pressure, while maintaining expression in the heart that relies on Ca_L channels for cardiac contraction. Furthermore, as the types of β and $\alpha_{2\delta}$ regulatory subunits in VSMCs become known, a number of new paradigms for reducing Ca_L channel expression or gating may emerge that will promote the design of new antihypertensive agents. First, unique CCBs that bind to the β or $\alpha_{2\delta}$ subunits to reduce Ca_L channel expression or gating may enact potent relaxation of VSMCs. Second, mutated β subunits have been designed that co-assemble with the α_{1C} subunit in the endoplasmic reticulum but prevent its translocation to the plasma membrane. In cardiac cells, these agents reduce the excitation–contraction coupling that relies on Ca^{2+} entry through Ca_L channels, but do not disrupt the kinetics of contraction or relaxation (Fan et al., 2003). Exciting findings such as these indicate that the development of new classes of antihypertensive drugs will rely on identifying the subunit basis of Ca_L channels in the vasculature, and designing drugs to reduce their expression or activity.

9. Summary

Reports from a number of laboratories concur that voltage-gated Ca^{2+} influx through Ca_L channels is elevated in VSMCs from several rat models of hypertension. In the same rat strains, an overabundance of the Ca_L channel α_{1C} subunits has been observed in several vascular beds that are involved in blood pressure regulation. Interestingly, it appears that high blood pressure per se promotes the expression of Ca_L channels in the VSMC membrane, and that even short-term rises in blood pressure in vivo are capable of increasing the number of Ca_L channels in small resistance arteries. A recent study suggests that the pressure-induced depolarization of VSMCs, which occurs during the development of hypertension, may provide an electrophysiological signal that triggers the upregulation of vascular Ca_L channels. Thus, blood pressure appears to represent an endogenous promoter of Ca_L channel expression in the arterial circulation, and new efforts are focused on identifying the molecular processes that mediate this event. These future studies, combined with a detailed knowledge of Ca_L channel structure in VSMCs, will set the stage for developing new antihypertensive drugs that reduce the expression or activity of the Ca_L channel to attenuate the anomalous vascular tone that is the hallmark finding of hypertensive disease.

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Fig. 1.

(A) Proposed membrane topology of the L-type Ca^{2+} (Ca_L) channel. The central α_1 subunit is a single large polypeptide of 4 repeat domains (I–IV). The intracellular linkers and N- and C-termini of the α_1 subunit interact with β subunits and intracellular signaling molecules. Genes for four β subunits (β_1 , β_2 , β_3 , β_4) have been identified, and four splice variants of the $\alpha_2\delta$ subunit exist. A γ subunit has been detected in skeletal muscle and brain. Abbreviations: AID, alpha interactive domain; BID, beta interactive domain; CaM, calmodulin; PKA, protein kinase A; PKC, protein kinase C. (B) Linear model of amino acids in the domains of a β subunit. The β subunits possess a guanylate kinase (GK) region that contains the beta interactive domain (BID), and a Src homology 3 (SH3) domain that may interact with the GK region to permit binding between the α_1 and β subunits.

Fig. 2.

Immunoblot showing that the α_{1C} subunit (200–240 kDa) is more highly expressed in the mesenteric, femoral and renal arteries of SHR compared to WKY rats. The doublet bands represent the long and short forms of the subunit. α -Actin was used as a loading control and appears as a 45-kDa band.

Fig. 3.

Renal arteries of SHR show an increased expression and function of the Ca_L channel α_{1C} subunit. (A) The α_{1C} subunit is more highly expressed in renal arteries of SHR compared to WKY rats. (B) Whole-cell Ca^{2+} currents elicited by 8-mV steps from -70 mV to +20 mV were higher in renal VSMCs of SHR compared to WKY rats. (C) Tension-recording traces in isolated renal arteries of WKY and SHR maintained in drug-free physiological salt solution (PSS). The arteries of SHR developed more Ca^{2+} -dependent tone, which was reversed by 1 $\mu\text{mol/L}$ nifedipine. Adapted with permission from Pratt et al. (2002).

Fig. 4.

Aortas of Sprague–Dawley rats were banded between the renal arteries to establish high blood pressure in the right renal circulation proximal to the banded site (inset). Four weeks later, renal arteries were microdissected from the right and left kidneys of the same animal for analysis. (A) The α_1C subunit was more highly expressed in the right renal (RR) arteries exposed to high blood pressure than in the left renal (LR) arteries. (B) Whole-cell Ca^{2+} currents elicited by 8-mV steps from -70 mV to $+50$ mV were higher in renal VSMCs of RR compared to LR arteries. (C) The RR arteries developed more Ca^{2+} -dependent tone than the LR arteries in drug-free physiological salt solution (PSS), which was reversed by $1 \mu\text{mol/L}$ nifedipine. Adapted with permission from Pesic et al. (2004).

Fig. 5.

Isolated renal arteries of control Sprague–Dawley rats were incubated for 48 h in control (Ctrl) or depolarizing (Depol.) media. (A) The Ca_L channel α_{1C} subunit was more highly expressed in the arteries depolarized for 48 h. (B) Whole-cell Ca^{2+} currents elicited by 8-mV steps from -70 mV to +50 mV were higher in renal VSMCs of depolarized compared to control arteries. (C) Cartoon suggesting that depolarization results in the upregulation of α_{1C} subunits in VSMC membranes. The mechanism has not been delineated. Adapted with permission from Pesic et al. (2004).

Table 1Transcript and protein expression of L-type Ca^{2+} (Ca_L) channel subunits detected in the vasculature

Subunit	Human chromosomal location	Vascular expression		
		mRNA	Protein	Preparation
α_{1C} ($\text{Ca}_V1.2$)	12p13.3	Yes		Human aorta ^{a,b}
		Yes	Yes	Mouse aorta ^{c,d}
		Yes	Yes	Rat aorta ^{e,f}
		Yes	Yes	Rat mesenteric artery ^g
		Yes	Yes	Rat femoral artery ^g
		Yes	Yes	Rat renal artery ^{h,i}
		Yes	Yes	Pig coronary artery ^j
			Yes	Pig pulmonary artery ^k
				*Not inclusive of all studies
β_1	17q21–q22	Yes		Rat renal artery ⁱ
β_2	10p12	Yes	Yes	Mouse aorta ^c
		Yes		Rat renal artery ⁱ
		Yes	Yes	Pig pulmonary artery ^k
β_3	12q13	Yes	Yes	Mouse aorta ^c
		Yes	Yes	Rabbit aorta ^l
		Yes		Rat renal artery ⁱ
β_4	2q22–q23	Yes		Rat renal artery ⁱ
$\alpha_2\delta-1$	7q21–q22	Yes	Yes	Human aorta ^{m,n}
$\alpha_2\delta-2$	3p21.3	Yes	Yes	Human aorta ^m
$\alpha_2\delta-3$	3p21.1			???
$\alpha_2\delta-4$	12p13.3			???

^aPang et al. (2003).^bSaada et al. (2003).^cMurakami et al. (2003).^dSafa et al. (2001).^eKoch et al. (1990).^fSaada et al. (2005).^gPratt et al. (2002).^hPesic et al. (2004).ⁱPratt et al. (2003).^jBowles et al. (2004).

k Pesic et al. (2005).

l Hullin et al. (1992).

m Gong et al. (2001).

n Klugbauer et al. (2003).

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Table 2Cardiovascular effects of deletion of specific Ca²⁺ channel subunits in mice

Subunit deletion	Survival	Cardiovascular effect
α_{1C} (smooth-muscle specific, inducible knockout)	Yes ^a	Tibial arteries show loss of Ca _L current in VSMCs, loss of myogenic tone and depolarization-induced contraction; blood pressure is low
β_1 (conventional knockout)	Die at birth of asphyxia ^b	?????
β_2 (conventional knockout)	Embryonic lethal ^c	?????
β_2 (retain expression in heart)	Yes ^c	?????
β_3 (conventional knockout)	Yes ^d	Reduced Ca _L current in aortic VSMCs, blood pressure is normal

^aMoosmang et al. (2003).^bGregg et al. (1996).^cBall et al. (2002).^dMurakami et al. (2003).