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Solubilization of the Cannabinoid Receptor from Rat Brain and Its Functional Interaction with Guanine Nucleotide-Binding Proteins

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SUMMARY

The present investigation was undertaken to characterize cannabinoid receptor binding in the absence of the membrane environment, inasmuch as cannabinoid drugs have been noted to influence the behavior of integral membrane proteins. The zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was able to solubilize the cannabinoid receptor from rat brain membranes, with the greatest yield and specific activity being obtained at a detergent/protein ratio of 0.5:1. [^{3}H]CP-55940 bound to a single class of binding sites in the CHAPS extract, which exhibited a K_d of 0.94 nM as determined by nonlinear regression analysis of equilibrium binding data. The order of potency for cannabinoid agonists in heterologous equilibrium binding studies was CP-55244 ≥ desacetyllevonantradol > Δ^9-tetrahydrocannabinol > cannabinol ≫ cannabidiol, consistent with the relative affinities for these agonists in brain membrane preparations. CP-55243, the biologically inactive enantiomer of CP-55244, competed for binding of [^{3}H]CP-55940 by <50% at 1 μM, similar to its poor affinity for the receptor in membranes. The CHAPS-solubilized cannabinoid receptor exhibited functional interactions with guanine nucleotide-binding proteins (G proteins). GTP and nonhydrolyzable analogs decreased [^{3}H]CP-55940 binding by 75%. The concentration-effect curves for guanine nucleotides exhibited a potency order similar to that observed for other G protein-linked receptors. Kinetic analyses indicated that GTP analogs increased the rate of agonist dissociation, decreasing the $t_{1/2}$ from 60 min at 0–4° to a multiphasic dissociation that exhibited a component having a $t_{1/2}$ of <1 min. The cannabinoid agonist desacetyllevonantradol was able to reduce pertussis toxin-catalyzed ADP-ribosylation of G proteins by 50%, demonstrating a receptor effect on G protein functions. These studies demonstrate that the membrane environment is not necessary for agonist binding to the cannabinoid receptor. Furthermore, the cannabinoid receptor maintains its functional interactions with pertussis toxin-sensitive G proteins in detergent solution.

The cannabinoid receptor, which binds central nervous system-active compounds from extracts of *Cannabis sativa* as well as synthetic bicyclic and tricyclic cannabinoid compounds, is a member of the superfamily of G protein-coupled receptors [reviewed by Howlett *et al.* (1)]. Cannabinoid receptors regulate adenylate cyclase via G_i to inhibit cAMP production. This has been demonstrated by guanine nucleotide and divalent cation requirements in neuroblastoma membranes (2) and by attenuation of the response in cells and membranes that had been treated with pertussis toxin (3). Pertussis toxin also attenuated cannabinoid inhibition of cAMP production in striatal slice preparations (4) and Chinese hamster ovary cells transfected with the cannabinoid receptor (5). This cannabinoid receptor-mediated second messenger regulation can be pharmacologically correlated with several *in vivo* activities of cannabinoid drugs, including analgesia, hypothermia, catalepsy (rigid immobility), and spontaneous activity (6, 7), and with recognition of tetrahydrocannabinol-like compounds in drug-discrimination studies (8). However, it cannot be assumed that these responses in animals are entirely attributed to decreased adenylate cyclase activity in target neurons, inasmuch as the activity of ion channels may also be regulated by cannabinoid receptors via a G protein-coupled mechanism (9, 10).

Cannabinoid compounds are quite lipophilic (11). Δ^9-Tetrahydrocannabinol exhibits a membrane/medium partition coefficient in excess of 12,000 (12). Cannabinoid compounds have been shown to alter membrane fluidity parameters with differing abilities depending upon the structure of the molecule and

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ABBREVIATIONS: G protein, guanine nucleotide-binding protein; BSA, bovine serum albumin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DALN, desacetyllevonantradol; GTPγS, guanosine 5′-*O*-(3-thió)triphosphate; Gpp(NH)p, guanyl-5′-yl imidodiphosphate; G_i, guanine nucleotide-binding protein mediating inhibition of adenylate cyclase; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

the nature of the model or physiological membrane (13–19). Membrane fluidity changes may be responsible for a number of effects on integral membrane proteins observed *in vitro* [see review by Martin (20)]. These might include alterations of the binding parameters observed in ligand binding assays for several G protein-coupled receptors (21–23), as well as stimulation of adenylate cyclase in some membranes (24, 25). Thus, the effect of the membrane environment on cannabinoid receptor binding parameters has been of concern for receptor-ligand interactions as well as receptor-G protein interactions. In an effort to address these issues, the present investigation has characterized the cannabinoid receptor in detergent solution. The receptor-ligand interactions in detergent solution and the receptor-G protein coupling have been examined using conditions under which the role of the membrane environment is not a factor.

Experimental Procedures

Materials. CHAPS detergent was obtained from Boehringer Mannheim. Frozen rat brains were from Pel-Freez. [^{3}H]CP-55940 was from DuPont-NEN and [^{32}P]NAD was from ICN. Cannabinoid ligands were donated by Pfizer, Inc., or the National Institute on Drug Abuse. Pertussis toxin was purchased from List Biologicals. All other chemicals were obtained from Sigma Chemical Co.

Membrane preparation and solubilization. Membranes were prepared from frozen rat brains as described previously (26). Membranes were stored at −80° in 25 mM Tris·HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA (TME). Protein concentrations were determined by the method of Bradford (27). Cannabinoid receptor binding activity in membranes was determined by incubating 20–50 g of membrane protein with 0.7 nM [^{3}H]CP-55940 in TME buffer at 30° for 60 min. Bound ligand was separated from free ligand by filtration over GF/C filters (Whatman) as described previously (28). Detergent solubilization of cannabinoid receptors was accomplished by adjusting membranes to a protein concentration of 10 mg/ml with 30 mM Tris·HCl, pH 7.4, 5 mM $MgCl_2$ (TM buffer). Reagents were added to achieve final concentrations of 20% glycerol, 10 mM $MgCl_2$, and 0.5 g/g of protein for the zwitterionic detergent CHAPS. The concentration of CHAPS was 8 mM unless indicated otherwise. After addition of detergent, the membranes were slowly stirred on ice for 30 min, followed by centrifugation at 100,000 × *g* for 40 min at 4°. The supernatant containing solubilized receptor was removed without disturbing the pellet and was stored frozen in aliquots at −80°. Stocks of solubilized receptor maintained stable agonist binding for at least 4 months.

Assay of solubilized receptor. Binding of the solubilized cannabinoid receptor was performed in Regisil-treated 12- × 75-mm borosilicate glass tubes. Cannabinoid ligand stocks were stored in ethanol at −80°. Before use the ethanol was evaporated under a stream of N_2 gas and the ligand was resuspended at a concentration of 100 μM in TM buffer containing 50 mg/ml fatty acid-free BSA. Dilutions of the stocks were made with TM buffer containing 1 mg/ml fatty acid-free BSA. The assay volume of 200 μl consisted of 60 μl (approximately 0.7 nM) of the cannabinoid agonist [^{3}H]CP-55940 (100 Ci/mmol), 40 μl of solubilized membrane extract (30 μg of protein), and 100 μl of TM buffer containing 1 mg/ml BSA (as vehicle) or the indicated drugs. To determine nonspecific binding, 100 μl of 400 nM DALN, a cannabinoid receptor agonist, were used. The assay mixture was incubated at 30° for 75 min. To separate free radioligand from that bound to receptor, a 150-μl aliquot from each tube was filtered at 4° over 0.7- × 6-cm columns of Sephadex G50 (medium) that had been equilibrated previously with TM buffer containing 10% glycerol and 0.1% CHAPS (TMGC buffer). Columns were suspended over 20-ml scintillation vials and, after the 150-μl assay aliquot was allowed to enter the gel, two aliquots of 150 μl of TMGC buffer were added, followed by a 1.3-ml addition. The entire eluate consisting of the column void volume (as determined by calibration with blue dextran) was collected, and 10 ml of scintillation fluid were added to each vial. Separation of blue dextran and bromophenol blue (markers for the void volume and included volume, respectively) required a minimum of 1 min after addition of elution buffer. Dissociation of bound receptor was not observed under these conditions. Data used in calculations were corrected for the partial aliquot filtered. Specific binding ranged from 65% to 80% of total binding and was linear with protein concentrations up to 0.4 mg/ml. Typical values for specific binding generally ranged from 150,000 to 200,000 dpm/mg of protein. Column blanks obtained in the absence of soluble receptor contained <5% of the total [^{3}H]CP-55940 present in the assay.

ADP-ribosylation of G proteins. CHAPS-solubilized extract was incubated for 75 min at 30° with 2.8 μg of pertussis toxin (which had been pretreated with 45 mM dithiothreitol) plus 5 μCi of [^{32}P]NAD. The reaction mixture (250 μl) also contained 30 mM Tris·HCl, pH 7.4, 5 mM $MgCl_2$, 0.5 mM EDTA, 10 mM thymidine, 1 mM ATP, 3 mM potassium phosphoenolpyruvate, 4 μg of pyruvate kinase, 10 μM NAD, 0.05 mg/ml fatty acid-free BSA, 3.2 mM CHAPS, and other additions as indicated. The reactions were stopped by precipitation of the proteins with 1 ml of 6% trichloroacetic acid at 4°, sedimentation of the pellets, and superficial washing of the pellets with absolute ethanol (twice). The pellets were resuspended in 150 μl of sample buffer, and 50-μl aliquots were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography as described previously (3). The absorbance of the bands was quantitated using a Bio-Rad video densitometer model 620 and the 1-D Analyst program.

Data analysis. Data obtained from equilibrium binding studies were analyzed using the computer software package Enzfitter (Elsevier-Biosoft) or Inplot (GRAPHpad) to compute nonlinear regression values. Heterologous equilibrium binding data were analyzed for both one- and two-site binding models using Inplot. Except where noted, binding parameters were best fit to a one-site binding model. IC_{50} values were converted to K_i values using the assumptions of Cheng and Prusoff (29).

Results

The zwitterionic detergent CHAPS has been successfully used to solubilize many of the G protein-linked receptors (30–33). Therefore, we pursued our initial solubilization experiments using various concentrations of this detergent. Optimal solubilization, defined as the greatest yield of binding activity with high specific binding, was obtained with a 0.5:1 (w/w) detergent/protein ratio (Table 1). Solubilization using this ratio resulted in a 2–3-fold increase in specific activity of cannabinoid receptor binding with nearly 60% of the receptor solubilized. Magnesium ion was found to be necessary, inasmuch as solubilization in the presence of excess EDTA yielded very low recovery of binding activity (data not shown). To ensure that the [^{3}H]CP-55940 binding was to solubilized receptor, an aliquot of the supernatant was passed through a 0.2-μm-pore diameter polysulfone filter (Gelman) before the radioligand binding assay. No difference in binding was observed between filtered and unfiltered supernatant. The final concentration of

TABLE I

Solubilization of the cannabinoid receptor using varying concentrations of CHAPS detergent

CHAPS/protein (w/w)	[CHAPS]	Protein solubilized	Receptor solubilized	Specific binding	Fold purification
	mM	%	%	%	
0.5:1	8	26.2 ± 8.8	58.0 ± 5.0	73.5 ± 8.5	2.7 ± 0.9
1:1	16	29.0 ± 8.5	40.3 ± 14.3	48.6 ± 11.3	1.5 ± 1.0
2:1	32	31.7 ± 9.4	12.9 ± 9.9	19.0 ± 15.1	0.5 ± 0.4

CHAPS during solubilization was 8 mM, which approximates the critical micellar concentration value of CHAPS (34). Concentrations of CHAPS as high as 5 mM in the assay had no effect on radioligand binding. To determine the effect of dilution on the solubilized binding activity, the detergent extract was diluted 5-fold with TM buffer and centrifuged at 100,000 × *g* for 30 min. No reduction in binding was observed in the supernatant, compared with controls that were not centrifuged, and no binding activity was precipitated as a result of dilution of the detergent.

Other detergents (Triton X-100, BIGCHAP, Zwittergent 3–12, and *n*-decylsucrose) were not able to solubilize [^{3}H]CP-55940 binding activity (data not shown). Earlier reports (35) indicated that addition of petroleum ether (20%) was necessary after the solubilization with CHAPS to observe optimal radioligand binding in the aqueous fraction. Subsequent studies found that this effect occurred at low (≤4 mM) concentrations of CHAPS. When the detergent concentration was increased to 8 mM, addition of petroleum ether had no effect on solubilized binding activity and so was eliminated from the solubilization protocol.

Characterization of the solubilized [^{3}H]CP-55940 binding site as the cannabinoid receptor. Equilibration of binding of the agonist [^{3}H]CP-55940 to the cannabinoid receptor in the detergent extract was essentially complete by 60 min at 30°, comparable to that observed in brain membranes (26). Nonlinear regression analysis of the hyperbolic data revealed a single class of binding sites exhibiting saturation kinetics, with a K_d of 0.94 ± 0.1 nM (Fig. 1). This value is identical to that obtained in parallel assays using rat brain membranes (35). Homologous equilibrium binding studies with increasing concentrations of CP-55940 yielded a curve having a K_i of 0.43 nM and a slope factor of 0.80 (data not shown), also consistent with a single class of binding sites.

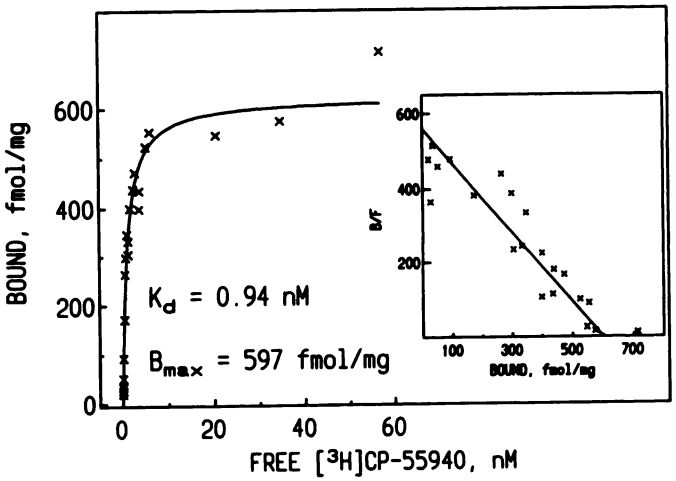

Fig. 1. [^{3}H]CP-55940 saturation curve in CHAPS-solubilized preparation derived from rat brain membranes. Increasing concentrations of radioligand were incubated with 100 μg of protein for 90 min at 30°. The concentration of total [^{3}H]CP-55940 was determined from a 20-μl aliquot taken from each assay tube before separation of free and bound radioligand. The free concentration of [^{3}H]CP-55940 was calculated by subtraction of bound radioligand from total radioligand. Nonspecific binding was determined in the presence of 200 nM DALN and the values were subtracted from total binding. Binding parameters were determined by nonlinear regression analysis according to a hyperbolic equation, using Enzfitter software. *Inset*, Scatchard transformation of the binding data. Data shown are combined from two separate experiments consisting of the average of triplicate determinations and are representative of two additional experiments.

To define further the [^{3}H]CP-55940 binding activity, several cannabinoid ligands were used in competition with the radioligand for binding. The order of potency for five well characterized cannabinoid agonists is shown in Fig. 2. Competition for the [^{3}H]CP-55940 binding site occurred in a dose-dependent manner with increasing concentrations of the ligands. Nonlinear regression analysis of heterologous equilibrium binding data indicated a single class of binding sites for the cannabinoid agonists (slope factors ranging from 0.88 to 1.27). The relative order of potency of these compounds for binding in detergent extracts is identical to that seen with the membrane-bound cannabinoid receptor (26), indicating that the site of ligand binding is the same in both membrane and solubilized preparations.

Interaction of the cannabinoid receptor with G proteins in detergent solution. The presence of guanine nucleotides is known to result in a decrease in maximal binding of agonists to G protein-coupled receptors (36). Previous characterization of the cannabinoid receptor in rat brain membranes provided evidence that guanine nucleotides can modulate agonist binding to the cannabinoid receptor (26). The observation of high affinity, specific binding of the radiolabeled agonist in detergent extracts indicated that the cannabinoid receptor was functionally coupled to a G protein(s). The effect of GTP and the nonhydrolyzable analogs GTPγS and Gpp(NH)p on the binding of [^{3}H]CP-55940 to the solubilized receptor is shown in Fig. 3. Each guanine nucleotide resulted in a decrease of agonist ligand binding of approximately 75%. An interesting observation was the slightly better fit of the parameters to a two-site binding model, suggesting that possibly multiple types of G proteins are coupled to the cannabinoid receptor. Alternatively, detergent solubilization could have altered the stoichiometry of receptors and G protein components such that a new equilibrium of high and low affinity interactions exists at higher concentrations of guanine nucleotides. However, when

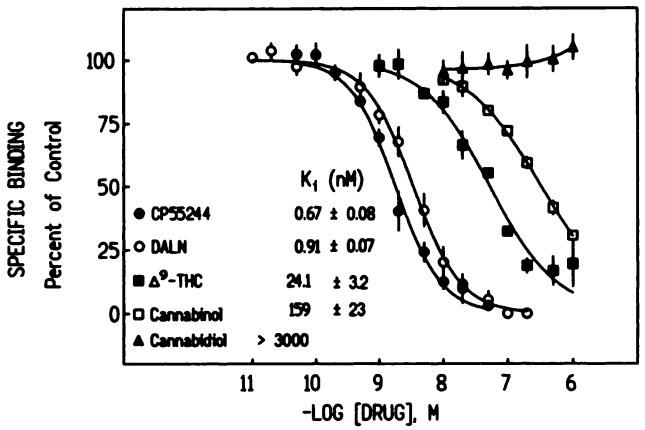

Fig. 2. Equilibrium binding of cannabinoid agonists to the solubilized cannabinoid receptor. Cannabinoid receptors were solubilized from rat brain membranes and then assayed for radioligand binding using 30 μg of protein/assay tube and a concentration of [^{3}H]CP-55940 of 0.7 nM. The assay was incubated at 30° for 90 min, after which bound and free radioligand were separated by gel filtration on Sephadex G50 at 4°. Nonspecific binding was determined in the presence of 200 nM DALN. Data were analyzed using nonlinear regression analysis (Inplot). Each point is the mean ± standard error of three individual experiments consisting of triplicate determinations. K_i values are reported as the mean ± standard error for the three experiments.

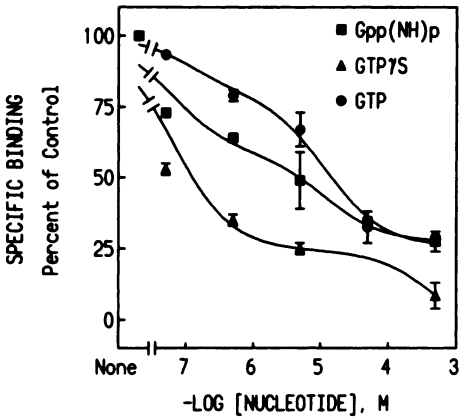

Fig. 3. Effect of increasing concentrations of guanine nucleotides on specific binding of [^{3}H]CP-55940 to the solubilized cannabinoid receptor. Equilibrium binding data were analyzed by nonlinear regression analysis according to a sigmoidal equation iterating on a one-site or two-site model. In all curves, binding parameters were slightly better fit to a two-site binding model. Each point is the average ± standard deviation of two individual experiments consisting of triplicate determinations.

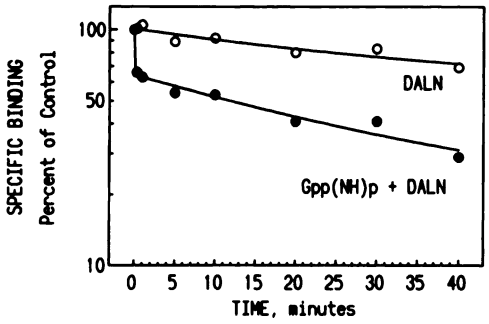

Fig. 4. Effect of Gpp(NH)p on DALN-induced dissociation of [^{3}H]CP-55940 from the solubilized cannabinoid receptor. [^{3}H]CP-55940 was allowed to equilibrate with solubilized receptor for 90 min at 30° in a total volume of 6 ml (concentrations of assay components were the same as in the standard assay), after which the reaction mixture was cooled to 4°. An aliquot (75 μl) was removed from the assay mixture and gel filtered to provide a control (time 0) before any additions. A 1-ml aliquot of the assay mixture was rapidly brought to a final concentration of 200 nM DALN alone or 200 nM DALN plus 100 μM Gpp(NH)p. At timed intervals, 75-μl aliquots were removed and filtered on G50 Sephadex columns. The void volume was eluted by a single 1.6-ml addition of column buffer. In this manner, multiple time courses could be completed from one stock of pre-equilibrated receptor. Each point is the mean of triplicate time courses and is similar to results from two additional experiments.

analyzed by use of an F test the difference between the curves generated by one- and two-site binding models was not statistically significant.

Occupation of G protein-coupled receptors by agonists results in an increase in the rate of exchange of GTP for GDP, with subsequent dissociation of the α and $\beta\gamma$ subunits of the G protein heterotrimer. This coincides with a decrease in the affinity of the receptor for agonist ligands (36). As further evidence for an association of G protein(s) with the cannabinoid receptor, solubilized receptor that had been previously equilibrated with [^{3}H]CP-55940 was mixed with the nonhydrolyzable guanine nucleotide Gpp(NH)p, and bound [^{3}H]CP-55940 was separated by gel filtration at timed intervals (Fig. 4). The addition of 200 nM DALN produced a slow ($t_{1/2}$ = 57 ± 11 min) dissociation of the radioligand from the receptor at 4°. When 100 μM Gpp(NH)p was included with the DALN, the rate of radioligand dissociation was biphasic, exhibiting one component with a significantly increased rate ($t_{1/2}$ < 1 min) and a second component with a rate comparable to that measured in the presence of DALN alone. Due to the procedure by which free radioligand and bound radioligand are separated, the Gpp(NH)p-induced increase in the rate of dissociation may be artifactually low, because ligand-bound receptor and Gpp(NH)p are not completely separated on the gel filtration columns for at least 1 min. Specific binding recorded 5 sec after addition of Gpp(NH)p was similar to the 1-min values.

The kinetic data have indicated that G protein(s) are able to regulate the affinity state of the cannabinoid receptor in detergent solution. We have conversely developed evidence that the cannabinoid receptor can modify the function of certain G proteins, as determined by their sensitivity to ADP-ribosylation by pertussis toxin. Fig. 5 demonstrates the pertussis toxin-dependent ADP-ribosylation of a band in the range of apparent molecular weight of 39,000–41,000 (Fig. 5, *lanes 2* and *3*), which appears upon densitometry to be a single peak with a shoulder on the higher molecular weight side. The density of this band was reduced by 44% when the incubation was conducted in the presence of GTP (Fig. 5, *lane 4*). This is consistent with the observation by others that the heterotrimer is the form that is ADP-ribosylated and that GTP or its analogs would allow the dissociation of the complex to a form that is no longer susceptible to pertussis toxin (37, 38). In the presence of the cannabinoid agonist DALN, the density of the band was reduced either in the absence (Fig. 5, *lane 5*) or in the presence (Fig. 5, *lane 6*) of GTP. These findings would be consistent with the cannabinoid agonist-receptor complex functionally interacting with a population of G proteins in detergent solution to promote the dissociation of the heterotrimer. The decrease in ADP-ribosylation observed with DALN in the absence of GTP might be explained if phosphorylation of tightly bound GDP occurred during the incubation. It is possible that nucleotide diphosphate kinase activity may utilize the ATP present in the incubation mixture for such a conversion. Alternatively, the binding of the agonist-receptor complex to the G protein heterotrimer may stabilize the complex in the absence of GTP and block the site of ADP-ribosylation near the carboxyl terminus of the G protein α subunit.

Discussion

The hydrophobic nature of cannabinoid drugs has promoted the notion that membrane interactions are a necessary component of the ligand-receptor interaction. Investigations of cannabinoid effects on ligand binding assays within the G

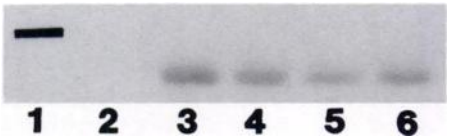

Fig. 5. ADP-ribosylation of pertussis toxin substrates in the CHAPS extract of brain membranes. ADP-ribosylation was carried out with the following additions to the reaction mixture: pertussis toxin, *lanes 3–6*; 100 μM GTP, *lanes 4* and *6*; 1 μM DALN, *lanes 5* and *6*. *Lane 1*, low molecular weight marker standards (Bio-Rad); the position of hen egg white ovalbumin (M_r 45,000) is marked. Areas under the curve of absorbance units were calculated after subtraction of the absorbance value from *lane 2*. These values were as follows: *lane 3*, 1.829; *lane 4*, 1.215; *lane 5*, 0.865; *lane 6*, 1.278. This experiment was repeated, with qualitatively similar results.

protein-coupled receptor class have uncovered what appears to be an influence of these drugs that is attributable to alterations in membrane fluidity or some other hydrophobic interaction and is apparent at high concentrations of these compounds (21–23). Clearly, cannabinoid compounds are able to influence cell membrane fluidity, as demonstrated by fluorescence polarization determinations using multiple probes (14), and these effects can influence the coupling of receptors to effectors including adenylate cyclase (24, 25). Small-angle X-ray diffraction and solid-state ^2H NMR analyses of cannabinoid interactions with model membranes have demonstrated that cannabinoid ligands are positioned with the long axis of the tricyclic molecule perpendicular to the lipid chains, being constrained at the polar interface by hydroxyl groups (17, 18, 39). These studies have led to a proposal that cannabinoid ligands must attain a permissible conformation within the membrane bilayer in order to promote a functional interaction with the cannabinoid receptor (19, 40). Such a model assumes that the ligand-receptor interaction occurs at the receptor-lipid interface and that the ligand-membrane interaction is necessary for the ligand-receptor interaction.

Data presented here demonstrate that cannabinoid binding occurs in the absence of the membrane environment. The properties of the ligand-receptor interaction in solution do not differ from structure-function relationships observed for the cannabinoid receptor in its membrane-associated state. This indicates that this receptor recognizes specific ligands by the same criteria irrespective of the extraprotein environment. Furthermore, detergent solubilization could be expected to perturb the annular lipids surrounding the receptor protein. If these tightly associated lipids were integral to the binding of cannabinoid ligands or to the mechanism whereby the ligand regulates receptor-G protein coupling, one would have expected an alteration of the binding properties in the presence of detergent. Many other G protein-coupled receptors interact with their ligands via contact within the pore formed within the seven transmembrane segments [see review by Dohlman *et al.* (41)].

G protein-coupled receptors have been solubilized using various types of detergent and conditions differing in ionic strength and the presence or absence of Mg^{2+}. In most instances of successful receptor solubilization, the selection of an appropriate detergent is most important. This choice is perhaps more important when applied to the cannabinoid receptor. The absence of high affinity antagonists that could be used to assay the receptor regardless of the degree of coupling to G proteins imposes a limitation on the method of solubilization. Association of the receptor with G proteins in detergent solution must be maintained for high affinity [^3H]CP-55940 binding to occur and for subsequent evaluation of the biochemical and pharmacological properties of the solubilized receptor. The selection of CHAPS as the detergent of choice for our studies is based on the successful solubilization of several other G protein-receptor complexes (30–33, 42–44). In many instances, soluble receptors exhibiting high affinity binding could not be obtained unless the receptor was prelabeled with an agonist ligand (45–47). Apparently, receptor occupation by agonist ligands stabilizes the receptor-G protein complexes during the solubilization procedures, after which the ligand can be removed from the receptor by dialysis or gel filtration. In one instance, the A_1 adenosine receptor was solubilized in the presence of excess EGTA and still exhibited high affinity agonist binding (43). However, membrane preparations produce substantial quantities of adenosine, which may have some affinity for the receptor even in the absence of Mg^{2+} and could offer some stabilization during detergent extraction. Our data indicate that prior equilibration of membranes with cannabinoid ligands is not necessary to obtain soluble cannabinoid receptor in the high affinity binding state.

Effective solubilization of the cannabinoid receptor has demonstrated that the protein can functionally interact with G proteins in CHAPS detergent solution. The heterogeneity observed in the kinetic studies suggests that the interaction of solubilized rat brain cannabinoid receptor with G protein becomes more complex upon solubilization. Evidence suggests that these G proteins could comprise G_i subtype(s) that regulate adenylate cyclase (2, 3) and pertussis toxin-sensitive G proteins that regulate Ca^{2+} (10) or K^+ (9) currents.

One question that arises is whether cannabinoid receptors are solubilized in a state of physical association with the G protein. Ligand affinity purification of certain G protein-coupled receptors has indicated that G proteins can remain tightly associated with their receptors and coelute from affinity columns upon the addition of competing ligands or guanine nucleotides (43, 44, 48). It is clear from the present data that uncoupling of the receptor from its attendant G protein by incubation in the presence of a GTP analog disrupts the receptor-ligand interaction. Guanine nucleotides appear to decrease ligand binding to the soluble cannabinoid receptor (75%) more so than to the membrane-bound receptor (40%) as shown for other receptor systems (42, 49). However, it is evident that a percentage of the population of receptors bind to the ligand in the presence of excess guanine nucleotide. Perhaps this represents a new equilibrium of ligand binding to receptors in a low affinity state. Because the presence of the high affinity state is important for optimal assay of the receptor through purification steps, the development of protocols for purification may be restricted to those conditions that preserve the receptor-G protein copurification. Modification of receptor solubilization protocols may be of interest for several additional reasons. Isolation of a receptor-G protein complex can determine the specificity of receptor-G protein interactions. Agonist binding is often complicated by differences in binding affinity states. The identification of the G protein composition associated with these different states could give further insight into the mechanisms of action of the cannabinoid receptor.

In this report we have provided the first evidence that the cannabinoid receptor can be solubilized in a form that retains high affinity binding. Our data, based on kinetic and pharmacological evidence, indicate that the [^3H]CP-55940 binding activity in solution is identical to that found in rat brain membranes. Our studies also indicate that this receptor is solubilized along with its associated G protein(s). In addition, we have described a rapid facile assay by which further characterization of the solubilized receptor can be accomplished. The solubilization of the cannabinoid receptor is an important initial step in the eventual purification of this receptor, as well as in furthering studies on structure-activity relationships between cannabinoids and the receptor without the constraints of the lipophilic membrane environment.

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