Minireview

The Dihydropyridine-sensitive Calcium Channel of the Skeletal Muscle: Biochemistry and Structure

W. NASTAINCZYK, A. LUDWIG and F. HOFMANN

Institut für Medizinische Biochemie, Medizinische Fakultät, Universität des Saarlandes, D 6650 Homburg/Saar, Federal Republic of Germany

Abstract. The dihydropyridine-sensitive calcium channel of the rabbit skeletal muscle is the first voltage-gated calcium channel which has been purified and biochemically characterized. The α_1-subunit, a 165 kDa protein, of the purified dihydropyridine receptor contains all regulatory sites of a L-type calcium channel and the calcium conducting unit. The purpose of this review is to summarize and discuss recent findings on the structure and possible function of the skeletal muscle calcium channel subunits.

Key words: Calcium channel — Skeletal muscle — Subunit structure

Introduction

Regulation of the influx of calcium through voltage-dependent channels is important for the control of many cellular functions. Well-known examples of cellular functions regulated by the influx of calcium include excitation-contraction coupling in cardiac and smooth muscle as well as hormone and transmitter release in neurons and other secretory cells (Reuter 1983; Tsien et al. 1987; Hofmann et al. 1987). Three classes of calcium channels (L-, N- and T-types) have been identified which differ in their electrophysiological properties, specific sensitivity to organic calcium channel blockers, and their ability to be regulated by the membrane potential and by hormone receptors (Nowycky et al. 1985). The electrophysiological properties of calcium channels have been studied in great detail in many cell types. However, their biochemical characterization has been limited in general to the skeletal muscle, where L-type channel like structures, i.e. the charge carriers have a particular high density (Galizzi et al. 1986). Mammalian skeletal muscles contain at least two separate

Presented at the microsymposium Calcium Transport Systems in Excitable Cells, Bratislava, Feb. 7, 1990

calcium channel subtypes (Cognard et al. 1986). One class of channels is activated at −65 mV and produces a transient inward current. This channel is not sensitive to organic calcium channel blockers. The activation of the second, a L-type channel, leads to a large and long lasting conductance and inactivates slowly. This channel is blocked by organic calcium channel antagonists (Almers et al. 1985). Adult skeletal muscle contains a third structure, the charge carrier, which is blocked by the organic channel blockers and its function appears to be essential for excitation-contraction coupling (Rios and Brum 1987; Adams and Beam 1989). The availability of organic calcium channel blockers and their radiolabeled forms have allowed the identification, purification and biochemical characterization of the L-type calcium channel of the skeletal muscle, the subject of this review.

Purification and functional reconstitution of the L-type Ca^{2+} channel

Voltage-dependent calcium channels in the skeletal muscle have been identified in vitro by three separate, allosteric linked drug binding sites which are specific for calcium antagonist drugs such as 1,4-dihydropyridines, phenylalkylamines and benzothiazepines (Hofmann et al. 1987). The skeletal muscle receptor for 1,4-dihydropyridine is localized at the transverse tubular membrane which contains high densities of these sites (Jorgensen et al. 1989). The receptor has been purified from rabbit skeletal muscle by several laboratories (Curtis and Catterall 1984; Flockerzi et al. 1986a; Takahashi et al. 1987; Leung et al. 1988). The purified receptor contains three polypeptides of 165 (α_1), 55 (β) and 32 (γ) kDa. A stoichiometric ratio of 1:1:1 was determined for the α-, β- and γ-subunits, suggesting that they may belong to the same molecular structure (Sieber et al. 1987). A single 170 kDa glycoprotein that upon reduction forms two polypeptides of 135 (α_2) and 32 (δ) kDa was also identified in the purified receptor complex. The relationship of this protein to the other three subunits is unknown.

The purified receptor complex reconstitutes to a L-type calcium channel of 20 pSi (Flockerzi et al. 1986b; Pelzer et al. 1988; Talvenheimo et al. 1987). This calcium channel is blocked by organic and inorganic calcium channel blockers. The open probability of the channel is increased by the calcium channel activator Bay K 8644 and by phosphorylation with cAMP-kinase (Nunoki et al. 1989; Hymel et al. 1988; Pelzer et al. 1988; Flockerzi et al. 1986b). A second channel with a lower conductance has also been observed, eventually indicating the presence of several distinct calcium channels (Ma and Coronado 1988). Binding experiments with dihydropyridines, phenylalkylamines and diltiazem have shown that the reconstituted receptor complex contains the binding sites for all three major classes of calcium channel antagonists (Flockerzi et al. 1986a; Striessnig et al. 1986; Sieber et al. 1987).

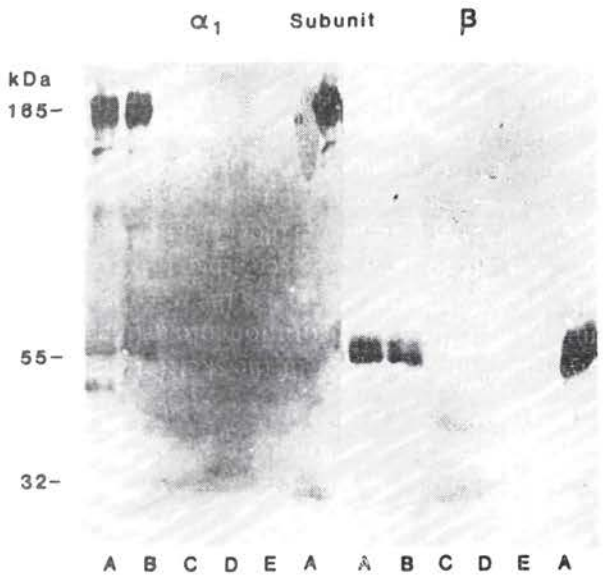

Fig. 1. Rabbit microsomes from various tissues and purified calcium channel of the rabbit skeletal muscle were subjected to SDS-PAGE under reducing conditions. Proteins were then transferred to an Immobilon (PVDF) membrane and stained with anti α_1-subunit mAB-8B7 (*left panel*) of anti β-subunit mAb-7C3 and goat anti mouse IgG alkaline phosphatase conjugate (*right panel*). *A*: pure calcium channel (0.4 μg), *B*: skeletal muscle (12.5 μg), *C*: heart (303 μg), uterus (500 μg) and trachea (500 μg).

Biochemistry of the L-type calcium channel subunits

α_1-subunit

The α_1-subunit has an apparent molecular mass of 165 kDa on SDS-PAGE. The protein molecule is poorly glycosylated. The α_1-subunit contains the specific sites for calcium channel blockers as determined by labeling with photoaffinity ligands for dihydropyridines, phenylalkylamines and benzothiazepines (Sieber et al. 1987; Striessnig et al. 1987; Takahashi et al. 1987). The α_1-subunit contains also specific phosphorylation sites, which in vitro are phosphorylated by cAMP-kinase, protein kinase C, Ca^{2+}-calmodulin-kinase, casein kinase II and cGMP-kinase (Röhrkasten et al. 1988; Jahn et al. 1988). The primary amino acid sequence of the α_1-subunit has been deduced from its cDNA and is consistent with a 212 kDa protein (Tanabe et al. 1987; Ellis et al. 1988). It contains four repeated transmembrane units of high homology, which are composed of six

Table 1. Immuno cross-reactivity of monoclonal antibodies against the α_1- or β-subunit of the rabbit skeletal muscle calcium channel with skeletal muscle microsomes from different species

	Human	Rabbit	Rat	Mouse	G. pig	Sheep	Cow	Pig	Chick	Frog
anti α_1 mAb										
6C4	−	+	+	−	+	−	+	−	−	−
3B5	−	+	+	+	+	−	+	−	−	−
7G11	−	+	+	+	+	+	−	−	−	−
7D11	−	+	+	+	+	+	+	+	−	−
8B7	+	+	+	+	+	+	+	+	+	+
anti β mAb										
7C3	+	+	+	+	+	+	+	+	−	−

Microsomes were prepared from the skeletal muscles by ultracentrifugation. The microsomal proteins were separated by SDS-Page under reducing conditions. The proteins were then transferred to a Immobilon (PVDF) membrane and stained with the monoclonal antibodies as described in legend to Fig. 1

transmembrane α-helices. The S4 segment contains a positively charged helix and could be the voltage sensor of the calcium channel. This unit is also found in other ion channels. The deduced amino acid sequence has a high degree of homology to other voltage-dependent ion channels, particularly to the sodium channel. The functional importance of the α_1-subunit for the calcium channel activity was shown in dysgenic muscle cells of mice where the α_1-subunit is greatly reduced. Injection of cDNA for the α_1-subunit restores a dihydropyridine- sensitive slow calcium channel in these cells (Tanabe et al. 1987). Moreover, one monoclonal antibody to the α_1-subunit inhibited the dihydropyridine-sensitive calcium channel in cultured muscle cells (Morton et al. 1988). These results show that the α_1-subunit is the channel forming protein, containing all regulatory sites known to effect the L-type calcium channel in vivo, i.e. sites for organic calcium channel blockers, activators and cAMP-dependent phosphorylation. The α_1-subunit of the skeletal muscle channel differs from that of other excitable tissues. Thus, Northern blots carried out with an oligonucleotide probe obtained from the cDNA of the skeletal muscle α_1-subunit show crosshybridization to 8.2 kb mRNA in brain, heart and smooth muscle and to a 6.3 kb mRNA in skeletal muscle (Mikami et al. 1989; Biel et al. 1989). Similarly, monoclonal antibodies against the α_1-subunit do not detect the α_1-subunit in other tissues including heart, brain, lung, trachea, and uterus (Fig. 1). In contrast, these antibodies detect the α_1-subunit in the skeletal muscle from human, rabbit, mouse, rat, guinea pig, hamster, sheep, cow, pig, chick, and frog (Table 1) (Schmid et al. 1986; Norman et al. 1987). This suggests that differences exist in the primary sequence of the α_1-subunit from different tissues.

β-subunit

The β-subunit has an apparent molecular mass of 55 kDa. The protein is apparently not glycosylated (Takahashi et al. 1987). The β-subunit contains several specific phosphorylation sites, which are phosphorylated by cAMP-kinase (at Ser 182) and other sites may be phosporylated by protein kinase C and cGMP-kinase (Jahn et al. 1988). The primary amino acid sequence of the β-subunit has recently been deduced from its cDNA which codes for a 57 kDa protein (Ruth et al. 1989). It contains four hydrophilic α-helices, each consisting of a homologous unit of eight amino acids. The deduced sequence is compatible with a peripheral membrane protein. A monoclonal antibody to the β-subunit can activate the calcium channel reconstituted into a planar lipid bilayer and can modulate the calcium channel of cultured cells (Vilven et al. 1988). These results and the phosphorylation studies support a modulatory role for the β-subunit. It is not known as yet, whether the β-subunit is present in other tissues. A monoclonal antibody to the β-subunit does not detect the subunit in other tissues including heart, brain, lung, trachea and uterus. But this antibody cross-reacts with a protein similar to the β-subunit in human, rabbit, rat, guinea pig, cow, pig, mouse and sheep (Table 1). However, other results suggested that mRNA and a protein similar to the β-subunit of the skeletal muscle may be present in brain. It is therefore possible that β-subunit like proteins are also present in some tissues.

γ-subunit

The γ-subunit of the dihydropyridine-sensitive calcium channel is a heavily glycosylated protein with an apparent molecular mass of 32 kDa on SDS-PAGE (Flockerzi et al. 1986a; Takahashi et al. 1987). Although, the carbohydrate content of the γ-subunit is 30 %, the subunit is very hydrophobic. An antibody to the γ-subunit inhibited the calcium channel activity, suggesting that the γ-subunit has also modulatory functions (Campbell et al. 1988). The primary structure of the γ-subunit is not known.

α_2- and δ-polypeptide

Previously it was suggested that the α_2-polypeptide was the receptor for calcium channel blockers (Barhanin et al. 1987). It is now known that the protein does not bind calcium channel blockers nor does it have the primary structure of an ion channel protein. The α_2-peptide is heavily glycosylated (Takahashi et al. 1987). After reduction, its apparent molecular mass decreases from 165 kDa to 135 kDa due to dissociation of disulfide linked proteins of a molecular mass of about 28 kDa. The 28 kDa protein has been termed δ-subunit (Leung et al.

1987). The primary sequence of the α_2-polypeptide has been deduced from its corresponding cDNA. The deduced sequence of the α_2-protein is compatible with that of a heavily glycosylated membrane protein (Ellis et al. 1988). The α_2-polypeptide and the attached δ-polypeptide are not phosphorylated in vitro. The functional role of these proteins is not yet known. Antibodies against the α_2-protein of the rabbit skeletal muscle specifically detect an identical or similar protein in a wide variety of tissues including brain, heart, skeletal muscle and smooth muscle (Schmid et al. 1986; Norman et al. 1987). Similarly, the cDNA of the α_2-protein hybridizes with the mRNA of other tissues in Northern blots (Ellis et al. 1988). Therefore, it is clear that an identical α_2-protein is present in most tissues. This result supports the notion that the α_2-protein may not be an essential part of the calcium channel.

Conclusion

The purified 1,4-dihydropyridine receptor of the skeletal muscle forms a functional L-type calcium channel after reconstitution into artifical membranes. The functional unit of the purified calcium channel is the α_1-subunit. The α_1-subunit has most properties of a dihydropyridine-sensitive L-type calcium channel: binding sites for the organic calcium channel blockers, i.e. 1,4-dihydropyridines, phenylalkylamines and benzothiazepines, phosphorylation sites for cAMP-kinase and protein kinase C, the primary structure of a voltage-dependent ion channel and forms a L-type calcium channel after reconstitution. The α_1-subunit of heart and smooth muscle are similar proteins. However, they differ considerably in those parts which are exposed to the intracellular compartment (Mikami et al. 1989). Antibodies raised against these structures do not crossreact with the α_1-subunit from different tissues. This suggests that different genes code for the α_1-subunit of skeletal muscle and heart or smooth muscle. The role of the β- and γ-subunit is unclear. But, several lines of evidence suggest that they are part of the calcium channel and modulate the channel function. Thus, the β-subunit contains specific phosphorylation sites for cAMP-kinase and protein kinase C. Antibodies against the β- or γ-subunit modulate *in vivo* and *in vitro* the dihydropyridine-sensitive calcium channel of the skeletal muscle. It is unlikely that the same proteins are present in other tissues. This and other evidence raise the possibility that these proteins may be essential for the function of the α_1-subunit as charge carrier in skeletal muscle. This interpretation would be in line with the finding that antibodies against the α_1-subunit stain an identical protein at two distinct localizations in skeletal muscle (Jorgensen et al. 1989). Further work is required to support this notion.

References

Adams B. A., Beam K. G. (1989): A novel calcium channel in dysgenic skeletal muscle. J. Gen. Physiol. **94,** 429–444

Almers W., McCleskey E. W., Palade P. T. (1985): Calcium channels in vertebrate skeletal muscle. In: Calcium in Biological Systems (Ed. R. P. Rubin, G. B. Weiss, and J. W. Putney, Jr.), pp. 321–330, plenum Press, New York

Barhanin, J., Coppola T., Schmid A., Borsotto M., Lazdunski M. (1987): The calcium channel antagonist receptor from rabbit skeletal muscle. Eur. J. Biochem. **167,** 525–31

Biel M., Ruth P., Flockerzi V. (1989): Cloning of a calcium channel from rabbit smooth muscle. Biol. Chem. Hoppe-Seyler **370,** 777

Campbell K. P., Leung A. T., Sharp A. H., Imagawa T., Kahl S. D. (1988): Calcium channel antibodies: subunit-specific antibodies as probes for structure and function. In: The Calcium Channel: Structure, Function and Implications (Eds. Morad M., Nayler W. G., Kazda S., Schramm M.), pp. 586–600, Springer, Berlin Heidelberg New York Tokyo

Cognard C., Lazdunski M., Romey G. (1986): Different types of calcium channels in mammalian skeletal muscle cells in culture. Proc. Nat. Acad. Sci. USA **83,** 517–521

Curtis B. M., Catterall W. A. (1984): Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. Biochemistry **23,** 2113–2118

Ellis S. B., Williams M. E., Ways N. R., Brenner R., Sharp A. H., Leung A. T., Campbell K. P., McKenna E., Koch W. J., Hui A., Schwartz A., Harpold M. M. (1988): Sequence and expression of mRNAs encoding the α_1 and α_2 subunits of a DHP-sensitive calcium channel. Science **241,** 1661–1664

Flockerzi V., Oeken H.-J., Hofmann F. (1986a): Purification of a functional receptor for calcium channel blockers from rabbit skeletal muscle microsomes. Eur. J. Biochem. 161, 217–224

Flockerzi V., Oeken H.-J. Hofmann F., Pelzer D., Cavalie A., Trautwein W. (1986b): The purified dihydropyridine binding site from skeletal muscle T-tubulus is a functional calcium channel. Nature **323,** 66–68

Galizzi, D. P., Borsotto M., Barhanin J., Fosset M., Lazdunski M. (1986): Characterisation and photoaffinity labeling of receptor sites for the calcium channel inhibitors d-cisdiltiazem, bepridil, desmethoxyverapamil, and (+)-PN200-110 in skeletal muscle transverse tubule membranes. J. Biol. Chem. **261,** 1393–1397

Hofmann F., Nastainczyk W., Röhrkasten A., Schneider T., Sieber M. (1987): Regulation of the L-type calcium channel. Trends Pharmacol. Sci. **8,** 393–398

Hymel L., Striessnig J., Glossmann H., Schindler H. (1988): Purified skeletal muscle 1,4-dihydropyridine receptor forms phosporylation-dependent oligomeric calcium channels in planar bilayers. Proc. Nat. Acad. Sci. USA **85,** 4290–4294

Jahn H., Nastainczyk W., Röhrkasten A., Schneider T., Hofmann F. (1988): Site specific phosphorylation of the purified receptor for calcium channel blockers by cAMP-, cGMP-dependent proteinkinase, protein kinase C, calmodulin-dependent protein kinase II, and casein kinase II. Eur. J. Biochem. **178,** 535–542

Jorgensen A. O., Shen A. C.-Y., Arnold W., Leung A. T., Campbell K. P. (1989): Subcellular distribution of the 1,4-dihydropyridine receptor in rabbit skeletal muscle in situ: An immunofluorescence and immunocolloidal gold-labelling study. J. Cell Biol. **109,** 135–147

Leung A. T., Imagawa T., Block B., Franzini-Armstrong C., Campbell K. P. (1988): Biochemical and ultrastructural characterization of the 1,4-dihydropyridine receptor from rabbit skeletal muscle. J. Biol. Chem. **263,** 994–1001

Leung A. T., Imagawa T., Campbell K. P. (1987): Structural characterization of the 1,4-dihydropy-

ridine receptor of the voltage-dependent calcium channel from rabbit skeletal muscle. Eur. J. Biol. Chem. **262,** 7943—7946

Ma J., Coronado R. (1988): Heterogeneity of conductance states in calcium channels of skeletal muscle. Biophys. J. **53,** 387—395

Mikami A., Imoto K., Tanabe T., Niidome T., Mori Y., Takeshima H., Narmiya S., Numa S. (1989): Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. Nature **340,** 230—233

Morton M. E., Caffrey J. M., Brown A. M., Froehner S. C. (1988): Monoclonal antibodies against the 1,4-dihydropyridine-binding complex inhibits calcium currents in BCH3H1 myocytes. J. Biol. Chem. **263,** 613—616

Norman R. I., Burgess A. J., Allen E., Harrison T. M. (1987): Monoclonal antibodies against the 1,4-dihydropyridine receptor associated with voltage-sensitive calcium channels detect similar polypeptides from a variety of tissues and species. FEBS Lett. **212,** 127—132

Nowycky M. C., Fox A. P., Tsien R. W. (1985): Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature **316,** 440—443

Nunoki K., Florio V., Catterall W. A. (1989): Activation of purified calcium channels by stoichiometric protein phosphorylation. Proc. Nat. Acad. Sci. USA **86,** 6816-6820

Pelzer D., Cavalie A., Flockerzi V., Hofmann F., Trautwein W. (1988): Reconstition of solubilized and purified dihydropyridine receptor from skeletal muscle microsomes has two single calcium channel conductances with different function properties. In: The Calcium Channel: Structure, Function and Implication. (Eds. Morad M., Nayler W. G., Kazda S., Schramm M.), pp. 217—230, Springer, Berlin—Heidelberg—New York—Tokyo

Reuter H. (1983): Calcium channel modulation by neurotransmitter, enzymes and drugs. Nature **301,** 569—574

Rios E., Brum G. (1987): Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. Nature **325,** 717—720

Röhrkasten A., Meyer H., Nastainczyk W., Sieber M., Hofmann F. (1988): cAMP-dependent protein kinase rapidly phosphorylates Ser 687 of the rabbit skeletal muscle receptor for calcium channel blockers. J. Biol. Chem. **263,** 15325—15329

Ruth P., Röhrkasten A., Biel M., Bosse E., Regulla S., Meyer H. E., Flockerzi V., Hofmann F. (1989): Primary structure of the β-subunit of the DHP-sensitive calcium channel from skeletal muscle. Science **245,** 1115—1118

Schmid A., Barhanin J., Coppola T., Borsotto M., Lazdunski M. (1986): Immunochemical analysis of subunit structures of 1,4-dihydropyridine receptor associated with voltage-dependent calcium channels in skeletal, cardiac, and smooth muscles. Biochemistry **25,** 3492—3495

Sieber M., Nastainczyk W., Žúbor V., Wernet E., Hofmann F. (1987): The 165-kDa peptide of the purified skeletal muscle dihydropyridine receptor contains the known regulatory sites of the calcium channel. Eur. J. Biochem. **167,** 117—122

Striessnig J., Goll A., Moosburger K., Glossmann H. (1986): Purified calcium channels have three allosterically coupled drug receptors. FEBS Lett. **197,** 204—210

Striessnig J., Knaus H.-G., Grabner M., Moosburger K., Seitz W., Lietz H., Glossmann H. (1987): Photoaffinity labelling of the phenylalkylamine receptor of the skeletal muscle transverse-tubule calcium channel. FEBS Lett. **212,** 247—253

Takahashi M., Seagar M. J., Jones J. F., Reber B. F. X., Catterall W. A. (1987): Subunit structure of dihydropyridine-sensitive calcium channel from skeletal muscle. Proc. Nat. Acad. Sci. USA. **84,** 5478—5482

Talvenheimo J. A., Worley III J. F., Nelson M. T. (1987): Heterogeneity of calcium channels from a purified dihydropyridine receptor preparation. Biophys. J. **52,** 891—899

Tanabe T., Takeshima H., Mikami A., Flockerzi V., Takahashi H., Kangawa K., Kojima M.,

Matsuo H., Hirose T., Numa S. (1987): Primary structure of the receptor for calcium channel blockers from skeletal muscle. Nature **328,** 313—318

Tsien R. W., Hess P., McCleskey E. W., Rosenberg R. L. (1987): Calcium channels in excitable cell membranes. Annu. Rev. Biophys., Biophys. Chem. **16,** 265—290

Vilven J., Leung A. T., Imagawa T., Sharp A. H., Campbell K. P., Coronado R. (1988): Interaction of calcium channels of skeletal muscle with antibodies specific for its dihydropyridine receptor. Biophys. J. **53,** 556a

Final version accepted February 27, 1990