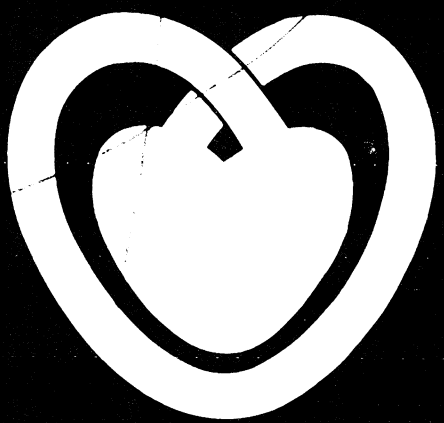

European Heart Journal

European Heart Journal

the journal of the European Society of Cardiology

Volume 12
Supplement D
Pages 1–205

European Heart Journal (1991) **12** (*Supplement D*), 95-98

High voltage activated calcium channels: molecular composition and function

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KEY WORDS: Ca^{2+} channel, cDNA cloning, phosphorylation, expression.

Voltage-activated calcium channels comprise a group of similar yet distinct proteins or protein complexes that differ in electrophysiological properties, modulation by phosphorylation and GTP-binding proteins and in their relative sensitivity to organic calcium channel blockers. Cloning of the cDNA of L-type calcium channels from skeletal muscle, heart and smooth muscle opens the way to understanding the molecular basis of channel function and regulation and provides means of studying calcium channels in other tissues.

Introduction

Voltage activated calcium channels are membrane-spanning proteins that allow the controlled entry of calcium ions into the cytoplasm of cells and thereby contribute to the genesis of action potentials[1-4]. By raising $[Ca]_i$, calcium channels transduce electrical signals to chemical signals that command changes in secretion, metabolism, contraction or excitability when decoded by appropriate calcium receptor proteins such as calmodulin, troponin, and calcium-activated potassium channels. Calcium channels are vital for several processes of the cardiovascular system. In the healthy heart, they are essential for generation of normal cardiac rhythm, to induce propagation through the atrioventricular node, and for contraction in atrial and ventricular muscle. In diseased myocardium, calcium channels can contribute to abnormal impulse generation and cardiac arrhythmias. In blood vessels they provide a direct supply of activating calcium, which controls smooth muscle contraction and vascular tone. At least three types of voltage-activated calcium channels have been distinguished on the basis of their voltage dependence, time dependence, conductance and pharmacology: namely, L-, T- and N-type channels (Table 1)[1-3]. L-type channels are virtually ubiquitous and are the major pathway for voltage-gated calcium entry in heart and most kinds of smooth muscle. The L-type channel is high voltage activated, has a high Ca^{2+} conductance contributing to a long-lasting current and is readily blocked by calcium channel blockers such as nifedipine and verapamil. In cardiac muscle, β-adrenergic agonists increase the probability of this channel type being open,[5] either by phosphorylation of the channel itself or by stabilizing the open state via the α subunit of the GTP-binding protein Gs[3,6]. Although work on many cell systems has contributed to current understanding of calcium channel function the structural properties of the channel have been investigated most thoroughly in skeletal muscle which is particularly rich in the specific high affinity receptor for calcium channel blockers (CaCB receptor)[7].

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Biochemistry of the skeletal muscle CaCB receptor/calcium channel

When purified from rabbit skeletal muscle, the calcium channel consists of three main subunits with molecular masses of 165 000 (CaCB receptor or α_1 subunit), 55 000 (β) and 32 000 Da (γ) (Table 2)[8-12]. A further polypeptide consisting of a disulphide-linked dimer of a 130 000 (α_2) and a 28 000 (δ) protein is present in this preparation at a variable concentration[12]. The subunits have been reconstituted to functional calcium channels which were modulated by phosphorylation[13] and by monospecific antibodies for the α_1, β and γ subunits[14,15]. The CaCB receptor is the principal transmembrane unit, which forms the ion-conducting pore. This protein binds calcium channel blockers[4,12,16,17] such as dihydropyridines and phenylalkylamines with 1:1 stoichiometry (for each compound one site per CaCB receptor) and is phosphorylated in vitro at Ser_{687} by cAMP-dependent protein kinase[18]. The β subunit, but not the γ subunit, contains multiple phosphorylation sites[19]. Neither of the subunits binds calcium channel blockers. Attempts to isolate only the CaCB receptor under non-denaturing conditions have not been successful so far, suggesting that the β and γ subunit stabilize the channel in a high-affinity CaCB-binding conformation. At present, it is not known whether the 130/28 kDa protein belongs to this structure[11] or is only a contaminant.

Molecular biology of the skeletal muscle calcium channel

Complementary DNAs for the skeletal muscle calcium channel were isolated on the basis of peptide sequences derived from the purified proteins[17,20-22]. The cDNA of the CaCB receptor encodes a large polypeptide (212 kDa) which is structurally similar to voltage activated sodium channels. As with the sodium channel, the

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Table 1 Properties of the three types of mammalian voltage activated calcium channels

Channel type	T	N	L
electrophysiology			
threshold potential	low	high	high
inactivation	fast	intermediate	slow
pharmacology			
dihydropyridine-sensitive	–	–	+
phenylalkylamine block	(+)	–	+
* Ω-conotoxin block	(+)	+	+
inorganic ion block	Ni^{2+}	Cd^{2+}	Cd^{2+}
occurence	ubiquitous	neurons	ubiquitous

* Omega-conotoxin appears to bind to N-type calcium channels in brain thereby blocking channel function (McCleskey, EW, Fox AP, Feldman DH *et al.* 1987; Proc Natl Acad Sci USA 84: 4327-31).

Table 2 Subunit structure of the skeletal muscle calcium channel

subunit	M_r (kDa)	CaCB	Phosphorylation	Glycosylation	Primary structure	Possible function
α_1 (CaCB-receptor)	165	dhp paa dil	Ser_{687}	weak	1873 aa	ion-conducting voltage sensor unit
β	55	—	Ser_{182} Thr_{205}	no	524 aa	regulatory
γ	32	—	—	strong	222 aa	regulatory
α_2	130	—	—	strong	1106 aa	?
δ	28	—	—	strong	n.d.	?

Abbreviations: CaCB, calcium channel blocker; dhp, dihydropyridine; paa, phenylalkylamine; dil, diltiazem; aa, amino-acid; n.d., not determined.

CaCB receptor possesses four internal repeats which are 48 to 55% homologous (Fig. 1). Each repeat is composed of five putative transmembrane α-helices and one amphophilic segment, S4, which contains five or six positively charged amino-acids. Homologous S4 segments are present in the potassium and sodium channels from a variety of different species suggesting that this highly conserved segment is an essential part of a voltage activated cation channel. It is thought that S4 responds to a change in the membrane potential with a slight intramembrane shift of its positive charges, and thereby induces a conformational change in the protein which leads to channel opening or closing[23]. The deduced amino-acid sequence of the skeletal muscle CaCB receptor contains seven potential phosphorylation sites for cAMP-dependent protein kinase[17]. One of these sites, Ser_{687}, is readily phosphorylated by cAMP kinase in vitro[18]. Microinjection of an expression plasmid carrying the CaCB receptor cDNA induces a high voltage activated calcium current in dysgenic muscle[24] and in non-excitable mouse L cells[21,22]. In addition, it restores excitation contraction coupling in myocytes of dysgenic (mdg) mice[24], suggesting that the skeletal muscle CaCB receptor functions both as a calcium channel and as a voltage sensor coupling extra-cellular excitation to the release of calcium from the sarcoplasmic reticulum. It is not known whether these two functions require the presence of the other subunits.

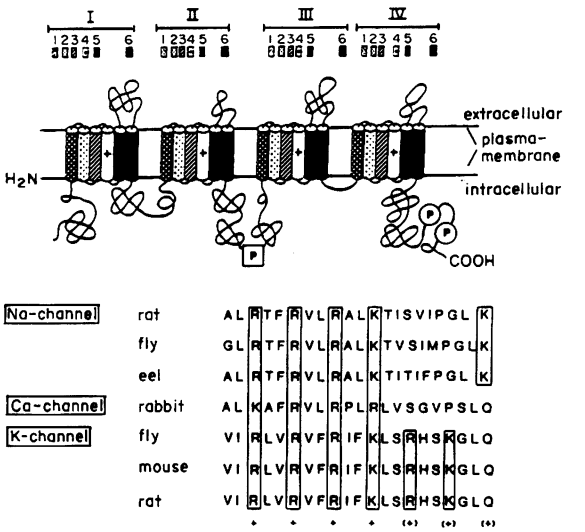

Figure 1 Transmembrane folding model of the CaCB subunit (upper part) and conservation of the amino-acids of the positively charged segment S4 among voltage activated cation channels (lower part). The internal repeats I, II, III and IV each composed of six transmembrane segments are shown. P indicates phosphorylation site which is phosphorylated in vitro in the skeletal muscle CaCB receptor (□) and which is conserved in the skeletal, cardiac and smooth muscle CaCB receptor (○). Alignment of S4 segments from voltage activated Na+, Ca2+ and K+ channels. The one letter code for amino-acids is used. R, Arg; K, Lys.

The β subunit consists of 524 amino-acids[25]. It contains four homologous α helical segments. In contrast to the CaCB receptor, the β subunit is substantially hydrophilic suggesting that it is a peripheral membrane protein. Further analysis of its primary structure reveals multiple potential phosphorylation sites, two of which, Ser_{182}[25] and Thr_{205}[26], are readily phosphorylated by cAMP-dependent protein kinase in vitro.

The γ subunit consists of 222 amino-acids[27,28]. It is very hydrophobic and contains four typical membrane spanning regions. Thus it is reasonable to assume that the amino and carboxy termini are localized intracellularly whereas the four hydrophobic segments cross the cell membrane[27]. This model (Fig. 2) is consistent with the two potential N-glycosylation sites being located on the extracellular site. In vivo the γ subunit is highly glycosylated, and this accounts for the difference in molecular mass between the natural γ subunit ($\approx$32 kDa) and the predicted mature unglycosylated polypeptide ($\approx$25 kDa). The primary structure of the γ subunit shows no homology to other known protein sequences. However, 34·1% of a sequence of 44 amino-acids within the γ subunit are identical with a similar sequence of the multidrug resistance protein 2 (mdr 2 or P-glycoprotein)[29]. Interestingly, mdr2 binds calcium channel blockers[30]. Although the γ subunit does not bind these compounds by itself, it cannot be excluded that the similar amino-acid sequences are indirectly involved in calcium channel blocker binding to the CaCB receptor and the mdr2 protein, respectively.

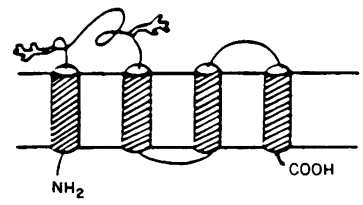

Figure 2 Transmembrane folding model of the γ subunit of the skeletal muscle calcium channel. The four transmembrane segments are indicated. Amino and carboxy termini are localized intracellularly. Potential N-glycosylation sites (Asn 43 and Asn 79) are indicated in the first extracellular loop.

L-Type calcium channel proteins in smooth and cardiac muscle

Complementary DNAs for the CaCB receptor have been isolated from rabbit heart[31] and rabbit smooth muscle[32] on the basis of sequence homology with their skeletal muscle counterpart. Both the cardiac and smooth muscle cDNAs encode large polypeptides (2171 and 2166 amino-acids, respectively) showing an overall homology of 66% (cardiac) and 65% (smooth muscle) to the skeletal muscle CaCB receptor. The amino-acid sequence of the smooth muscle CaCB receptor differs from the rabbit heart receptor at four sites comprising the amino terminus, segments IS6 and IVS3 and an intervening sequence between repeats I and II[32]. Both channel proteins are differentially expressed. The mRNA of the cardiac channel is exclusively expressed in heart whereas the mRNA of the smooth muscle channel is present in airway and vascular smooth muscle cells, which exist in lung, trachea, heart, aorta and brain (Table 3)[32]. Cardiac ventricular and smooth muscle myocytes express mainly high voltage activated calcium channels, with the slow inactivating properties classified as L-type, which are sensitive to calcium channel blockers. In agreement with this, microinjected synthetic RNA derived from the cloned cardiac and smooth muscle CaCB receptor cDNA directs the synthesis of similar channels in Xenopus oocytes[31,32]. These results indicate that the cardiac and smooth muscle CaCB receptors alone are sufficient to induce calcium channel activity.

Conclusion

In vivo, the skeletal muscle CaCB receptor functions both as voltage sensor, which directly controls release of calcium from the sarcoplasmic reticulum, and as calcium channel.The similar tissue specific expression of the mRNA encoding the skeletal muscle CaCB receptor (α_1), β and γ subunit (Table 3) suggests that the three proteins contribute to these functions. In contrast to the skeletal muscle CaCB receptor, the cardiac receptor functions only as calcium channel and, when injected into myotubes of dysgenic mice, it releases calcium by an indirect calcium-dependent mechanism[33]. Hence, different CaCB receptor proteins and their association with other components, such as β and γ subunits, may

Table 3 Hybridization specificity of the calcium channel subunits

cDNA probe	smooth	cardiac	skeletal
CaCB receptor			
common	8·9/(15·5)	8·9/(15·5)	6·5/(8·9)
smooth	8·9/(15·5)	8·9/(15·5)	—
cardiac	—	8·9/(15·5)	—
skeletal	—	—	6·5
α_2 subunit	8·0	8·0	8·0
β subunit	—	—	1·9
γ subunit	(1·3)	—	1·3

Numbers indicate length of hybridizable mRNAs of rabbit smooth, cardiac, and skeletal muscle in kilobases using the respective cDNA probes (for details see refs 17, 20, 25, 27, 28, 31, 32).

be the structural basis for differences in function among various L-type calcium channels. This hypothesis is strongly supported by the isolation of partial cDNA clones encoding various CaCB receptor-like proteins in rat aorta[34] and brain[35], suggesting that at least a portion of calcium channel diversity is the result of the expression of distinct CaCB receptors.

In vivo, L-type calcium channels from cardiac and smooth muscle have similar electrophysiological properties[1,2]. The primary sequences of the cardiac and lung CaCB receptor contain identical sites that might be phosphorylated by cAMP-dependent protein kinase in vivo[31,32]. However, the biochemical modulation of both channels appears to be different. cAMP-dependent phosphorylation increases the cardiac calcium current[5,36], whereas it has little or no effect on the smooth muscle current[37]. Therefore, stimulation of the calcium current might be not due to phosphorylation of the CaCB receptor itself. Further work involving the stable expression of the CaCB-receptor in cells exhibiting appropriate signal transduction pathways will be required to test this hypothesis.

We thank R. Nagel for expert technical assistance and H. Siepmann for the graphical work. This work was supported by Deutsche Forschungsgemeinschaft, Thyssen, and Fond der Chemischen Industrie.

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