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Identification of the binding subunit of the σ-type opiate receptor by photoaffinity labeling with 1-(4-azido-2-methyl[6-^{3}H]phenyl)-3-(2-methyl[4,6-^{3}H]phenyl)guanidine

(1,3-di-*o*-tolylguanidine/benzomorphan opiates/phencyclidine/psychotomimesis)

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ABSTRACT **The σ-type opiate receptor is a distinct binding site in the brain that may mediate some of the psychotomimetic effects caused by benzomorphan opiates and phencyclidine in humans. We have developed a synthetic, highly selective ligand for this receptor, 1,3-di-*o*-tolylguanidine (DTG). To identify the binding protein(s) of the σ receptor, we have now synthesized a radiolabeled azide derivative of DTG, 1-(4-azido-2-methyl[6-^{3}H]phenyl)-3-(2-methyl[4,6-^{3}H]phenyl)-guanidine ([^{3}H]N_3DTG). In guinea pig brain membrane binding assays conducted in the dark, [^{3}H]N_3DTG bound reversibly, selectively, and with high affinity (K_d = 10 nM) to σ receptors. The drug specificity profile of reversible [^{3}H]-N_3DTG binding was identical to that of [^{3}H]DTG and ^{3}H-labeled (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine binding indicating that [^{3}H]N_3DTG is a selective σ receptor ligand. Guinea pig brain membranes were photoaffinity-labeled with [^{3}H]N_3DTG. NaDodSO$_4$/PAGE of detergent-solubilized membrane extract identified a single 29-kDa radioactive band. Sepharose Cl-6B gel chromatography of photolabeled brain membranes solubilized with the nondenaturing detergent sodium cholate showed a radioactive complex with a Stoke's radius of 4.6 nm (M_r, 150,000) that may represent the intact σ receptor complex. NaDodSO$_4$/PAGE of this complex showed that the radiolabeled material was a 29-kDa polypeptide that may be the binding subunit of the σ receptor. The specific σ receptor photoaffinity ligand described here should be a useful tool for purifying and characterizing the σ receptor.**

Synthetic morphine derivatives of the benzomorphan class cause hallucinations, disorientation, drunkenness, and other psychotomimetic effects in humans (1, 2). These effects are similar to those seen in phencyclidine (PCP or "angel dust") intoxication (3). In addition, benzomorphans and PCP have similar behavioral and autonomic effects in animals (4–9). The molecular characterization of the brain receptors with which benzomorphans and PCP interact is of considerable importance since the psychotomimetic symptoms caused by these drugs are similar to those seen in paranoid schizophrenia. Indeed, PCP has been proposed to be a useful drug model for schizophrenia (3).

Radioligand binding studies on rodent brain membrane suspensions have identified the existence of two separate drug binding sites that are candidates for mediating some of the psychotomimetic effects of benzomorphans and PCP. These binding sites are the σ receptor (10–14) and the PCP receptor, respectively (14–17). Both sites interact with PCP and benzomorphans. However, the σ receptor has a higher affinity for benzomorphans than for PCP, and the PCP receptor has a higher affinity for PCP than for benzomorphans. The main pharmacological difference between the two sites is that the σ receptor binds haloperidol as well as many other neuroleptic drugs, whereas the PCP receptor is insensitive to neuroleptics.

Some light has been shed on the physiological function of PCP and, possibly, σ receptors: The PCP receptor may be identical to a Na^+/Ca^{2+}-type ion channel that is coupled to the *N*-methyl-D-aspartate-type glutamate receptor, since it has been shown that PCP and related drugs block this channel (18). In addition, PCP and *N*-methyl-D-aspartate binding sites have been shown to have a nearly identical neuroanatomical distribution (19). The function of the σ receptor, on the other hand, is less clear. However, it has been shown that σ receptor-selective drugs cause an increase in the electrically stimulated twitch response of isolated guinea pig (20) and mouse (21) vasa deferentia; in addition, it has been shown that σ receptor-selective drugs cause an increase in the electrically stimulated release of norepinephrine from the mouse vas deferens raising the possibility that σ receptors may be involved in stimulating catecholamine release (21).

To characterize the molecular properties of the σ receptor, we have synthesized a radiolabeled photoaffinity ligand, 1-(4-azido-2-methyl[6-^{3}H]phenyl)-3-(2-methyl[4,6-^{3}H]phenyl)guanidine ([^{3}H]N_3DTG), derived from the σ receptor ligand 1,3-di-*o*-tolylguanidine or 1,3-di-(2-methylphenyl)-guanidine (DTG). We report here that [^{3}H]N_3DTG selectively labels a single polypeptide of M_r 29,000 in guinea pig brain membranes as determined by NaDodSO$_4$/PAGE followed by fluorography. Under nondenaturing conditions on a Sepharose 6B column this polypeptide elutes as part of a M_r ≈150,000 complex. These results suggest that the binding subunit of the σ receptor is a distinct, brain membrane-associated binding protein and that this binding protein, as part of a larger complex, may participate in mediating some of the central actions of drugs that interact with σ receptors.

MATERIALS AND METHODS

Synthesis of N_3-Labeled DTG and [^{3}H]N_3DTG. The synthesis schemes used are shown in Fig. 1.

Synthesis of 1-(4-azido-2-methylphenyl)-3-(2-methylphenyl)guanidine (compound 8, Fig. 1). 1-(4-Amino-2-methylphenyl)-3-(2-methylphenyl)guanidine (compound **6**, Fig. 1) was prepared from compounds **1** and **2** by way of compound **3** (22) and then was converted into the dihydro-

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Abbreviations: DTG, 1,3-di-(2-methylphenyl)guanidine or 1,3-di-*o*-tolylguanidine; [^{3}H]N_3DTG, 1-(4-azido-2-methyl[6-^{3}H]phenyl)-3-(2-methyl[4,6-^{3}H]phenyl)guanidine; PCP, phencyclidine.

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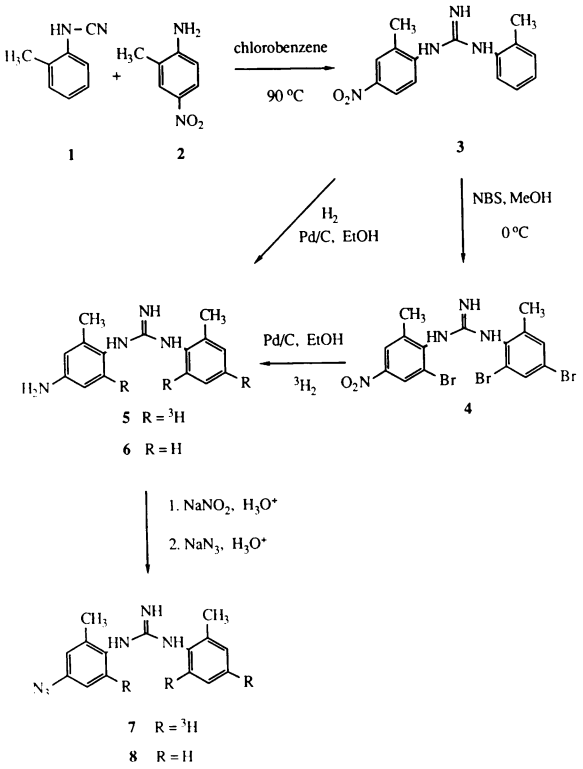

FIG. 1. Synthesis and structure of N_3-labeled DTG and [^{3}H]N_3DTG.

chloride, mp 285–288°C. A 412-mg (1.25 mmol) sample of the dihydrochloride was dissolved in water (4 ml) and concentrated dihydrochloric acid (0.385 ml, 4.60 mmol). The solution was cooled in an ice bath, and solid $NaNO_2$ (142 mg, 2.69 mmol) was added in a single portion. The now yellow solution was stirred at 0°C for 1 hr and then cooled to −10°C. Next, a solution of NaN_3 (109 mg, 1.69 mmol) in water (1.5 ml) was added dropwise over 15 min. The chilled solution was allowed to stir for 45 min and then to warm to 25°C. This solution was extracted with ether, and then the aqueous phase was basified with 5 M NaOH (1.54 ml, 7.6 mmol), causing a yellow precipitate to form. The mixture was extracted with ether, and the combined extracts were washed with brine, dried ($MgSO_4$), and concentrated to dryness to give an orange oil. This was dissolved in ethanol (10 ml), and then water (8 ml) was added, precipitating the crude azide (215 mg, 65%) as a tan powder, mp 116–118°C. A 180-mg portion was crystallized from ethanol/water, 1:1 (vol/vol) (6 ml). The needle crystals were collected, dissolved in ethanol (2 ml), and filtered through a 0.5-cm column of activated charcoal on a bed of Celite. On diluting the filtrate with water (0.5 ml), the analytical sample of compound **8** (34 mg; 12% based on compound **6**) crystallized as off-white needles, mp 123–124°C (dec). ^{1}H NMR ($C^2H_3O^2H$) δ 2.269 (s, 3), 2.272 (s, 3), 6.848 (dd, 1, J = 2.7, 8.4 Hz), 6.914 (d, 1, J = 2.1 Hz), 7.004 (td, 1, J = 1.5, 7.2 Hz), 7.136–7.229 (m, 4); IR (KBr) 2123 cm^{-1} (strong, azide). Anal. Calcd. for $C_{15}H_{16}N_3$: C, 64.27; H, 5.75; N, 29.98. Found: C, 63.87; H, 5.59; N, 29.67.

Synthesis of 1-(6-bromo-2-methyl-4-nitrophenyl)-3-(4,6-dibromo-2-methylphenyl)guanidine (compound 4, Fig. 1). *N*-Bromosuccinimide (425 mg, 2.39 mmol) was added to a stirred suspension of 1-(2-methyl-4-nitrophenyl)-3-(2-methylphenyl)guanidine (compound **3**, Fig. 1) [222 mg, 0.781 mmol; prepared as described (22)], in MeOH (3 ml) at 0°C. After stirring for 2 hr the brown slurry was diluted with MeOH (1 ml) and stirred at 25°C for 1 hr. Filtration gave a brown solid (192 mg), a 131-mg portion of which was crystallized from acetone by dropwise addition of water, to give compound **4** (77 mg, 28%) as brown needles, mp 198–200°C. Sublimination of these crystals at 155–160°C/0.01 mm gave compound **4** (40 mg) as a bright yellow powder, mp 210–213°C. ^{1}H NMR ($C^2H_3O^2H$) Δ 2.357 (s, 3), 2.488 (s, 3), 7.444 (d, 1, J = 1.5 Hz), 7.669 (d, 1, J = 1.8 Hz), 8.033 (d, 1, J = 2.1 Hz), 8.267 (d, 1, J = 2.4 Hz). Anal. Calcd. for $C_{15}H_{13}Br_3N_4O_2$: C, 34.58; H, 2.52; N, 10.75. Found: C, 34.64; H, 2.47; N, 10.65.

Synthesis of (4-amino-2-methyl[6-^{3}H]phenyl)-3-(2-methyl-[4,6-^{3}H]phenyl)guanidine (compound 5, Fig. 1). A 25-mg sample of the tribrominated nitro derivative (compound **4**) was submitted to Amersham for catalytic reduction in the presence of 50 Ci (1 Ci = 37 GBq) of $^3H_2$ gas. This replaced the bromide atoms by ^{3}H and at the same time reduced the nitro group to an amino group. Excess concentrated HCl was added to convert the resulting ^{3}H-labeled amine (compound **5**) into its chemically more stable dihydrochloride for storage. The sample was stored in ethanol/water, 1:1 (vol/vol), at −70°C.

Two- to 5-mCi portions of the crude radioactive product were purified by reversed-phase HPLC on a Vydac 218TP54 C_{18} column (The Separations Group, Hesperia, CA) by using a 30-min linear gradient of 0–50% CH_3CN in 0.1% trifluoroacetic acid. The flow rate was 1 ml/min, and 1-min fractions were collected with a fraction collector. The HPLC equipment consisted of two Waters model 510 pumps, a Waters gradient controller, and a Kratos Spectroflow 757 flow-through spectrophotometer operating at 220 nm. A UV-absorbing radioactive peak eluted at 15.2 min, identical to the elution time of the unlabeled amine (compound **6**) (22). The specific radioactivity of the amine (compound **5**) was found to be 102 Ci/mmol, based on the amount of material under the UV-absorbing peak as determined by spectrophotometry and on the radioactivity associated with this peak as determined by liquid scintillation spectrometry.

Synthesis of [^{3}H]N_3DTG (compound 7, Fig. 1). A 2-mCi portion of the HPLC-purified ^{3}H-labeled amine (compound **5**) was evaporated to dryness, dissolved in 40 μl of 3 M acetic acid, and cooled to 0°C. A solution of 10 μg of $NaNO_2$ in 2 μl of 3 M acetic acid was added. After incubation on ice for 15 min, 10 μg of NaN_3 in 2 μl of 3 M acetic acid was added, and the reaction was allowed to proceed in the dark on ice for another 15 min. The resulting solution was then subjected to reversed-phase HPLC purification with the same chromatography conditions described above for the purification of the ^{3}H-labeled amine (compound **5**). A major radioactive peak coincided with a major UV-absorbing peak eluting at 26.6 min. The fraction containing this peak was collected in subdued light and stored in the dark at −70°C until used.

To confirm the identity of this peak, 1 mg of unlabeled amine (compound **6**) was subjected to a scaled-up azide conversion protocol as described above. Reversed-phase HPLC purification of the reaction mixture yielded a UV-absorbing peak with the same retention time as that of the radiolabeled product. The 300-MHz ^{1}H NMR spectrum of this material was identical to that of the analytical sample of unlabeled azide (compound **8**).

σ Receptor Reversible Binding Assays. The basic binding assay protocol used to characterize the reversible binding of [^{3}H]N_3DTG has been described (12). Briefly, frozen guinea pig brains (Biotrol, Indianapolis) were homogenized with a Polytron homogenizer in 10 vol (wt/vol) of ice-cold 0.32 M sucrose. The homogenate was spun for 10 min at 900 × *g* at 4°C in a Sorvall RC5B centrifuge. The supernatant was collected and recentrifuged at 4°C at 13,700 × *g* for 40 min. The pellet was washed with 50 mM Tris·HCl (pH 7.4) (Tris) and centrifuged again at 13,700 × *g*. The final pellet was resuspended in Tris at a protein concentration of 3 mg/ml

and stored frozen at −70°C. For binding assays, the frozen membrane suspension was thawed and diluted 1:6 with Tris. To a 12 × 75 mm culture tube was added 800 μl of the membrane suspension, 100 μl of [^{3}H]N_3DTG in Tris, and 100 μl of Tris alone or Tris with known concentrations of various unlabeled drugs. The final protein concentration was 400 μg/ml. Nonspecific binding was defined as that remaining in the presence of 10 μM haloperidol. After incubation in the dark at room temperature for 2 hr, the membrane suspension was diluted to 5 ml and rapidly filtered over Whatman GF/B glass fiber filters followed by two washes with 5 ml of Tris by using a Brandel (Gaithersburg, MD) 48-well cell harvester. Filters were dissolved in 10 ml of Cytoscint (Westchem Products, San Diego, CA), and radioactivity was measured in a liquid scintillation counter with an efficiency of 50%. Scatchard analyses were done on an IBM AT personal computer by using the EBDA data analysis program (23).

Photoaffinity Labeling of Guinea Pig Brain σ Receptors. The brain membrane suspensions used for photoaffinity labeling of σ receptors were prepared as described above except that the final protein concentration was 800 μg/ml. The presence of protease inhibitors (1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, leupeptin at 5 μg/ml, and bacitracin at 2.5 μg/ml) throughout the membrane preparation and incubation had no effect on labeling efficiency or specificity. In a typical experiment, 1-ml aliquots of membrane suspension were incubated in the dark for 2 hr at room temperature with 10 nM [^{3}H]N_3DTG alone or in the presence of various unlabeled drugs to determine specificity of photoaffinity labeling. The suspensions were then rapidly filtered through a 2.4-cm Whatman GF/B glass fiber filter by using a Hoefer (San Francisco) FH224V filtration apparatus and washed with two 5-ml aliquots of Tris. The filters were then placed on ice and exposed to long wavelength (366 nm) UV light by using a 500-W UV lamp (Sunjet 400T deluxe, Electrolux-Kern GmbH, Göttingen, F.R.G.) at a distance of 10 cm for 15 min. Membrane proteins were solubilized from the filters by incubating in 2 ml of either 0.2% $NaDodSO_4$ or 20 mM sodium cholate in Tris for 4 hr at 4°C with constant shaking. The detergent-solubilized material was then centrifuged at 105,000 × *g* for 1 hr.

$NaDodSO_4$/PAGE and Sepharose Cl-6B Chromatography. Following photoaffinity labeling, $NaDodSO_4$-solubilized membrane proteins were subjected to electrophoresis in a 12% discontinuous gel (24). Gels were electrophoresed at 30 mA for 4 hr followed by fixing in a solution of 50% (vol/vol) methanol and 10% (vol/vol) acetic acid and staining with Coomassie blue R-250. The fixed and stained gels were impregnated with EN^{3}HANCE (New England Nuclear) and exposed to x-ray film (Kodak X-Omat AR). Alternatively, the distribution of radioactivity in the gels was determined by counting 5-mm gel slices after a 24-hr incubation in Protosol (New England Nuclear) at 50°C. Gel permeation chromatography of cholate-solubilized membrane proteins was done on a 2 × 100 cm Sepharose Cl-6B column (Pharmacia) equilibrated at room temperature in Tris containing 20 mM sodium cholate at a flow rate of 20 ml/hr. Fractions (2.5 ml) were collected, 1-ml aliquots were dissolved in 12 ml of Aquasol scintillation fluid (New England Nuclear), and radioactivity was measured.

RESULTS AND DISCUSSION

In the dark [^{3}H]N_3DTG bound reversibly, selectively, specifically, saturably, and with high affinity to guinea pig brain membrane suspensions. In a typical binding assay with 95,000 cpm of [^{3}H]N_3DTG, 15,000 cpm was bound, whereas in the presence of 10 μM haloperidol 1500 cpm remained bound indicating that specific binding was ≥90% of total binding. No difference in nonspecific binding was seen by using 10 μM DTG as nonspecific control. Equilibrium of [^{3}H]N_3DTG binding was reached by 2 hr at room temperature. Equilibrium saturation binding analysis showed that [^{3}H]N_3DTG bound saturably to guinea pig brain membrane suspensions (Fig. 2). Scatchard analysis of the binding resulted in a linear Scatchard plot with a K_D of 10 nM and a B_{max} of 5.3 pmol/mg of protein.

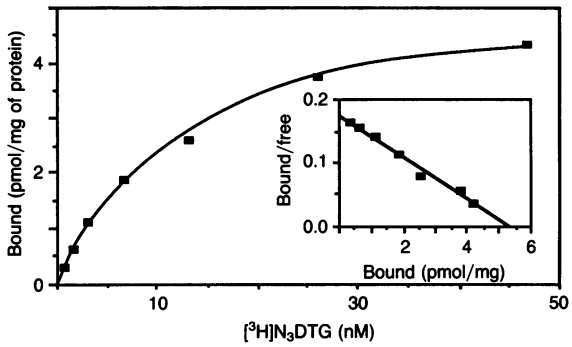

FIG. 2. Equilibrium saturation binding of [^{3}H]N_3DTG to guinea pig brain membranes. Membranes were incubated in the dark for 2 hr at room temperature with various concentrations of ligand. Values are means of triplicate determinations. (*Inset*) Scatchard plot of saturation binding data.

A drug specificity profile of [^{3}H]N_3DTG binding by using various unlabeled drugs considered to be typical σ-receptor ligands (12) showed that [^{3}H]N_3DTG labels the same binding sites as [^{3}H]DTG: As shown in Table 1, the K_i values of the various drugs in displacing [^{3}H]N_3DTG binding were very similar to those found for displacement by the same drugs of [^{3}H]DTG. Indeed, the correlation of the K_i values of the various drugs in the two binding assays was high (r = 0.97). A high correlation (r = 0.92) was also observed between [^{3}H]N_3DTG and the σ-selective ligand ^{3}H-labeled (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine (data not shown).

Table 1. K_i values of σ receptor active drugs against [^{3}H]N_3DTG and [^{3}H]DTG

Drug	K_i, nM: Against [^{3}H]N_3DTG	Against [^{3}H]DTG
N_3-labeled DTG	10 ± 0.7*	19 ± 0.4
Haloperidol	5 ± 0.3	5 ± 0
DTG	27 ± 2	26 ± 2*
Perphenazine	123 ± 15	41 ± 10
(+)-Pentazocine	45 ± 9	42 ± 2
(−)-Pentazocine	128 ± 13	131 ± 4
(+)-Butaclamol	1877 ± 196	2087 ± 240
(−)-Butaclamol	229 ± 32	514 ± 89
(+)-3-PPP	61 ± 2	74 ± 4
(−)-3-PPP	332 ± 20	272 ± 21
(+)-Cyclazocine	123 ± 16	317 ± 42
(−)-Cyclazocine	2554 ± 223	2573 ± 240
(+)-SKF 10,047	554 ± 43	607 ± 86
(−)-SKF 10,047	3815 ± 446	3884 ± 549
TCP	946 ± 50	1577 ± 395
PCP	938 ± 33	1020 ± 103
Trifluprom azine	566 ± 39	588 ± 65
Chlorpromazine	553 ± 75	1432 ± 257
Amitriptyline	280 ± 24	292 ± 8
Imipramine	379 ± 35	505 ± 14

Values are mean ± SEM, n = 3 or 4. (+)- and (−)-3-PPP, (+)- and (−)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; TCP, *N*-[1-(2-thienyl)cyclohexyl]piperidine; SKF 10,047, *N*-allylnormetazocine.
*K_d values.

Thus, under reversible binding conditions, [^{3}H]N_3DTG is a highly specific σ receptor radioligand.

Having established the binding selectivity of [^{3}H]N_3DTG, we tested the ability of [^{3}H]N_3DTG to covalently label σ receptor binding protein(s) in guinea pig brain membranes. Incubation of brain membranes with [^{3}H]N_3DTG followed by washing and exposure to UV light was performed. Solubilization of the derivatized membranes followed by $NaDodSO_4$ electrophoresis and fluorography of the gels revealed that the photolabel was selectively incorporated into a single band of M_r 29,000 (Fig. 3).

Labeling of this band was unaffected by the presence of 10 μM morphine in the incubation mixture but could be completely blocked by the σ ligands haloperidol, (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine, DTG, or (+)-pentazocine at 10 μM (Fig. 3). Dopamine, serotonin, scopolamine, and γ-aminobutyric acid were ineffective at blocking incorporation of label at the same concentration (data not shown).

When the sodium cholate-solubilized photolabeled brain membrane extracts were chromatographed on a Sepharose Cl-6B sizing column in 20 mM sodium cholate as described above, the radioactivity was associated with a major peak containing molecules with a Stoke's radius of 4.6 nm (M_r, 150,000, uncorrected for bound detergent) (Fig. 4). Some noncovalently associated [^{3}H]N_3DTG or a breakdown product eluting in the total volume of the column was also detected. When the material under the M_r ≈150,000 peak was concentrated and subjected to $NaDodSO_4$/PAGE, and the radioactivity in the gel slices was measured, the radioactivity was predominantly associated with a peak of M_r 29,000 (Fig. 5). No M_r ≈150,000 peak was seen under those conditions.

These results demonstrate that the binding subunit of the σ receptor is a distinct M_r 29,000 polypeptide. This polypeptide is apparently membrane bound as detergent is required to solubilize it. This is an important observation since it raises the possibility that this subunit may participate in mediating some of the actions of σ-receptor-active drugs in the brain.

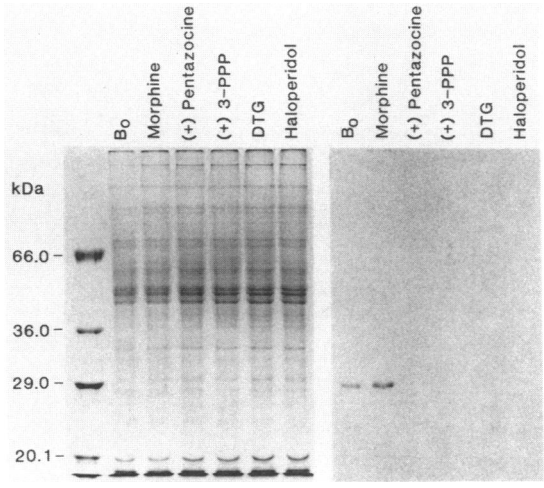

FIG. 3. $NaDodSO_4$/polyacrylamide gel electrophoresis of solubilized guinea pig brain membrane proteins following photoaffinity labeling as indicated. Aliquots (1 ml, 800 μg of protein) were incubated with 10 nM [^{3}H]N_3DTG in the presence or absence of various unlabeled drugs at a concentration of 10 μM, followed by washing, irradiation, and solubilization with $NaDodSO_4$. (*Left*) Coomassie blue staining. (*Right*) Fluorography of gel after treatment with EN^{3}HANCE and exposure to Kodak X-Omat AR film for 14 days at −70°C, showing selective incorporation of label into a M_r 29,000 polypeptide. Molecular weight markers (Sigma) were trypsin inhibitor (20.1 kDa), carbonic anhydrase (29 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), and bovine serum albumin (66 kDa). (+)-3-PPP, (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine.

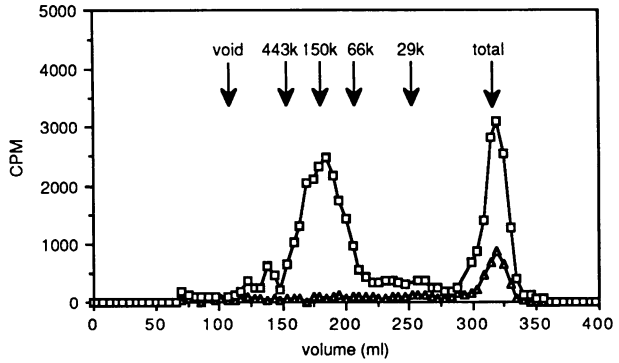

FIG. 4. Sepharose Cl-6B chromatography of photoaffinity labeled guinea pig brain membrane proteins showing specific labeling of a M_r ≈150,000 complex. Proteins were solubilized after labeling with 20 mM sodium cholate/Tris. Membranes were incubated with 10 nM [^{3}H]N_3DTG in the presence (△) or absence (□) of 10 μM haloperidol. Molecular weight standards were tryptophan, carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), apoferritin (443 kDa), and blue dextran (Sigma).

The results presented here also suggest that the M_r 29,000 protein is part of a larger protein complex, since under nondenaturing conditions, this polypeptide migrates as part of a M_r ≈150,000 protein complex. Treatment with $NaDodSO_4$ causes separation of the M_r 29,000 polypeptide from its other associated protein(s). This finding suggests that the σ receptor binding subunit is noncovalently assoc ated with other subunit(s). No disulfide bridges seem to involved here since $NaDodSO_4$ electrophoresis of photol ed membrane extract without prior reduction shows ame M_r 29,000 polypeptide as seen under reducing con s (data not shown). It will be important to determine wh the M_r ≈150,000 complex corresponds to the function ctive σ receptor.

The σ receptor whose binding subunit has be ntified here can be pharmacologically distinguished fr e PCP receptor (25, 26). Both binding sites are can tes for mediating some of the psychotomimetic effects benzomorphans and PCP. A ^{3}H- and azido-labeled PCP-derived photoaffinity ligand has been shown to label a number of polypeptides in rat brain membrane suspensions (27). These included a 90-kDa polypeptide whose labeling was potently blocked by PCP-related drugs as well as a 33-kDa polypep-

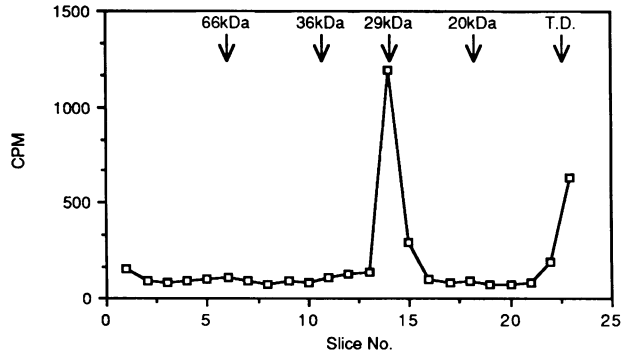

FIG. 5. $NaDodSO_4$/polyacrylamide gel electrophoresis demonstrating that photoaffinity-labeled cholate-solubilized receptor is composed of radiolabeled M_r 29,000 polypeptide in a noncovalently associated complex. The M_r ≈150,000 radioactive peak fractions from Sepharose Cl-6B chromatography (Fig. 4) were pooled, concentrated, and electrophoresed on a 12% discontinuous gel. Gel slices (5 mm) were incubated in 10% Protosol in Aquasol (New England Nuclear) overnight at 50°C, and then radioactivity was measured.

tide whose labeling was blocked less effectively by PCP-related drugs but was strongly inhibited by the benzomorphan (±)-*N*-allylnormetazocine, for which the σ receptor has a higher affinity (27). It is not clear at present whether the 33-kDa PCP binding polypeptide from rat brain corresponds to the 29-kDa σ binding subunit identified here in guinea pig brain by using [^{3}H]N_3DTG. This ligand should be a useful tool to further characterize the structural and molecular properties of the σ receptor and its associated proteins and to investigate its relation to PCP binding sites in the brain.

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