Biochimica et Biophysica Acta 863 (1986) 205–212
Elsevier

BBA 73167

Rapid incorporation of the solubilized dihydropyridine receptor into phospholipid vesicles

William A. Horne, Gregory A. Weiland, Robert E. Oswald and Richard A. Cerione

Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (U.S.A.)

(Received 23 June 1986)

Key words: Dihydropyridine receptor; Reconstitution; Solubilization; Ca^{2+} antagonist; Phospholipid vesicle

We describe the rapid incorporation of the CHAPS solubilized dihydropyridine receptor into phospholipid vesicles. A series of sucrose gradient sedimentation experiments demonstrate that the (+)-[^{3}H]PN200-110-labeled dihydropyridine receptor is associated with lipid vesicles following detergent removal by Extracti-gel chromatography. Solubilization of the receptor results in a loss of (+)-[^{3}H]PN200-110 binding affinity relative to that observed in native membranes; the high affinity binding of (+)-[^{3}H]PN200-110 can be restored upon reincorporation of the receptor into phospholipid vesicles. Similarly, the incorporation of the receptor restores its stability to incubation at 37°C relative to that of the detergent solubilized receptor, thereby mimicking the properties of the membrane bound form of the receptor. The dissociation rate of (+)-[^{3}H]PN200-110 from the reconstituted receptor is shown to be allosterically regulated by verapamil and diltiazem, indicating that the binding sites for these calcium antagonists have been inserted along with the dihydropyridine receptor into phospholipid vesicles. The results presented in this report, thus demonstrate the successful reconstitution of the dihydropyridine receptor into phospholipid vesicles by a variety of criteria. The reconstitution method described here is rapid and efficient, and should now facilitate structure-function studies of this receptor and its interrelationships with other regulatory components of the voltage-sensitive calcium channel system.

Introduction

An increasing amount of effort is currently being devoted toward understanding the mechanism of action of voltage sensitive calcium channels because of their involvement in the regulation of a number of important cellular functions, including neurotransmitter release, hormone secretion, and excitation-contraction coupling [1]. Structure-function studies of these channels have only recently become possible with the discovery that a number of different compounds can directly influence calcium fluxes by binding to specific receptor sites associated with the calcium channel [2]. Dihydropyridine derivatives comprise one such group of compounds which bind to a receptor site thought to be an integral component of the calcium channel and thereby act as either antagonists (nitrendipine, PN200-110) or agonists (Bay K 8644) in regulating calcium channel function [3]. The mechanism of binding of the dihydropyridines has recently been studied in detail [4]. Other compounds known to be calcium antagonists have been shown to either enhance (diltiazem) or in-

Correspondence: Dr. W.A. Horne, Department of Pharmacology, NYSCVM, Cornell University, Ithaca, NY 14853, U.S.A.

hibit (verapamil) dihydropyridine binding through allosteric interaction from distinct receptor sites also thought to be integrally associated with the calcium channel [5].

The dihydropyridine receptor has recently been solubilized and partially purified from heart [6,7] and skeletal muscle [8,9]. Radiation inactivation studies have suggested a size range of 180000–280000 for the membrane bound receptor [10], while hydrodynamic studies of the solubilized receptor have yielded a molecular weight of 370000 [6]. The receptor appears to be comprised of at least three polypeptide components; these being designated as α ($M_r = 145000–170000$), β ($M_r = 30000–50000$), and γ ($M_r = 30000$) [8]. Photoaffinity labeling has demonstrated that the binding site for dihydropyridines, as well as the site for verapamil and diltiazem, are associated with the large molecular weight subunit [11]. The 50 kDa component appears to be the site of cyclic AMP-dependent protein kinase phosphorylation [12].

The molecular details regarding the mechanisms of regulation of voltage-sensitive calcium channels by pharmacological agents, as well as by other agents (hormones, kinases), remain to be elucidated. It has been shown electrophysiologically that β-adrenergic agonists increase the probability that calcium channels will open at a given membrane potential [13]. This most likely reflects a direct phosphorylation of the channel by the cyclic AMP-dependent protein kinase, which is activated by the cyclic AMP generated from β-adrenergic stimulation of adenylate cyclase. Recently, noradrenaline and γ-aminoisobutyric acid have been shown to inhibit calcium channel function in dorsal root ganglion cells [14]. This inhibition appears to be independent of cyclic AMP levels and thus may reflect either receptor-mediated activation of another signal pathway (such as that regulating protein kinase C activity [15]), or a more direct effect by these receptors on the channel itself via the inhibitory GTP binding protein, G_i, or some G_i-like protein (i.e., G_o [16,17]). A thorough understanding of the interrelationships between these different regulatory pathways and calcium channel function will require the isolation and functional reconstitution of these different components in a lipid milieu. As an initial step toward the development of such a system, we describe here the successful incorporation of the dihydropyridine receptor into phospholipid vesicles.

Experimental Procedures

Materials. (+)-[^{3}H]PN200-110 (spec. act. 76.0 Ci/mmol) was obtained from New England Nuclear. [^{3}H]Phosphatidylcholine (spec. act. 60 Ci/mmol) was a gift from Dr. D.B. Zilversmit (Cornell University, Ithaca, NY). Nitrendipine was supplied by Miles Institute for Preclinical Pharmacology (New Haven, CT), diltiazem from Marion Laboratories (Kansas City, MO), and sucrose from Bethesda Research Laboratories (Bethesda, MD). Extracti-gel D was obtained from Pierce Chemical Company (Rockford, IL). All other materials were obtained from Sigma.

Preparation of transverse-tubule membranes. Male New Zealand rabbits (2.0–2.5 kg) were euthanized by intravenous injection of sodium pentobarbital. Microsomes of white skeletal muscle from the hind limbs and lumbar region were prepared as described by Fernandez et al. [18]. Transverse tubules were purified according to the procedure of Rosemblatt et al. [19]. Purified membranes were suspended in 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) and stored in liquid nitrogen for later use.

Preparation of soluble dihydropyridine receptors. For those experiments requiring prelabeled receptor, membranes (1.0 mg of protein/ml) were incubated for 90 min at 20°C in 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) with 3.0 nM (+)-[^{3}H]PN200-110 and 100 μM diltiazem in borosilicate glass test tubes. Nonspecific binding was determined by labeling the membranes in the presence of 1 μM nitrendipine. Incubation was performed under a sodium vapor light to avoid breakdown of the dihydropyridines.

Membranes, labeled or unlabeled, were washed once with 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) (100000 × g for 20 min). The pellet was resuspended in 1.0% (w/v) CHAPS in 150 mM KCl, 1 mM $CaCl_2$, 1 mM PMSF, 25 mM Hepes-NaOH (pH 7.4), to a final detergent/protein ratio of 10 : 1. The suspension was agitated for 5 min at 24°C then allowed to stand at 4°C for 25 min without exposure to light. The suspen-

sion was then centrifuged for 1 h at 100 000 × *g* and the supernatant containing solubilized material was used in subsequent studies.

Incorporation of dihydropyridine receptors into phospholipid vesicles. Soybean phosphatidylcholine (100 μl of a 20 mg/ml sonicated solution) was added to 400 μl soluble receptor in 150 mM KCl, 1 mM $CaCl_2$, 1 mM PMSF, 25 mM Hepes-NaOH (pH 7.4) containing 1.0% CHAPS, and the mixture was incubated on ice for 2 min. Fifty μl of 100 mM $MgSO_4$ was then added, and the total mixture (0.55 ml) was kept on ice for 30 min. Following incubation, the mixture was applied to an Extracti-gel column (1 ml) at 4°C which was pretreated with 4 volumes of 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) containing 2 mg/ml of bovine serum albumin and then equilibrated with 2 volumes of 150 mM KCl, 1 mM $CaCl_2$, 10 mM $MgSO_4$, 1 mM PMSF, 25 mM Hepes-NaOH (pH 7.4). Elution of the protein-lipid fractions was performed using 1.5 ml of the latter buffer. Typically, eluates from three Extracti-gel columns were combined for binding or sedimentation experiments.

Density gradient centrifugation. Linear 32-ml sucrose gradients (10–25%) in H_2O containing 150 mM KCl, 1 mM $CaCl_2$, 100 μM diltiazem, 1 mM PMSF, 25 mM Hepes-NaOH (pH 7.4) were prepared in 2.5 × 8.9 cm Beckman polyallomer Quick-Seal centrifuge tubes. In addition, the gradients contained either 1.0% CHAPS or 10 mM $MgSO_4$. Four ml of either solubilized material or lipid vesicles containing 50 μl of sucrose (1.0 mg/ml) were carefully layered on top of a gradient. An additional 4 ml of buffer was layered on top of the sample and the tubes were heat sealed. The gradients were centrifuged at 200 000 × *g* in a Beckman VTi50 vertical rotor for 2 h at 4–6°C. The tubes were drained from the bottom and 0.6 ml fractions were collected. In experiments where either the dihydropyridine receptor or lipid vesicles had been prelabeled, fractions were assayed for radioactivity. In other experiments, only lipid fractions (which formed an obvious band at the top of the gradient) were collected for subsequent binding studies.

(+)-[^{3}H]PN200-110 binding assays. Assay of membrane bound and reconstituted dihydropyridine receptors was performed as previously described [4] with only slight modification. Membranes (0.09 mg protein/ml) or vesicles (0.25 mg protein/ml) were incubated at 8°C for 90 min in 150 mM KCl, 10 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) with (+)-[^{3}H[PN200-110 (0.1–3.0 nM) in a total volume of 0.5 ml and 0.25 ml, respectively, in borosilicate glass test tubes. Nonspecific binding was determined by the inclusion of 1 μM unlabeled nitrendipine in the reaction mixture over the same concentration range of (+)-[^{3}H]PN200-110. In the case of membranes, the reaction was terminated by dilution of the reaction mixture with 4 ml of 150 mM KCl, 10 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4 at 8°C) and rapid filtration through Whatman GF/B filters. The filters were washed twice with 4 ml of the same buffer. In the case of vesicles, 0.25 ml of 20% (w/v) PEG 8000 in 150 mM KCl, 10 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) was added, and the reaction mixture was kept on ice for 20 min. The mixture was then diluted with 4 ml 10% (w/v) PEG 8000 in 150 mM KCl, 10 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) and filtered through Whatman GF/F filters. The filters were washed twice with 4 ml of the same buffer.

Assay of soluble receptor was performed as previously described [6] with only slight modification. Aliquots (0.25 ml) of solubilized material were incubated with 75 μl of γ-globulin (10 mg/ml) and 0.3 ml 20% (w/v) PEG 8000 in 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) on ice for 20 min. Samples were then filtered through Whatman GF/C filters. The filters were washed three times with 4 ml of 10% (w/v) PEG in 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4).

In each case, the filters were allowed to dry, and the amount of radioactivity remaining on the filters was determined using a Xylene-based fluor (ACS, Amersham) in a Beckman liquid scintillation spectrometer at an efficiency of approx. 38%. Specific binding was determined by subtracting nonspecific binding from total binding. Nonspecific binding was determined by the amount of radioactivity retained on the filter when unlabeled nitrendipine was included in the reaction mixture, while total binding was that measured in the absence of unlabeled nitrendipine. Equilibrium bind-

ing isotherms were analyzed by a nonlinear least-squares fitting routine performed on a Micro Vax I computer.

Results and Discussion

Solubilization and incorporation of the dihydropyridine receptor into phospholipid vesicles

The primary aim of this work was to insert the solubilized dihydropyridine receptor into phospholipid vesicles using procedures which could accomodate other important regulatory proteins of the calcium channel system, including adrenergic receptors, GTP binding proteins, and various kinases. The receptor preparation used in these studies represent solubilized fractions from purified T-tubule membranes (15 pmol of (+)-[^{3}H]PN200-110 binding sites per mg of protein) which were obtained following the incubation of the membranes with 1.0% CHAPS. This treatment generally resulted in 80% solubilization of total membrane protein, and 20% recovery of (+)-[^{3}H]PN200-110 binding sites (3.0 pM/mg protein). As described in a previous report [20], a 2- to 3-fold increase in the recovery of (+)-[^{3}H]PN200-110 binding sites could be achieved by the addition of 10% glycerol to the solubilization mixture.

The first step in the reconstitution protocol was to incubate the solubilized receptor (typically 3 pmoles in 1.0% CHAPS) with soybean phosphatidylcholine (1.7 mg) for a brief period of time ($\leq$ 30 min) at 4°C. This mixture was then subjected to detergent removal by Extracti-gel D chromatography using approaches similar to those developed for the functional insertion of adrenergic receptors and GTP binding proteins coupled to adenylate cyclase [21,22]. The lipid vesicles, which in effect are re-formed as a result of the detergent removal step, can be visualized by electron microscopy (Fig. 1) and appear to be similar in size distribution (500–1000 Å) to those formed using other types of detergent removal procedures [23]. Inclusion of 10 mM $MgSO_4$ is required both in the initial incubation and during the elution from the Extracti-gel column in order to obtain optimal insertion of the receptor. This most likely reflects the ability of the divalent cation to catalyze vesicle reformation during the detergent removal step [24]. The recoveries of dihydropyridine receptor activity from the Extracti-gel column range from 25–50% and are quite similar to those obtained for a wide range of receptors and GTP binding proteins using a similar approach [21,22].

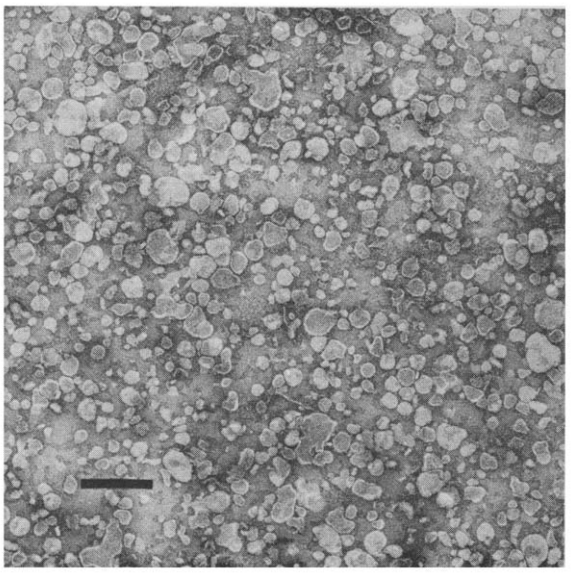

Fig. 1. Electron micrograph of negatively stained vesicles containing reconstituted dihydropyridine receptors. Vesicles can be seen to range in size from 500 to 1000 Å. Magnification, 47250×. The bar indicates 200 nm. Electron microscopy was performed by John Telford, Cornell University.

Sucrose gradient sedimentation of the soluble and reconstituted dihydropyridine receptor

A series of sucrose gradient sedimentation experiments were performed in order to assess the effectiveness of the above described insertion of the dihydropyridine receptor into lipid vesicles. These results are summarized in Fig. 2. Specifically, the gradient profile shown in panel 2A demonstrates that the soluble dihydropyridine receptor, prelabeled with (+)-[^{3}H]PN200-110, sediments as a single peak of radioactivity (fraction 28) in gradients prepared in 1.0% CHAPS. Panel 2B shows that when the same experiment is performed in gradients without detergent, the prelabeled receptor aggregates and sediments to the bottom of the centrifuge tube. (Note that the symbols simply serve to identify each line and that the line itself reflects data points taken from each fraction). In either instance, a peak of micelle-as-

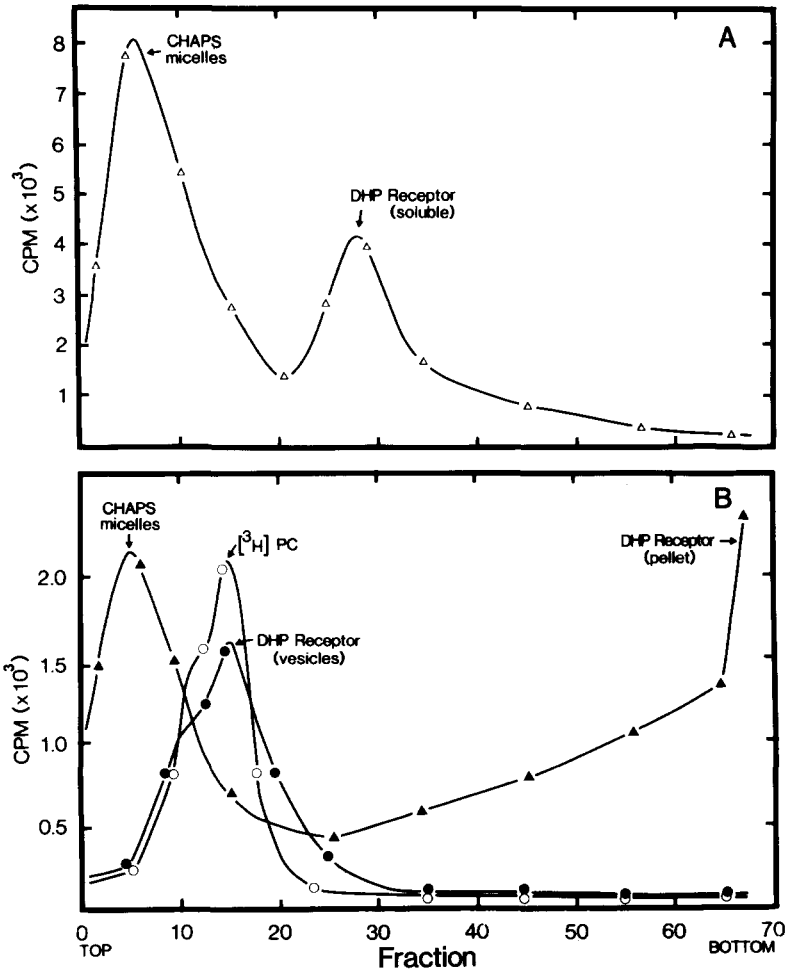

Fig. 2. Sucrose gradient sedimentation of the soluble and reconstituted dihydropyridine receptor. 32-ml linear (10–25%) sucrose/H_2O density gradients containing 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4), and either 1.0% CHAPS (A) or 10 mM $MgSO_4$ (B) were prepared as described. Samples were sedimented for 2 h at 200000 × *g* in a Beckman VTi-50 vertical rotor at 4.0 °C. Fractions (0.6 ml) were collected from the bottom of the tube and assayed for radioactivity. (A) Sedimentation of the soluble dihydropyridine (DHP) receptor. 4 ml of 1.0% CHAPS solubilized material containing the (+)-[^{3}H]PN200-110-labeled dihydropyridine receptor were layered on a gradient containing 1.0% CHAPS and sedimented as described above. Micelle associated (+)-[^{3}H]PN200-110 runs at the top of the gradient. The (+)-[^{3}H]PN200-110-labeled dihydropyridine receptor peak is indicated by an arrow. (B) Sedimentation of the reconstituted dihydropyridine receptor. 4 ml of lipid vesicles containing the (+)-[^{3}H]PN200-110-labeled dihydropyridine receptor were layered on a gradient and sedimented as described above. The (+)-[^{3}H]PN200-110-labeled dihydropyridine receptor associated with lipid vesicles (●) is indicated by an arrow. The results of sedimentation experiments in which lipid vesicles were labeled with [^{3}H]phosphatidylcholine (○) are also shown in the figure. The [^{3}H]phosphatidylcholine peak coincides with the (+)-[^{3}H]PN200-110-labeled reconstituted dihydropyridine receptor peak, as indicated by an arrow. Sedimentation of the (+)-[^{3}H]PN200-110-labeled soluble dihydropyridine receptor in the absence of lipid and detergent (▲) is shown to result in the formation of a pellet containing aggregated receptor. Micelle associated (+)-[^{3}H]PN200-110 runs at the top of the gradient. In this figure, lines represent data collected from each fraction, whereas symbols serve to distinguish between lines.

sociated (+)-[^{3}H]PN200-110 runs at the top of the gradient. Panel 2B also illustrates that when the prelabeled dihydropyridine receptor is subjected to the reconstitution protocol outlined above and run on a gradient in the absence of detergent, it does not aggregate but sediments to a position corresponding to that observed with lipids alone. The presence of two components for both the lipid

vesicles, and the receptor vesicles, is probably due to some heterogeneity in the lipid composition of the vesicles prepared from crude soybean phosphatidylcholine since only a single component is observed when more pure egg yolk lipids are used (data not shown). Overall, these experiments indicate that the soluble dihydropyridine receptor is in fact associated with the lipid vesicle fraction following Extracti-gel chromatography.

Equilibrium binding of (+)-[^{3}H]PN200-110 to membrane-bound, soluble, and reconstituted receptor

The specific binding of (+)-[^{3}H]PN200-110 to the membrane-bound, soluble, and reconstituted forms of the dihydropyridine receptor was demonstrated to be saturable and of high affinity by methods described under Experimental Procedures. Fig. 3 illustrates that Scatchard plots for (+)-[^{3}H]PN200-110 binding to each form of the receptor are linear, which is consistent with a single population of noninteracting binding sites. A significant difference is observed in the (+)-[^{3}H]PN200-110 binding affinity to the soluble (K_d = 6.8 ± 0.96 nM) and the membrane bound (K_d = 0.78 ± 0.28 nM) forms of the receptor. When the dihydropyridine receptor is reincorporated into phospholipid vesicles, following detergent removal, the binding affinity (K_d = 1.1 ± 0.34 nM) is again similar to that observed for the membrane-bound form. The loss in affinity which occurs upon solubilization of the receptor appears to be due to a nonspecific interaction between the ligand and the detergent micelles (which effectively reduces the concentration of free ligand) rather than being due to differences in the tertiary conformation of the lipid-associated and deter-

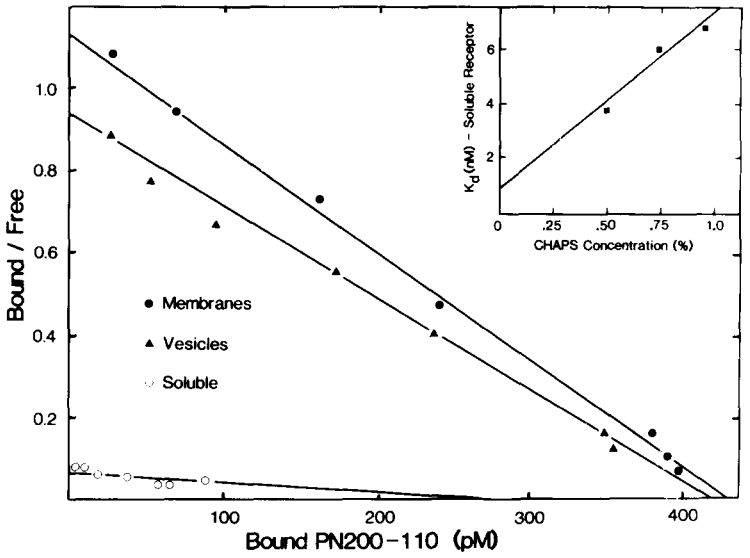

Fig. 3. Scatchard plots of (+)-[^{3}H]PN200-110 binding to membrane bound, soluble, and reconstituted dihydropyridine receptor. T-tubule membranes, soluble receptor, and reconstituted receptor were prepared as described. Binding to T-tubule membranes (●) and vesicles (▲) was performed in 150 mM KCl, 1 mM $CaCl_2$, 10 mM $MgSO_4$, 25 mM Hepes-NaOH (pH 7.4) at 8.0°C. Binding to soluble receptor (○) was performed in 1.0% CHAPS in 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) at 8.0°C. Data shown are representative of six experiments done with each receptor preparation. The protein concentration varied among the three groups; i.e., membrane bound, 0.09 mg protein/ml; soluble, 0.32 mg protein/ml; and reconstituted, 0.25 mg protein/ml. These protein concentrations were chosen to best illustrate the data. K_d values for (+)-[^{3}H]PN200-110 determined in these representative plots: membranes, K_d = 0.78 ± 0.28 nM; vesicles, K_d = 1.1 ± 0.34 nM; soluble, K_d = 6.8 ± 0.96 nM. Inset: (+)-[^{3}H]PN200-110 binding to soluble receptor at varying concentrations of detergent. T-tubule membranes were solubilized with 1.0% CHAPS in 150 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4) at 8°C and the resulting solubilized material was then diluted to the indicated concentration of detergent. [^{3}H]PN200-110 binding was done at 8.0°C. The plot shown is an extrapolation to zero concentration of detergent from points chosen above the critical micellar concentration of CHAPS (0.3–0.4%). Data shown are average values of two saturation isotherms done at each detergent concentration.

gent-solubilized forms of the receptor. As shown in the inset to Fig. 3, the K_d value for (+)-[^{3}H]PN200-110 binding to the soluble receptor is markedly dependent on the concentration of CHAPS present in the binding assay. The concentrations of detergent chosen for these experiments were above the critical micellar concentrations for CHAPS (which is necessary to effectively solubilize the receptor) and it can be seen that extrapolation to zero from K_d values determined at these points yields a K_d value for (+)-[^{3}H]PN200-110 binding to the soluble receptor which is similar to that of the membrane bound form of the receptor.

In order to determine whether receptor sites for other calcium antagonists had been incorporated into phospholipid vesicles along with the dihydropyridine receptor, the effects of diltiazem and verapamil on the dissociation rate of (+)-[^{3}H]PN200-110 were examined as described under Experimental Procedures. As demonstrated in Fig. 4, dissociation of (+)-[^{3}H]PN200-110 from the reconstituted form of the dihydropyridine receptor occurs slowly at 8°C. Two hours after a 10-fold dilution into binding media containing 1 μM nitrendipine, 50% of the radioligand remains bound. As shown in the figure, 100 μM diltiazem reduces, whereas 100 μM verapamil enhances the dissociation rate of (+)-[^{3}H]PN200-110. These results are similar to those demonstrated for the membrane bound receptor [5,6] and thus provide evidence that these allosteric regulatory sites have been incorporated together with the dihydropyridine receptor into phospholipid vesicles.

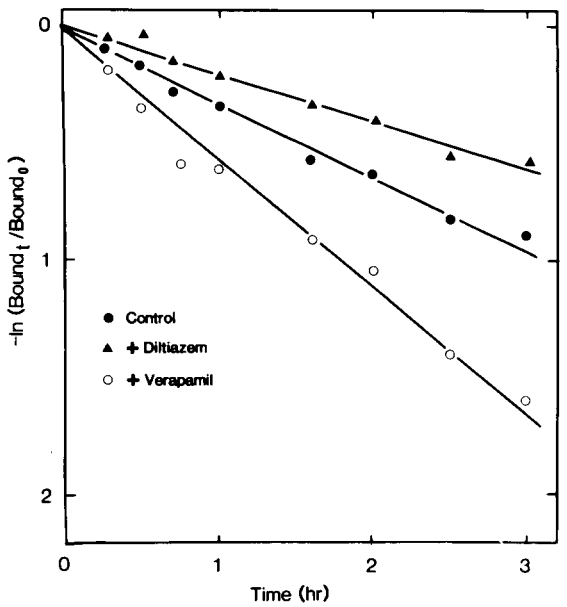

Fig. 4. Effect of diltiazem and verapamil on the dissociation of (+)-[^{3}H]PN200-110 from the reconstituted dihydropyridine receptor. Lipid vesicles containing the reconstituted dihydropyridine receptor were prepared as described. Vesicles were labeled with 3.0 nM (+)-[^{3}H]PN200-110 in the presence or absence of 1 μM nitrendipine and diluted 10-fold into 150 mM KCl, 1 mM $CaCl_2$, 10 mM $MgSO_4$, 25 mM Hepes-NaOH (pH 7.4) at 8.0°C containing 1 μM nitrendipine (●), 100 μM diltiazem and 1 μM nitrendipine (▲), or 100 μM verapamil and 1 μM nitrendipine (○), and assayed by filtration at the indicated time points. Specifically bound (+)-[^{3}H]PN200-110 was determined as the difference between samples labeled in the presence and absence of 1 μM nitrendipine.

Temperature stability of the membrane bound, soluble and reconstituted dihydropyridine receptor

The restoration of temperature stability following solubilization has been used previously as evidence for the successful reincorporation of recep-

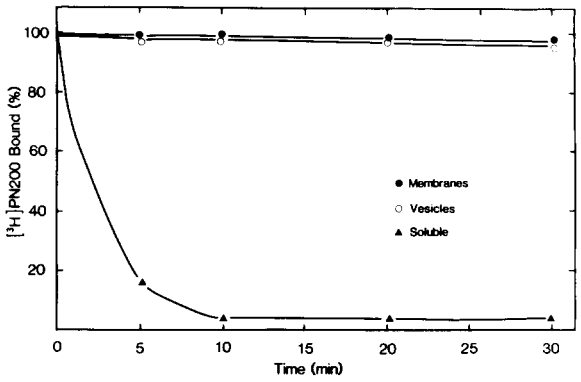

Fig. 5. Temperature stability of the membrane bound, soluble, and reconstituted dihydropyridine receptor. T-tubule membranes, soluble receptor, and reconstituted receptor were prepared as described. T-tubule membranes (●) and vesicles (○) were diluted 5-fold into 150 mM KCl, 1 mM $CaCl_2$, 10 mM $MgSO_4$, 25 mM Hepes-NaOH, (pH 7.4 at 37°C) and incubated at 37°C for the indicated time prior to the measurement of (+)-[^{3}H]PN200-110 binding. Soluble receptor (▲) was diluted 5-fold into 150 mM KCl, 1 mM $CaCl_2$, 1.0% CHAPS, 25 mM Hepes-NaOH (pH 7.4 at 37°C) and incubated at 37°C for the indicated time prior to the measurement of (+)-[^{3}H]PN200-110 binding. Binding was performed at 8°C in the presence of 3.0 nM (+)-[^{3}H]PN200-110 as described under Experimental Procedures. Specifically bound (+)-[^{3}H]PN200-110 was determined as the difference between samples labeled in the presence and absence of 1 μM nitrendipine.

tor proteins into phospholipid vesicles [25]. In the present study, temperature stability was assessed by the ability of the dihydropyridine receptor to retain (+)-[^{3}H]PN200-110 binding during incubation at 37°C. Fig. 5 shows that the CHAPS-solubilized dihydropyridine receptor is very labile at 37°C, where it loses over 80% of its (+)-[^{3}H]PN200-110 binding activity within 5 min. However, as is the case for the membrane-bound receptor, the reconstituted dihydropyridine receptor is stable for at least 30 min at this temperature. This provides further evidence that the dihydropyridine receptor has been reincorporated into phospholipid vesicles following Extracti-gel chromatography.

In summary, the results presented here demonstrate the successful incorporation of the dihydropyridine receptor into phospholipid vesicles as assessed by a variety of criteria. The relative ease and rapidity of this method should make it a useful assay tool in the purification of the dihydropyridine receptor-calcium channel complex. An added advantage is that this method can also be used to insert a wide variety of membrane components suspected to be involved in the regulation of calcium channel function. Thus future studies will be aimed at probing the functional interaction of these components, and their regulatory effects on calcium flux, in a lipid vesicle system.

Acknowledgement

This work was supported in part by NIH Grant 1 K11 HL01415-01.

References

1 Hagiwara, S. and Byerly, L. (1981) Annu. Rev. Neurosci. 4, 69–125
2 Fleckenstein, A. (1983) Circ. Res. 52, Suppl. 1, 3–16
3 Janis, R.A. and Triggle, D.J. (1984) Drug Dev. Res. 4, 257–274
4 Weiland, G.A. and Oswald, R.E. (1985) J. Biol. Chem. 260, 8456–8464
5 Murphy, K.M.M., Gould, R.J., Largent, B.L. and Snyder, S.H. (1983) Proc. Natl. Acad. Sci. USA 80, 860–864
6 Horne, W.A., Weiland, G.A. and Oswald, R.E. (1986) J. Biol. Chem. 261, 3588–3594
7 Rengasamy, A., Ptasienski, J. and Hosey, M. (1985) Biochem. Biophys. Res. Commun. 126, 1–7
8 Curtis, B.M. and Catterall, W.A. (1984) Biochemistry 23, 2113–2118
9 Borsotto, M., Barhanin, J., Norman, R.I. and Lazdunski, M. (1984) Biochem. Biophys. Res. Commun. 122, 1357–1366
10 Ferry, D.R., Goll, A. and Glossmann, H. (1983) EMBO J. 2, 1729–1732
11 Galizzi, J.P., Borsotto, M., Barhanin, J., Fosset, M. and Lazdunski, M. (1986) J. Biol. Chem. 261, 1393–1397
12 Curtis, B.M. and Catterall, W.A. (1985) Proc. Natl. Acad. Sci. USA 82, 2528–2532
13 Reuter, H. (1983) Nature 301, 569–574
14 Holz IV, G.G., Rane, S.G. and Dunlap, K. (1986) Nature 319, 670–672
15 Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315–321
16 Sternweis, P.C. and Robishaw, J.D. (1984) J. Biol. Chem. 259, 13806–13813
17 Neer, E.J., Lok, J.M. and Wolf, L.G. (1984) J. Biol. Chem. 259, 14222–14229
18 Fernandez, J.L., Rosemblatt, M. and Hidalgo, C. (1980) Biochim. Biophys. Acta 599, 552–568
19 Rosemblatt, M., Hidalgo, C., Vergara, C. and Ikemoto, N. (1981) J. Biol. Chem. 256, 8148–8148
20 Borsotto, M., Norman, R.I., Fosset, M. and Lazdunski, M. (1984) Eur. J. Biochem. 142, 449–455
21 Cerione, R.A., Codine, J., Benovic, J.L., Lefkowitz, R.J., Birnbaumer, L. and Caron, M.G. (1984) Biochemistry 23, 4519–4525
22 Cerione, R.A., Regan, J.W., Nakata, H., Codine, J., Benovic, J.L., Giershik, P., Somers, R.L., Spiegel, A.M., Birnbaumerr, L., Lefkowitz, R.J. and Caron, M.G. (1986) J. Biol. Chem. 261, 3901–3909
23 Anholt, R., Fredkin, D.R., Deerinck, T., Ellisman, M., Montal, M. and Lindstrom, J. (1982) J. Biol. Chem. 257, 7122–7134
24 Kelleher, D.J., Rashidbaigi, A., Ruoho, A.E. and Johnson, G.L. (1983) J. Biol. Chem. 258, 12881–12885
25 Tamkun, M.M., Catterall, W.A. (1981) J. Biol. Chem. 256, 11457–11463