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Identification of an α Subunit of Dihydropyridine-Sensitive Brain Calcium Channels

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Voltage-sensitive calcium channels in different tissues have diverse functional properties. Polyclonal antibodies (PAC-2) against the α subunits of purified rabbit skeletal muscle calcium channels immunoprecipitated calcium channels labeled with the dihydropyridine PN200-110 from both skeletal muscle and brain. The immunoreactivity of PAC-2 with the skeletal muscle channel was greater than that with the brain calcium channel and was absorbed only partially by prior treatment with the brain channel. PAC-2 specifically recognized a large peptide in synaptic plasma membranes of rabbit brain with an apparent molecular size of 169,000 daltons. This protein resembles an α subunit of the skeletal muscle calcium channel in apparent molecular weight, antigenic properties, and electrophoretic behavior after reduction of disulfide bonds. Thus, the dihydropyridine-sensitive calcium channel of rabbit brain has an α subunit that is homologous, but not identical, to those of the skeletal muscle calcium channel. The different functional properties of these two calcium channels may result from minor variations in structurally similar components.

THE VOLTAGE-SENSITIVE CALCIUM channel is one of the key factors in the control of calcium-linked cellular functions such as action-potential generation, muscle contraction, and secretion of hormones and neurotransmitters (*1*). In neurons, three different classes of Ca^{2+} channels have been described in electrophysiological experiments (*2, 3*). One of these, termed the L channel, mediates slowly activated, long-lasting Ca^{2+} currents that are blocked by dihydropyridine Ca^{2+} channel antagonists and enhanced by dihydropyridine Ca^{2+} channel agonists (*3*). Dihydropyridine-sensitive Ca^{2+} channels also mediate Ca^{2+} entry during the action potential in muscle tissues. High-affinity dihydropyridine receptors have been identified in skeletal, smooth, and cardiac muscles and in brain (*4*). They have been successfully solubilized (*5–8*) and purified to near homogeneity from skeletal muscle T-tubular membranes (*6, 9*). The purified dihydropyridine receptors consist of a noncovalent complex of α, β, and γ subunits having apparent molecular sizes of 160,000, 50,000, and 33,000 daltons, respectively (*6*). The apparent size of a fraction of the α subunits is reduced to 135,000 daltons by cleavage of disulfide bonds (*6, 10*). The purified dihydropyridine receptor from skeletal muscle has also been incorporated into phospholipid vesicles and bilayers and been shown to mediate dihydropyridine-sensitive Ca^{2+} conductance, which provides evidence that the three subunits are sufficient to mediate the physiological functions of the Ca^{2+} channel (*10*).

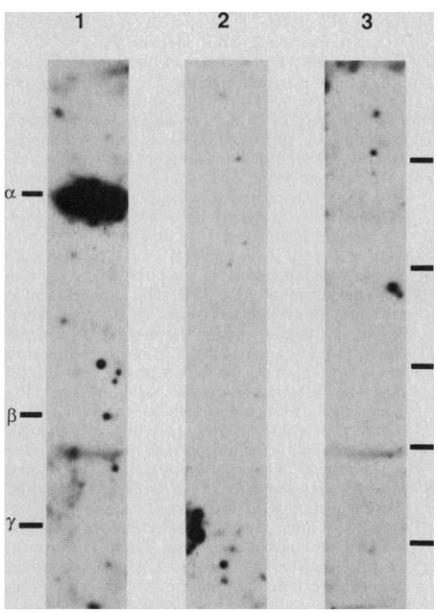

Fig. 1. Immunoblotting of T-tubule membranes from rabbit skeletal muscle by PAC-2. T-tubule membrane proteins (190 μg per lane) were transblotted from SDS–polyacrylamide gel to a nitrocellulose sheet and immunostained by PAC-2 (lane 1), preimmune serum (lane 2), and PAC-2 absorbed with purified rabbit skeletal muscle Ca^{2+} channel (lane 3). The concentrations of all the antisera were 0.3% by volume. The migration positions of α, β, and γ subunits of the skeletal muscle Ca^{2+} channel are indicated on the left. The migration positions of standard proteins indicated by horizontal bars correspond, from top to bottom, to the following molecular weights: 200,000, 116,000, 68,000, 42,000, and 30,000.

Although the Ca^{2+} channels of skeletal muscle T-tubules have served as a valuable model system for biochemical studies, Ca^{2+} channels in other tissues have many different properties. For example, dihydropyridine-sensitive Ca^{2+} channels in neurons have a higher affinity for dihydropyridine Ca^{2+} antagonists (*11*), high sensitivity to inhibition by ω-conotoxin (*12*), higher single-channel conductance (*3, 13*), faster kinetics of opening and closing (*2, 3, 14*), and different regulation by protein phosphorylation (*1, 10, 15*). In view of the importance of Ca^{2+} channels in neurons and the many functional differences between Ca^{2+} channels in neurons and skeletal muscle, it is of considerable interest to determine the molecular properties of dihydropyridine-sensitive Ca^{2+} channels in the brain. Here we describe polyclonal antibodies that recognize the α subunits of the purified skeletal muscle Ca^{2+} channel and use those antibodies to identify and compare a corresponding polypeptide component of the dihydropyridine-sensitive Ca^{2+} channels in the brain.

As a first step, we purified the brain dihydropyridine-sensitive Ca^{2+} channel by applying the methods used for T-tubule Ca^{2+} channels (*6*) (Table 1). The synaptic plasma membrane fraction purified from rabbit brain homogenate specifically bound 0.16 pmol of [^{3}H]PN200-110 per milligram of protein at saturation. Treatment with 1% digitonin solubilized 46% of the protein and 32% of the [^{3}H]PN200-110–channel complex (*5*). The solubilized channel was purified by chromatography on wheat germ agglutinin (WGA)–Sepharose and velocity sedimentation through a sucrose density gradient. The specific activity of final preparation was 2.91 pmol of [^{3}H]PN200-110 bound per milligram of protein, representing an overall 18.2-fold purification from the synaptic plasma membranes. If the molecular weight of dihydropyridine receptor in brain is 210,000 [as determined by radiation inactivation (*16*)], the specific activity of a homogeneous preparation would be 4760 pmol per milligram of protein, indicating that 1600-fold further purification is required to achieve homogeneity. Evidently the low concentration of dihydropyridine-sensitive Ca^{2+} channels in

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the brain necessitates the use of affinity chromatographic methods for purification.

Antibodies against the purified Ca^{2+} channel from skeletal muscle T-tubules can be used as reagents for identification and affinity purification of the Ca^{2+} channel from brain if there is immunological cross-reaction between these two channels. To utilize this approach, we raised polyclonal antisera in several BALB/c mice by repeated injection of purified skeletal muscle Ca^{2+} channel (*17*), and the antiserum with the highest titer against rabbit brain Ca^{2+} channels in radioimmune assay was selected for further study. In order to identify the antigenic site of the resulting antiserum (PAC-2), T-tubule membrane proteins were separated by SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose, and immunostained with PAC-2. As shown in lane 1 of Fig. 1, PAC-2 stained a protein band with an apparent molecular weight of 160,000, which is the same value as that of α subunits of purified Ca^{2+} channels. This staining was not observed with preimmune serum (lane 2) or with PAC-2 preabsorbed with the purified skeletal muscle Ca^{2+} channel (lane 3), which indicates that this band contains the α subunits of the skeletal muscle channel. PAC-2 did not bind to proteins corresponding to β and γ subunits of the Ca^{2+} channel. Although a small peptide of 40,000 daltons was immunostained by PAC-2 (lane 1), this staining was not blocked after the absorption of PAC-2 with the purified skeletal muscle Ca^{2+} channel (lane 3). Therefore, we conclude that the α subunits of skeletal muscle Ca^{2+} channels contain the major recognition site of PAC-2 in T-tubule membranes and that recognition is specifically blocked by prior treatment of PAC-2 with the purified channel.

To determine the cross-reactivity of PAC-2 with the brain channel, we labeled the Ca^{2+} channels in microsomal membranes from rabbit skeletal muscle and synaptic plasma membranes from brain with [^{3}H]PN200-110, solubilized with 1% digitonin and purified by chromatography on WGA-Sepharose. The labeled channels were incubated with increasing amounts of PAC-2 or preimmune serum, and the antigen-antibody complexes were precipitated by adding complexes of protein A–Sepharose and antibody to mouse immunoglobulin of classes G, A, and M [Ig(G+A+M)]. The concentration dependence of immunoprecipitation of the [^{3}H]PN200-110–labeled Ca^{2+} channels by PAC-2 is shown in Fig. 2A. Substantial immunological reactivity with both the skeletal muscle and brain Ca^{2+} channel was observed. The [^{3}H]PN200-110–labeled channels recovered in the precipitate increased with increasing PAC-2 concentration, reaching a maximum value of 60% of the total [^{3}H]PN200-110–labeled channels added. Normal serum precipitated only the background level of radioactivity, even with the maximum amount of serum tested. The immunoreactivity of PAC-2 with the skeletal muscle Ca^{2+} channel was greater than with the channel from brain. Half-maximal immunoprecipitation of skeletal muscle channels was obtained at an antiserum concentration of 0.04% (by volume). In contrast, a concentration of 0.33% (by volume) was required to obtain equivalent immunoprecipitation of the [^{3}H]PN200-110–channel complex from brain. Thus, the dihydropyridine-sensitive Ca^{2+} channels in brain and skeletal muscle are antigenically related but not identical.

The difference in immunoreactivity of brain and skeletal muscle Ca^{2+} channels in Fig. 2A might result from a reduced average affinity of PAC-2 antibodies for the brain channel or from the presence of antibody populations that recognize epitopes on the skeletal muscle Ca^{2+} channel exclusively. To determine whether brain Ca^{2+} channels could react with all the PAC-2 antibodies against skeletal muscle Ca^{2+} channels, we phosphorylated the purified skeletal muscle Ca^{2+} channel with ^{32}P on the α and β subunits as described (*18*) and then immunoprecipitated with PAC-2 that had been previously incubated with increasing amounts of partially purified unlabeled Ca^{2+} channels from either skeletal muscle or brain. Immunoprecipitation of the ^{32}P-labeled channel was progressively decreased by increasing the amount of skeletal muscle Ca^{2+} channels used for absorption and was completely blocked by 1 pmol of Ca^{2+} channel per microliter of PAC-2 (Fig. 2B). Complete block of the immunoreactivity of PAC-2 was also achieved by treating with highly purified skeletal muscle Ca^{2+} channel. In contrast, immunoprecipitation was

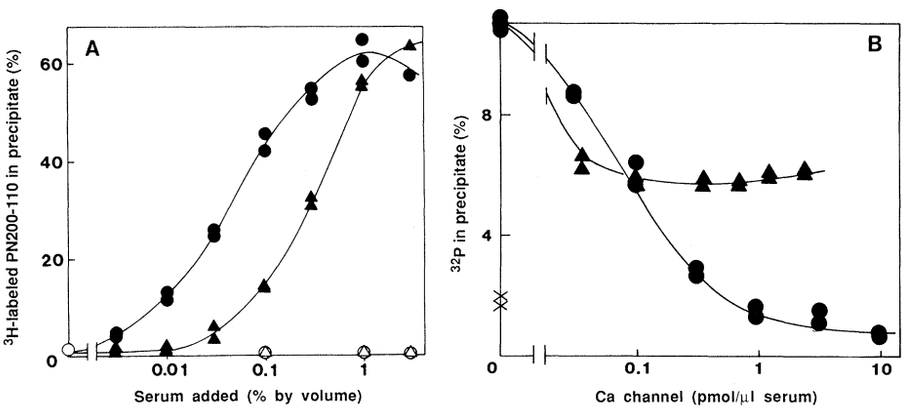

Fig. 2. (**A**) Immunoprecipitation of Ca^{2+} channels labeled with [^{3}H]PN200-110 and solubilized from rabbit skeletal muscle T-tubules and brain by polyclonal antibody PAC-2. A BALB/c mouse was immunized with biweekly injections of 10 μg of purified rabbit skeletal muscle Ca^{2+} channel (*6, 17*), and the resulting antiserum was designated PAC-2. Microsomal membranes from skeletal muscle and synaptic plasma membranes from brain were labeled with [^{3}H]PN200-110, solubilized (*5*), and partially purified as described in the legend to Table 1. Equal amounts of [^{3}H]PN200-110–labeled Ca^{2+} channels were incubated with the indicated amounts of PAC-2 or preimmune serum in 0.5% digitonin in NPT buffer for 4 hours at 4°C to allow antigen-antibody interaction. A 10-μl portion of rabbit antiserum to mouse Ig(G+A+M) (Zymed) and 2 mg of protein A–Sepharose CL-4B (Sigma) were mixed in 0.1% bovine serum albumin and phosphate-buffered saline. After being mixed for 2 hours, the resulting complex was washed twice with 0.5% digitonin in NPT buffer by sedimentation in a microfuge and added to the antigen-antibody sample. After being mixed for an additional 2 hours at 4°C, the antigen-antibody-protein A–Sepharose complex was collected by sedimentation and washed twice in 0.5% digitonin in NPT buffer; the radioactivity recovered in the precipitate was expressed as the percentage of total [^{3}H]PN200-110–labeled Ca^{2+} channels. Symbols: ● and ○, skeletal muscle; ▲ and △, brain. Filled symbols, immunoprecipitation by PAC-2; open symbols, immunoprecipitation by preimmune serum. The values are typical results of four different experiments. (**B**) Absorption of the immunoreactivity of PAC-2 by preliminary treatment with skeletal muscle or brain Ca^{2+} channels. Skeletal muscle Ca^{2+} channel was purified from rabbit T-tubules and phosphorylated with ^{32}P by catalytic subunit of cyclic adenosine monophosphate (cAMP)–dependent protein kinase as described (*10*). [^{3}H]PN200-110–labeled Ca^{2+} channels were partially purified from skeletal muscle microsomes and synaptic plasma membranes as described in the legend to Table 1. PAC-2 at a final concentration of 0.01% was mixed with various amounts of the partially purified skeletal muscle (●) or brain (▲) channel in 75 m*M* NaCl, 2.5 m*M* EDTA, 50 m*M* NaH_2PO_4, 25 m*M* tris, 20 m*M* KF, and 1% Triton X-100, *p*H 7.4, at 4°C. The concentration of partially purified channels was determined from the amount of [^{3}H]PN200-110 bound. After overnight incubation, ^{32}P-labeled Ca^{2+} channel (4500 cpm per sample) was added and incubated for 4 hours at 4°C. The antigen-antibody complex was precipitated by protein A–Sepharose antiserum to mouse Ig(G+M+A) complex as described in the legend to Fig. 1. The ^{32}P recovered in the precipitate was determined by liquid scintillation counting. The background immunoprecipitation without antiserum was determined in parallel experiments (**X**). Values are typical results of four different experiments.

reduced to 50% of the maximum level by 0.1 pmol of brain Ca^{2+} channel per microliter of PAC-2, but the extent of inhibition was not increased further with increasing amounts of the brain channel. In four separate experiments, 43 ± 4% (mean ± SEM) of the immunoreactivity against skeletal muscle Ca^{2+} channel was blocked by prior treatment with brain Ca^{2+} channels. These results show that only a fraction of the immunoreactivity of PAC-2 can be blocked by brain Ca^{2+} channels, which suggests that only part of the structure of skeletal muscle Ca^{2+} channel is immunologically homologous to the brain channel.

To identify the peptide recognized by PAC-2, purified preparations of skeletal muscle and brain dihydropyridine-sensitive Ca^{2+} channels were labeled with ^{125}I, immunoprecipitated by PAC-2, and analyzed by SDS–polyacrylamide gel electrophoresis. When the purified skeletal muscle Ca^{2+} channel was iodinated by the Bolton-Hunter method (*19*), the α and β subunits were clearly identified after immunoprecipitation (Fig. 3A, lane 1). Without disulfide reduction, the apparent molecular sizes of the iodinated α and β subunits were 163,000 and 54,000, respectively, in close agreement with previous estimates of 160,000 and 50,000 (*6*). The γ subunit was so poorly labeled that its identification was difficult by this method. The 163,000- and 54,000-dalton polypeptides were not immunoprecipitated by preimmune serum, which confirmed the identification of these polypeptides as α and β subunits of the Ca^{2+} channel (Fig. 3A, lane 2).

When the purified skeletal muscle Ca^{2+} channel was labeled with ^{125}I by the Iodobead method (*20*), immunoprecipitated, and analyzed by SDS–gel electrophoresis after disulfide reduction with dithiothreitol, we observed a diffuse zone of ^{125}I-labeled protein migrating in the region of the α subunits and two faint protein bands of low molecular weight migrating in the region of the γ subunit (Fig. 3A, lane 3). The labeled α subunits migrated as a leading band of 135,000 daltons with a diffuse trailing zone spreading to 180,000 daltons (Fig. 3A, lane 3). This behavior, which was observed only with samples iodinated by this procedure, may result from oxidative intramolecular cross-linking of α subunits giving species with a range of apparent molecular weights similar to those observed with and without reduction of disulfide bonds (135,000 to 162,000) (*6*). The high and low molecular-weight bands were not immunoprecipitated by preimmune serum (Fig. 3A, lane 4) or by PAC-2 that had been absorbed with purified Ca^{2+} channel (Fig. 3A, lane 5), which confirms identification of these bands as Ca^{2+} channel components. A labeled protein band corresponding to the β subunit of the purified Ca^{2+} channel was not observed in Iodobead-labeled samples because of a low level of iodination by this method and dissociation of this subunit by the harsh reaction conditions.

To identify the immunoreactive polypeptides in the rabbit brain, synaptic plasma membrane glycoproteins were solubilized in digitonin, purified by WGA-Sepharose chromatography as described above, and iodinated with ^{125}I by either the Bolton-Hunter or Iodobead methods. In the preparation labeled with Bolton-Hunter reagent, a sharp protein band with a molecular size of 169,000 daltons was specifically immunoprecipitated by PAC-2 (Fig. 3B, lane 1). The size of this polypeptide is similar to that of the α subunits of the skeletal muscle Ca^{2+} channel. This polypeptide has a small apparent size of 140,000 daltons after reduction of disulfide bonds (Fig. 3B, lane 2). Its immunoprecipitation is not observed with preimmune serum (Fig. 3B, lane 3). In the preparation labeled with Iodobeads, a similar sharp protein band of 169,000 daltons was specifically immunoprecipitated (Fig. 3B, lane 4). Its apparent size was reduced to 140,000 daltons by reduction of disulfide bonds (Fig. 3B, lane 5). Immunoprecipitation of this protein band was not observed with preimmune serum (Fig. 3B, lane 6) and was markedly reduced in intensity (Fig. 3B, lane 7) with PAC-2 absorbed with purified skeletal muscle Ca^{2+} channel. Thus, the immunoreactive component of the brain dihydropyridine-sensitive Ca^{2+} channel is a glycoprotein with the apparent molecular size and the electrophoretic behavior upon reduction of disulfide bonds that are characteristic of an α subunit of the skeletal muscle Ca^{2+} channel.

The α subunits of the purified skeletal muscle Ca^{2+} channel are noncovalently associated with two smaller polypeptides, β and γ (*6*). Although our antibodies recognize only α subunits, brain polypeptides analogous to the β and γ subunits might be specifically coprecipitated with the α subunits. Such specifically associated polypeptides were not clearly identified in these studies (Fig. 3B). However, the presence of a β subunit–like protein would have been obscured by nonspecific immunoprecipitation of a diffuse labeled band with an apparent molecular size of 50,000 to 60,000 daltons in samples labeled by Bolton-Hunter reagent (Fig. 3B, lanes 1 through 3). The γ subunits in skeletal muscle are not well labeled by the Bolton-Hunter method and would not be expected to be observed in

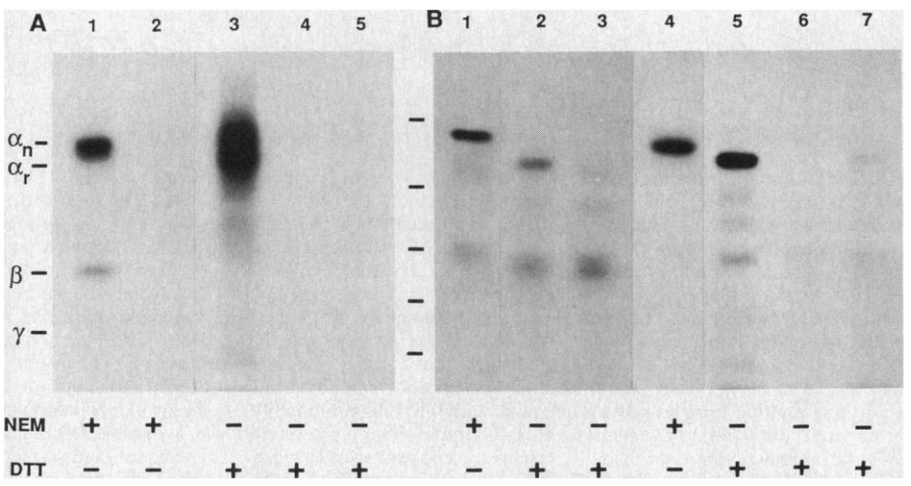

Fig. 3. Immunoprecipitation of ^{125}I-labeled α subunit of Ca^{2+} channels from skeletal muscle and brain by PAC-2. (**A**) Purified skeletal muscle Ca^{2+} channel was iodinated by either the Bolton-Hunter (lanes 1 and 2) (*19*) or Iodobead (lanes 3 to 5) (*20*) method. Iodinated proteins were immunoprecipitated with PAC-2 (lanes 1 and 3), preimmune serum (lanes 2 and 4), and PAC-2 preabsorbed with purified skeletal muscle Ca^{2+} channel (lane 5). (**B**) Partially purified [^{3}H]PN200-110–labeled Ca^{2+} channels from rabbit brain were iodinated by the Bolton-Hunter (lanes 1 to 3) or Iodobead (lanes 4 to 7) method and immunoprecipitated by PAC-2 (lanes 1, 2, 4, and 5), preimmune serum (lanes 3 and 6), and PAC-2 absorbed with purified skeletal muscle Ca^{2+} channel (lane 7). For immunoprecipitation, the iodinated proteins were incubated with PAC-2 or preimmune serum at a concentration of 0.3% in 75 m*M* NaCl, 2.5 m*M* EDTA, 50 m*M* NaH_2PO_4, 25 m*M* tris, 20 m*M* KF, 1% Triton X-100 at *p*H 7.4 for 4 hours at 4°C. The resulting antigen-antibody complex was precipitated by protein A–Sepharose (5 mg per sample). After boiling in SDS in the presence of 20 m*M* *N*-ethylmaleimide (NEM) or 20 m*M* dithiothreitol (DTT), the solubilized proteins were analyzed by SDS–gel electrophoresis in a discontinuous 5 to 15% (weight to volume) gradient polyacrylamide gel system as described (*6*). Gels were stained with Coomassie blue, destained, dried, and exposed to Kodak X-Omat film. The migration positions of standard proteins indicated by horizontal bars between (A) and (B) correspond, from top to bottom, to the following molecular sizes: 200,000, 116,000, 68,000, 42,000, and 30,000.

Table 1. Partial purification of the dihydropyridine-sensitive Ca^{2+} channel from rabbit brain. A synaptic plasma membrane fraction was prepared from rabbit forebrain according to the procedure of Jones and Matus (*23*). The measurement of [^{3}H]PN200-110 binding to the membrane-bound Ca^{2+} channels was performed as described (*5, 6*). The membranes were labeled with a saturating concentration (2 n*M*) of [^{3}H]PN200-110 (New England Nuclear) and solubilized in 1% digitonin with a 5:1 (by weight) ratio of detergent to protein in 75 m*M* NaCl and 10 m*M* Hepes-tris, *p*H 7.4, at 4°C for 30 minutes. Insoluble material was removed by centrifugation at 175,000*g* for 45 minutes at 4°C. The solubilized material was diluted 1:1 with 75 m*M* NaCl and 10 m*M* Hepes-tris, *p*H 7.4, to which had been added a 7-ml packed volume of WGA-Sepharose. After a 30-minute equilibration, the WGA-Sepharose was packed in a 2 cm by 10 cm column and washed with 0.1% digitonin in NTP buffer (75 m*M* NaCl, 50 m*M* NaH_2PO_4, 25 m*M* tris, *p*H 7.4), and adsorbed glycoproteins were eluted with 100 m*M* *N*-acetylglucosamine in 0.1% digitonin in NPT. The specific activity (picomoles of [^{3}H]PN200-110 bound per milligram of protein) of the pooled fraction was determined from the specifically bound [^{3}H]PN200-110 (*5, 6*). The values are typical results of two different experiments. Negligible dissociation of [^{3}H]PN200-110 occurred during the purification process as assessed by polyethyleneglycol precipitation assays (*5, 6*) or sucrose gradient analysis. Peak [^{3}H]PN200-110–labeled fractions were pooled and frozen in liquid nitrogen for use in immunoprecipitation assays or for further purification by velocity sedimentation through linear 5 to 20% sucrose gradients for 1.5 hours at 210,000*g* as described (*5, 6*). Throughout the preparation, all solutions contained 1 m*M* phenylmethanesulfonyl fluoride and 1 μ*M* pepstatin A. Protein concentrations were determined according to the method of Bradford (*24*), with bovine serum albumin used as the standard.

Purification step	[^{3}H]PN200-110 bound		Protein		Specific activity (pmol/mg)	Purification (*x*-fold)
	pmol	%	mg	%		
Synaptic plasma membranes	11.5	100	72	100	0.160	
Digitonin extract	3.70	32	33	46	0.112	0.7
WGA-Sepharose	1.71	14.8	2.7	3.8	0.627	3.9
Sucrose gradient	0.60	5.2	0.21	0.29	2.91	18

these samples. In samples labeled with the Iodobead method, several minor components were observed when disulfide bonds were reduced before electrophoresis (Fig. 3B, lane 5). None of these corresponded to the β and γ polypeptides of the skeletal muscle channel. Preparative purification of a functionally active form of the dihydropyridine-sensitive Ca^{2+} channel from brain is required before its complete subunit composition can be established.

Our results identify a polypeptide component of a neuronal Ca^{2+} channel and show that this protein subunit of the dihydropyridine-sensitive Ca^{2+} channel from brain is similar to an α subunit of the purified skeletal muscle Ca^{2+} channel in apparent molecular size, antigenic properties, and electrophoretic behavior upon reduction of disulfide bonds [see note added in proof and (*21*)]. Other biochemical studies have also suggested that dihydropyridine-sensitive Ca^{2+} channels in different tissues are structurally similar despite their considerable functional diversity. The dihydropyridine-sensitive Ca^{2+} channels of skeletal, smooth, and cardiac muscles and brain are all glycoproteins (*5–9*) that have target sizes for radiation inactivation between 200,000 and 300,000 daltons (*16*) and similar sedimentation coefficients in detergent (*5–8*). In other experiments, we have found PAC-2 also cross-reacts with the dihydropyridine-sensitive Ca^{2+} channels of rabbit heart (*17*) and smooth muscle (*8*), indicating that the dihydropyridine-sensitive Ca^{2+} channels of all four tissues examined to date contain at least one antigenic site with similar immunoreactivity. With PAC-2, an α subunit of the rabbit heart dihydropyridine receptor was identified as a polypeptide with apparent molecular size of 170,000 daltons and 141,000 daltons before and after reduction of disulfide bonds (*17*). Using a polyclonal antibody raised against a 32,000- to 34,000-dalton component of the skeletal muscle Ca^{2+} channel, Schmid *et al.* identified a protein of 170,000 to 176,000 daltons in skeletal, smooth, and cardiac muscles under nonreducing conditions (*22*). Considered together with our data, these results suggest that the dihydropyridine-sensitive Ca^{2+} channels of all excitable tissues contain large α subunits having similar size and partially homologous structure.

Immunoreactivity of PAC-2 with the skeletal muscle Ca^{2+} channel was absorbed only partially by the pretreatment with Ca^{2+} channels of brain (Fig. 2) and heart (*17*), suggesting that α subunits have tissue-specific regions in their structure. The immunoreactivity of PAC-2 varied among various tissues: skeletal muscle > cardiac muscle ≈ smooth muscle > brain (*9, 17*) (Fig. 2A). Therefore, it is likely that homology of α subunits among muscle tissues is greater than that between skeletal muscle and brain. These structural differences within the α subunits may be the origin of the differences in the properties of Ca^{2+} channels among excitable tissues.

Note added in proof: Since submission of this manuscript, we have found that the skeletal muscle Ca^{2+} channel contains two different α subunits: α_1 of 170,000 daltons, and α_2 of 140,000 daltons disulfide-linked to δ of 24,000 to 26,000 daltons (*21*). The α of the brain Ca^{2+} channel identified in this study appears analogous to the α_2 subunit of the skeletal muscle Ca^{2+} channel.

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