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Purified skeletal muscle 1,4-dihydropyridine receptor forms phosphorylation-dependent oligomeric calcium channels in planar bilayers

(ion channel/reconstitution/transverse tubule/cooperativity/membrane-protein association)

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ABSTRACT **The purified 1,4-dihydropyridine receptor from skeletal muscle has been incorporated into planar bilayers, and its channel characteristics have been investigated. Conductances showed the characteristics of an L-type Ca^{2+} channel: divalent cation selectivity ($P_{Ba}/P_{Na} \approx 30$), blockage of Na^+ conductance by micromolar Ca^{2+}, and blockage of the Ca^{2+} channel by D890 and by Cd^{2+}. The α_1 subunit of the receptor must be phosphorylated by the cAMP-dependent protein kinase to give channel activity. BAY K 8644 did not activate nonphosphorylated channels, and (+)-PN200-110 caused dramatic prolongation of mean open times when applied after phosphorylation. Channel properties were found to be dependent on association of receptor molecules in the bilayer. Single receptor molecules form channels of 0.9 pS (100 mM Ba^{2+}) and show no voltage-dependent gating. Upon association, both voltage-dependent gating and higher conductance events are recovered; stabilized conductance levels assume values of even multiples of 0.9 pS, predominately 7.5 and 15 pS and multiples of these values up to 60 pS. Thus, individual channels become functionally coupled (synchronous opening and closing) with association, reinstating the characteristics of one larger unitary channel. It is concluded that the L-type Ca^{2+} channel represents an oligomer of 1,4-dihydropyridine–receptor protein complexes, each of which constitutes a channel, where the array of channels (oligochannel) opens and closes in concerted action.**

Ca^{2+} channels play a central role in the physiology and pharmacology of excitable cells. The L-type Ca^{2+} channels have different classes of allosterically interacting drug-receptor sites—e.g., for 1,4-dihydropyridines (DHPs), the phenylalkylamines, and the benzothiazepines. These drug receptors have been purified from the richest mammalian source, the skeletal muscle transverse (T)-tubule membrane (1–5). Upon reconstitution of the purified Ca^{2+} antagonist receptor in planar membranes, L-type Ca^{2+} channel activity was observed (5–7). However, in intact skeletal muscle fibers, a large apparent discrepancy is observed between the number of DHP binding sites and the apparent number of T-tubule Ca^{2+} channels (8). An involvement of the receptor protein in charge movement, relevant to excitation–contraction coupling (9), has been suggested (10–12). The "feet structures," which bridge the sarcoplasmic reticulum (SR) and T-tubule membranes, are now known to be identical with the ryanodine receptor (13, 14), which recently has been shown to form the Ca^{2+}-release channel when incorporated into planar bilayers (15). The "feet structures" consist of an oligomeric associate of 12–16 single polypeptides (M_r = 360,000) (14), and the purified ryanodine receptor forms arrays of synchronized Ca^{2+} channels in the bilayer (15). We now find oligomer formation of the purified DHP receptor in planar bilayers to be essential for establishing channel properties expected for the L-type Ca^{2+} channel of T-tubule (16), suggesting a model of directly coupled SR and T-tubule Ca^{2+} channel oligomers at the triad junction. Preliminary reports have appeared (17, 18).

METHODS

Purification and Reconstitution of DHP Receptor. Highly purified T-tubule membranes were prepared as described (19). DHP receptor from guinea pig hind-limb muscle was purified as described (3). Samples were used immediately or stored at −70°C until needed, with no significant differences observed. The purified protein was incorporated into vesicles for bilayer formation by using the "fast dilution" method (20, 21): 10–50 μl of purified receptor [≈10 μg of protein per ml of buffer containing 0.1% digitonin, 0.5 M NaCl, and 50 mM Tris chloride (pH 7.4)] was rapidly diluted with bath sonication into 20 ml of a dilute suspension (≈10 μg of lipid per ml) of crude soybean phospholipid (Sigma; type II-S) in 110 mM NaCl containing 10 mM Hepes/Tris (pH 7.4; reconstitution buffer). Vesicles were prepared by swirling this suspension with glass beads over a dried film of 18 mg of acetone-washed soybean phospholipid and 3 mg of cholesterol. Pure lipid vesicles were prepared by the same method.

Planar Bilayer Formation and Channel Analysis. Bilayers were derived from the vesicle suspensions at room temperature by the SVB technique (*s*eptum-supported, *v*esicle-derived *b*ilayer) (21, 22). The number of DHP receptor binding sites per bilayer $N_{s,b}$, (usually 100) was estimated (21) from the relation $N_{s,b} = N_{l,b}/(N_{l,v}/N_{s,v})$; $N_{l,v}/N_{s,v}$ is the number of lipid molecules per DHP binding site in the vesicle suspension (adjustable by dilution with lipid vesicles), and $N_{l,b} \approx \pi d^2/4A_l$ in which d is the bilayer diameter (70-, 100-, or 160-μm apertures were used) and A_l is the area per lipid (A_l = 65 Å^2). $N_{s,b}$ can be estimated in this way because the purified receptors are monodispersed and expected to distribute randomly in the reconstituted vesicles [$N_{l,v}/N_{s,v} \geq 10^6$ (i.e., much less than one DHP site per vesicle)] and resulting bilayers ("initial distribution assay"; refs. 20, 21, and 23). Recovery of channel activity is estimated from the ratio $G_{max}/g_l N_{s,b}$, where G_{max} is the maximum conductance and g_l is the single-channel conductance (0.9 pS in this study).

After member formation, $BaCl_2$ was added to give (in addition to reconstitution buffer) 100 mM on the cis side of

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Abbreviations: DHP, dihydropyridine; SR, sarcoplasmic reticulum; T-tubule, transverse tubule; *I–V*, current–voltage; $N_{s,b}$, number of DHP sites per bilayer; $N_{l,b}$, number of lipid molecules per bilayer; $N_{s,v}$, number of DHP sites per vesicle; $N_{l,v}$, number of lipid molecules per vesicle.

the bilayer only (medium A) or on both cis and trans sides (medium B). Receptor phosphorylation was performed either (*i*) with T-tubule vesicles or reconstituted vesicles or (*ii*) directly in the bilayer cell (cis side) after bilayer formation in medium A containing 5 μM BAY K 8644, 1 mM $MgCl_2$, 1 mM adenosine (5′-O^3)-3-thiotriphosphate, and 250 units of the catalytic subunit of the cAMP-dependent protein kinase per ml. The potential difference across the bilayer was controlled by a voltage clamp circuit with the solution on the trans side maintained at ground by an operational amplifier with a 5-GΩ feedback resistor (21). Throughout this paper, voltage is expressed as the voltage applied to the cis side. Conductance scales were calculated by assuming an ohmic current–voltage (*I–V*) relation. The output signal was filtered at 3 kHz, digitized, and stored on tape. A B-Scope computer system (Med-Natic, Munich) was used for data analysis. The resolution product, time resolution × amplitude resolution, was 3×10^{-15} A·sec for 160-μm apertures and 1×10^{-15} A·sec for 100-μm apertures.

RESULTS

Purified DHP receptors induced Ca^{2+}- or Ba^{2+}-selective conductances when incorporated into vesicle-derived planar bilayers. In medium A, the reversal potential was -56 ± 8 mV ($n = 7$), which corresponds to P_{Ba}/P_{Na} of 30 when using a constant field equation (24). Conditions were such that a certain number of receptor sites (usually 100) were expected to be incorporated and randomly distributed in the plane of the bilayer.

Among the earliest conductances seen in the course of an experiment were surprisingly small events of only 0.9 pS (Fig. 1*A*, trace 1). This value is far lower than that reported for T-tubule Ca^{2+} channels inserted into planar bilayers—either ≈10 pS (25) or ≈20 pS (16). However, beginning several minutes after bilayer formation, events of increasingly higher conductance were observed. Fig. 1*A* shows representative examples for this development. Transitions within the burst-like events tended to increase in rate (flickering) with higher conductance levels and usually occurred among several not clearly resolvable substates (trace 3). Occasionally the activity stabilized to homogeneous bursts of two-state transitions (trace 4). Among the more stabilized events, even multiples of the smallest level (0.9 pS) were observed, such as 3.5, 7, and 15 pS (traces 2–4). Amplitude histograms of events with highly stabilized levels showed jumps of 4, 8, or often 16 times the elementary conductance of 0.9 pS (see effect of (+)-PN200-110, Fig. 3*D*).

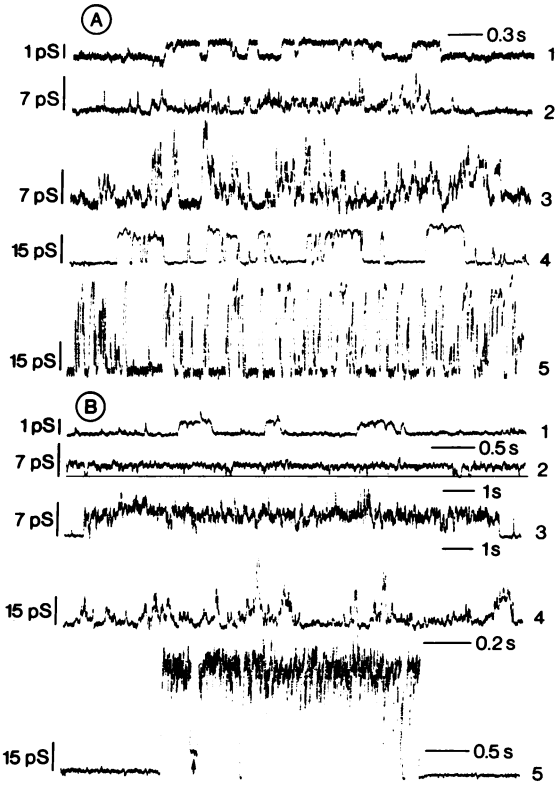

FIG. 1. Single-channel conductance states of purified DHP receptor (*A*) and T-tubule membranes (*B*) in planar bilayers. T-tubule vesicles were phoshorylated and diluted with lipid vesicles to give 100 DHP binding sites per bilayer in medium A. Reconstitution of purified receptor was in medium B. Holding potentials (mV) were 50 (A5), 100 (B4 and A2-4), 150 (B2, 3, 5, and A1), and 250 (B1).

The amount of activity at steady state increased with increasing number of receptor sites per bilayer ($N_{s,b}$), and the time to reach steady-state activity decreased with increasing $N_{s,b}$ (not shown). However, there was a large scatter of activity from sample to sample, some showing scarce or no activity. On the average, only a few percent of the expected channel activity was recovered, and considerable decay of activity occurred within a few hours of preparation. Similarly, sparse activity was observed from reconstituted T-tubule vesicles in planar bilayers. However, activity increased dramatically after phosphorylation by the catalytic subunit of the cAMP-dependent protein kinase (Fig. 1*B*). While small conductances were occasionally observed (traces 1 and 2), the majority of conductance levels fell into the 7- to 15-pS range (traces 3 and 4). All but the smallest events showed considerable fluctuation in the open state (traces 3 and 5) or a mixture of conductance states (trace 4). Clearly defined stable substates also were observed (see the arrow in trace 5; less clearly defined substates are observable in traces 3 and 4). Therefore, we tested the effects of phosphorylation on the purified receptor.

The purified DHP receptor used for these studies showed a simple, characteristic subunit composition (Fig. 2*A*). Phosphorylation of the preparation with the cAMP-dependent

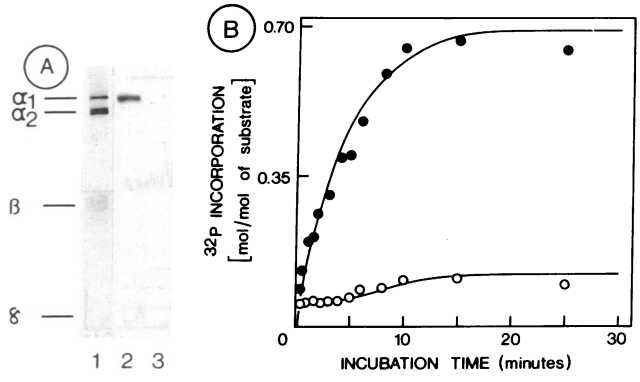

FIG. 2. Phosphorylation of the purified DHP receptor. (*A*) Lanes: 1, silver stain of the purified Ca^{2+} antagonist receptor preparation [1300 pmol of tritiated (+)-PN200-110 binding sites per mg of protein; 1 μg of protein was separated under reducing conditions (27)]; 2, fluorogram of the tritiated (−)-azidopine photoaffinity-labeled purified DHP receptor preparation; 3, same as lane 2 but with 1 μM (+)-PN200-110 present. (*B*) Time course of the phosphorylation of the purified DHP receptor preparation with the catalytic subunit of the cAMP-dependent protein kinase. Phosphorylation of 4.5–6 nM solubilized DHP receptor was at 30°C in 50 mM Tris chloride, pH 7.4/6 mM $MgCl_2$/6 mM EGTA at 3 μM [γ-^{32}P]ATP (5550 dpm/pmol)/500 units of catalytic subunit (Sigma; P2645) per ml. The reaction was terminated after the indicated times by transfer of 50-μl aliquots into boiling sample buffer for gel electrophoresis. Silver-stained α_1 (●) and α_2 (○) bands were cut out from the gel (3), and ^{32}P was quantitated by scintillation counting.

protein kinase revealed that the phosphorylation site is exclusively on the α_1 polypeptide (M_r = 170,000 in both reducing and nonreducing gels), which is almost stoichiometrically phosphorylated. The same subunit contains the binding sites for DHPs and phenylalkylamines, as clearly shown with photoaffinity probes (4, 26, 27).

Phosphorylation of the purified DHP receptor in the bilayer induced a high degree of activation for every preparation tried (n = 4) and at every attempt (n = 12), with recovery values of at least 50%. An example is shown in Fig. 3. Phosphorylation increased the time-averaged Ba^{2+} current of the membrane from a basal value of 0.34 pA (Fig. 3*A*) to 5.91 pA (Fig. 3*B*), an increase of 17.4-fold, within 1 min of adding the protein kinase. The major effect of phosphorylation was a dramatic increase in the number of channels observed, rather than a change in channel characteristics (compare Fig. 3*C* with trace 3 in Fig. 1*A*). There may be some quantitative effects on the mean open time that are difficult to assess in view of the complexity of the activity.

The effects of DHPs on Ca^{2+} channel activity were also investigated. When added to a bilayer containing receptors that had not been previously phosphorylated, BAY K 8644 alone was unable to stimulate activity (Fig. 3*A*). Moreover, phosphorylation in either the presence or absence at the cis side of 5 μM BAY K 8644 yielded no dramatic differences (not shown). Channel activity detected without prior phosphorylation was not inhibited by addition at the cis side of 10 μM (+)-PN200-110, a potent and specific calcium antagonist (not shown). However, when (+)-PN200-110 was added to a bilayer containing phosphorylated channels, it produced a dramatic morphogenesis of channel activity after about 10 min (Fig. 3*D Left*), leading to the appearance of an "S" or sublevel gating mode characterized by long, stabilized open states (range of seconds). Interestingly, the conductance jump heights in the S mode were usually multiples of 4, 8, or 16 times the smallest observed conductance, 0.9 pS (Fig. 3*D Right*). This mode of the channel was stable and continued for the life of the membrane.

The combined effects of phosphorylation and (+)-PN200-110 enabled a more reproducible study of the reconstituted

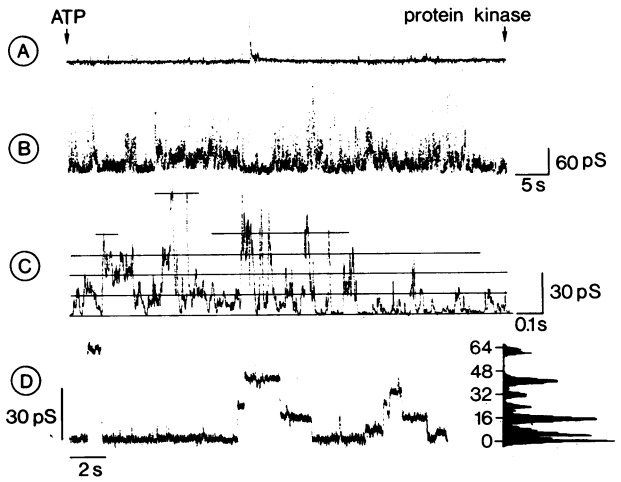

FIG. 3. Channel activation by phosphorylation (*A–C*) and effect of (+)-PN200-110 (*D*) on 100 DHP sites per bilayer. (*A*) Activity without prephosphorylation (1 mM adenosine (5′-O^3)-3-thiotriphosphate, 1 mM $MgCl_2$, and 5 μM BAY K 8644 were already present). (*B*) Activity 1 min after phosphorylation was initiated by addition of 250 units of catalytic subunit per ml. (*C*) Trace in B with expanded time scale. The experiment was in medium A at 0-mV holding potential. The reversal potential was −55 mV. Integrated channel currents were 27.9 pA·sec (*A*) and 381.2 pA·sec (*B* and *C*). (*D Left*) Trace with the same membrane as in *A–C* 10 min after addition of 10 μM (+)-PN200-110. (*D Right*) Amplitude histogram in units of 0.9 pS.

Ca^{2+} channel, especially of the changes in channel characteristics with time after membrane formation (t = 0). One example is shown in Fig. 4*A–C*, with 100 receptor molecules ($N_{s,b}$ = 100) initially distributed at random in the bilayer plane. Shortly after bilayer formation (Fig. 4*A Upper*), a noisy steady-state conductance was observed. The noise had a Gaussian distribution about a mean conductance ($\bar{G}$) of 23 pS with a standard deviation of 4.5 pS, corresponding to a variance σ^2 = 18 pS^2, when corrected for the baseline value (σ^2 baseline = 2 pS^2). This noise fulfills the relation $g_1 = \sigma^2/G + G/N_{s,b}$ for g_1 = 1.0 pS. The agreement between this value and the observed 0.9-pS smallest channel-conductance level (Fig. 1*A*, trace 1) indicates that the noise originates from independent activity of, on the average, about 20 such channels. Since the receptor molecules are randomly distributed, we assign the 0.9-pS channel to a single DHP receptor molecule. Most interestingly, this elementary channel seems not to be voltage-gated because the *I–V* relation of the ensemble of channels was approximately linear (see Fig. 4*A* and legend for details).

In the course of time, the noise of the trace in Fig. 4*A Upper Left* disappeared, and larger "single channel" events appeared instead, as exemplified by the trace in Fig. 4*B Upper*, taken at t = 10 min. The corresponding *I–V* curve (Fig. 4*B Lower*) is clearly nonlinear. Steady-state conductance at $t >$ 15 min was characterized by overlapping events with amplitude similar to that in Fig. 3*D* (measured under the same conditions). During voltage ramps, prominent levels could be

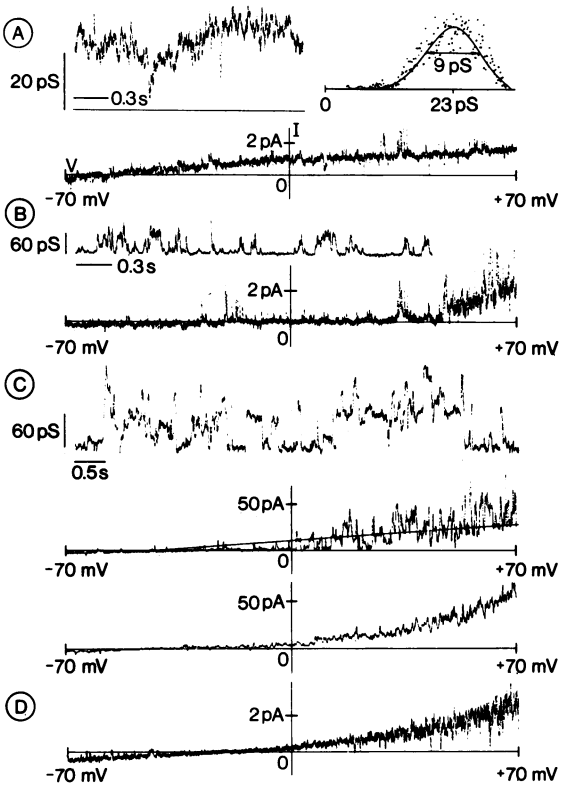

FIG. 4. Conductance and *I–V* relation of the purified DHP receptor channel as a function of time after bilayer formation (t = 0) with 100 DHP binding sites. (*A Upper*) Conductance trace at t = 2.0 min and its amplitude histogram. (*A Lower*) Average response to three voltage ramps taken just after the trace. (*B Upper*) Conductance trace at t = 12.7 min. (*B Lower*) Average response to three ramps taken just after the trace. (*C Top*) Conductance trace at t = 20.0 min. (*C Middle*) Response to a single voltage ramp. (*C Bottom*) Average response to eight ramps recorded at t = 22.0–24.4 min. The voltage ramp speed in *A–C* was 8 mV/sec. Holding potentials were +50 mV (*A* and *B*) and +70 mV (*C*). (*D*) *I–V* relation for bilayer-incorporated phosphorylated T-tubule membranes (voltage ramp speed = 12 mV/sec).

traced linearly down to the reversal potential, as indicated in the *I–V* curve in Fig. 4*C Middle*. The *I–V* curve in Fig. 4*C Bottom* represents the average response to eight consecutive voltage ramps. Its nonlinear shape reflects voltage-dependent gating (i.e., voltage dependence of open-state probability). Remarkable agreement was found when these *I–V* characteristics were compared with those obtained from T-tubule membrane vesicles under similar conditions (Fig. 4*D*). While voltage-dependent gating developed, the reversal potential remained unchanged within experimental accuracy (cf. Fig. 4*A* and *C*) at about −50 to −60 mV.

The DHP receptor/Ca^{2+} channel would be expected to exhibit sensitivity to known modulators of L-type Ca^{2+} channels. Fig. 5*A* shows that the membrane-impermeable phenylalkylamine D890 (5 μM cis side) is capable of fully inhibiting the macroscopic current of a membrane containing phosphorylated Ca^{2+} channels in the presence of 5 μM BAY K 8644. A further property of the L-type Ca^{2+} channel (and perhaps of all Ca^{2+} channels) is its ability to conduct monovalent cations in the absence of Ca^{2+} and the blockage of monovalent cation current by low concentrations of Ca^{2+} (28). This relates to the proposed permeation mechanism of the channel, which is thought to possess two high-affinity binding sites for Ca^{2+} (29, 30). This effect could be demonstrated for the purified skeletal muscle DHP receptor (Fig. 5*B*). Another characteristic of specifically L-type Ca^{2+} channels is their high sensitivity to blockage by Cd^{2+} in the micromolar range (30). This is shown in Fig. 5*C*.

DISCUSSION

We have studied the purified skeletal muscle DHP receptor in planar bilayers at a defined low density of receptor sites (about one DHP binding site per 100 μm^2) using a random initial distribution, which is enabled by the vesicle-derived bilayer technique (21, 22). Just after bilayer formation, when the receptor molecules are expected to be singly distributed over the bilayer plane, clearly resolvable 0.9-pS channel events were observed. Noise analysis under highly activating conditions revealed that this value is representative for the entire observed ensemble of single dispersed DHP receptors. From this we conclude that the channel constituted by the monomeric DHP receptor has a conductance of 0.9 pS in 100 mM $BaCl_2$. The channel is divalent-cation selective ($P_{Ba}/P_{Na} \approx 30$), and its open-channel conductance as well as its open-state probability is approximately independent of voltage. For further discussion we shall refer to this channel as the "monochannel" with conductance g_1.

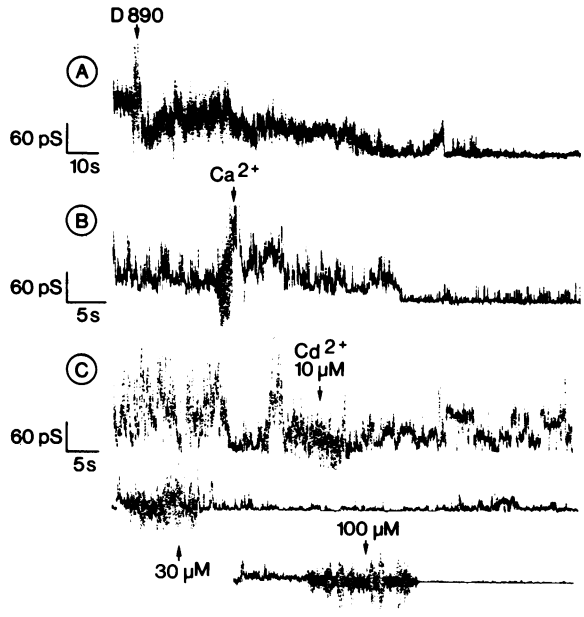

FIG. 5. Block of purified DHP receptor channels by phenylalkylamines and divalent cations. (*A*) Effect of 5 μM D890 (on the cis side of the bilayer) at 100 binding sites per bilayer in medium A plus 5 μM BAY K 8644 (cis side). The receptor was phosphorylated from the cis side 22 min before D890 addition. (*B*) Effect of Ca^{2+} (100 μM $CaCl_2$) on Na^{2+} conductance in buffer containing (cis and trans sides) 100 mM NaCl and 10 mM Hepes/Tris (pH 7.4). (*C*) Effect of cis-side $CdCl_2$ addition in medium B (noise during addition is from stirring). Holding potentials were +50 mV (*A* and *B*) and +100 mV (*C*).

The low conductance value and voltage independence appear inconsistent with the growing evidence that DHP receptors form "L-type" Ca^{2+} channels with a conductance in the range of 8–24 pS—for example ≈10 pS (25), ≈12 pS (37), ≈10 and ≈20 pS (5, 6, 16, 32, 33), and 8 and 15 pS (34), most showing pronounced voltage dependence of open-state probability. However, with increasing time after membrane formation, higher conductance levels appeared as single events (hereafter termed "oligochannels") at the expense of monochannel events. Resolvable conductance values of oligochannels often corresponded to even integer multiples of the monochannel conductance (g_1), predominantly 4, 8, 16, and multiples of 16 up to 64). These values more than cover the range of reported conductances, and the often-seen 7.5- and 15-pS events match the values found in smooth muscle cells (34). Most remarkably, oligochannels showed marked voltage dependence in contrast to monochannels, closely matching the expectation from incorporated T-tubule membranes, whereas Ba^{2+} selectivity remained unchanged during the transition from monomeric to oligomeric channels. Other evidence supports the view that the oligochannels correspond to the physiologically observed Ca^{2+} channels. (*i*) They demonstrated a high Na^+ conductivity that was blocked by low Ca^{2+}. (*ii*) All conductance levels were completely blocked by D890 in the expected concentration range (35). Flockerzi and co-workers (5, 6) reported a similar block only for 20-pS events but not for other conductance levels, whereas Smith *et al.* (7) found modulation of 12- to 14-pS channels by DHPs. (*iii*) The channels were blocked by Cd^{2+} in the expected concentration range (30). (*iv*) Finally, the channels responded to phosphorylation by the cAMP-dependent protein kinase.

Phosphorylation was required to activate channels to a high recovery value (>50% on the basis of 0.9 pS per DHP binding site). This statement applies to all conductance states. Basal activities observed without prephosphorylation were generally low and highly variable and thus can be attributed to a residual fraction of phosphorylated receptors after purification. This finding is in accord with the recent report by Armstrong and Eckert (36), who showed that L-type Ca^{2+} channels in GH_3 cells must be phosphorylated to be active. In the same study, it was proposed that BAY K 8644 stabilizes the phosphorylated state of the channel against dephosphorylation, shifting the equilibrium *in vivo* to more active channels. We also find no direct channel activation by BAY K 8644 alone without prephosphorylation. Our unexpected finding that (+)-PN200-110 does not block the channel at 10 μM but stabilizes conductance fluctuations in oligochannel events may also relate to phosphorylation if, for example, the effect of (+)-PN200-110 *in vivo* was to facilitate dephosphorylation or block phosphorylation. Since the phosphorylation and DHP binding sites are located on the α_1 subunit of the channel (refs. 4, 5, and 11 and this paper), it is clear from the strong dependence on phosphorylation for activity that the α_1 subunit is a necessary and constituent part of the channel-forming molecular complex.

The change in conductance characteristics with time is likely due to the formation of stable receptor oligomers, whereby the protomeric channels become functionally cou-

pled. Oligomeric associates are formed by collisions (resulting from lateral diffusion) starting from randomly distributed receptor molecules. This is inferred from three lines of evidence. (*i*) The time for the functional change to occur was dependent on receptor density (for 1000 sites per bilayer oligochannels were observed immediately) (*ii*) The increase in conductance yields integer multiples of g_1. (*iii*) Monomeric and oligomeric channels show the same selectivity of Ba^{2+} over Na^+. Although a rigorous conclusion would require more quantitative studies (relationship between association time and receptor density; statistical analysis of sublevels), we believe the present qualitative level of evidence suffices to rule out the other logical possibility—namely, that all functional properties are manifested in single receptor molecules. Rather, it appears that ensemble properties (channel association) lead to functional coupling—i.e., concerted opening and closing, as well as voltage dependence of gating.

The degree of channel coupling in oligochannels varied. Complete coupling (all-or-none cooperativity), giving the appearance of a single channel (see trace 1 in Fig. 4), was less frequently observed than incomplete coupling (graded cooperatively; I see trace 3 in Fig. 1*A*). This variation in the degree of coupling may relate to certain constraints on organization and number of protomers needed in an oligomer for strict coupling to take effect. During association from single proteins, such particularly ordered structures may occur with low probability or may be formed by slow rearrangements. Alternatively, an additional component or anchorage at the membrane surface may be required. It is to be expected that channel associate topology is lost in whole or in part during membrane dissociation and channel purification. In terms of incomplete coupling, "flickering" might correspond to fast variation in the degree of channel coupling, and the resolved substates might correspond to occasional resting periods.

In summary, reconstitution from single DHP receptor molecules yields phosphorylation-dependent channels with essentially the characteristics of the L-type Ca^{2+} channel, provided that the receptor proteins occur in oligomeric association. Consequently, we conclude that the L-type Ca^{2+} channels observed in patch clamp studies represent oligomeric channels with strict functional coupling among associated monochannels. The new aspect of the present study in this respect lies in the effort to reestablish oligochannels from purified single DHP receptor proteins, which at present results in less complete channel coupling than prevails in the native membrane. Interestingly, time-correlated transitions between several sublevels, which can be taken to indicate incomplete coupling in a oligochannel complex, have been observed for L-type calcium channels expressed in oocytes (37).

Oligochannels have, necessarily, a large number of DHP binding sites. This is consistent with the as-yet-unexplained findings of 35–50 times more DHP binding sites than 20-pS Ca^{2+} channels in intact skeletal muscle fibers (8). Existence of multiple binding sites infers a complex relationship between occupancy of binding sites and channel activity, which is still to be investigated. Identification of the T-tubule Ca^{2+} channel as an oligochannel compliments a recent finding (15) that the sarcoplasmic reticulum Ca^{2+}-release channel is also an oligochannel showing a similar sequence of even-integer multiples of a monochannel conductance. The structure of the ryanodine receptor/Ca^{2+}-release channel is consistent with a tetragonal oligomer of ≈16 high molecular weight polypeptides (M_r = 360,000) (14), identical to the "feet structures" that bridge the T-tubule and SR membranes (38). This invites the speculation that the anchorage of the "foot structure" to the T-tubule membrane is actually to the DHP receptor, with coalignment of the two different oligomeric channels in tetragonal arrays of 16 channels each, constituting the machinery of excitation–contraction coupling.

Finally, it should be added that two other channels, Omp F porin (23) and acetylcholine receptor (20), have shown channel cooperativity upon protein reassociation. There is also increasing evidence from patch-clamp studies of diverse channel types for equally spaced sublevels in channel events with conservation of ion selectivity (recently reviewed in ref. 39), which indicates that the oligochannel concept may have general applicability, offering a genuine challenge for further reconstitution studies using initial distribution assays.

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