# The biochemistry and molecular biology of the dihydropyridine-sensitive calcium channel

Kevin P. Campbell, Albert T. Leung and Alan H. Sharp

*Calcium channels are known to exist in muscle, neuronal and secretory cells. The 1,4-dihydropyridines are potent blockers of L-type $Ca^{2+}$ channels, and have been used as specific probes in the study of dihydropyridine-sensitive $Ca^{2+}$ channels. The receptor for the 1,4-dihydropyridines has been purified from skeletal muscle in order to characterize the biochemistry and molecular biology of the dihydropyridine-sensitive $Ca^{2+}$ channel. This review summarizes recent findings on the subunit composition of the dihydropyridine-sensitive $Ca^{2+}$ channel, and discusses the structure and possible function of the individual subunits.*

*Kevin P. Campbell, Albert T. Leung and Alan H. Sharp are at the Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242, USA.*

$Ca^{2+}$ ions play an important role in the regulation of many cellular functions. $Ca^{2+}$ influx across the surface membrane via voltage-dependent $Ca^{2+}$ channels and $Ca^{2+}$ efflux from internal stores via $Ca^{2+}$ release channels contribute to the rise of the free $Ca^{2+}$ concentration that occurs within the cytoplasm of various cells upon stimulation (for recent reviews, see Refs 1-8). Voltage-dependent $Ca^{2+}$ channels are known to exist in muscle, neuronal and secretory cells[1-5]. Recent electrophysiological studies (see R. W. Tsien *et al.*, this issue) have provided evidence that there are at least three types of voltage-dependent $Ca^{2+}$ channels (L-, N- and T-types). The various types of voltage-dependent $Ca^{2+}$ channels are distinguished by several properties, including then-different sensitivities to the 1,4-dihydropyridines. The L-type $Ca^{2+}$ channel, but neither the T- nor the N-type, is sensitive to the dihydropyridine $Ca^{2+}$ channel blockers and activators. Although dihydropyridine-sensitive $Ca^{2+}$ channels exist in many cells, their biochemical characterization has generally been limited to skeletal muscle, where the receptor for the dihydropyridines is highly enriched. There are at least two types of $Ca^{2+}$ channels in skeletal muscle[9-11], but only the L-type is sensitive to the dihydropyridines and thus is the subject of this review.

## Transverse tubular dihydropyridine-sensitive $Ca^{2+}$ channel

Voltage-dependent $Ca^{2+}$ channels, including dihydropyridine-sensitive $Ca^{2+}$ channels, are localized to the transverse tubular membrane in adult skeletal muscle[10]. The receptor for the 1,4-dihydropyridines in skeletal muscle is also localized to the transverse tubular membrane, and isolated transversar tubule vesicles contain the highest density of dihydropyridine receptors[12,13]. Dihydropyridine-sensitive $Ca^{2+}$ channels are also inhibited by the phenylalkylamines and benzothiaeepines. The same compounds bind with high affinity to transverse tubular membranes and allosterically regulate dihydropyridine binding[14-17]. Therefore, the dihydropyridine-sensitive $Ca^{2+}$ channel from skeletal muscle transverse tubules contains at least three distinct, allosterically regulated binding sites for $Ca^{2+}$ channel blockers.

## Purification and subunit composition of the dihydropyridine-sensitive $Ca^{2+}$ channel

The dihydropyridine receptor has been purified from skeletal muscle by several laboratories[18-26]. Lectin affinity chromatography is a major tool in the purification of the dihydropyridine receptor[18]. Curtis and Catterall[18] and Borsotto *et al.*[19] were the first to purify the dihydropyridine receptor from detergent-solubilized transverse tubule membranes isolated from rabbit skeletal muscle. The purified receptor bound with high affinity to dihydropyridines and the binding of the dihydropyridines was allosterically coupled to the sites for verapamil and diltiazem. The original report of Curtis and Catterall[18] described an α-subunit of 160 kDa, a β-subunit of 50 kDa and a γ-subunit of 32 kDa in the purified dihydropyridine receptor. The most distinguishing feature of the a-subunit was that its apparent molecular mass shifted to 130 kDa upon reduction. Borsotto *et al.*[19,20] reported the purification of functional dihydropyridine receptors from CHAPS-solubilized transverse tubule membranes from both fresh and frozen rabbit skeletal muscle. A single 170 kDa polypeptide that forms two polypeptides of 140 kDa and 32 kDa upon reduction was identified in this preparation. The dihydropyridine receptor has recently been purified from skeletal muscle triads[22] or skeletal muscle microsomes[21,24,25] in the presence of protease inhibitors by several laboratories. The subunit composition of these preparations of receptor appears to be more complex than the initial descriptions[18-20]. Figure 1A shows our preparation of dihydropyridine receptor purified from digitonin-solubilized triads[22]. Four polypeptides of 175 kDa, 170 kDa, 52 kDa and 32 kDa are clearly identified by Coomassie Blue staining of SDS-polyacrylamide gels under non-reducing conditions. The 175 kDa protein shifts to 150 kDa under reducing conditions. The four polypeptides have been referred to as the α-subunit (170 kDa polypeptide), the $\alpha_2$-subunit, formerly called α (175/150 kDa polypeptide), the β-subunit (52 kDa polypeptide) and the γ-subunit (32 kDa polypeptide)[22,24-28]. In previous publications[28-30], we referred to the 170 kDa polypeptide as the δ-subunit. A stoichiometric ratio of 1:1:1:1 was obtained for the $\alpha_1$-, $\alpha_2$-, β- and γ-subunits, respectively, after taking into consideration the glycoprotein nature of the $\alpha_2$-subunit[28]. This observation strongly suggests that all four subunits are integral components of the dihydropyridine receptor. Subsequently, Takahashi *et al.*[27] described the observation of δ-subunits of 24-27 kDa that are formed by the reduction of the $\alpha_2$ subunit. (See Refs 18-40 and Table I for a summary of the subunit composition of the dihydropyridine-sensitive $Ca^{2+}$ channel and of the properties of the individual subunits.)

### *$\alpha_1$-Subunit*

It is now clear that the α-subunit of the dihydropyridine-sensitive $Ca^{2+}$ channel is in fact composed

  

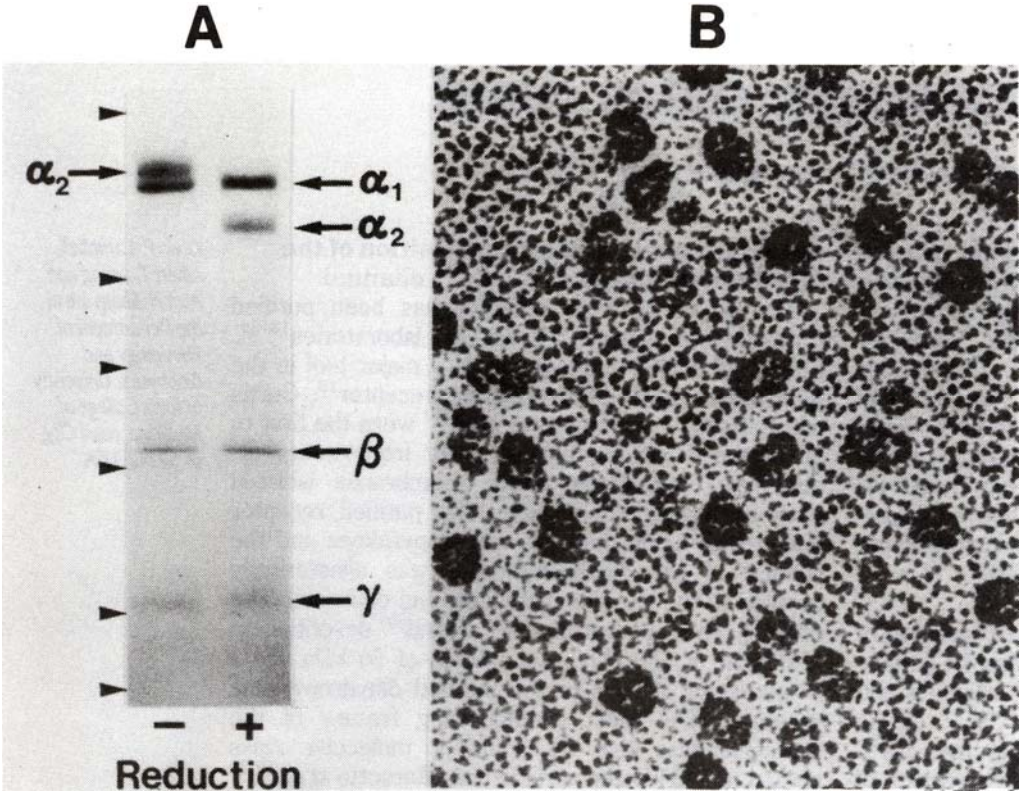


**Fig. 1.** *Biochemical and ultrastructural characterization of the dihydropyridine-sensitive $Ca^{2+}$ channel.* **(A)** *SDS-polyacrylamide gel electrophoresis of the purified dihydropyridine-sensitive $Ca^{2+}$ channel. The dihydropyridine receptor was purified and separated on a 5-16% SDS-polyacrylamide gradient gel (10 μg perlane) under non-reducing (–) (10 mM* N*-ethylmaleimide) or reducing (+) (5 mM ditbiothreitol) conditions as described by Leung* et al.[28]. *Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R and extensively destained with 10% methanol, 5% acetic acid. The $\alpha_1$-subunit (170 kDa), $\alpha_2$-subunit (175 kDa/150 kDa), β-subunit (52 kDa) and γ-subunit (32 kDa) are indicated by arrows. δ-subunits are not visible with Coomassie Blue staining. The arrowheads indicate the positions of the molecular mass standards: (from top to bottom) 200; 97.4; 68; 43; 25.7; and 18.4 kDa.* **(B)** *Electron micrograph of rotary-shadowed dihydropyridine-sensitive $Ca^{2+}$ channel. The purified dihydropyridine-sensitive $Ca^{2+}$ channel (0.5-1.0 μg) was freeze-dried, rotary-shadowed with carbon-platinum and imaged in an electron-microscope as described by Leung* et al.[28]. *The micrographs reveal a homogeneous preparation: 16 x 22 nm ovoid-shaped particles with two symmetrical halves separated by a central cleft. The two halves of the complex may represent the two larger polypeptides in association with the two smaller polypeptides.*

of two distinct proteins, the $\alpha_1$-subunit and the $\alpha_2$-subunit[22,26,27]. The $\alpha_1$-subunit has an apparent molecular mass of 155-200 kDa on SDS-PAGE under both non-reducing and reducing conditions distinguishing it from the $\alpha_2$-subunit which undergoes a characteristic decrease in apparent molecular mass upon reduction. The $\alpha_1$-subunit possesses many of the properties that are expected for the dihydropyridine-sensitive $Ca^{2+}$ channel. Photoaffinity labelling with the dihydropyridines [$^3$H] azidopine[26,27,29,32-34] and [$^3$H] PN200–110 (Ref. 29), and the arylalkylamine [$^3$H] LU49888 (Refs 26, 32–34) have shown that the $\alpha_1$-subunit contains the binding sites for both of these classes of $Ca^{2+}$ channel blockers. The $\alpha_1$-subunit is a substrate for cAMP-dependent protein kinase[30,35-38], a $Ca^{2+}$/calmodulin-dependent protein kinase[38], protein kinase C[35] and a protein kinase intrinsic to skeletal muscle triads[30]. Unlike the $\alpha_2$-subunit, the $\alpha_1$-subunit is either not glycosylated or only lightly glycosylated[22,27]. Monoclonal and polyclonal antibodies to the $\alpha_1$-subunit have been produced by various laboratories[22,24,27] and none of the antibodies reported so far have been shown to cross-react with the other subunits of the dihydropyridine receptor. One monoclonal antibody to the $\alpha_1$-subunit has been shown to inhibit dihydropyridine-sensitive $Ca^{2+}$ channels in cultured muscle cells[39]. The $\alpha_1$-subunit is also absent or greatly reduced in mice with muscular dysgenesis, which lack dihydropyridine-sensitive $Ca^{2+}$ channels[41]. It is interesting that the $\alpha_1$-subunit, which contains many of the properties of the dihydropyridine-sensitive $Ca^{2+}$ channel, was not seen in the original preparations of receptor even though these preparations were active in dihydropyridine binding. The most likely explanation for these early results is that the $\alpha_1$-subunit was present but partially degraded and therefore not recognized on SDS-PAGE[26].

The primary ammo acid sequence of the $\alpha_1$-subunit has recently been deduced from its cDNA and is consistent with a 212 kDa transmembrane protein with five potential *N*-glycosylation sites and seven potential cAMP-dependent phosphorylation sites[40]. The $\alpha_1$-subunit has four repeated units of high homology and each unit contains six presumably α-helical membrane-spanning segments (S1-S6). The S4 segment contains an ordered pattern of positive charges and could be the voltage sensor for the $Ca^{2+}$ channels. Thus, the structure of the $\alpha_1$-subunit is consistent with it being the ion-conducting and voltage-sensing unit of the dihydropyridine-sensitive $Ca^{2+}$ channel. Also, the $\alpha_1$-subunit has a high degree of homology to the α-subunit of the $Na^+$ channel[40-42] and putative $K^+$channel[43].

*$\alpha_2$-Subunit*

The $\alpha_2$-subunit of the dihydropyridine receptor was originally identified simply as 'α' before recognition of the $\alpha_1$-subunit. The $\alpha_2$-subunit is the only known subunit that has been identified in intact form in all purified preparations of the dihydropyridine receptor reported so far. This is probably due to resistance of this protein to proteolytic degradation. The $\alpha_2$-subunit is easily identified by its characteristic increase in mobility on SDS-PAGE after treatment with sulfhydryl reducing agents. The apparent decrease in molecular mass from 165-220 kDa to 130-150 kDa after reduction of disulfide bonds is possibly due to dissociation of one or more disulfide-linked proteins of 24-33 kDa which have been termed the δ-subunit[27]. It is now apparent that the 170 kDa complex of 140 kDa and 32 kDa disulfide-linked proteins isolated by Lazdunski and colleagues[20] is the $\alpha_2\delta$ complex. The $\alpha_2$-subunit is heavily glycosylated, binds wheat germ agglutinin (WGA) and concanavalin A[22,27,44], and is decreased in apparent molecular mass

by treatment with various glycosidases including neuraminidase[27], endoglycosidase F[27,36], and glycopeptidase F[27,36,45]. Removal of the *N*-linked carbohydrate by exhaustive digestion with glycopeptidase F[27,36,45] or neuraminidase followed by endoglycosidase F, results in an apparent molecular mass of 105-112 kDa[27]. These results indicate that the $\alpha_2$-subunit probably has an extensively glycosylated extracellular domain. In addition, the $\alpha_2$-subunit is weakly labelled by a hydrophobic photoaffinity probe[27], indicating possible membrane-spanning regions. Therefore, the $\alpha_2$-subunit probably consists of a large glycosylated extracellular domain and a relatively small transmembrane domain. Polyclonal antibodies produced by Barhanin *et al.*[45] against the non-reduced $\alpha_2\delta$ complex identify the δ-subunit on immunoblots after reduction of disulfide bonds as well as the reduced form of the $\alpha_2$-subunit. Antibodies against the $\alpha_2$-subunit do not react with the $\alpha_1$-, β- or γ-subunits[27,48], showing that the $\alpha_2$-subunit is distinct from these proteins. In addition, the N-terminal sequence of the $\alpha_2$-subunit[23] is different from the sequence of the $\alpha_1$-subunit[40]. Since the $\alpha_2$-subunit is not phosphorylated and does not appear to contain binding sites for the dihydropyridine or phenylalkylamine $Ca^{2+}$ channel blockers, the functional role of the $\alpha_2$-subunit is as yet unclear.

### β-*Subunit*

The β-subunit of the dihydropyridine-sensitive $Ca^{2+}$ channel has an apparent molecular mass of 52-65 kDa on SDS-PAGE which is insensitive to reduction. It is a substrate for cAMP-dependent protein kinase[30,35,37], protein kinase C[35] and a protein kinase intrinsic to skeletal muscle triads[30]. A monoclonal antibody specific for the β-subunit does not cross-react with any other subunits of the dihydropyridine receptor and is capable of immunoprecipitating the [$^3$H]PN200-110 labelled dihydropyridine receptor from digitonin-solubilized membranes[28]. Since the $\alpha_1$-subunit of the dihydropyridine receptor contains the dihydropyridine binding site (see above), the immunoprecipitation of dihydropyridine binding activity by an antibody specific to the β-subunit demonstrates that the β-subunit and $\alpha_1$-subunit are associated in digitonin-solubilized membranes. The β-subunit also co-purifies with the $\alpha_1$-subunit throughout the entire purification process, demonstrating the tight association between these two proteins[28]. A monoclonal antibody to the β-subunit has been shown to be capable of activating the $Ca^{2+}$ channel reconstituted into planar lipid bilayers47. This result and the phosphorylation of the β-subunit suggest that the β-subunit is involved in the regulation of $Ca^{2+}$ channel activity.

### γ-*Subunit*

The γ-subunit of the dihydropyridine receptor is a glycoprotein with an apparent molecular mass of 30-33 kDa on SDS-PAGE[18,21,22]. This protein has not been observed in purified preparations of the dihydropyridine receptor in all laboratories. It appears to be heavily glycosylated because it can be labelled by $^{125}$I-WGA[27] and its apparent molecular mass decreases to 20 kDa after digestion with neuraminidase followed by endoglycosidase F[27]. In addition, the γ-subunit is heavily labelled by a hydrophobic photoaffinity probe[27]. Therefore, this protein probably has both extracellular and transmembrane domains. Preliminary results from our laboratory have shown that polyclonal antibodies against this subunit do not cross-react with other subunits, indicating that it is distinct from and not a proteolytic degradation product of a larger subunit[48]. In addition, these antibodies were found to inhibit $Ca^{2+}$ channel activity[47], suggesting that the γ-subunit is a tightly associated regulatory subunit of the $Ca^{2+}$ channel.

**TABLE I.** Subunit composition of dihydropyridine-sensitive $Ca^{2+}$ channel[a]

| Subunit | $\alpha_1$ | $\alpha_2$ | β | γ | δ |
|---|---|---|---|---|---|
| **Structural properties** | | | | | |
| Molecular mass (kDa) | | | | | |
| Non-reduced | 155–200 | 165–200 | 52–65 | 30–33 | – |
| Reduced | 155–200 | 130–150 | 52–65 | 30–33(?) | 24–33 |
| Stoichiometry | 1 | 1 | 1 | 1 | ? |
| Glycoprotein | – (?) | +++ | – | + | + |
| Primary structure | Complete, from cDNA | N-terminal | ND | ND | ND |
| Disulfide linkage | – | +(δ) | – | – | +($\alpha_2$) |
| Membrane structure | | | | | |
| Transmembrane domains | +++ | + | – | +++ | + |
| Cytoplasmic domains | +++ | ? | +++ | ? | ? |
| Extracellular domains | +++ | +++ | ? | + | + |
| **Functional properties** | | | | | |
| DHP binding | + | – | – | – | – |
| Arylalklamine binding | + | – | – | – | – |
| Phosphorylation | + | – | – | – | – |
| $Ca^{2+}$ channel function | Ion-conducting and voltage-sensing unit | ? | Regulatory unit? | Regulatory unit? | ? |

[a] Summarized from Refs 18-40, Abbreviation: ND, not determined.

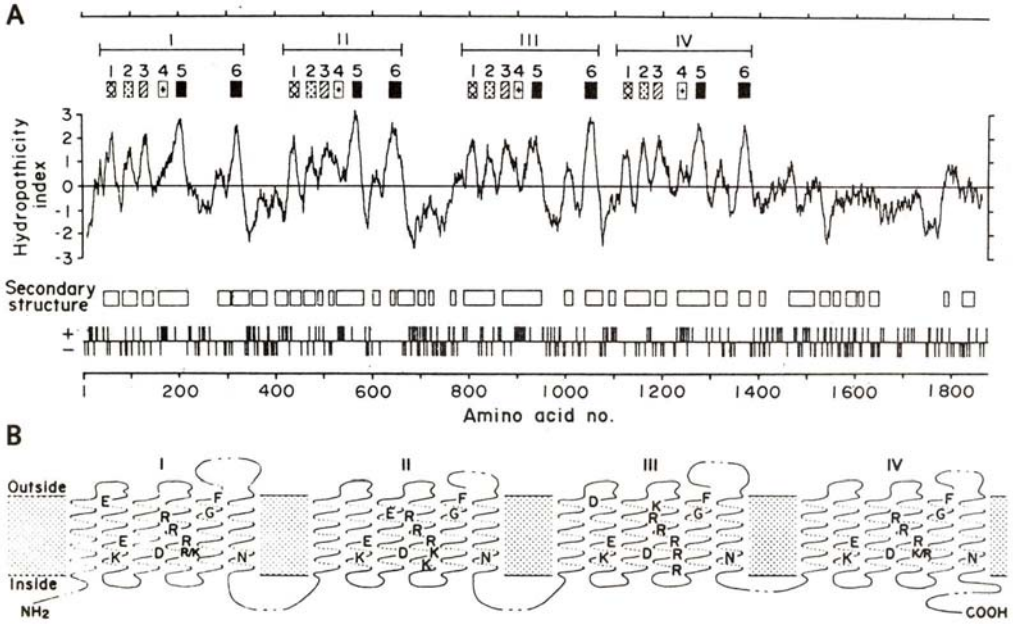


**Fig. 2.** *Primary and secondary structure of the $\alpha_1$-subunit of dihydropyridine-sensitive $Ca^{2+}$ channel from rabbit skeletal muscle as deduced from cloning and sequence analysis of cDNA complementary to its mRNA.* **(A)** *Hydropathicity plot showing four internal homology units (I-IV) each containing six transmembrane $\alpha$-helical segments (S1-S6). Amino acid sequences having a predicted $\alpha$-helix and/or $\beta$-sheet structure are indicated by the open boxes. Positions of positively charged residues (Lys and Arg) and negatively charged residues (Asp and Glu) are indicated by upward (+) and downward (–) vertical lines, respectively.* **(B)** *Schematic representation of structural characteristics common to the $\alpha_1$-subunit of the dihydropyridine-sensitive $Ca^{2+}$ channel and the $\alpha$-subunit of the voltage-dependent $Na^+$ channel. The conserved amino acids are shown in one-letter code.* (Taken, with permission, from Ref. 40.)

### δ-*Subunit*

The δ-subunit has not been observed by all laboratories, but seems to consist of one or more small proteins of 24–33 kDa which appear on SDS-PAGE following the reduction of the receptor. These subunits may account for the decrease in apparent molecular mass of the $\alpha_2$-subunit that occurs on reduction. For example, Fig. 1A clearly shows the mobility change in the $\alpha_2$-subunit upon reduction, but the δ-subunit is not observed in the reduced sample. It is likely that the small size of the δ-subunit results in poor staining by Coomassie Blue and a large amount of the peptide(s) is required for visualization on SDS-PAGE. However, δ-subunit(s) has been identified and characterized by the laboratories of Lazdunski[45,46] and Catterall[27]. Schmid *et al.*[46] identified three proteins of 32 kDa, 29 kDa and 25 kDa disulfide-linked to a protein of 140 kDa. The polyclonal antibodies against the 32 kDa protein labelled the 32 kDa, 29 kDa and 25 kDa proteins on immunoblots under reducing conditions and the 170 kDa protein under non-reducing conditions. Furthermore, one-dimensional peptide maps also suggested that these proteins are related to each other[45]. The apparent molecular mass of the 32 kDa protein was decreased by treatment with glycopeptidase F[45]. Takahashi *et al.*[27] showed that proteins of 27 kDa and 24 kDa can be labelled with $^{125}$I-WGA and with a hydrophobic photoaffinity probe. These proteins were apparent on SDS-PAGE only after treatment of the receptor preparation with disulfide reducing agents. Thus, it appears that the δ-subunit contains a hydrophobic transmembrane domain, is disulfide-linked to the $\alpha_2$-subunit and is glycosylated and therefore exposed to the extracellular medium. While the above experimental evidence tends to suggest that δ-subunit peptides are released from the $\alpha_2$-subunit upon reduction of disulfide bonds, several questions remain unanswered. For example, are the δ-subunit peptides products of the same gene that encodes the $\alpha_2$-subunit? If they are translated on the same polypeptide as the $\alpha_2$-subunit, are the δ-subunit peptides the product of normal post-translational processing or are they artifacts of the purification process? Furthermore, the stoichiometric ratios of putative δ-subunit proteins to the other subunits of the dihydropyridine receptor have not been determined. And finally, a functional role for this subunit has yet to be identified.

## Ultrastructure of the purified dihydropyridine-sensitive $Ca^{2+}$ channel

Electron microscopic characterization of the purified dihydropyridine-sensitive $Ca^{2+}$ channel has provided structural information on the size and shape of the $Ca^{2+}$ channel complex[28]. Rotary shadowed micrographs of the freeze-dried dihydropyridine receptor revealed a homogeneous preparation of ovoidal particles 16 × 22 nm in size (Fig. 1B), demonstrating that the protein components of the purified dihydropyridine receptor exist as a single complex. Each ovoidal particle appears to be primarily composed of two halves of similar size, separated by a small central gap. The ultrastructural data are consistent with the subunit composition of the receptor indicating an oligomeric complex of ~430 kDa. The two halves of the complex may represent the two larger polypeptides in association with the two smaller polypeptides. The ultrastructural data are also consistent with the hydrodynamic studies of the dihydropyridine receptor[49], in which it was reported to be a large ellipsoidal transmembrane protein.

## Reconstitution of the dihydropyridine-sensitive $Ca^{2+}$ channel

The purified dihydropyridine receptor has been reconstituted into phospholipid vesicles and lipid bilayers[45,50-52]. The reconstituted protein complex has been shown to contain the binding sites for the dihydropyridines[45,50-52], arylalkylamines[45,50] and diltiazem[45,50]. When the dihydropyridine receptor was purified in the presence of Bay K8644, the reconstituted receptor was capable of conducting $Ca^{2+}$ as determined by $^{45}Ca^{2+}$ flux studies with phospholipid vesicles[50] and electrophysiological studies with lipid bilayers[51,52]. However, in the flux studies using phospholipid vesicles, only a small percentage of the purified dihydropyridine receptors function as $Ca^{2+}$ channels and in the bilayer experiments only a small number of $Ca^{2+}$ channels are studied from the very

large number of receptors that are added to the bilayer solution. Although the reconstitution experiments are promising, more work is clearly required to characterize the purified channel and to identify the subunit(s) required for $Ca^{2+}$ channel function.

**Oligomeric subunit structure of the dihydropyridine receptor**

Figure 3 presents a model of the dihydropyridine-sensitive $Ca^{2+}$ channel and its oligomeric subunit structure. Four subunits ($\alpha_1$, $\alpha_2$, $\beta$, and $\gamma$) comprise the $Ca^{2+}$ channel complex. The $\alpha_1$-subunit is the major transmembrane component of the receptor and the $\alpha_2$-subunit is the major glycoprotein component of the receptor, being mostly exposed on the extracellular face of the membrane. The small transmembrane domain shown as $\alpha_2$ may be disulfide-linked to the extracellular domain and may represent the $\delta$-subunit. The $\beta$-subunit is mostly cytoplasmic, but may be tightly associated to the membrane or other subunits. Finally, the $\gamma$-subunit is embedded in the membrane and is a glycoprotein. In addition to the observation of co-purification of the $\alpha_1$-, $\alpha_2$-, $\beta$-, $\gamma$- and $\delta$-subunits of the dihydropyridine receptor, several pieces of evidence suggest that the association of these subunits is very tight, highly specific and possibly necessary for ligand binding and $Ca^{2+}$ channel activity. The dihydropyridine- and WGA-binding activity of the dihydropyridine receptor are located on separate subunits of the receptor. However, both of these properties of the dihydropyridine receptor can be co-isolated on the same complex using WGA-Sepharose or monoclonal antibodies to the $\alpha_1$- and $\beta$-subunits of the receptor[22,27-29]. Individual subunits can be dissociated from the receptor complex only by using strong detergent solutions such as 1% SDS, which also results in the loss of ligand binding[27-29]. Expression of functional $Ca^{2+}$ channels from the cloned $\alpha_1$ cDNA has yet to be reported. These results suggest that the interaction of multiple subunits may be required for ligand binding and $Ca^{2+}$ channel functions. Furthermore, results from Coronado's laboratory, using subunit-specific antibodies, indicate that the $\beta$-subunit and $\gamma$-subunit may modulate the $Ca^{2+}$ channel activity[47], which is postulated to reside on the $\alpha_1$-subunit[40].

**The dihydropyridine receptor's role in excitation—contraction coupling**

The exact physiological role of the dihydropyridine receptor in skeletal muscle and whether it functions exclusively as a $Ca^{2+}$ channel remains to be elucidated. It has been suggested that the dihydropyridine receptor acts as a voltage sensor in excitation-contraction coupling to release $Ca^{2+}$ from the sarcoplasmic reticulum (SR). This hypothesis is supported by the observations that the number of functional $Ca^{2+}$ channels represents less than five per cent of the dihydropyridine receptor found in skeletal muscle[53] and dihydropyridines inhibit charge movement in the transverse tubule membranes and the release of $Ca^{2+}$ from the SR with a similar dose-dependence[54]. The $Ca^{2+}$ release channel of the SR has recently been purified from isolated skeletal muscle triads[55,56] and shown to be identical to the ryanodine receptor and 'SR feet' (see also R. Coronado, this issue). Isolated triads are enriched in both dihydropyridine-[22] and ryanodine-binding

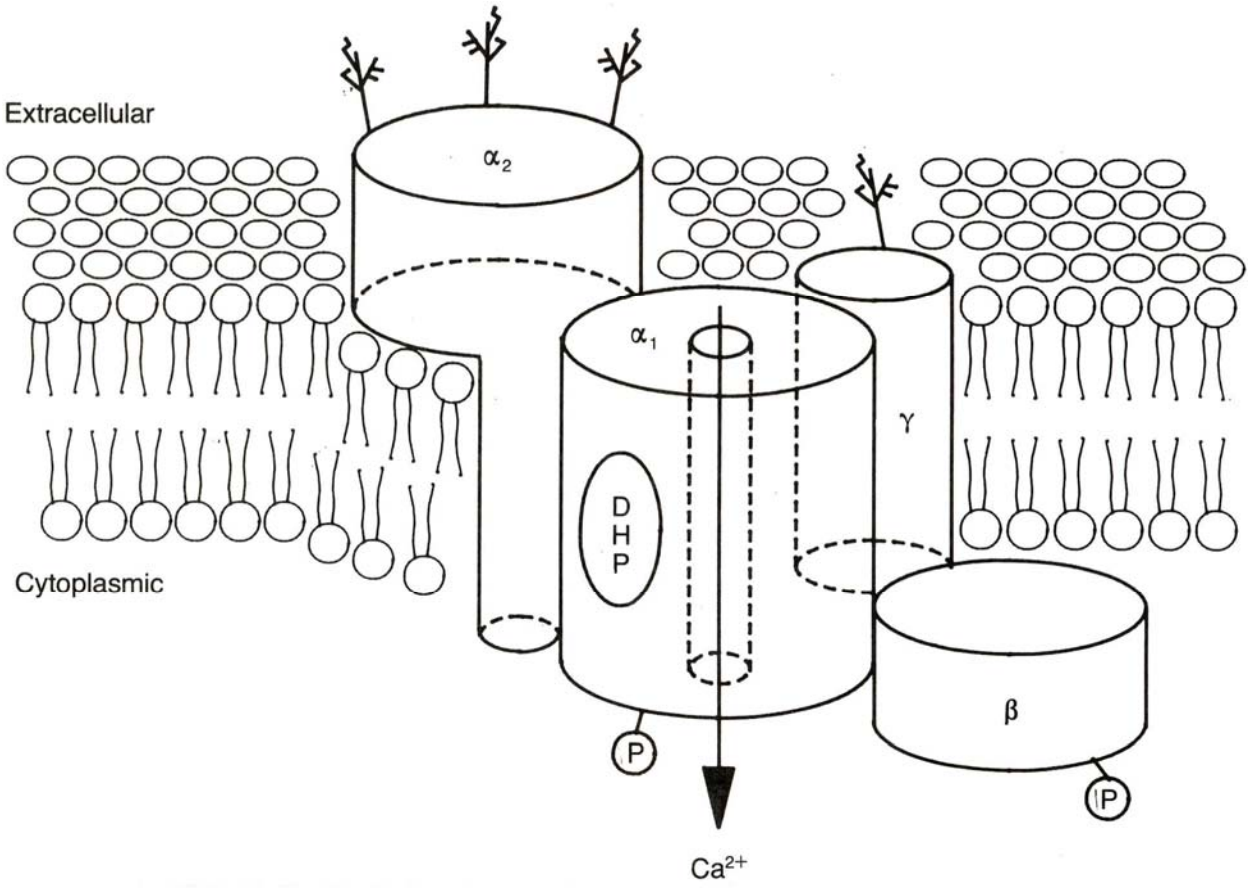


**Fig. 3.**
*Model of the dihydropyridine-sensitive $Ca^{2+}$ channel. The proposed model of the subunit structure of the dihydropyridine receptor is based on Refs 18-29.*
*Symbols: ⚡, phosphorylation; P, N-glycosylation sites.*

*Acknowledgements*
*We acknowledge the American Heart Association and the National Institutes of Health grants HL-37187, HL-39265 and HL-14388for support of this research.*

activity[57], suggesting that the dihydropyridine receptor might be in close proximity to and capable of communicating with the ryanodine-sensitive $Ca^{2+}$ release channel of the sarcoplasmic reticulum. Morphological data suggest that the dihydropyridine receptor is represented by a distinctive set of intramembranous particles that are the sole components of the junctional transverse tubule membrane and are present in diamond-shaped clusters that correspond exactly in position to the subunits of the 'SR feet' (Ref. 28 and Block, B. A., Imagawa, T., Campbell, K. P. and Franzini-Armstrong, C., unpublished observations). Preliminary results from our laboratory suggest that a complex of dihydropyridine receptor and ryanodine receptor can be isolated[58]. All these results suggest the presence of a large junctional complex, consisting of the dihydropyridine and ryanodine receptors, spanning the triadic junction. Excitation-contraction coupling could then occur through the direct allosteric coupling between the dihydropyridine receptor of the transverse tubule membrane and the ryanodine receptor of the junctional SR. While the information presented in this review supports the possible dual role for the dihydropyridine receptor ($Ca^{2+}$ channel and excitation-contraction voltage sensor), it also raises many questions. Most importantly, what is the biological mechanism that effects this dual function of the dihydropyridine receptor? Are there two forms of the dihydropyridine receptor, one that functions as a $Ca^{2+}$ channel and another that functions as a voltage sensor? Are there post-translational modifications of the receptor protein or endogenous modulators that affect the function of the dihydropyridine receptor? Finally, have the biochemical and molecular biological studies presented been performed on the $Ca^{2+}$ channel and/or voltage sensor?

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