Solubilization and Reassembly of the Mitochondrial Benzodiazepine Receptor†

Robert R. H. Anholt,‡ Ueli Aebi,§ Peter L. Pedersen,|| and Solomon H. Snyder*,‡

Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, of Cell Biology and Anatomy, and of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received November 5, 1985

ABSTRACT: We have solubilized and reassembled the peripheral-type benzodiazepine receptor, a component of the mitochondrial outer membrane, from rat adrenal gland mitochondria. The ligand binding site of this receptor undergoes denaturation during solubilization in digitonin, Triton X-100, or 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate at detergent concentrations above 0.1%, which is evident from the loss of high-affinity binding of [^{3}H]PK11195, a ligand selective for the mitochondrial benzodiazepine receptor. The conformation of the binding site for PK11195 can be stabilized during solubilization in sodium cholate by relatively low concentrations of supplementary soybean lipid. Drug displacement studies demonstrate that the pharmacological properties of the receptor are preserved under these conditions. Electron micrographs of the solubilized preparation show a heterogeneous population of many small particles (<100 Å) and some larger membranous aggregates (up to 500 Å). Sucrose gradient centrifugation indicates that these lipoprotein complexes are of high buoyant density. They can be incorporated in liposomes via cholate dialysis in the presence of additional supplementary lipid. The behavior of the mitochondrial benzodiazepine receptor during solubilization and reassembly suggests that it is an integral protein of the outer membrane.

The anxiolytic and anticonvulsant actions of benzodiazepines are widely appreciated. The therapeutic effects of these drugs are due to modulation of GABAergic neurotransmission and are mediated via specific high-affinity binding sites in the central nervous system (Braestrup & Squires, 1977; Möhler & Okada, 1977; Tallman et al., 1978, 1980; Olsen, 1981; Richards & Möhler, 1984). Besides these "central-type benzodiazepine receptors", binding sites for benzodiazepines have also been identified in a large number of peripheral tissues (Braestrup & Squires, 1977; Taniguchi et al., 1980; Wang et al., 1980; Marangos et al., 1982; Schoemaker et al., 1983; Benavides et al., 1983a, 1984; Anholt et al., 1984, 1985; De Souza et al., 1985). These binding sites, initially designated "peripheral-type benzodiazepine receptors", differ from the central-type receptors in their pharmacological properties. The affinities with which benzodiazepines and related drugs bind to the peripheral receptor do not correlate with their therapeutic potencies as anxiolytic agents (Braestrup & Squires, 1977; Möhler & Okada, 1977; Tallman et al., 1980; Marangos et al., 1982; Schoemaker et al., 1983; Benavides et al., 1983a, 1984; Anholt et al., 1984; De Souza et al., 1985). Furthermore, peripheral-type benzodiazepine receptors are not linked to GABA receptors as are their central-type counterparts (Marangos et al., 1982; Schoemaker et al., 1983). Autoradiographic studies on whole body sections of neonatal rats, using the high affinity ligand [^{3}H]Ro5-4864,[1] revealed that in many tissues the pattern of peripheral-type benzodiazepine binding sites resembles the histochemical pattern of cytochrome oxidase activity, suggesting that these benzodiazepine receptors are associated with mitochondria (Anholt et al., 1985). Subcellular fractionation studies on the adrenal gland using [^{3}H]PK11195 as the ligand provided evidence that these receptors are indeed localized to the mitochondria (Anholt et al., 1986). Moreover, titration of the mitochondria with digitonin, which separates the outer membrane from the inner membrane, demonstrated that the peripheral-type benzodiazepine binding site is part of a prominent component of the mitochondrial outer membrane (Anholt et al., 1986).

Solubilization of the mitochondrial benzodiazepine receptor may help to elucidate its exact biochemical nature and, hence, its biological function. Here, we report the solubilization and reassembly of benzodiazepine receptors from rat adrenal gland mitochondria using cholate as the detergent. The binding site for PK11195 appears to be labile in detergent solution, and its conformation can be stabilized by supplementary lipids. The behavior of the mitochondrial benzodiazepine receptor in detergent solution suggests that it is an integral membrane protein, the conformation of which is critically dependent on the lipid environment.

Materials and Methods

Preparation of Mitochondria. Sprague-Dawley rats (150–175 g; Hilltop Laboratories, Scottdale, PA) were killed by a blow behind the neck followed immediately by decapitation. The adrenal glands were removed, carefully freed from surrounding adipose tissue, and incubated for 15 min in ice-cold buffer A (2 mM HEPES, 70 mM sucrose, and 0.21 M D-mannitol, pH 7.4). Typically, about 0.8 g wet weight of adrenal glands was homogenized in 2 mL of ice-cold buffer A in a glass homogenizer by four slow up and down strokes with a loose-fitting Teflon pestle with multiple radial serrations.

† This work was supported by USPHS Grant DA-00266. U.A. is supported by NIH Grant GM-31940 and is the recipient of a research award from the Maurice Müller Foundation of Switzerland. P.L.P. is supported by NIH Grant CA-32742, and S.H.S. is the recipient of Research Scientist Award DA-00074.

* Author to whom correspondence should be addressed.

‡ Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences.

§ Department of Cell Biology and Anatomy.

|| Department of Biological Chemistry.

[1] Abbreviations: PK11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide; Ro5-4864, 7-chloro-1,3-dihydro-1-methyl-5-(*p*-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one; Ro15-1788, ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate; buffer A, 2 mM HEPES, 70 mM sucrose, and 0.21 M D-mannitol, pH 7.4; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

0006-2960/86/0425-2120$01.50/0

Then, an additional 6 mL of ice-cold buffer A was added, and the homogenate was centrifuged for 15 min at 635g at 4 °C in the SM24 rotor of a refridgerated Sorvall RC-5B centrifuge. The supernatant was collected and centrifuged for 15 min at 6500g at 4 °C. The mitochondrial pellet resulting from this centrifugation was gently homogenized into a paste with a test tube filled with ice, and the final volume was adjusted to 2 mL with buffer A. After centrifugation of the mitochondrial suspension for 15 min at 10000g at 4 °C, the pellet was homogenized as before, this time in a final volume of 1 mL. After repeated centrifugation for 15 min at 10000g, the washed mitochondrial fraction was suspended in 1 mL of buffer A. Protein was determined according to the method of Lowry et al. (1951), with bovine serum albumin as standard. The mitochondria were used fresh or after storage at −20 °C.

Receptor Binding Assays. Peripheral-type benzodiazepine receptors were assayed with [^{3}H]PK11195 (85.0 Ci/mmol; New England Nuclear, Boston, MA), an isoquinoline carboxamide derivative that binds to these receptors selectively and with high affinity (Benavides et al., 1983b; Le Fur et al., 1983). For binding assays, 400 μL of samples diluted in 50 mM Tris-HCl buffer, pH 7.7, to a concentration of 20–100 μg of protein/mL was incubated with 50 μL of [^{3}H]PK11195 at a final concentration between 1.0 and 2.0 nM and 50 mL of distilled water or 10 μM unlabeled PK11195 to assess the extent of nonspecific binding. Unlabeled PK11195 was donated by Dr. G. Le Fur (Pharmuka Laboratories, Gennevillers, France). The mixtures were incubated for 1 h in an ice–water bath. The assays were terminated by the rapid addition of 2 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.7, followed immediately by vacuum filtration through Schleicher & Schuell No. 32 glass fiber filters, presoaked in 0.5% poly(ethyleneimine). The filters were washed with 5 mL of ice-cold buffer, extracted in Formula 963 scintillation cocktail (New England Nuclear, Boston, MA), and counted in a liquid scintillation counter at 47% efficiency. All assays were performed in triplicate. Nonspecific binding was defined as binding of [^{3}H]PK11195 in the presence of 1 μM unlabeled PK11195 and was usually less than 15% of the total binding. Specific binding was calculated by subtracting the nonspecific binding from the total binding. Ro5-4864, clonazepam, flunitrazepam, diazepam, and Ro15-1788 were a gift from Dr. Peter Sorter (Hoffmann-La Roche, Nutley, NJ).

Solubilization of Peripheral-Type Benzodiazepine Receptors. Cholic acid, Triton X-100, and CHAPS were obtained from Sigma (St. Louis, MO). Cholic acid was recrystallized according to the method of Kagawa and Racker (1971) and used as the sodium salt. Digitonin was obtained from Fisher Scientific Co. (Fair Lawn, NJ) and was dissolved by warming. A fresh stock solution was prepared before each use. Soybean lipid (asolectin) was obtained from Associated Concentrates (Woodside, NY) and used without further purification. Stock suspensions of 100 or 10 mg/mL soybean lipid were prepared by repeated freezing and thawing. Lipid suspensions prepared by sonication apparently resulted in the formation of mixed micellar configurations with cholate, which were approximately 35% less effective in stabilizing the binding site for PK11195 than lipid suspensions prepared by repeated freezing and thawing.

Mitochondria were solubilized at 4 °C at a protein concentration of 1.2–1.7 mg/mL in 10 mM sodium phosphate buffer–100 mM NaCl, pH 7.4, containing the desired concentration of detergent and lipid. The mixtures were incubated for 30 min at 4 °C on an end-over-end shaker and centrifuged for 30 min in an Eppendorf microcentrifuge. The supernatants were collected and assayed for [^{3}H]PK11195 binding.

Reassembly of Peripheral-Type Benzodiazepine Receptors in Liposomes. Receptors were solubilized in the presence of 2% sodium cholate and 2 mg/mL soybean lipid. Supplementary soybean lipid was added such that the reconstitution mixture contained 2% cholate, 25 mg/mL soybean lipid, and 1 mg/mL mitochondrial protein in 10 mM sodium phosphate buffer–100 mM NaCl, pH 7.4. The mixture was incubated for 30 min at 4 °C on an end-over-end shaker. Liposomes were formed by removal of the cholate via dialysis for 16–18 h against two changes of 500 volumes of 10 mM sodium phosphate buffer–100 mM NaCl, pH 7.4. The reconstituted preparations were subjected to a freeze–thaw cycle by placing them in a freezer at 20 °C followed by thawing at room temperature to promote sealing and fusion of the reconstituted vesicles (Anholt et al., 1982). Liposomes without solubilized mitochondrial protein were prepared in parallel as controls.

The apparent internal volume of the reconstituted vesicles was measured by equilibration with $^{45}Ca^{2+}$ (New England Nuclear, Boston, MA) at a concentration of 16.5 μCi/mL for 48 h at 4 °C. Internal $^{45}Ca^{2+}$ was separated from external $^{45}Ca^{2+}$ by cation exchange. Aliquots (25 μL) of the incubation mixture were passed through Dowex 50W-8X in the Tris form, equilibrated, and eluted as described by Gasko et al. (1976). The eluates were counted in a scintillation counter in Formula 963 scintillation cocktail (New England Nuclear, Boston, MA) at 50% efficiency. All measurements were performed in triplicate.

Preparation of Mitochondrial Outer Membranes. Mitochondrial outer membranes were prepared by treatment of the mitochondria with digitonin, essentially as previously described (Anholt et al., 1986). An equal volume of a digitonin solution in buffer A was added rapidly to freshly isolated mitochondria at a protein concentration of 2 mg/mL, such that the final concentration of digitonin was 0.065%. The suspension was incubated for 30 min at 4 °C on an end-over-end shaker, diluted 3-fold with buffer A, and centrifuged for 30 min at 10000g at 4 °C. The supernatant was collected and centrifuged for 1 h at 310000g in a Beckman SW55Ti rotor at 4 °C to pellet the isolated outer membranes.

Sucrose Gradient Centrifugation. Aliquots (100 μL) of solubilized preparations containing 2% cholate and 2 mg/mL soybean lipid were applied to 25-μL cushions of sucrose at varying densities in 10 mM sodium phosphate, 100 mM NaCl, 2% cholate, and 2 mg/mL soybean lipid, pH 7.4, in Beckman airfuge tubes. Reconstituted preparations or mitochondrial outer membranes were applied to sucrose cushions in 10 mM sodium phosphate buffer and 100 mM NaCl, pH 7.4. The tubes were centrifuged in a Beckman airfuge at ambient temperature at 100000g for 1 h. The supernatants were collected and assayed for [^{3}H]PK11195 binding to assess the fraction of peripheral-type benzodiazepine receptors that did not pellet through the sucrose cushion. Monoamine oxidase in the supernatants was assayed at 37 °C according to the method of Wurtman and Axelrod (1963) employing [^{3}H]-tyramine (35.1 Ci/mmol, New England Nuclear, Boston, MA) as the substrate.

Electron Microscopy. For electron microscopy, samples were diluted 25–250-fold with the appropriate buffer. Aliquots (1–3 μL) were then injected into a 3-μL drop of buffer that was placed on a carbon-coated 400 mesh/in. copper grid made hydrophilic by glow discharge in a reduced atmosphere of air. After 30 s, the grid was blotted with filter paper and washed for 15 s upside down on several drops of buffer and subsequently water. Finally, it was negatively stained for 15 s upside

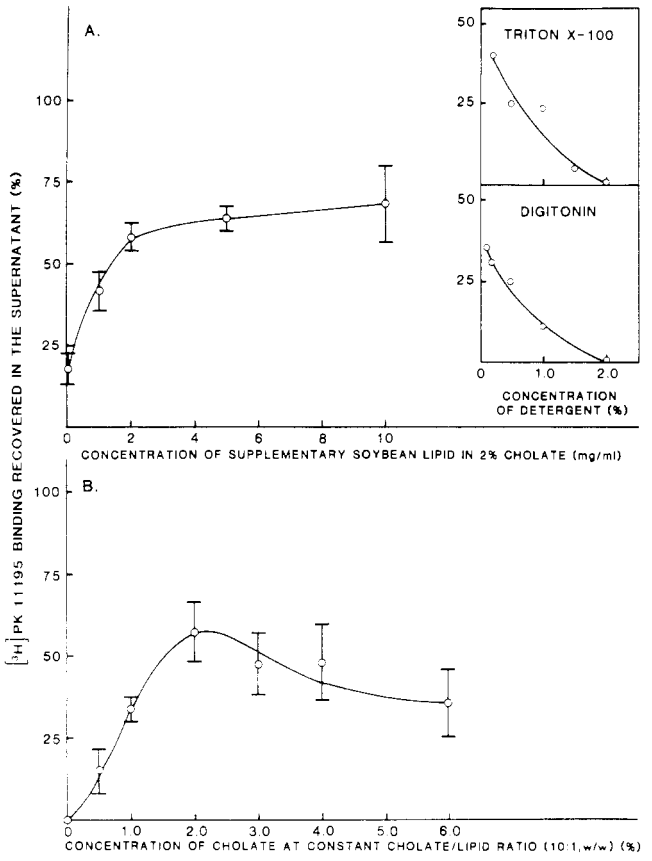

FIGURE 1: Solubilization of benzodiazepine receptors from rat adrenal gland mitochondria. (A) Stabilization of the binding site for [^{3}H]-PK11195 by supplementary soybean lipid in cholate solution. (B) Stability of the binding site for [^{3}H]PK11195 over a range of cholate concentrations at a constant cholate/lipid ratio (10:1 w/w). The insets in panel A show the denaturing effects of Triton X-100 and digitonin on the binding of [^{3}H]PK11195. Data points are the average of three independent experiments. Binding assays were performed in the presence of 1.0 nM [^{3}H]PK11195. The protein concentration in the initial solubilization mixtures was 1.7 mg/mL.

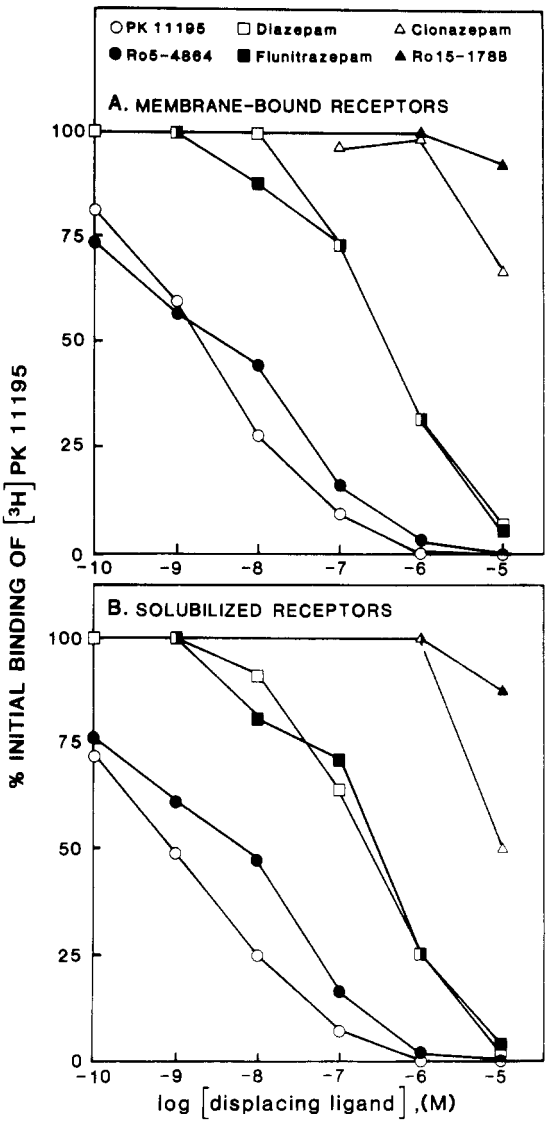

FIGURE 2: Pharmacological characterization of [^{3}H]PK11195 binding sites in intact mitochondria (panel A) and after solubilization in 2% cholate–2 mg/mL soybean lipid (panel B). Binding assays were performed with 6.4×10^{-10} M [^{3}H]PK11195 in the presence of the indicated concentration of displacing ligand. Amplitudes of maximal specific binding of [^{3}H]PK11195 amounted to 5533 cpm in panel A and 4630 cpm in panel B. Data points are the average of two independent experiments, each of which consisted of triplicate measurements. Standard errors are within 15% of the mean.

down on several drops of 0.75% uranyl formate, pH 4.25. Before the grid was allowed to dry, excess stain was blotted with filter paper and further drained off by gentle suction with a capillary.

Specimens were examined in a Zeiss EM10C transmission electron microscope operated at an acceleration voltage of 80 kV. Electron micrographs were recorded at either 25000× or 50000× nominal magnification under minimal dose conditions (Williams & Fisher, 1970) on Kodak SO-163 electron image film and developed for 4 min in 3× diluted Kodak D-19 developer. Magnification was calibrated with negatively stained catalase crystals as described previously (Wrigley, 1968).

Results

In a previous study, we localized the peripheral-type benzodiazepine receptor to the mitochondrial outer membrane via the use of low concentrations of digitonin (<0.1%), which separate the mitochondrial outer membrane from the inner membrane (Anholt et al., 1986). Concentrations of digitonin above 0.1% disrupt the outer membrane and denature the binding site for [^{3}H]PK11195 (inset to Figure 1A). The same effect is observed with the nonionic detergent Triton X-100 (inset to Figure 1A) or the zwitterionic detergent CHAPS (data not shown).

Protection of protein conformation by lipids in detergent solution has been reported for a number of membrane proteins, including the nicotinic acetylcholine receptor (Anholt et al., 1981), the sodium channel (Agnew et al., 1978), and the IgE receptor (Rivnay and Metzger, 1982). On the basis of this precedence, we attempted to solubilize the mitochondrial benzodiazepine receptor in mixed micelles of cholate and soybean lipid. Supplementary soybean lipids stabilize the binding site for PK11195 during solubilization in 2% sodium cholate (Figure 1A). About 18% of the binding activity survives in 2% cholate in the absence of supplementary lipid, presumably due to protection by endogenous membrane components. However, in the presence of 2 mg/mL soybean lipid about 60% of the initial activity can be recovered. Increasing the cholate concentration at a constant detergent/lipid ratio to 6% cholate and 6 mg/mL soybean lipid leads to loss of about 40% of the activity recovered under optimal conditions (2% cholate and 2 mg/mL soybean lipid, Figure 1B). Half-maximal solubilization of [^{3}H]PK11195 binding sites occurs at 0.8–1.0% cholate, reflecting the critical micellar concentration of the detergent under the conditions used (Figure 1B).

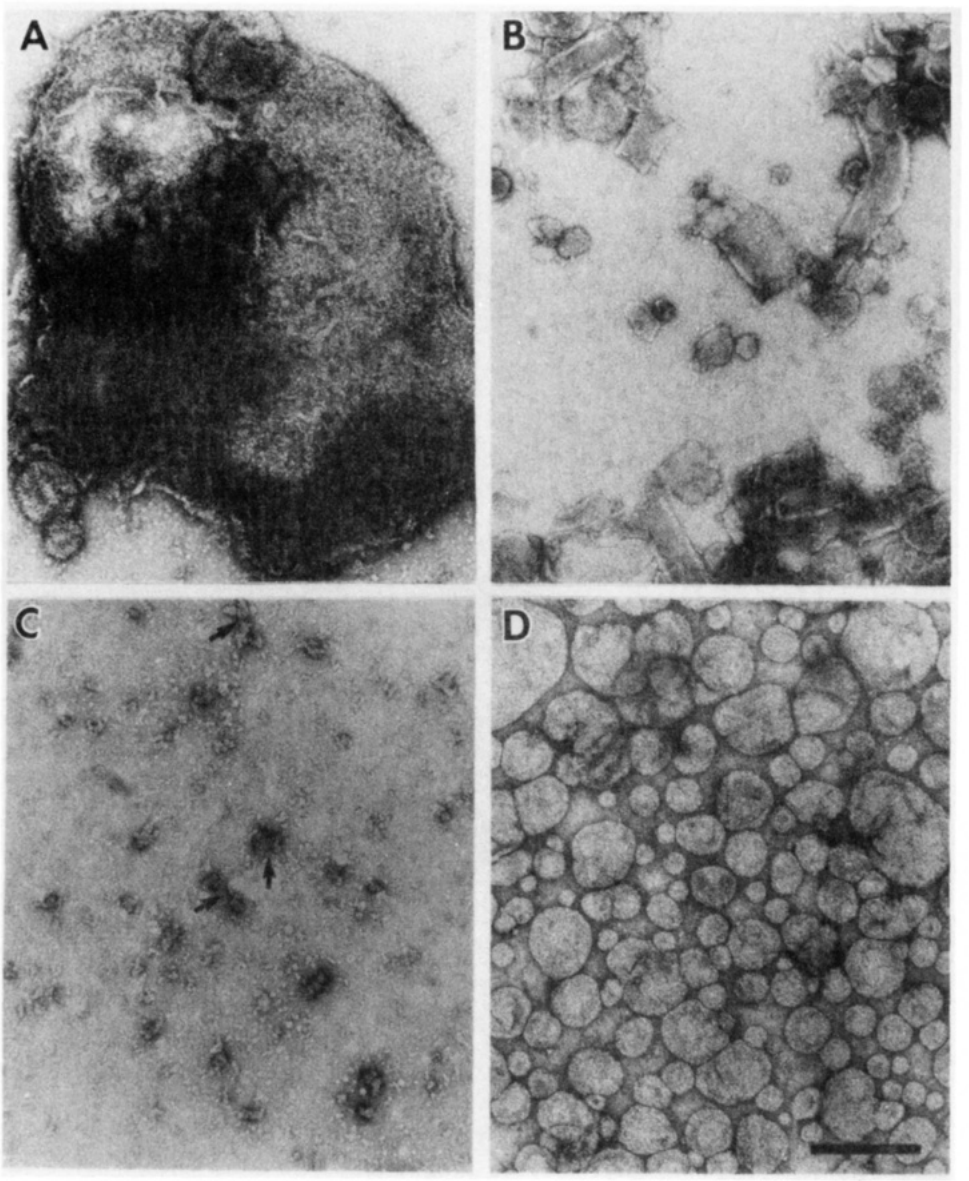

FIGURE 3: Electron micrographs of negatively stained mitochondrial preparations. (A) A representative mitochondrion. This nearly intact mitochondrion appears to be bursting, allowing observation of the highly vesiculated inner membrane, characteristic of mitochondria from the adrenal cortex. (B) Mitochondrial outer membranes isolated after treatment with digitonin. (C) A cholate/lipid extract. Notice the numerous small particles and membrane patches which display a tendency to form aggregates (arrows). (D) The same extract shown in panel C after addition of supplementary lipid followed by removal of the cholate via dialysis, which results in the formation of reconstituted vesicles. The liposomes range in diameter from ~350 to ~1800 Å. Many of the small liposomes may be devoid of protein (Anholt et al., 1982). All electron micrographs are shown at the same magnification. The scale bar in panel D represents 2000 Å.

To confirm the pharmacological integrity of the solubilized receptors, we performed drug displacement studies. The benzodiazepine receptor from rat adrenal gland binds PK11195 and Ro5-4864 with nanomolar affinity while diazepam and flunitrazepam are less potent, as previously reported (Figure 2A; Benavides et al., 1983a; De Souza et al., 1985; Anholt et al., 1986). The potent anticonvulsant drug clonazepam and the central-type benzodiazepine antagonist Ro15-1788 do not displace [^{3}H]PK11195 from the mitochondrial benzodiazepine receptor over the range of concentrations studied (Figure 2A). This pharmacological profile, characteristic for the peripheral-type benzodiazepine receptor, is preserved in cholate/lipid solution (Figure 2B).

Electron microscopic examination of the solubilized material after negative staining reveals the presence of a heterogeneous population of many small (less than 100 Å) particles, which display a tendency to aggregate. In addition, some larger membranous aggregates, up to 500 Å in size, are present (Figure 3C). Analysis of the extract by sucrose gradient centrifugation shows that it consists of dense lipoprotein complexes, which band at 30–35% (w/w) sucrose (Figure 4A). These high-density aggregates do not consist exclusively of benzodiazepine receptors but include other mitochondrial membrane components as well, since monoamine oxidase activity in the extract appears to band at the same high sucrose density (Figure 4A). Lipid-rich mitochondrial outer membranes prepared by digitonin treatment, shown in Figure 3B, band at 15–20% (w/w) sucrose (data not shown).

Removal of the cholate by dialysis after supplementing the preparation with additional lipid (25 mg/mL) results in the formation of reconstituted vesicles (Figure 3D; Anholt et al., 1981, 1982). These vesicles range from ~350 to ~1800 Å in diameter (average diameter = 560 Å, n = 309). Vesicle formation is accompanied by an increase in the apparent internal volume of the preparation from 0.32 ± 0.08 μL/mg of lipid (n = 3) before dialysis to 1.78 ± 0.24 μL/mg of lipid (n = 3) after cholate dialysis. Control liposomes formed in the absence of protein have an apparent internal volume of 0.68

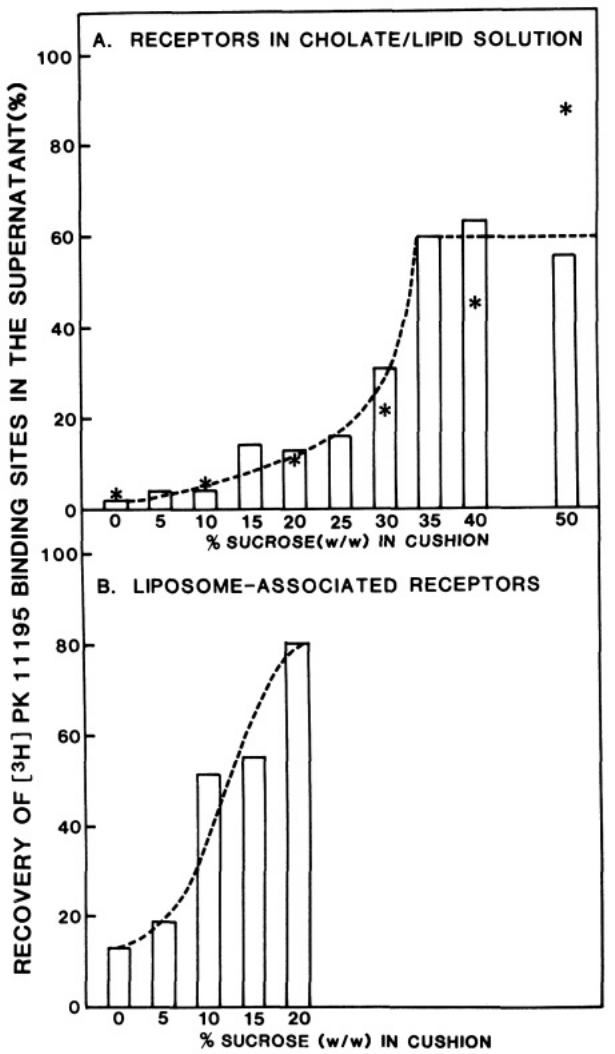

FIGURE 4: Sucrose gradient centrifugation of mitochondrial benzodiazepine receptors in cholate/lipid solution (panel A) and after incorporation in liposomes (panel B). Centrifugation was performed on sucrose cushions in Beckman airfuge tubes as described under Materials and Methods. The supernatants were collected and assayed for [^{3}H]PK11195 binding at a concentration of 1.5 nM [^{3}H]PK11195. The asterisks in panel A indicate the recovery of monoamine oxidase activity in the supernatants. About 57% of the total monoamine oxidase activity is recovered in the initial extract.

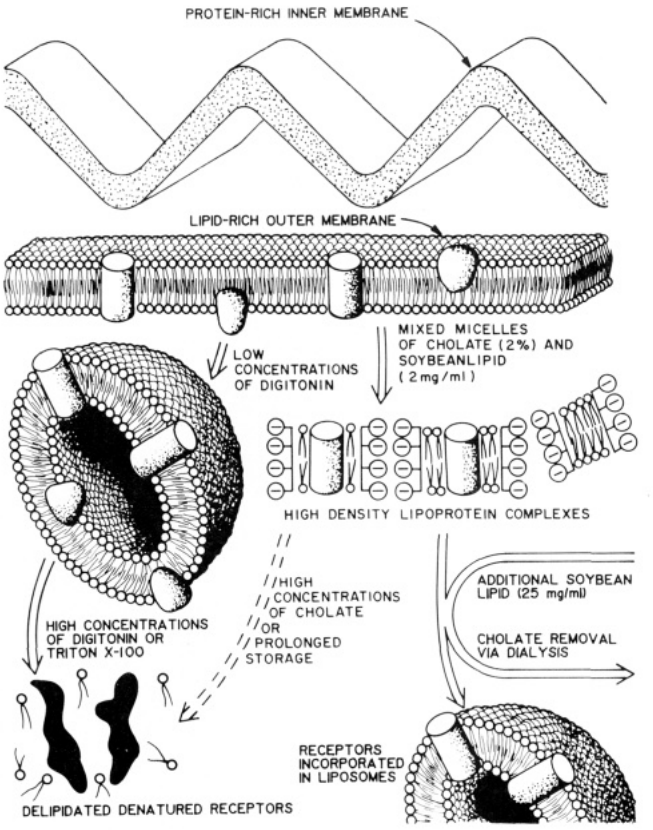

FIGURE 5: Schematic representation of solubilization of benzodiazepine receptors from mitochondrial outer membrane and their reassembly in liposomes. The benzodiazepine receptors are indicated as cylindrical transmembrane structures.

$\pm$ 0.10 μL/mg of lipid (n = 3) and diameters ranging from 250 to $\sim$1000 Å (average diameter = 480 Å, n = 144) [see also Anholt et al. (1982)]. Incorporation of the mitochondrial benzodiazepine receptor in reconstituted vesicles is further demonstrated by sucrose gradient centrifugation (Figure 4B). Reconstituted receptors band at 10–15% (w/w) sucrose, indicating reassembly of the receptors with the soybean lipid as low-density liposomes (Figure 4B).

The stability of the benzodiazepine binding site is greatly decreased after solubilization. About 29% of the binding of [^{3}H]PK11195 is lost after 1 day of storage at 4 °C in cholate/lipid solution, and about 85% of the activity is lost after storage under these conditions for 1 week. In contrast, no loss of activity is apparent when mitochondrial benzodiazepine receptors are stored for 1 week at 4 °C after reconstitution.

Discussion

We have demonstrated that the binding site for [^{3}H]-PK11195 can be protected during extraction of the mitochondrial benzodiazepine receptor in cholate by supplementary soybean lipid. This lipid dependence is quantitatively similar to that previously observed for solubilized acetylcholine receptors from *Torpedo* electric organ membranes (Anholt et al., 1981). Under optimal stabilizing conditions, the detergent is present in 20-fold molar excess over the lipid. For the acetylcholine receptor, it has been suggested that the bipolar cyclopentenophenanthrene rings of the cholate molecules leave one or more lipid annuli around the protein intact and carry the receptor as a lipoprotein complex in the detergent solution (Anholt et al., 1981). At elevated concentrations of cholate, about 40% of the binding activity observed under optimal conditions is lost (Figure 1B). The same experiment performed under similar conditions with the nicotinic acetylcholine receptor led to a loss of 85% of the channel activity recovered under optimal conditions (Anholt et al., 1981). Thus, it appears that in cholate solution the annular lipid is bound with higher affinity by the protein and, hence, is less easily displaced by excess detergent in the case of the mitochondrial benzodiazepine receptor than in the case of the nicotinic acetylcholine receptor.

Figure 5 presents a hypothetical scheme of the solubilization and reassembly processes described above. Low concentrations of digitonin or Triton X-100 fractionate the mitochondrial outer membrane in the form of small vesicles, which can be sedimented by centrifugation for 1 h at 310000g (Schnaitman & Greenawalt, 1968; Anholt et al., 1986; Figure 3B). Martini et al. (1983) reported the solubilization of benzodiazepine receptors from rat kidney in Triton X-100, and Benavides et al. (1985) reported the solubilization of benzodiazepine receptors from rat adrenal gland in digitonin. The apparent discrepancy between these reports and our observations may be explained by the possibility that these investigators obtained under their experimental conditions mitochondrial outer membrane vesicles, shown in Figure 3B, rather than recep-

tor–detergent micelles. High concentrations of these detergents result in delipidation of the receptor and the loss of high-affinity binding of [^{3}H]PK11195 (Figures 1 and 5). The binding site for PK11195 is stabilized in mixed micelles of sodium cholate and soybean lipid. Under these conditions, the receptors are carried by the detergent as lipoprotein complexes that have a higher buoyant density than the native lipid-rich mitochondrial outer membrane (Figures 4 and 5). These lipoprotein complexes, shown in Figure 3C, appear to include other mitochondrial membrane proteins such as monoamine oxidase in addition to the benzodiazepine receptor. Removal of the detergent via dialysis results in the reassembly of these complexes with the lipid as reconstituted vesicles (Figures 3D and 5). It should be noted, however, that in the absence of a defined transmembrane function of the mitochondrial benzodiazepine receptor reconstitution studies should be considered preliminary. The absolute dependence of the integrity of the ligand binding site of the mitochondrial benzodiazepine receptor on supplementary lipid in cholate solution suggests that it is an integral protein of the outer membrane possibly involved in the regulation of a transmembrane function.

ACKNOWLEDGMENTS

We thank Dawn C. Dodson for secretarial assistance.

REFERENCES

Agnew, W. S., Levinson, S. R., Brabson, J. S., & Raftery, M. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2606–2610.

Anholt, R., Lindstrom, J., & Montal, M. (1981) *J. Biol. Chem. 256*, 4377–4387.

Anholt, R., Fredkin, D. R, Deerinck, T., Ellisman, M., Montal, M., & Lindstrom, J. (1982) *J. Biol. Chem. 257*, 7122–7134.

Anholt, R. R. H., Murphy, K. M. M., Mack, G. E., & Snyder, S. H. (1984) *J. Neurosci. 4*, 593–603.

Anholt, R. R. H., De Souza, E. B., Oster-Granite, M. L., & Snyder, S. H. (1985) *J. Pharmacol. Exp. Ther. 233*, 517–526.

Anholt, R. R. H., Pedersen, P. L., De Souza, E. B., & Snyder, S. H. (1986) *J. Biol. Chem. 261*, 576–583.

Benavides, J., Malgouris, C., Imbault, F., Begassat, F., Uzan, A., Renault, C., Dubroeucq, M. C., Gueremy, C., & Le Fur, G. (1983a) *Arch. Int. Pharmacodyn. Ther. 266*, 38–49.

Benavides, J., Quarteronet, D., Imbault, F., Malgouris, C., Uzan, A., Renault, C., Dubroecq, M. C., Gueremy, C., & Le Fur, G. (1983b) *J. Neurochem. 41*, 1744–1750.

Benavides, J., Quarteronet, D., Plouin, P.-F., Imbault, F., Phan, T., Uzan, A., Renault, C., Dubroeucq, M.-C., Gueremy, C., & Le Fur, G. (1984) *Biochem. Pharmacol. 33*, 2467–2472.

Benavides, J., Menager, J., Burgevin, M. C., Ferris, O., Uzan, A., Gueremy, C., Renault, C., & Le Fur, G. (1985) *Biochem. Pharmacol. 34*, 167–170.

Braestrup, C., & Squires, R. F. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3805–3809.

De Souza, E. B., Anholt, R. R. H., Murphy, K. M. M., Snyder, S. H., & Kuhar, M. J. (1985) *Endocrinology (Baltimore) 116*, 567–573.

Gasko, O. D., Knowles, A. F., Shertzer, H. G., Suolinna, E. M., & Racker, E. (1976) *Anal. Biochem. 72*, 57–65.

Kagawa, Y., & Racker, E. (1971) *J. Biol. Chem. 246*, 5477–5487.

Le Fur, G., Perrier, M. L., Vaucher, N., Imbault, F., Flamier, A., Benavides, J., Uzan, A., Renault, C., Dubroeucq, M. C., & Gueremy, C. (1983) *Life Sci. 32*, 1839–1847.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Marangos, P. J., Patel, J., Boulenger, J.-P., & Clark-Rosenberg, R. (1982) *Mol. Pharmacol. 22*, 26–32.

Martini, C., Giannaccini, G., & Lucacchini, A. (1983) *Biochim. Biophys. Acta 728*, 289–292.

Möhler, H., & Okada, T. C. (1977) *Science (Washington, D.C.) 198*, 849–851.

Olsen, R. W. (1981) *J. Neurochem. 31*, 1–13.

Richards, J. G., & Möhler, H. (1984) *Neuropharmacol. 23*, 233–242.

Rivnay, B., & Metzger, H. (1982) *J. Biol. Chem. 257*, 12800–12808.

Schnaitman, C., & Greenawalt, J. W. (1968) *J. Cell Biol. 38*, 158–175.

Schoemaker, H., Boles, R. G., Horst, W. D., & Yamamura, H. I. (1983) *J. Pharmacol. Exp. Ther. 225*, 61–69.

Tallman, J. F., Thomas, Y. J., & Gallagher, D. W. (1978) *Nature (London) 274*, 383–385.

Tallman, J. F., Paul, S. M., Skolnick, P., & Gallager, D. W. (1980) *Science (Washington, D.C.) 207*, 274–281.

Taniguchi, T., Wang, J. K. T., & Spector, S. (1980) *Life Sci. 27*, 171–178.

Wang, J. K. T., Taniguchi, T., & Spector, S. (1980) *Life Sci. 27*, 1881–1888.

Williams, R. C., & Fisher, H. W. (1970) *J. Mol. Biol. 52*, 121–123.

Wrigley, N. (1968) *J. Ultrastruct. Res. 24*, 454–464.

Wurtman, R. J., & Axelrod, J. A. (1963) *Biochem. Pharmacol. 12*, 1439–1441.