Tissue-Specific Expression of Calcium Channels

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The high-voltage-activated calcium channel is a multimeric protein complex containing α_1, α_2/δ, β, and γ subunits. The α_1 subunit is the ion conduction channel and contains the binding sites for calcium channel blockers and toxins. Three genes code for distinct L-type, dihydropyridine-sensitive α_1 subunits; one gene codes for the neuronal P-type (Purkinje) α_1 subunit; and one gene codes for the neuronal N-type α_1 subunit. The smooth and cardiac muscle L-type calcium channel α_1 subunits are splice variants of the same gene. The α_1 subunits are coexpressed with a common α_2/δ subunit and tissue-specific β subunits (at least three genes). The γ subunit apparently is expressed only in skeletal muscle. The properties of these cloned and expressed calcium channels are discussed here. (Trends Cardiovasc Med 1993;3:48–53)

Calcium entry across the plasma membrane in response to hormonal or electrical stimuli represents a major pathway of cellular control of calcium. The voltage-dependent calcium channels, activated and inactivated at a high membrane potential, are the best-characterized plasmalemmal calcium entry pathway, primarily because of the existence of powerful and specific channel-blocking agents. By the use of the organic calcium channel blockers (CaCBs) originally introduced by Fleckenstein et al. (1967), and several neurotoxins, these channels have been subdivided into three distinct classes, the L-, N-, and P-type calcium channels (Figure 1). In contrast, no specific ligands are presently available to characterize the T-type calcium channel, which is low voltage activated and present in a wide variety of excitable and nonexcitable cells. L-, N-, and P-type calcium channels are activated at a high membrane potential (around –30 mV), inactivate slowly (long lasting), and are expressed in neuronal and nonneuronal cells (Tsien et al. 1991). N- and P-type calcium channels are blocked specifically by ω-conotoxin GVIA and the funnel web spider toxin ω-Aga-IVA, respectively (Mintz et al. 1992). Both channels have been identified only in neurons and neuroendocrine cells. L-type channels are readily blocked by the classic CaCBs nifedipine (a 1,4-dihydropyridine, DHP), verapamil (a phenylalkylamine, PAA), and diltiazem (a benzothiazepine) (Glossmann and Striessnig 1988, Hofmann et al. 1990). L-type calcium channels are expressed in neuronal and endocrine cells and in cardiac, smooth, and skeletal muscle. In skeletal muscle, they are essential for excitation–contraction coupling, which does not require calcium influx through the channel. In the normal heart, they are necessary for the generation and propagation of electrical impulses and for the initiation of contraction in atrial and ventricular muscle. In smooth muscle, they are involved in tension development for which process they provide part of the necessary calcium.

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• Composition of the Calcium Channel

Initially, the L-type calcium channel was purified from skeletal muscle. The purified complex contains four proteins (Figure 2): the α_1 subunit [212,018 Daltons (D)], which contains the binding sites for all known CaCBs and the calcium-conducting pore; the intracellularly located β subunit (57,868 D); the transmembrane γ subunit (25,058 D); and the α_2/δ subunit, a disulfide-linked dimer of 125,018 D [see Catterall et al. (1988), Hofmann et al. (1990), and references cited there]. Reconstitution of the purified complex into phospholipid bilayers results in functional calcium channels that are reversibly blocked by CaCBs and are modulated by cAMP-dependent phosphorylation (Flockerzi et al. 1986, Hymel et al. 1988, Nunoki et al. 1989, Mundiña-Weilenmann et al. 1991). The primary sequences of these proteins have been deduced by cloning their corresponding cDNAs from rabbit skeletal muscle (Tanabe et al. 1987, Ellis et al. 1988, Ruth et al. 1989, Bosse et al. 1990, Jay et al. 1990). By using these cDNAs as probes, different α_1 and β subunits have been cloned from heart, smooth muscle, endocrine, and neuronal cells.

• Subunits of the Calcium Channel

The α_1 Subunit

Complete cDNA clones of α_1 subunits that direct the expression of functional calcium channels in *Xenopus* oocytes or cell culture cells have been isolated from skeletal, cardiac, smooth muscle, endocrine glands, and brain. The primary sequences of these cDNAs are homologous to one another and encode proteins of predicted molecular masses of 212–273 kD and homologies of 41%–70%. Hydropathicity analysis of all α_1 subunits predicts a transmembrane topology similar to that of other voltage-dependent ion channels with four homologous repeats, each containing five hydrophobic putative transmembrane α helices and one amphipathic segment (S4) (Figure 2). By functional expression of chimeras of the skeletal

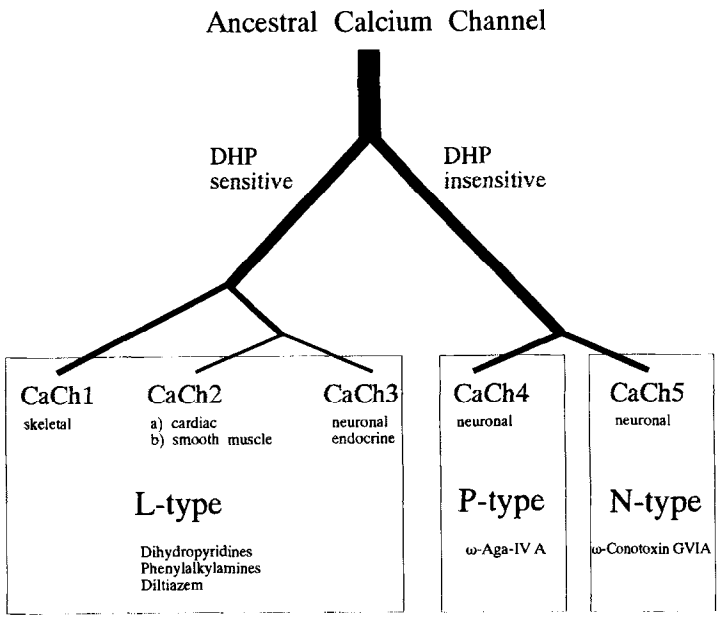

Figure 1. A putative developmental tree of the dihydropyridine-sensitive and -insensitive calcium channel genes. The numbering system of the calcium channel α_1 subunit follows the order of publication of functional clones [see also Hofmann et al. (1993)]. The Snutch et al. (1990) nomenclature for brain calcium channels does not include the CaCh1 gene. The CaCh2, CaCh3, CaCh4, and CaCh5 genes correspond to Snutch genes C, D, A, and B, respectively.

and the cardiac muscle α_1 subunit, specific properties of the calcium channel were assigned to distinct parts of the ion-conducting pore: repeat I determines the activation time of the chimeric channel, that is, slow activation upon membrane depolarization with the repeat from skeletal muscle and rapid activation with that from cardiac muscle (Tanabe et al. 1991); the putative cytoplasmic loop between repeats II and III determines the type of excitation–contraction coupling. The loop from the skeletal muscle calcium channel α_1 subunit induces contraction in the absence of calcium influx, whereas the loop from the cardiac calcium channel α_1 subunit induces contraction only in the presence of calcium influx (Tanabe et al. 1990). The "extracellular" loop between transmembrane helices 5 and 6 (SS1–SS2 region) is predicted to fold into the membrane, to form part of the pore of the channel (Guy and Conti 1990), and to take part in the control of ion selectivity (Heinemann et al. 1992). Photoaffinity labeling of skeletal muscle α_1 subunit followed by limited proteolysis and immunoprecipitation indicates that the DHP-binding site is localized close to the SS1–SS2 region of repeat III (Striessnig et al. 1991, Nakayama et al. 1991) and to a sequence following the IVS6 segment (Regulla et al. 1991), whereas the PAA-binding site has been located directly after the IVS6 segment (Striessnig et al. 1990) (Figure 2). Binding studies with radiolabeled DHPs demonstrate that the stably expressed α_1 subunits from skeletal and smooth muscle alone contain the allosterically coupled binding sites for the known CaCBs (Kim et al. 1990, Bosse et al. 1992).

The so-far cloned cDNAs for the α_1 subunits are encoded by five different genes (CaCh1–5) (Figure 1). The product of the CaCh1 gene occurs in skeletal muscle in two isoforms: a minor form (~5%) of 212 kD containing the complete amino acid sequence encoded by α_1 mRNA, and a major form (~95%) of 190 kD that is derived from the full-length product by posttranslational proteolysis close to amino acid residue 1690 (De Jongh et al. 1991). Presumably, the shorter form is involved functionally at the triad in excitation–contraction coupling of the skeletal muscle. The short and the long forms are phosphorylated rapidly in vitro by cAMP-dependent protein kinase at Ser-687 (Röhrkasten et al. 1988), which is located at the cytosolic loop between repeats II and III, and Ser-1854 (Rotman et al. 1992), which is present only in the larger untruncated form. The α_1 subunits from cardiac (CaCh2a) (Mikami et al. 1989) and smooth muscle (CaCh2b) (Biel et al. 1991) are splice products of the second gene (CaCh2), which is also expressed in other tissues. The homology of the deduced amino acid sequence of CaCh2a to CaCh2b is ~95%. A major difference between the cardiac and the smooth muscle α_1 subunits is the different IVS3 segment that results from alternative splicing of the primary transcript from the CaCh2 gene (Perez-Reyes et al. 1990). The two alternative splice variants CaCh2a and CaCh2b have been expressed transiently and stably (Biel et al. 1991, Singer et al. 1991, Bosse et al. 1992). No major differences have been observed in basic electrophysiologic and pharmacologic properties of the two isoforms, including the amplitude of inward current, steady-state activation and inactivation, and DHP sensitivity (Welling et al. 1992b). However, Northern-blot and polymerase chain reaction (PCR) analyses show that both splice variants are differentially expressed in heart and smooth muscle (Biel et al. 1991) and during cardiac development (Diebold et al. 1992).

cDNA of the third gene (CaCh3) was isolated from neural and endocrine tissues and represents a neuroendocrine-specific L-type calcium channel (Williams et al. 1992a, Seino et al. 1992), whereas the gene products of the fourth and fifth genes (CaCh4 and CaCh5) have been found exclusively in neural tissues. Calcium channels transiently expressed from cRNA of CaCh4 induce high-voltage-activated calcium currents that are insensitive to nifedipine and ω-conotoxin but are inhibited by a mixture of toxins from the funnel web spider that characterize this channel as a P-type calcium channel (Mori et al. 1991). The gene product of CaCh5 binds and is irreversibly blocked by picomolar concentrations of ω-conotoxin, identifying the CaCh5 protein as a neuronal N-type calcium channel (Dubel et al. 1992, Williams et al. 1992b).

The α_2/δ Subunit

The skeletal muscle α_2/δ subunit (CaA1) is a glycosylated membrane protein of

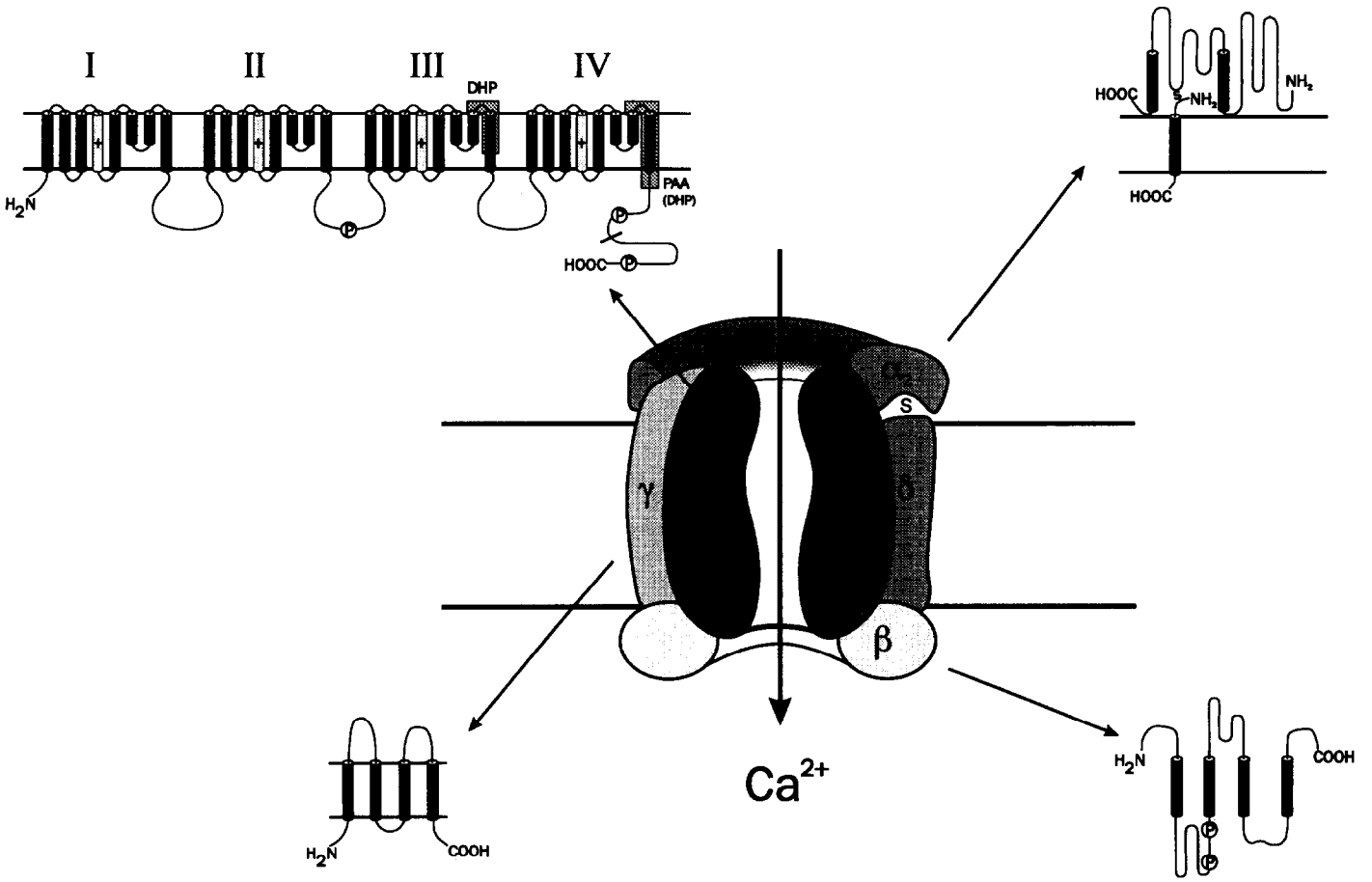

Figure 2. Proposed structure of the skeletal muscle calcium channel. The putative transmembrane configuration of individual subunits is taken from the hydropathicity analysis of the primary sequences: I, II, III, and IV are proposed repeats of calcium channel α_1 subunit; +, proposed transmembrane amphipathic α helix and the proposed voltage-sensing helix of the channel, respectively; P, sites phosphorylated in vitro by cAMP kinase; s, disulfide bridge between the transmembrane δ and the extracellular located α_2 subunit; the SS1–SS2 region is a suggested part of the channel pore containing part of the DHP-binding site; PAA, phenylalkylamine-binding site; and the *dash* at the carboxy-terminal part of α_1 subunit indicates the area where the α_1 subunit is processed posttranslationally. The extracellular space is above the *horizontal lines*.

125,018 D (Ellis et al. 1988), which is apparently highly conserved in most tissues. In the skeletal muscle, the primary protein product of the α_2/δ gene is processed posttranslationally by proteolysis resulting in an α_2 protein containing amino acids 1–934 and a δ protein containing amino acids 935–1080 (De Jongh et al. 1990). The transmembrane δ subunit anchors the extracellular located α_2 protein by disulfide bridges to the plasma membrane (Jay et al. 1991). Immunoblots (Norman et al. 1987) and Northern blots (Ellis et al. 1988, Biel et al. 1990) show that similar or identical α_2/δ subunits exist in skeletal muscle, heart, brain, vascular, and intestinal smooth muscle. Two identical α_2/δ cDNAs have been isolated from rabbit skeletal muscle and human brain (Williams et al. 1992a), whereas the α_2/δ cDNA isolated from rat brain (Kim et al. 1992) predicts an identical δ protein and a splice variant of the processed α_2 protein.

The β Subunit

The skeletal β subunit (CaB1) is an intracellularly located membrane protein consisting of 524 amino acids (Ruth et al. 1989). Its deduced amino acid sequence contains stretches of heptad repeat structure that are characteristic for cytoskeletal proteins. Two other genes (CaB2 and CaB3) encoding β proteins different from the skeletal muscle β subunit have been isolated from a cardiac cDNA library (Hullin et al. 1992). Their deduced amino acid sequences show an overall homology to CaB1 of 71% (CaB2) and 66.6% (CaB3). Differential splicing of the primary transcript of CaB1 results in at least four isoforms: CaB1a–CaB1d (Ruth et al. 1989, Pragnell et al. 1991, Williams et al. 1992a). CaB1a is expressed in skeletal muscle whereas the other isoforms are expressed in brain. Four different splice variants have been characterized for the CaB2 gene (CaB2a–CaB2d); CaB2a and CaB2b have been isolated from a rabbit cardiac cDNA library whereas CaB2c and CaB2d have been cloned from rabbit and rat brain libraries (Hullin et al. 1992, Perez-Reyes et al. 1992). Like the CaB1 gene, the CaB2 and CaB3 genes are tissue-specifically expressed with transcripts of CaB2 existing abundantly in heart and to a lower degree in aorta, trachea, and lung; whereas transcripts of CaB3 genes are expressed in brain and smooth-muscle-containing tissues such as aorta, trachea, and lung (Hullin et al. 1992). This suggests that the CaB3 gene product may be expressed predominantly in neuronal and smooth muscle cells.

The γ Subunit

The γ subunit (CaG1) consists of 222 amino acids and is an integral membrane protein (Bosse et al. 1990, Jay et al. 1990). Its deduced amino acid sequence contains four putative transmembrane domains and two glycosylation sites that are located at the extracellular side. Northern and PCR analysis have not identified the presence of γ subunit in other tissues, suggesting that this protein may be specific for skeletal muscle.

- **Functional Interaction of the Calcium Channel Subunits**

With the exception of the skeletal muscle α_1 subunit, all other cDNAs of Figure 1 have been expressed singly or in combination with other subunits in *Xenopus* oocytes as functional ion channels. Transient expression in *Xenopus* oocytes of CaCh2a cRNA (Mikami et al. 1989) and CaCh2b cRNA (Biel et al. 1990) induces DHP-sensitive currents with electrophysiologic properties similar to those reported from cardiac and smooth muscle. Heterologous coexpression of the cardiac α_1 subunit together with the skeletal muscle β subunit and α_2/δ subunit enhanced consistently the inward current to amplitudes >1 μA/oocyte (Singer et al. 1991). The α_2/δ or β subunits alone or the combination of both decreased the activation time of the barium current twofold (Singer et al. 1991, Wei et al. 1991). Oocytes containing all four subunits (α_1, α_2/δ, β, and γ) had fast-inactivating barium currents. The coexpression of the γ subunit shifted the steady-state inactivation of I_{Ba} by 40 mV to negative membrane potentials (Singer et al. 1991). Under each condition, inward currents were increased several-fold by the calcium channel agonist BayK 8644. Homologous coexpression of the cardiac α_1 subunit with the cardiac β (CaB2) or the neuronal/smooth muscle β subunit (CaB3) with or without the α_2/δ subunit results in an increase in the amplitude of I_{Ba} as well as in an acceleration of channel activation (Hullin et al. 1992).

The three neuronal α_1 subunit cDNAs—that is, the neuroendocrine L-type CaCh3 gene, the neuronal P-type CaCh4 gene, and the neuronal N-type CaCh5 gene—induce barium currents only when coexpressed with the α_2/δ and β subunits (Mori et al. 1991, Williams et al. 1992a and b). The increase in current occurred always in the presence of the β subunit most likely by an increased number of plasmalemmal calcium channel molecules (see also below). These results suggest that the skeletal muscle β subunit interacts with different α_1 subunits by a common interaction site and mechanism. Similar effects of the subunits were obtained by stable coexpression of the skeletal muscle α_1 and β subunits in mouse fibroblasts (L cells), which do not contain an endogenous calcium channel. The β subunit decreased the activation time of the expressed channel >50-fold, and increased the number of DHP-binding sites twofold (Lacerda et al. 1991). In contrast to these results, it has been reported that coexpression of all four skeletal muscle subunits in L cells resulted in a decreased amplitude of the barium current and in a diminished response toward the calcium channel agonist BayK 8644 (Varadi et al. 1991). This phenomenon has not been observed with other L-type calcium channels expressed in *Xenopus* oocytes or CHO cells.

The smooth muscle α_1 (CaCh2b) subunit induces barium currents in CHO cells that are identical to those of native smooth muscle: the single-channel conductance was 26 pSi in the presence of 80 mM Ba^{2+}, the open probability increased with membrane depolarization, and the voltage dependence of activation and inactivation was similar to that of the native smooth muscle channel (Bosse et al. 1992). Stable expression of the CaCh2b with the skeletal muscle β gene (CaB1) increased in parallel the number of DHP-binding sites and the amplitude of whole cell barium current, suggesting that the amplitude of the inward current is directly related to the number of expressed α_1 protein molecules (A. Welling et al., unpublished results). In addition, the coexpression of the β subunit decreased the channel activation time twofold and shifted the voltage dependence of steady-state inactivation by 18 mV to –13 mV, without affecting the sensitivity toward the DHP agonist BayK 8644. The expression of the cardiac α_1 subunit (CaCh2a) in the same cells induces currents that are indistinguishable from that induced by the smooth muscle α_1 subunit. The only difference noted was a faster activation of the cardiac channel. This electrophysiologic similarity is not surprising, since the primary sequence of both channels is 95% identical (Biel et al. 1990).

- **Hormonal Modulation of the Cardiac and Smooth Muscle Calcium Channels**

Activation of the L-type calcium channel is voltage dependent, yet the response to changes in the membrane potential is modulated by hormones. The β-adrenergic receptor agonist isoproterenol increases the cardiac calcium current three- to sevenfold either by cAMP-dependent phosphorylation of the channel (Osterrieder et al. 1982, Hartzell et al. 1991), by the activated α subunits of the trimeric GTP-binding protein G_s (Yatani and Brown 1989), or by a combination of the activated α subunits of the trimeric GTP-binding protein G_s and the active cAMP kinase (Cavalié et al. 1991) (Figure 3). The L-type calcium current of isolated tracheal smooth muscle cells is stimulated also by activation of the β-adrenergic receptor (Welling et al. 1992a). This β-receptor effect is mediated directly by a G protein and not by cAMP-kinase activation. These results suggest that the CaCh2 gene product may be regulated in vivo by the α subunit of a G protein and by cAMP-dependent phosphorylation. The primary sequences of cardiac and smooth muscle α_1 subunits are almost identical and contain identical potential phosphorylation sites. It is therefore conceivable that the cAMP-dependent stimulation of the cardiac calcium channel depends not solely on the phosphorylation of the α_1 subunit but also on the tissue-specific coexpression of other proteins, for example, the β subunit. In support of this idea, it has been reported recently that cAMP increases barium currents in *Xenopus* oocytes expressing the cardiac α_1 only in the presence of the skeletal muscle β subunit (Klöckner et al. 1992). The reported inward currents were small and therefore did not exclude the possibility that the β subunit stimulated in the presence of cAMP the endogenous *Xenopus* oocyte-specific calcium channel (Dascal et al. 1992). Whatever the correct interpretation of these results is, it is evident that the hormonal control of the cardiac calcium channel may be exerted not through the α_1 subunit alone but by tissue-specific expression of various β subunits. The deduced amino acid sequence of the skeletal muscle β subunit

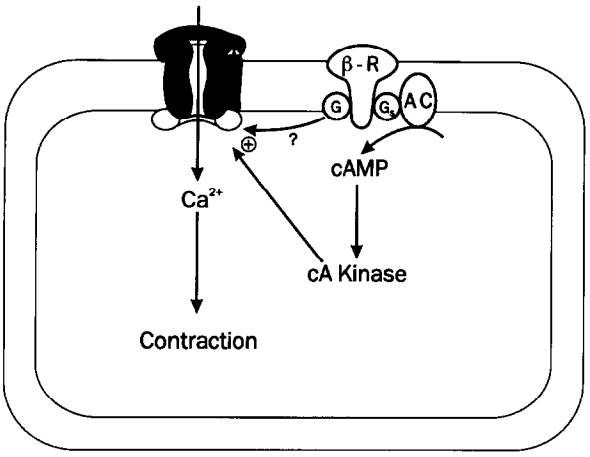

Figure 3. Suggested regulation of the cardiac calcium channel. Note that the cardiac calcium channel does not contain a γ subunit. β-R, β-adrenergic receptor; G, trimeric GTP-binding protein; and AC, adenylyl cyclase.

(CaB1) contains several phosphorylation sites. Two of these sites, Ser-182 and Thr-205, are phosphorylated in vitro by cAMP-dependent protein kinase (Ruth et al. 1989, De Jongh et al. 1989). The equivalent to Thr-205 is conserved in the "cardiac" β subunit (Thr-165 in CaB2a and Thr-191 in CaB2b) but is not present in the "smooth muscle" β subunit CaB3. The sequence following this potential phosphorylation site is highly variable and determines several splice variants (Hullin et al. 1992). This variable region may be responsible for the tissue-specific regulation of the L-type calcium currents by hormones and neurotransmitters.

• Conclusion

High-voltage-activated calcium channels are oligomeric complexes of four different subunits: α_1, α_2/δ, β, and γ. So far, five different genes encoding α_1 subunits and three distinct genes encoding β subunits have been isolated from various tissues. The specific electrophysiologic and pharmacologic characteristics of calcium currents in different cells result from the expression of tissue-specific subunits of the calcium channel leading to differences in functional interaction. This genetic polymorphism explains also the different regulatory mechanisms and possibly offers a chance for a therapy with more specific drugs than those on hand in the future.

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