Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle

(calcium antagonists/protein phosphorylation/glycoproteins/ion channels)

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ABSTRACT Purified dihydropyridine-sensitive calcium channels from rabbit transverse-tubule membranes consist of three noncovalently associated classes of subunits: α (167 kDa), β (54 kDa), and γ (30 kDa). Cleavage of disulfide bonds reveals two distinct α polypeptides and an additional component, δ. The α subunit, a 175-kDa polypeptide that is not N-glycosylated, contains the dihydropyridine binding site, cAMP-dependent protein kinase phosphorylation sites(s), and substantial hydrophobic domain(s). α2, a 143-kDa glycoprotein, has none of the properties characteristic of α1 but binds lectins and contains about 25% N-linked carbohydrate. α2 is disulfide-linked to δ, a 24- to 27-kDa glycoprotein. β (54 kDa) contains a cAMP-dependent phosphorylation site but is not N-glycosylated and does not have a hydrophobic domain. γ (30 kDa) has a carbohydrate content of about 30% and extensive hydrophobic domain(s). Precipitation with affinity-purified anti-α1 antibodies or α2-specific lentil lectin-agarose demonstrated that α2βγδ behaves as a complex in the presence of digitonin or 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, whereas the αβδ complex dissociates from αβγ in the presence of Triton X-100. A model for subunit interaction and membrane insertion is proposed on the basis of these observations.

Dihydropyridine (DHP)-sensitive calcium channels have been solubilized from skeletal muscle transverse tubules (T-tubules) by treatment with digitonin (1–3) or 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (4) and purified by conventional methods using high-affinity binding of radiolabeled derivatives of the DHP calcium channel antagonists nitrendipine or PN200-110 as a specific assay (1–4). The channel protein purified in the presence of digitonin contained three classes of noncovalently associated polypeptides when analyzed by NaDodSO4/PAGE without reduction of disulfide bonds: α (160 kDa), β (53 kDa), and γ (33 kDa) (1). Photoreactive calcium antagonists specific for the allosterically coupled DHP, benzothiazepine, and phenylalkylamine binding sites can be covalently attached to polypeptide(s) of 145-170 kDa in T-tubule membranes, suggesting that α subunits of the purified calcium channel contain all three drug receptor sites (5, 6). A complex of the α, β, and γ polypeptides mediates DHP-sensitive calcium conductance when reconstituted in phospholipid vesicles or planar bilayers (7, 8). The α and β polypeptides are good substrates for cAMP-dependent protein kinase (9), and phosphorylation of these subunits is accompanied by an increased probability of calcium-channel opening (8). These results are consistent with the conclusion that a complex of α, β, and γ classes of polypeptides is sufficient to form a functional calcium channel that can be modulated by agonist and antagonist drugs and by cAMP-dependent phosphorylation in a manner similar to native calcium channels.

In previous studies in this laboratory (1, 10), the α subunit of the calcium channel was found to behave anomalously in NaDodSO4/PAGE after reduction of disulfide bonds. This behavior was initially ascribed to partial reduction of intra-peptide disulfide bonds whose cleavage and reformation resulted in a variable fraction of the protein with smaller apparent size. We now describe use of subunit-specific antibodies and a battery of specific labeling methods to show that the α-protein band contains two calcium channel subunits, α1 and α2, that have similar size but clearly different properties. We propose a model of DHP-sensitive calcium channel structure in which the α1 subunit is the central transmembrane component with three independently associated subunits. Preliminary reports of some aspects of this work have been presented (11, 12).

MATERIALS AND METHODS

Materials. [3H]Azidopine (53 Ci/mmol; 1 Ci = 37 GBq), [3H]PN200-110 (70 Ci/mmol), [γ-32P]ATP (3000 Ci/mmol), 125I-labeled Bolton-Hunter reagent, and endoglycosidase F were obtained from New England Nuclear. Neuraminidase was from Sigma and lentil lectin-agarose from Vector Laboratories (Burlingame, CA).

Purification of T-Tubule Membranes and Calcium Channels. Rabbit skeletal muscle T-tubule membranes were prepared according to Rosemblatt et al. (13). Calcium channels were purified from T-tubules or skeletal muscle microsomes as described (1). Protein concentrations were determined by the method of Peterson (14).

NaDodSO4/Polyacrylamide Gel Electrophoresis. NaDodSO4-denatured proteins were analyzed in a discontinuous gel system, according to Laemmli (15), consisting of a stacking gel of 3% acrylamide and a running gel containing a 5–15% (wt/vol) acrylamide gradient (1). Proteins in sample buffer (1) were treated with either 20 mM N-ethylmaleimide (MalNEt) to alkylate sulphydryl groups or 20 mM dithiothreitol (DTT) to reduce disulfide bonds, as indicated below each gel lane in the figures. The gels were silver-stained according to the method of Oakley et al. (16). In some cases, the gel was soaked in 0.1% (wt/vol) K2Cr2O7 and 0.013% H3PO4 for 10 min before silver staining.

Abbreviations: DHP, dihydropyridine; T-tubule, transverse tubule; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; MalNEt, N-ethylmaleimide; DTT, dithiothreitol; TID, 3-(trifluoromethyl)-3-(m-iodophenyl)diazirine; WGA, wheat germ agglutinin.

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**Immunological Methods.** Polyclonal antibodies (PAC-10) were obtained from the ascites fluids of a SJL/J mouse (17) immunized with purified skeletal muscle calcium channel and were purified by protein A-Sepharose chromatography. Anti-\( \alpha_1 \) antibodies were affinity-purified from PAC-10 by the method of Olmsted (18). The purified calcium channel was treated with 0.5% (wt/vol) Triton X-100. After removal of dissociated \( \alpha_2 \beta \) subunits by adsorption to lentil lectin-agarose, the remaining subunits were separated by NaDodSO4/PAGE and transblotted onto a nitrocellulose sheet. The portion containing the \( \alpha_1 \) subunit was cut out and used for affinity purification. A blank nitrocellulose strip was processed identically and used as a control.

**RESULTS AND DISCUSSION**

**Polypeptide Composition of the DHP-Sensitive Calcium Channel.** Calcium channels were solubilized and purified by previously reported methods (1) and then analyzed by NaDodSO4/PAGE and silver staining (Fig. 1, lanes 1 and 2). Under alkylation conditions (lane 3), three classes of polypeptides that have been designated \( \alpha \) (167 kDa), \( \beta \) (54 kDa), and \( \gamma \) (30 kDa) (1) were detected. When disulfide bonds were cleaved with DTT (lane 2), the \( \alpha \) band split into two clearly resolved protein populations of 175 kDa and 143 kDa. Reduction also led to the appearance of a new family of low molecular mass polypeptides (24–27 kDa) clearly distinct from the \( \gamma \) subunit. These polypeptides were poorly visualized by the silver stain but were labeled clearly by lectins (see Fig. 3). The 175-kDa and 143-kDa polypeptides showed different coloration when gels were silver-stained, suggesting that they were distinct molecular species. Furthermore, these two polypeptides could be distinguished immunologically. In immunoblots, a polyclonal antibody (PAC-10) against the native calcium channel selectively labeled the 167-kDa band before reduction of disulfide bonds (Fig. 1, lane 3) but only the 175-kDa polypeptide after reduction (lane 4). No immunolabeling was observed with preimmune serum (lane 5) or with PAC-10 that had been preabsorbed with purified calcium channel (lane 6).

Since these observations indicate that the 175-kDa and 143-kDa components are different polypeptides, we designate them \( \alpha_1 \) and \( \alpha_2 \), respectively. The 24- to 27-kDa components appear to be disulfide-linked to the \( \alpha_2 \) subunit. The 24-kDa peptide may be proteolytically derived from the 27-kDa peptide (20), so we refer to them collectively as the \( \delta \) subunit. In all of our figures, the migration positions of the \( \alpha \) protein band and the \( \beta \) and \( \gamma \) subunits under alkylation conditions are indicated on the scale labeled MalNEt, and the migration positions of all the subunits under reducing conditions are indicated on the scale labeled DTT.

**Differential Labeling of Calcium Channel Subunits.** \([\text{3H}]\)-PN200-110 and \([^{125}\text{I}]\)labeled polypeptides have been shown to covalently label a 145- to 170-kDa polypeptide in T-tubule membranes (5, 6) and purified calcium channels (3) that presumably corresponds to one of the two \( \alpha \) subunits. In our preparations, \([^{125}\text{I}]\)labeled polypeptide was incorporated by UV photolysis into a polypeptide that migrated as a band at 167 kDa before reduction of disulfide bonds (Fig. 2, lane 1) and 175 kDa after reduction of disulfide bonds (lane 2). The electrophoretic behavior of this polypeptide identifies it as the \( \alpha_1 \) subunit. No labeling was observed in the presence of 2 \( \mu \text{M} \) PN200-110 (lane 3).

In previous work, \( ^{32}\text{P} \) (from \( [\gamma^{32}\text{P}]\text{ATP} \)) was stoichiometrically incorporated into the \( \alpha \)-protein band and the \( \beta \) subunit by the catalytic subunit of cAMP-dependent protein kinase (9). Comparison of the electrophoretic mobility of the phosphorylated bands before and after reduction of disulfide bonds (Fig. 2, lanes 4 and 5) showed that the \( \alpha_1 \) subunit is a good substrate for this enzyme, whereas the \( \alpha_2 \) subunit is not labeled. The \( \beta \) subunit was more weakly labeled at the low ATP concentrations used (9), while no phosphorylation of the \( \gamma \) or \( \delta \) subunits was detected.

Ion channel-forming polypeptides should contain hydrophobic segments, which may be detected by use of the hydrophobic probe 3-(trifluoromethyl)-3-\( m^-{125}\text{I} \)iodo-phenyl)diazirine (\([^{125}\text{I}]\text{TID} \)). This photoreactive compound partitions into free detergent micelles and detergent-associated with the major hydrophobic domains of integral membrane proteins and is specifically incorporated into these regions by photolysis (22). The \( \alpha_1 \) and \( \gamma \) subunits were prominently labeled by \([^{125}\text{I}]\text{TID} \), with a much lower level of incorporation into \( \alpha_2 \) and \( \delta \) (Fig. 2, lanes 6 and 7). The \( \beta \) subunit was not detectably labeled. Quantitation of \([^{125}\text{I}]\text{TID} \) in excised protein bands showed that the \( \alpha_1 \) and \( \gamma \) subunits incorporated 10-fold more TID per unit mass than the \( \alpha_2 \) or **Fig. 1.** Polypeptide composition of the DHP-sensitive calcium channel. Lanes 1 and 2: purified calcium channels were analyzed by NaDodSO4/PAGE and silver staining. Lanes 3–6: polypeptides separated by NaDodSO4/PAGE with or without reduction of disulfide bonds as indicated below each lane. Lanes 3–6: polypeptides transferred to nitrocellulose sheets and immunolabeled by incubation with PAC-10 (lanes 3 and 4), preimmune serum (lane 5), or PAC-10 that had been preabsorbed with purified calcium channel (lane 6), followed by incubation with \([^{125}\text{I}]\)-labeled protein A, washing, and autoradiography as described (19).

**Fig. 2.** Differential labeling of calcium channel subunits. (**Left** Photoaffinity labeling. T-tubule membranes (0.4 mg/ml) in 25 mM Hepes/1 mM CaCl\(_2\) adjusted to pH 7.5 with Tris base were incubated with 6 nM \([^{125}\text{I}]\)labeled in the absence (lanes 1 and 2) or in the presence (lane 3) of 2 \( \mu \text{M} \) PN200-110 and were irradiated for 15 min at 4°C with a 30-watt UV source (\( \lambda_{\text{max}} \) 356 nm). The membranes were solubilized in 1% digitonin/10 mM Hepes/185 mM NaCl/0.5 mM CaCl\(_2\)/0.1 mM phenylmethylsulfonyl fluoride/1 \( \mu \text{M} \) cycloheximide A adjusted to pH 7.5 with Tris base, and calcium channels were partially purified by chromatography on WGA-Sepharose (I) and analyzed by NaDodSO4/PAGE and fluorography. (**Center** Phosphorylation. Purified calcium channel was incubated with 0.3 \( \mu \text{M} \) cAMP-dependent kinase catalytic subunit and 0.12 \( \mu \text{M} \) carrier-free \( [\gamma^{32}\text{P}]\text{ATP} \) for 15 min at 37°C as described (9) (lanes 4 and 5). (**Right** Hydrophobic labeling. \([^{125}\text{I}]\text{TID} \) (15 Ci/mmol) was prepared and purified calcium channel was labeled with \([^{125}\text{I}]\text{TID} \) (100 Ci/mmol) in a buffer containing 0.1% digitonin, as previously described (21) (lanes 6 and 7).
δ subunits, even though, as shown below, nearly all α1 and γ subunits are associated with an α2 subunit. These results indicate that the α1 and γ subunits are the most hydrophobic components of the purified calcium channel complex, suggesting that they are the principal transmembrane polypeptides.

**Subunit Glycosylation.** Solubilized [3H]DHP receptors specifically bind to various immobilized lectins (23), and affinity chromatography on wheat germ agglutinin (WGA)-Sepharose is the most efficient purification step (1-4). These results imply that at least one subunit is glycosylated. The oligosaccharide chains of the calcium channel were detected by separating the subunits by NaDodSO4/PAGE and probing the resolved polypeptides with 125I-labeled WGA or Con A. 125I-labeled WGA bound to the α-protein band (α3) and the γ subunit in gels run under alkylating conditions (Fig. 3A, lane 1) and to the α2, γ, and δ subunits in gels run under reducing conditions (Fig. 3A, lane 2). 125I-labeled Con A labeled only the α2 subunit (Fig. 3B, lanes 1 and 2). No labeling of α1 or β was detected with either lectin. An unidentified polypeptide of about 105 kDa was also labeled by radiiodinated WGA in reducing conditions but not by Con A. Since these results indicate that α2 and δ are glycosylated but α1 is not, the α1 subunit and the disulfide-linked α2δ complex are independent polypeptides and are not derived from another.

To determine the extent of glycosylation and the core polypeptide size of the calcium channel subunits, purified channel preparations were labeled with 125I (19), incubated with glycosidases to remove oligosaccharide chains, and analyzed by NaDodSO4/PAGE. Fig. 3C presents the change in electrophoretic mobility and apparent molecular mass of the α1, α2, β, and γ subunits resulting from sequential deglycosylation with neuraminidase and endoglycosidase F. Both neuraminidase and endoglycosidase F caused reduction in the apparent sizes of α1 and γ. A plateau was reached after 16 hr with endoglycosidase F, corresponding to core polypeptide sizes of 105 kDa for α2 and 20 kDa for γ. Poor iodination of the δ subunit prevented estimation of its carbohydrate content by this method. No shift in the mobility of the α1 or β subunits was noted. Since α1 and β are particularly sensitive to proteolysis (ref. 1 and unpublished results), the change in mobility of α2 and γ must result from deglycosylation and not peptide bond cleavage by contaminating proteases.

**Analysis of Noncovalent Subunit Interactions by Lentil Lectin-Agarose Chromatography.** The specific labeling experiments described above establish that α1 and α2 are independent polypeptides, with widely different core polypeptide sizes, that comigrate during NaDodSO4/PAGE in nonreducing conditions due to the high carbohydrate content of α2 and its disulfide linkage to δ. The α1 subunit has properties expected of the calcium channel, including a DHP binding site, at least one cAMP-dependent phosphorylation site, and an extensive hydrophobic domain(s). It is important to establish whether α2δ is a persistent impurity or a specifically associated component of the oligomeric calcium channel complex.

Experiments with 125I-labeled lectins established Con A as a specific probe for the α2 subunit (Fig. 3B). Lentil lectin has the same specificity as Con A, and its lower affinity facilitates elution of bound glycoproteins with methyl α-D-mannoside. The calcium channel was labeled with 125I by the Bolton-Hunter procedure (Fig. 4, lanes 1 and 2). The incorporation of 125I into α2 was anomalously low, presumably indicating a low content of accessible primary amines. When the calcium channel protein was denatured by treatment with 1% NaDodSO4 and then exchanged into 0.5% Triton X-100 by gel filtration, only α2 bound specifically to lentil lectin-agarose (Fig. 4, lane 10), while α1, β, and γ remained in the unbound fraction (Fig. 4, lane 9). This result confirms the conclusion that only α2 binds to lectins with the specificity of lentil lectin and Con A.

**Fig. 3. Glycosylation of the calcium channel subunits.** (A) Calcium channel subunits were separated by NaDodSO4/PAGE and labeled directly in the gel by binding of 125I-labeled WGA (prepared as in ref. 24) according to the method of Burridge (25) in the absence (lanes 1 and 2) or presence (lane 3) of 200 mM N-acetylglucosamine. (B) Separated calcium channel subunits were electrophoretically transferred to a nitrocellulose sheet to reduce background and then incubated with 125I-labeled Con A (prepared as in ref. 24) in the absence (lanes 1 and 2) or presence (lane 3) of 100 mM methyl α-D-mannoside. (C) 125I-labeled calcium channel was treated with neuraminidase (N, 1 unit) in 50 mM NaCl/25 mM sodium citrate/50 mM NaH2PO4/0.02% digitonin, pH 5.0, at 37°C. After 4 hr, the mixture was boiled for 2 min in the presence of 0.5% NaDodSO4 and 2% 2-mercaptoethanol, and 1 unit of endoglycosidase F (Endo F) with Triton X-100 and EDTA (final concentrations 2% and 50 mM, respectively) was added. After the indicated incubation periods, aliquots were removed, mixed with 3% NaDodSO4 to stop the reaction and applied to a Sephadex G-50 column pre-equilibrated with sample buffer (1). Material that was eluted at the void volume was analyzed by NaDodSO4/PAGE under reducing conditions and autoradiography, and the Rf and apparent molecular mass of the α1 (o), α2 (c), β (a), and γ (b) subunits were determined. Control samples (C) were incubated for the same time in identical buffers from which enzymes were omitted.

**Fig. 4. Lentil lectin affinity chromatography.** 125I-labeled calcium channel (100 ng) in 150 μl of 25 mM Hepes/150 mM NaCl/1 mM CaCl2/1 mM MgCl2/0.1 mM phenylmethylene sulfonyl fluoride/1 μM pepstatin A (adjusted to pH 7.5 with Tris base) containing detergent as indicated below was incubated for 90 min at 4°C with 50 μl of lentil lectin-agarose, equilibrated in the same buffer, under agitation. The resin was removed by centrifugation and the supernatant was collected. The resin was washed three times with 1 ml of buffer and resuspended in 150 μl of buffer containing 0.2 M methyl α-D-mannoside. The eluate was collected after a 90-min batch incubation. Eluate (lanes 4, 6, 8, and 10) and wash (lanes 3, 5, 7, and 9) samples were analyzed by NaDodSO4/PAGE and autoradiography. Lanes 1 and 2: 125I-labeled calcium channel with no treatment. Lanes 3 and 4: 0.1% digitonin. Lanes 5 and 6: 0.1% CHAPS. Lanes 7 and 8: 0.5% Triton X-100. Lanes 9 and 10: samples treated with 1% NaDodSO4 were exchanged into 0.5% Triton X-100 by filtration over a 2-ml Sephadex G-50 column and analyzed in 0.5% Triton X-100.
In addition, experiments in 1% Triton X-100 (lane 5) showed complete dissociation of the \(a_2\) subunit, in agreement with the results of lentil lectin chromatography. The \(b\) subunit and a small fraction of the \(\gamma\) subunit (not easily seen in Fig. 5B) were communoprecipitated with \(a_1\) in Triton X-100. The results of these immunoprecipitation experiments have been confirmed by selective elution of \(\alpha_2\) subunits from immune complexes. Purified calcium channel subunits were immunoprecipitated in 0.5% digitonin as in Fig. 5, lane 1. Resuspension in a buffer containing 1% Triton X-100 caused complete release of the \(\alpha_2\delta\) dimer and partial release of \(\gamma\) without loss of \(b\) from the precipitate (data not shown). These results show that the \(\alpha_2\delta\) dimer is specifically associated with a complex of the \(a_1\), \(b\), and \(\gamma\) subunits in detergents that preserve the functional properties of the calcium channel. We tentatively conclude that \(a_2\), \(b\), and \(\gamma\) all interact independently with the “central” subunit \(a_1\). The differing degrees of stability in Triton X-100 suggest subunit binding to \(a_1\) with increasing affinity in the order \(\alpha_2\delta < \gamma < b\).

An Oligomeric Model for the Subunit Structure of the DHP-Sensitive Calcium Channel. Since the initial report from this laboratory (1), studies on the polypeptide composition of the T-tubule calcium channel using digitonin extraction and similar purification techniques have confirmed both the existence of the \(a\), \(b\), and \(\gamma\) classes of subunits and the anomalous behavior of the \(\alpha\) protein band on disulfide reduction (2, 3, 8). In contrast to these results, purification of the calcium channel by a different procedure yielded only 140-kDa and 32- to 33-kDa polypeptides that were reported to be noncovalently associated (4), although subsequent immunochemical analyses suggested that these two polypeptides were disulfide-linked (20). These observations were inconsistent with the finding by the same authors that photoaffinity labeling of T-tubules identified, after disulfide reduction, a single 170-kDa band as containing the calcium antagonist binding sites (6). This apparent discrepancy led to the proposal that their purified protein consisting of a disulfide-linked dimer of 140-kDa and 32- to 33-kDa polypeptides resulted from proteolytic nicking of this single 170-kDa polypeptide labeled in T-tubules and represented the entire functional calcium channel including cAMP-dependent phosphorylation sites (27) and calcium antagonist binding sites (4). Our present results require an alternative interpretation of these data. The disulfide-linked dimer observed in these previous studies is likely to correspond to the \(\alpha_2\delta\) disulfide-linked glycoprotein dimer, which we show here to be a peripherally associated component of the calcium channel that has neither cAMP-dependent phosphorylation sites, DHP binding sites, nor extensive hydrophobic domains. Instead, these functional components of the calcium channel reside in the \(a_1\), \(b\), and \(\gamma\) subunits of the purified calcium channel. While the \(\alpha_2\delta\) complex appears to be quite resistant to proteolysis, the \(\beta\) subunit (1) and, to a lesser degree, the \(a_2\) subunit (unpublished results) are susceptible to proteolytic degradation during typical purification procedures, and special precautions are required to recover them intact (1). This differential susceptibility to proteolysis may explain why \(a_1\) and \(\beta\) were not detected in preparations made from frozen and thawed muscle and solubilized in the stronger detergent CHAPS (4).

On the basis of present knowledge of the structure of the DHP-sensitive calcium channel, and in analogy with current models of the structure of voltage-sensitive sodium channels (reviewed in ref. 28), we propose a model (Fig. 6) based on a central ion channel-forming element interacting with three other noncovalently associated subunits. The \(\alpha_1\) subunit, which contains the calcium antagonist binding sites, cAMP-dependent phosphorylation sites, and the largest hydrophobic domains, is proposed to be the central ion channel-forming component of the complex. Its apparent molecular

Fig. 5. Immunoprecipitation of calcium channel subunits by anti-\(\alpha_1\) antibodies. Affinity-purified anti-\(\alpha_1\) antibodies (●) and a blank control (▲) were incubated with [\(\text{125I}\)]PN200-110-labeled skeletal muscle calcium channel in 145 \(\mu\)l of 0.5% digitonin/75 mM NaCl/50 mM Na\(\text{H}_2\)PO\(\text{4}\)/25 mM Tris-HCl, pH 7.4, at 4°C for 4 hr. Antibody-antigen complexes were washed to protein A-Sepharose and washed by centrifugation, and the radioactivity was determined by scintillation counting. Results are expressed as the percentage of input radioactivity. (B) [\(\text{125I}\)]-labeled calcium channel was immunoprecipitated as described previously (19), using anti-\(\alpha_1\) antibodies (lanes 1, 3, 5 and 7) or the control preparation (lanes 2, 4, 6 and 8), in immunoprecipitation buffer containing the detergents indicated below, and the immunoprecipitates were analyzed by NaDodSO\(\text{4}/\text{PAGE}\) in the presence of DTT, followed by autoradiography. Lanes 1 and 2: 0.5% digitonin. Lanes 3 and 4: 0.1% CHAPS. Lanes 5 and 6: 1% Triton X-100. Lanes 7 and 8: samples incubated with 1% NaDodSO\(\text{4}\) for 2 min at 100°C and exchanged into 0.5% digitonin by gel filtration on a 2-ml Sephadex G-50 column.
mass of 175 kDa from NaDodSO\textsubscript{4}/PAGE is likely to be a reasonable approximation of the true polypeptide size, since no N-glycosylation was detected. This calcium channel subunit is therefore enough to contain four homologous transmembrane domains analogous to those of the rat brain sodium channel \(a\) subunit, whose mRNA alone encodes a functional ion channel (29, 30). Like \(\alpha\), the sodium channel \(\alpha\) subunit also contains cAMP-dependent phosphorylation sites (28) and extensive hydrophobic domains that are efficiently labeled by TID (21).

The \(\beta\) subunit is also a substrate for cAMP-dependent kinase (9), but hydrophobic labeling indicates that it does not interact with the membrane phase and it is not a glycoprotein. It is probably therefore tightly associated with an intracellular domain of \(\alpha\).

The \(\gamma\) subunit of 30 kDa interacts independently with \(\alpha\), contains at least one transmembrane segment, and consists of approximately 50% carbohydrate. All these properties are similar to those of the \(\beta\) subunit of the rat brain and skeletal muscle sodium channels (28). A polypeptide of similar size appears to be associated with the apamin-sensitive, calcium-activated potassium channel (31), and it is interesting to speculate that this subunit may be a conserved constituent of voltage- or calcium-dependent ion channels.

The \(\alpha_2\delta\) dimer appears to interact more weakly with \(\alpha\) than \(\alpha_1\), although the conditions necessary to achieve dissociation result in a loss of DHP-binding activity. The 105-kDa core polypeptide of \(\alpha_2\) contains a heavily glycosylated extracellular domain but displays weak hydrophobic labeling, indicating a limited intramembrane domain. For this reason it seems unlikely that the ion channel is formed jointly by \(\alpha_1\) and \(\alpha_2\) at their zone of interaction.

The proposed model assumes a complex containing one of each subunit type. Our present results and previous data showing quantitative binding of solubilized calcium channels to Con A (23) suggest that each complex contains at least one \(\alpha_1\) and one \(\alpha_2\) subunit but do not specify the stoichiometry of any subunits. \(\alpha_1\) and \(\alpha_2\) appear to be present in approximately equal amounts on silver stained gels, and the \(\alpha_1\) and \(\beta\) subunits incorporate approximately one mole of \(^{32}\text{P}\) per mole of complex (9). A complete hydrodynamic analysis of the skeletal muscle calcium channel has not been reported. However, a size of 370 kDa determined for the rat ventricular muscle DHP receptor (32) is within reasonable range of the predicted size of the complex represented in Fig. 6 (416 kDa).

Thus, an assumption of one mole of each subunit in the complex is plausible but requires direct experimental verification.

Our model emphasizes a central role for the \(\alpha_1\) subunit in calcium channel function. Further studies of pharmacological and physiological modulation of reconstituted calcium channels lacking individual subunits or of calcium channels specified by mRNA for individual subunits will be necessary to ascribe a functional role to the other subunits in the complex.

Note Added in Proof. A recent report (33) shows that a verapamil derivative labels \(\alpha_1\), but not \(\alpha_2\), subunits. In contrast, another recent paper (34) reports only a single intact \(\alpha_1\) subunit for calcium channels purified from frozen muscle, as found by Borsotto et al. (4).