Identification of a Novel 1,4-Dihydropyridine- and Phenylalkylamine-binding Polypeptide in Calcium Channel Preparations*

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Calcium channels have been purified from both skeletal muscle (1–6) and heart (7). These studies uniformly reveal the existence of a 130 to 150-kDa polypeptide, “α subunit” (1), in purified calcium channel preparations. This polypeptide has a characteristic behavior in sodium dodecyl sulfate-polyacrylamide gels: its apparent mass is reduced by 30–40 kDa from 160–190 kDa upon treatment with disulfide reducing agents such as dithiothreitol or β-mercaptoethanol (1). Borsotto et al. (3) suggest that purified voltage-dependent calcium channels consist of a 142, a 32, and a 33-kDa polypeptide. In contrast, others (1, 2, 4, 5) have identified 130 to 155-kDa (α subunit), 50 to 65-kDa (β subunit), and 31 to 33-kDa (γ subunit) subunits. Functional calcium channels present in the purified preparations have been proven in reconstitution experiments (8–11). The presence of a 1,4-dihydropyridine-binding site in the purified calcium channel preparations has been demonstrated by measurement of radioactivity after prelabeling the membrane-bound channel with a radiolabeled 1,4-dihydropyridine derivative (1–3, 6, 7) or by carrying out direct binding studies (3–5). Both of these procedures are useful for determining the specific activity in purified preparations, but they do not reveal the identity of the drug-binding subunit. Monoclonal antibodies directed against disulfide-linked 140- and 30-kDa polypeptides were capable of immunoprecipitating reversible 1,4-dihydropyridine and (–)-D 8881 binding from detergent-solubilized membranes (12). Vandaele et al. (12) conclude from these studies that a single disulfide bridge-containing 170-kDa polypeptide is the calcium channel and the drug receptor.

In order to convincingly identify the drug-binding polypeptide, however, stereoselective covalent labeling of the purified receptor protein is necessary. Previous studies demonstrated the existence of both low (13, 14) and high (5, 15–19) molecular weight polypeptides which can be photoaffinity labeled in partially purified calcium channel preparations. Recently, the phenylalkylamine receptor in a purified guinea pig skeletal muscle calcium channel preparation was found to be located on a 155-kDa polypeptide which did not change its electrophoretic mobility after reduction with dithiothreitol (18). Previous data showed that a 158-kDa polypeptide was photoaffinity labeled in guinea pig skeletal muscle transverse...
tubular membranes with (-)-[^3]H]azidopine under both reducing and alkylating conditions (16). Other studies suggest that the drug-binding 170-kDa polypeptide identified by photoaffinity labeling in rabbit skeletal muscle transverse tubular membranes is identical with the disulfide-linked 140- and 30-kDa polypeptides isolated by the same group and used in subsequent preparations (20). If this were the case, the drug-binding polypeptide would be either the 140- or the 30-kDa polypeptide under disulfide reducing conditions. Direct photoaffinity labeling results to prove this have not been presented. In order to test this possibility and to determine the reason for the contradictory results present in the literature, skeletal muscle calcium channels were purified by three different procedures from two different species in two different laboratories and were photoaffinity labeled by two specific probes: a 1,4-dihydropyridine, (-)-[^3]H]azidopine, (15), and a phenylalkylamine, [N-methyl-[^3]H]LU 48988 (18). These studies resulted in the discovery of a label 155 to 170-kDa, 1,4-dihydropyridine- and phenylalkylamine-binding polypeptide in purified calcium channel preparations which does not change its electrophoretic mobility under reduction with dithiothreitol and, therefore, is distinct from the previously described a subunit.

EXPERIMENTAL PROCEDURES

Membrane Preparations—Skeletal muscle membranes enriched in 1,4-dihydropyridine-binding sites were isolated from two species, i.e., rabbit and guinea pig. Transverse tubular membranes were isolated by the method of Nakayama et al. (6) and crude membranes were prepared as described by Glossmann and Ferry (21). Both fresh muscle and muscle kept at -70 °C for several weeks as described by Galizi et al. (22) were used for isolation of the membranes. When frozen muscle was used, 1.5- to 2.1 mg of muscle protein were thawed overnight in a refrigerator (at 4 °C) before using for membrane preparations (6).

Calcium Channel Purification—Three different procedures were employed for purification of guinea pig or rabbit skeletal muscle calcium channels from digitonin-solubilized membranes. Procedure 1 is a two-step purification using only a WGA-Sepharose affinity column and sucrose density gradient centrifugation as described by Striessnig et al. (5). Procedure 2 is a four-step purification which is based on the original method of Curtis and Catterall (1). This employs a WGA-Sepharose affinity column, DEAE ion-exchange chromatography, a second WGA-Sepharose affinity column and sucrose density gradient centrifugation as described by Nakayama et al. (6). Procedure 3 is the same as procedure 2 but the DEAE ion-exchange chromatography and the second WGA-Sepharose affinity chromatography were omitted. Procedures 1 and 3 are the same as far as the purification steps are concerned, but they are different in that different buffers and conditions were used. Briefly, procedure 3 is as follows. Previously isolated skeletal muscle membranes, which were stored at -70 °C, were thawed in a 25 °C water bath, rehomogenized, and then centrifuged at 100,000 × g for 30 min. The pellet was diluted to 2 mg of protein/ml concentration with a solubilization buffer containing 185 mM KCl, 1.5 mM CaCl₂, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM iodoacetamide, 1 µg/ml antipain, 1% digitonin, and 10 mM HEPES-Tris, pH 7.4, at 4 °C, and solubilized on ice for 40 min. The insoluble material was sedimented by centrifugation at 100,000 × g for 60 min. One hundred ml of digitonin extract (supernatant) was incubated for 2 h at 4 °C with ~33 ml of WGA-Sepharose which had previously been equilibrated with the solubilization buffer. The WGA-Sepharose was packed into a column and was washed with 80 ml of solubilization buffer containing 1% digitonin and then with 130 ml of solubilization buffer containing 0.1% digitonin. The glycoproteins adsorbed to the column were eluted with the column volume of buffer containing 0.1 M N-acetylglucosamine, 1 mM CaCl₂, 0.1% digitonin, 0.1 mM phenylmethanesulfonyl fluoride, 1 mM iodoacetamide, 1 µg/ml antipain, and 5 mM Tris-Cl, pH 7.4, at 4 °C. The protein concentration was determined by the Bio-Rad Protein Assay. The two peak protein fractions, approximately 1.5-2 ml each, were combined and applied to a 36-37 ml, 5-20% linear sucrose density gradient. The sucrose was dissolved in 5 mM MOPS-NaOH buffer, pH 7.4, at 4 °C containing 1 mM CaCl₂, 0.1 mM phenylmethanesulfonyl fluoride, 1 mM iodoacetamide, 1 µg/ml antipain, and 0.1% digitonin. The gradients were centrifuged in a VT520 vertical rotor at 210,000 × g for 90 min. The gradients were fractionated from the bottom of the quick-sealed tubes into 1.3-ml fractions. The protein concentration and specific (+)-[^3]H]PN200-110 binding were measured in each fraction and the fractions containing the highest binding site concentrations were used for subsequent experiments.

Radioligand Binding—The specific activities of the purified calcium channel preparations were determined by carrying out saturation binding experiments using (+)-[^3]H]PN200-110 as labeled ligand in the presence of 10−30 nM d-ct-diltiazem (21, 23). The specific activities were between 1 and 1.5 nmol/mg protein when previously frozen muscle was used and 1.5 and 2.1 nmol/mg protein when previously frozen skeletal muscle served as the starting material for purification.

Photoaffinity Labeling—Photoaffinity labeling of membranes was performed as previously described (16). The purified calcium channel protein, 2.5-10 µg/ml, was incubated with 7-10 nM (-)-[^3]H]azidopine or 12-20 nM [N-methyl-[^3]H]LU 48988 for 45 min at 25 °C. The final concentration of digitonin was 0.08-0.1% (w/v). Nonspecific binding (or incorporation) was measured in the presence of 1-3 µM (+)-PN200-110 or 3-10 µM (-)-desmethoxyverapamil, respectively. Fifty µl aliquots were assayed in duplicate using the polyethylene glycol precipitation technique (21) to determine the concentration of reversibly bound ligand prior to irradiation. The irradiation mixture was then irradiated on ice from a distance of 10 cm with ultraviolet light (Philips 38W/TL lamp) for 20 min. Aliquots of the irradiated samples were lyophilized and then reuspended in sample buffer contained either 10 mM dithiothreitol (which were either 10 mM dithiothreitol (reducing conditions). The samples were heated in the sample buffer for 15 min at 57 °C or for 10 min at 60 °C and electrophoresed on 8% straight or 5-15% gradient sodium dodecyl sulfate-polyacrylamide gels (5).

Gels were stained with amipril Blue or silver and were scanned using an LKB Ultrascan Laser Densitometer prior to determining the incorporated radioactivity by either cutting the gel into 2-mm slices followed by liquid scintillation counting (5) or by preparing fluorograms of the stained gels on Kodak XAR films (exposure time 1-5 weeks) after impregnation with EnlightenSM (Du Pont-New England Nuclear).

RESULTS

When membranes isolated from fresh (not previously frozen) skeletal muscle of rabbit or guinea pig and one of the three procedures described under "Experimental Procedures" were used for purification of calcium channels, four major polypeptides were present in sucrose density fractions containing the highest density of the 1,4-dihydropyridine-binding sites. Fig. 1 shows the polypeptide compositions of the purified guinea pig (A) and rabbit (B) skeletal muscle calcium channel obtained by using procedure 1 for purification. The four polypeptides consistently present in our preparations are termed a₁, a₂, β, and γ. Our nomenclature refers to the size of the subunits, determined under reducing conditions (presence of dithiothreitol), a₁ being the largest subunit. The molecular masses of these subunits are 155-170 kDa (a₁), 130-150 kDa (a₂), 50-65 kDa (β), and 30-35 kDa (γ). These 4 polypeptides co-purified with the (+)-[^3]H]PN200-110-binding activity and were missing from sucrose density gradient fractions which were devoid of 1,4-dihydropyridine binding. Occasionally, polypeptides that dominate sucrose density gradient fractions containing no specific 1,4-dihydropyridine-binding activity are also present in the 1,4-dihydropyridine-binding fractions. These polypeptides (103, 65, 25-30 kDa), if present, are considered contaminants. When the purified protein was solubilized before electrophoresis in the presence of 10 mM N-ethylmaleimide, i.e., under alkylating conditions, again, two large polypeptides were identified with apparent masses of 160-190 and 155-170 kDa (Fig. 1, A and B).

Membranes were also isolated from rabbit or guinea pig skeletal muscle previously kept frozen at -70 °C for several weeks. This procedure, originally suggested by Galizi (22), significantly increases the 1,4-dihydropyridine-binding activ-
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Two pg of protein was applied per lane. polypeptide in skeletal muscle membranes and purified skeletal muscle membranes and purified skeletal muscle calcium channel preparations. Two pg of protein was applied per lane. putative subunits of the calcium channel, while a1 corresponds to reducing conditions. The large polypeptide, which changes its electrophoretic mobility upon reduction, is present in this preparation (Fig. 1C). The lower labeling demonstrated under reducing conditions with (+)-[3H]azidopine (panels A and B) was still incomplete. However, the distribution of the specific incorporation radioactivity strictly followed the absorbance corresponding to the staining intensity of the α1 polypeptide. A nearly complete separation of the two polypeptides of varying number and size were labeled by both aryline alazide ligands (data not shown).

In order to identify the 1,4-dihydropyridine- and phenylalkylamine-binding subunit of the purified calcium channel, photoaffinity labeling was carried out using the aryline ligands (+)-[3H]azidopine and [N-methyl-[3H]LU 49888. When calcium channels purified from membranes derived from frozen tissue were photoaffinity labeled, 60 to 90-kDa polypeptides of varying number and size were labeled by both aryline ligands (data not shown).

An extensive search to determine the reason for the variability of these data revealed that identical polypeptides can be photoaffinity labeled both in the membranes used for purification and in the purified calcium channel preparations irrespective of the purification procedure used. When the membranes were isolated from fresh tissue, only one, a 155 to 170-kDa, polypeptide was photoaffinity labeled by both aryline ligands in both membranes (Fig. 1D, left panel and 14339 purified calcium channel preparations was also approximately an order of magnitude higher when (+)-[3H]azidopine (5) or [N-methyl-3H]LU 49888 (18) were used as labeled ligands. These data suggest that the affinity of calcium channel inhibitor drugs is decreased by approximately an order of magnitude upon solubilization and purification of the membrane-bound receptor.
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Fig. 2. Characterization of the α₁ and α₂ subunits of purified skeletal muscle calcium channels. Calcium channels purified using procedure 1 from fresh muscle of guinea pig (12.6 µg of protein) or rabbit (15.8 µg of protein) were incubated with (−)-[N-methyl-³H]LU 49888 (12.6 nM) or (−)-[¹⁹]azidopine (7.5 nM) in 5.0 ml of total incubation volume and photoaffinity labeled. Improved separation of the two α polypeptides was achieved by electrophoresis on 8% polyacrylamide gels at 45 mA constant current for 7 h until the 116-kDa marker (β-galactosidase) reached the bottom of the gel. The densitometric scans of the silver-stained bands are shown overlayed with the specifically incorporated [N-methyl-³H]LU 49888 (Panels A, C, and E) and (−)-[¹⁹]azidopine (Panels B, D, and F) recovered in the respective gel slices (open columns). Specifically incorporated radioactivity is total incorporated label minus that incorporated in the presence of the competing drug. Specific disintegrations/min were normalized to the maximal specific incorporation (100%), always coinciding with the absorption maximum of the α₁ band. Panels A and B, 2.1 µg of photolabeled protein from guinea pig skeletal muscle was applied to each lane after denaturation in the presence of 10 mM N-ethylmaleimide (NEM). Panels C and D, 4.2 µg of photolabeled protein from guinea pig skeletal muscle was applied to each lane after denaturation in the presence of 10 mM dithiothreitol (DTT). Panels E and F, 5.2 µg of photolabeled rabbit skeletal muscle calcium channel protein was applied to each lane after denaturation in the presence of 10 mM dithiothreitol. Panel G, silver stain of the gels prior to gel scanning and slicing. The lanes of the [N-methyl-³H]LU 49888-labeled proteins are shown: 1 and 2, guinea pig; 3 and 4, rabbit; 1 and 3 total incorporation lanes; 2 and 4, nonspecific incorporation lanes. The gel scans of Panels A, C, and E were obtained from lane 1 (alkylating) and lanes 1 and 3 (reducing), respectively. Maximal specific labeling (100%) was 914 dpm (A), 2223 dpm (B), 2102 dpm (C), 823 dpm (D), 1451 dpm (E), and 840 dpm (F). The incorporation efficiency was 4-6% for [N-methyl-³H]LU 49888 and 2% (reducing)-11% (alkylating conditions) for (−)-[¹⁹]azidopine.
was achieved in the presence of dithiothreitol, i.e., under reducing conditions. Under these conditions, the incorporation of [N-methyl-^3H]LU 49888 and (−)^[H]azidopine occurred exclusively within the α subunit polypeptide region. A shoulder of staining intensity and of specific incorporation of both photolabels was evident, under reducing conditions, towards the top of the α subunit. Therefore, we cannot exclude some microheterogeneity in the region of the α subunit. One explanation for this behavior might be the glycoprotein nature of the α polypeptides which could explain their diffuse migration pattern compared with the standard proteins run in parallel (Fig. 2G).

DISCUSSION

The data presented in this paper provide the first direct evidence for the existence of a novel 1,4-dihydropyridine- and phenylalkylamine-binding polypeptide (termed here α subunit) in purified rabbit and guinea pig skeletal muscle calcium channel preparations. The α subunit co-purified with the α, β, and γ subunits previously described by Curtis and Catterall (1) in both rabbit and guinea pig calcium channel preparations. The mass of the α subunit (155–170 kDa) is very similar to that of the α subunit (130–140 kDa) described by Curtis and Catterall (1) which we term “α1” in order to emphasize the difference between these polypeptides. The diffuse α1 and α2 bands frequently overlap each other on gradient gels and, therefore, their differentiation requires selective photoaffinity labeling and the use of disulfide reducing agents. While the α1 subunit can be photoaffinity labeled by the two radioabeled arylazide ligands employed in this study, neither α2, β, nor γ subunits were labeled by these same ligands. Furthermore, the α1 subunit did not display a different electrophoretic mobility under alkylating and reducing conditions, which is characteristic for the α2 subunit (1).

Skeletal muscle calcium channels were purified by several different groups (1–6) and none of them recognized the existence of distinct α1 and α2 polypeptides. In a previous work by Curtis and Catterall (1), however, one can see a faint, diffuse band probably corresponding to the α1 subunit described here (see Fig. 2B in Ref. 1). The existence of this polypeptide, however, was unrecognized. In a later study, Curtis and Catterall (2) noted that under alkylating conditions the phosphorylated α subunit migrated on sodium dodecyl sulfate gels slightly more rapidly than the majority of the nonphosphorylated α subunit. We believe that in their preparation it was not the α2 subunit, which runs above the α1 under alkylating conditions, but rather the unrecognized α1 subunit which was phosphorylated by the catalytic subunit of cAMP-dependent protein kinase. This is evident from our preliminary phosphorylation data. Recently, Flockeri et al. (4) reported the presence of a 142-kDa polypeptide in a purified rabbit skeletal muscle calcium channel preparation which did not change its electrophoretic mobility upon reduction with dithiothreitol and was phosphorylated by the catalytic subunit of cAMP-dependent protein kinase. This polypeptide, although its Mₐ is somewhat lower, is similar to the α1 subunit described in this study. The novel characteristics of this polypeptide which distinguish it from the previously described α subunit have remained largely unnoticed. Two polypeptides, corresponding to the α1 and α2 subunits demonstrated in this study, have recently been described in a purified guinea pig skeletal muscle calcium channel preparation (18). The one corresponding to α1 was photoaffinity labeled with [N-methyl-^3H]LU 49888 but the other one was not. However, (−)^[H]azidopine, the 1,4-dihydropyridine probe, was not used in those studies; therefore, it is not possible to determine whether the 1,4-dihydropyridine and phenylalkylamine receptor sites were on the same polypeptide.

The lack of the α1 subunit in purified calcium channel preparations in some studies (3, 6) in spite of the presence of 1,4-dihydropyridine-binding sites would appear to contradict our conclusion, namely, that both the 1,4-dihydropyridine- and phenylalkylamine-binding sites reside in the α1 subunit. To investigate this, purified calcium channel preparations that contained no α1 subunit but had a B_max of 2 nmol/mg protein for (±)-^[H]PN 200-110 binding were photoaffinity labeled with (−)^[H]azidopine. These preparations contained fragments of α1, often not readily detectable on silver-stained gels. These fragments retain their ability to bind drugs and are absent in preparations containing a discreet α1 band. We also investigated the reason for the degradation of the α1 subunit and found that it was not the use of different purification protocols or different membrane isolation protocols, but rather the use of frozen muscle. When fresh muscle was used for isolation of membranes, both a rapid procedure (21) and a more complex procedure (6) were equally adequate for obtaining preparations with an intact α1 subunit. We conclude that the original procedure described by Curtis and Catterall (1), the rapid two-step purification described by Striessnig et al. (5), and the two-step purification described under procedure 3 of this paper were all suitable for purification of skeletal muscle calcium channels. We also conclude that photoaffinity labeled polypeptides smaller than the α1 subunit represent either nonspecific labeling or degradation products of α1. It is apparent that the α1 subunit is much more labile than the α2 and is therefore subject to degradation and hence can easily be overlooked as being a discrete and important subunit of the calcium channel.

Purification procedures different from those involved in this study have been described by Lazdunski and his associates (3, 7, 20, 24–26). Interestingly, their skeletal and cardiac muscle calcium channel preparations revealed the same pattern (3, 7, 20, 24–26) and the only components that were described were 140- and 30-kDa polypeptides. The 140-kDa polypeptide described by these investigators (3, 7, 20, 24–26) displays characteristics very similar to the α1 subunit described here and the α subunit first identified by Curtis and Catterall (1).

Using polyclonal and monoclonal antibodies, it was demonstrated that the 170-kDa, disulfide bridge-containing protein is split into 140- and 30-kDa polypeptides upon treatment with disulfide reducing agents (12, 25–27). Both (±)-^[H]PN 200-110 and (−)^[H]desmethoxyverapamil binding were immunoprecipitated with monoclonal antibodies that identified a 170-kDa disulfide bridge-containing protein under alkylating conditions. It was therefore concluded that the purified skeletal muscle calcium channel consists of only two, a 140- and 30-kDa, disulfide-linked polypeptides (12, 20, 25, 26). This contradicts our conclusion, namely, that the 1,4-dihydropyridine- and phenylalkylamine-binding subunit of the skeletal muscle calcium channel is a 155 to 170-kDa polypeptide which does not change its electrophoretic mobility upon treatment with disulfide reducing agents. To resolve this contradiction, the differences in the calcium channel purification procedures must be investigated. The Lazdunski group (22, 28) uses either fresh rabbit skeletal muscle or muscle which was kept frozen at −70 °C for several weeks or months before preparation of membrane. Here we showed specific photoaffinity labeling that freezing and subsequent thawing of the muscle results in degradation of the drug-binding α, 2 F. Guba, Jr., P. L. Vaghy, E. McKenna, and A. Schwartz, unpublished data.
subunit (Fig. 1). Galizzi et al. (17) have also identified in rabbit skeletal muscle transverse-tubular membranes a polypeptide which was photoaffinity labeled with (+)-(3H)PN200-110 and has an apparent molecular mass of 155-170 kDa after treatment with disulfide reducing β-mercaptoethanol. This polypeptide seems to be very similar to the α1 subunit described in this study and is different from the 170-kDa disulfide bridge-containing protein which dissociates to a 140- and 30-kDa subunit upon treatment with β-mercaptoethanol. We speculate that the 155 to 170-kDa α1 subunit was present and photoaffinity labeled by Galizzi et al. (17) in those membranes which were isolated from fresh tissue using the procedure of Fosset et al. (28), but it was confused with the 170-kDa disulfide bridge-containing protein only present under non-reducing conditions. Furthermore, fragments of the drug-binding α1 subunit must have been present in calcium channel preparations derived from frozen and thawed skeletal muscle. Borsotto et al. (3, 24), in fact, described the presence of minor bands other than the 140- and 30kDa bands in purified calcium channel preparations. The amount of these bands was, however, highly variable from one preparation to another and, therefore, these bands were assumed to be contaminants. Photoaffinity labeling experiments to prove this have not been done by Borsotto et al. (3, 24).

Another major difference between the purification procedures employed here and those employed by Borsotto et al. (3, 24) is the detergent used for solubilization of the channel macromolecule from the muscle membranes. We and others (1, 2, 4-6) have used digitonin because direct radioligand-binding experiments, which are required for photoaffinity labeling, can easily be done in the digitonin-solubilized extracts. In contrast, it is difficult to demonstrate specific binding of (+)-(3H)JD 888 to CHAPS-solubilized membranes (12). Monoclonal antibodies directed against the 170-kDa disulfide bridge-containing protein immunoprecipitated different polypeptides from CHAPS- and digitonin-solubilized skeletal muscle membranes. From CHAPS-solubilized membranes only one, a 170-kDa polypeptide, was immunoprecipitated under alkylating conditions. This was split into 140- and 30-kDa polypeptides under reducing conditions (Fig. 2 in Ref. 12). In contrast, an additional 170-kDa polypeptide, which did not change its electrophoretic mobility upon reduction, was also immunoprecipitated from digitonin-solubilized membranes. Under alkylating conditions only a single, 170-kDa polypeptide was described. However, after careful inspection of the published autoradiogram, one can identify a very diffuse smear suggestive of multiple bands (Fig. 2B in Ref. 12). The 170 to 140-kDa doublet demonstrated under reducing conditions is, in fact, very similar to the α1 and α2 doublet described in this study. However, Vandeke et al. (12) did not consider the 170- and 140-kDa polypeptides to be two separate subunits of the calcium channel. Instead, they speculated that a proteolytic cleavage of the 170-kDa, disulfide bridge-containing polypeptide can occur without the separation of the fragments. (This would be the case if a pre-existing intrachain disulfide bridge became an interchain bridge and held the proteolytic fragments together.) It was assumed that this proteolytic cleavage was complete and produced a 140- and a 30-kDa fragment in CHAPS-solubilized membranes but was only partial in digitonin-solubilized membranes and, therefore, a 170-, a 140-, and a 30-kDa fragment resulted (12). This explanation was supported by the homology in the peptide maps of the 170- and 140-kDa polypeptides obtained from digitonin-solubilized muscle (12). Photoaffinity labeling experiments to prove that the 140-kDa and/or the 30-kDa fragments bind calcium channel modulator drugs have not been done.

The photoaffinity labeling experiments presented here show that the 155 to 170-kDa polypeptide, which does not change its electrophoretic mobility upon reduction, can be photoaffinity labeled by both (+)-(3H)azidopine and [N-methyl-3H]LI 49888, but the 130 to 150-kDa and the 30-kDa polypeptides cannot. These data suggest that the 155 to 170-kDa and 130 to 150-kDa polypeptides present in purified skeletal muscle calcium channel preparations are not identical but are two distinct subunits which are termed here α1 and α2.

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