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Molecular Mechanisms of Hormone Action

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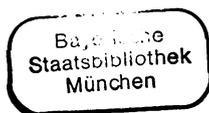
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The Structure of the Skeletal and Nonskeletal Muscle Calcium Channel

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1 Introduction

A number of fundamental biological processes such as mobility, secretion, and neurotransmission are regulated by a change in the cytosolic calcium concentration. The cytosolic calcium concentration is raised from around 0.1 to 1 μM either by a release from cellular stores or by an influx from the extracellular space through voltage-dependent calcium channels. These channels are located in the plasma and transverse tubular membranes of many cells and are classified into at least three types (Nowycky et al. 1985), the T- (*t*iny or *t*ransient), N- (*n*either T- nor L-, or *n*eural), and L- (*l*arge or *l*ong-*l*asting) channels. The L-type channels are the target of different organic drugs, the calcium channel blockers, which are used therapeutically in a variety of cardiovascular diseases. L- and N-type channels are not only regulated by the membrane potential or drugs but also by hormones through phosphorylation and/or G proteins. The electrophysiological properties of the L-type channel have been studied in great detail in cardiac, neuronal, and smooth muscle cells. In contrast, its biochemical and molecular structure has been elucidated up to now only in skeletal muscle.

2 Structure of the Skeletal Muscle Calcium Channel

The transversal tubular membranes of the skeletal muscle contain high affinity binding sites for dihydropyridines and phenylalkylamines (Galizzi et al. 1986). The high concentration of the transversal tubular binding sites has facilitated their purification to apparent homogeneity (Curtis and Catterall 1984; Flockerzi et al. 1986a; Takahashi et al. 1987; Leung et al. 1988). The purified channel contains three proteins of 165 kDa (α_1), 55 kDa (β), and 32 kDa (γ) which copurify in a constant 1:1:1 ratio (Sieber et al. 1987). A further glycoprotein which contains two disulfide-linked peptides of 135 kDa (α_2) and 28 kDa (δ) is present in the purified preparation. The relationship of this protein to the other three subunits is unknown.

The oligomeric structure of the channel is unclear at present. Electron microscopy of the purified channel proteins suggests that at least the α_1 -, β -, and γ -subunits are part of a higher ordered structure (Leung et al. 1988). The α_1 -subunit contains the binding sites for calcium channel blockers (Sieber et al. 1987; Striessnig et al. 1986a, 1987;

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Takahashi et al. 1987), is readily phosphorylated by cAMP-kinase at Ser 687 (Röhrkasten et al. 1988), and has the primary structure of a voltage-dependent ion channel (Tanabe et al. 1987; Ellis et al. 1988). Antibodies specific for the α_1 -, β -, and γ -subunit modulate the calcium channel of cultured cells and the reconstituted skeletal muscle channel supporting an oligomeric structure of the channel and a modulatory role for the β - and γ -subunit (Campbell et al. 1988; Morton et al. 1988; Vilven et al. 1988).

The primary sequence of the α_1 -, α_2 -, and β -subunit has been elucidated recently by cloning of the corresponding cDNAs. The structure of the γ -subunit is not known at present. The α_1 -subunit contains four transmembrane regions which are composed of six transmembrane α -helices (Tanabe et al. 1987). The same topology is found in other voltage-dependent ion channels. The deduced amino acid sequence of the α_2 protein is compatible with that of a peripheral membrane protein, although it contains three transmembrane α -helices (Ellis et al. 1988). In agreement with its behavior as glycoprotein several consensus sequences for glycosylation are present in a large extracellular domain of the protein. The β -subunit contains four hydrophilic, 27 to 42 amino acid long α -helices, each of which contains a homologous stretch of eight amino acids (Ruth et al. 1989). The deduced amino acid of this protein is compatible with that of a peripheral membrane protein. The deduced amino acid sequence contains several potential phosphorylation sites. One of these sites, Ser 182, is phosphorylated in vitro by cAMP-kinase. A second and third site may be phosphorylated by cGMP-kinase and protein kinase C which enzymes modify rapidly the β -subunit in vitro (Nastainczyk et al. 1987; Jahn et al. 1988).

3 Binding Characteristics of the Purified Channel

Only the 165 kDa (α_1) subunit binds the dihydropyridines, phenylalkylamines, and benzothiazepines with high affinity (Flockerzi et al. 1986a; Striessnig et al. 1986b; Sieber et al. 1987). The respective apparent binding constants of the purified receptor are two- to tenfold higher than those of the membrane-bound receptor. The decreased affinity is due mainly to the difficulty in separating rapidly bound and free ligand when the solubilized receptor is used. The binding of dihydropyridines and phenylalkylamines is modulated by calcium. The high affinity binding of PN 200-110, a dihydropyridine, occurs only in the presence of micromolar to millimolar concentrations of calcium. The binding of PN 200-110 is regulated allosterically further by diltiazem and verapamil. In contrast, binding of devapamil, a verapamil analog, occurs in the absence of calcium if carried out at 4°C. Micromolar concentrations of calcium inhibit the binding of devapamil at 4°C. However, if the binding reaction is carried out at 30°C, devapamil binds only in the presence of a micromolar concentration of calcium and binding is inhibited by millimolar calcium. Preliminary analysis suggests that 0.1 to 1.0 μ M calcium decreases the dissociation rate of devapamil at 30°C. Preincubation of the receptor for less than 20 min at 30°C in the absence of calcium and devapamil prevents binding of devapamil at 4°C. Apparently, a low concentration of calcium is necessary to stabilize the receptor at 30°C in a conformation which allows high affinity binding of devapamil. This apparent temperature dependence is reminiscent of the in vivo situation where the charge transfer of skeletal muscle is

blocked by calcium channel blockers only at low temperatures and/or after partial depolarization of the plasma membrane (Berwe et al. 1987; Lamb and Walsh 1987; Rios and Brum 1987). Inactivation of the L-type calcium channels, which is induced by an increase in cytosolic calcium, increases the affinity of calcium channel blockers by several orders of magnitude (Bean 1984). This suggests that the conformation of the purified receptor protein complex may fluctuate between two states which are analogous to an active and inactivated channel.

4 Reconstitution of a Calcium Channel

The T-tubular localization of the calcium channel blocker binding sites has prevented so far a clear identification of their function. These binding sites may function only as a charge carrier which links membrane depolarization with the intracellular calcium release. Myotubes and single cells contain calcium channels which are similar to L-type channels of cardiac myocytes. T-tubular membranes contain L-type channels if the membrane is reconstituted into a phospholipid bilayer. The open probability of these channels increases after the addition of the calcium channel agonist Bay K 8644, cAMP-kinase and ATP, and the α -subunit of the GTP-binding protein of G_s (Flockerzi et al. 1986b; Talvenheimo et al. 1987; Hymel et al. 1988; Ma and Coronado 1988; Pelzer et al. 1988; Yatani et al. 1988). The channels are blocked by organic and inorganic calcium channel blockers. Similarly, the purified receptor complex reconstitutes to an L-type calcium channel. The opening probability increases after the addition of the channel agonist Bay K 8644, cAMP-kinase, and ATP·Mg. The channel is blocked by organic and inorganic calcium channel blockers and has a single channel conductance of 20 pSi (Flockerzi et al. 1986b; Pelzer et al. 1988). However, the reconstituted proteins support also channels of lower conductance which are not regulated by phosphorylation (Pelzer et al. 1988). The channel-forming protein is the α_1 -subunit since (1) reconstitution of an isolated α_1 -subunit leads to the formation of an L-type calcium channel (Pelzer et al. 1988); (2) injection of the cDNA for the α_1 -subunit into dysgenic muscle cells, which lack the α_1 -subunit, restores E-C coupling and a dihydropyridine-sensitive slow calcium channel in these cells (Tanabe et al. 1988); (3) antisense RNA complementary to the cDNA of the skeletal muscle α_1 -subunit cDNA prevents the expression of cardiac muscle L-type channel in oocytes (Lotan et al. 1989). Thus, it is quite clear that the α_1 -subunit is a calcium channel. This channel has slower opening and closing kinetics than the channel from other tissues, suggesting that the skeletal muscle channel differs from that of other tissues.

5 Structure of Nonskeletal Muscle L-Type Calcium Channels

The identity of the L-type calcium channel in other tissues has not been elucidated completely. Photoaffinity labeling of purified protein fractions and membranes suggests that the cardiac muscle receptor for calcium channel blockers is slightly larger than that of skeletal muscle. Apparent molecular weights of 195 versus 165 kDa have been reported (Schneider and Hofmann 1988; Chang and Hosey 1988). A similar molecular weight has been observed for the brain receptor (Striessnig et al. 1988).

Northern blots carried out with an oligonucleotide probe derived from the cDNA of the skeletal muscle α_1 -subunit show cross-hybridization to an 8.2 kb mRNA in brain, heart, and smooth muscle and to a 6.3 kb mRNA in skeletal muscle, suggesting again that the skeletal muscle channel differs from that of other excitable tissues. Monoclonal antibodies against the α_1 -subunit of the rabbit skeletal muscle channel do not specifically label proteins of other tissues. These antibodies detect the α_1 -subunit in the skeletal muscle from human, rabbit, guinea pig, hamster, cow, pig, chick, and frog. In contrast, antibodies directed against the α_2 -proteins of the rabbit skeletal muscle receptor label an identical or similar protein in a wide variety of tissues, including skeletal muscle, heart, brain, 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). This supports again the notion that an identical α_2 -protein is present in all tissues, whereas the α_1 -subunit differs quite considerably from that of skeletal muscle. So far, it is unclear whether the β - and γ -subunit are part of the calcium channel of other tissues. Preliminary data suggest that mRNA and a protein similar to the β -subunit of skeletal muscle may be present in brain. It is therefore possible that both subunits are present in other tissues. The proteins associated with the N-type channel are distinct from these proteins. N-type channels bind specifically ω -conotoxin and have a reported molecular weight of above 200 kDa (Abe and Saisu 1987; Barhanin et al. 1988; Marqueze et al. 1988).

6 In Vivo Regulation and Conclusions

Calcium channels are regulated in vivo by three mechanisms: The membrane potential, protein kinases, and G proteins. The latter two mechanisms are of interest since they allow hormonal regulation of channel opening and closing. Cyclic AMP-dependent protein kinase increases the open probability of the cardiac and some neuronal L-type channels in vivo (Trautwein et al. 1986; Gray and Johnston 1987). The protein phosphorylated in vivo is unknown but the work with the skeletal muscle channel suggests that it may be the α_1 -subunit. The α -subunits of G_o , G_i , and G_s appear to regulate the opening and closing probability of calcium channels in neuronal, neurosecretory, and cardiac cells (Yatani et al. 1987; Rosenthal et al. 1988). The exact mechanism has not been determined in most cases. The opening probability of reconstituted cardiac and skeletal muscle calcium channels increases after addition of the α -subunit of G_s . However, it is unclear whether the α_1 -subunit of G_s directly affects a cardiac calcium channel in vivo, since inhibition of cAMP-kinase blocks the β -adrenergic stimulation of the cardiac calcium current. However, recently it has been found that the β -adrenergic receptor of isolated tracheal smooth muscle cells stimulates the calcium current (Felbel et al. 1989). This stimulation was not mimicked by the catalytic subunit of cAMP-kinase but was mimicked by GTP γ S. This finding suggests that in these smooth muscle cells the α -subunit of G_s may couple directly to the calcium channel. These results support therefore the hypothesis that L-type calcium channels are regulated by multiple mechanisms in vivo.

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