Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 594–598, January 1985
Neurobiology

Purification and characterization of the μ opiate receptor from rat brain using affinity chromatography

(receptor purification/hybromet)

RHODA MANECKJEE*, R. SUZANNE ZUKIN*†, SYDNEY ARCHER‡, JOSEPHINE MICHAEL‡, AND PETER OSEI-GYIMAH‡

*Departments of Biochemistry and Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461; and ‡Department of Chemistry, Cogswell Laboratory, Rensselaer Polytechnic Institute, Troy, NY 12181

Communicated by Michael V. L. Bennett, September 12, 1984

ABSTRACT **Opiate receptors have been solubilized from rat neural membranes and purified 500-fold (relative to the crude solubilized extract) by affinity chromatography. Active receptors were solubilized by using 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), a zwitterionic derivative of cholic acid. Affinity chromatography was carried out using Affi-Gel 401, a sulfhydryl derivative of agarose to which "hybromet," a newly synthesized opioid ligand with high affinity for the μ receptor, had been attached. Scatchard analysis of [^{3}H]etorphine binding to the purified receptor revealed a single class of high-affinity sites (K_d = 1.4 nM; B_{max} = 2800 fmol/mg of protein). Half-maximal binding was achieved at ≈1 nM. Activity was markedly inhibited by protein modifying reagents, findings which suggest that the sites are proteinaceous. Opiate binding activity was also inhibited by the guanyl nucleotide GTP. Electrophoresis of the purified material under denaturing conditions revealed three subunits of molecular weights 94,000, 44,000, and 35,000. The inhibitory guanyl nucleotide binding protein (N_i) implicated in opiate action has been shown to be comprised of two subunits of molecular weights 42,000 and 35,000. Thus, the opiate receptor may be an aggregate of multiple protein components that may include a guanyl nucleotide binding protein.**

Biochemical, pharmacological, and behavioral studies provide compelling evidence that the actions of opiates and opioid peptides upon nervous tissue are mediated by interactions with μ, δ, and κ receptors (for reviews, see refs. 1 and 2). The receptors are membrane-associated and their integrity appears dependent upon protein (3), carbohydrate (4), and phospholipid (5) components. The molecular dimensions of the receptors have been estimated in prelabeling experiments and more directly in partially purified preparations. Experiments in which opiate receptors were prelabeled with the potent agonist [^{3}H]etorphine (6) or with μ-selective radiolabeled enkephalin derivatives (7) prior to solubilization afforded a M_r estimate for the native receptor complex of 300,000–400,000. More recently, solubilization studies of an active receptor species from rat brain (8–10), toad brain (11), and cell culture (12, 13) have provided similar molecular weight estimates and suggest that the receptor is an aggregate of multiple protein components.

Relatively little is known about the specific molecular properties of the three opiate receptor subtypes. Studies from our laboratory (10) indicate that the native μ and κ receptor complexes differ significantly in the macromolecular properties, including size, as estimated by molecular exclusion chromatography. In a study involving a highly selective, irreversible δ ligand, Klee *et al.* (13) showed that the active subunit of the δ receptor under denaturing conditions has a M_r of 58,000. On the basis of these studies, it is not possible to determine whether the μ, δ, and κ receptors represent distinct polypeptide species or different aggregational states of the same protein component.

Purification and characterization of brain opiate receptors provides a direct means to elucidate the molecular properties that distinguish the receptor subtypes. However, this has been difficult to achieve in part because of the lack of a tissue source highly enriched in these receptors. Attempts to purify δ opiate receptors from a neuronal cell line (13) and κ and μ receptors from toad and mammalian brain using a wheat germ agglutinin column (14) and from rat brain using an affinity column (9) have been reported. In the present study we have used the zwitterionic detergent 3-[(3-chol amidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) to solubilize opiate receptors in relatively high yield from rat neural membranes. We have achieved an ≈500-fold purification of the μ opiate receptor (relative to the crude CHAPS extract) by affinity chromatography.

MATERIALS AND METHODS

Materials. Male Sprague–Dawley rats were purchased from Charles River Breeding Laboratories. Etorphine, normorphine, and [^{3}H]etorphine (18 Ci/mmol; 1 Ci = 37 GBq) were generously donated by the National Institute on Drug Abuse. [^{3}H]Etorphine was shown to be >97% pure by thin-layer chromatography. Levorphanol was a gift from Hoffman–La Roche. Trypsin, polyethylene glycol (PEG) 6000, and GTP were purchased from Sigma; CHAPS was from Calbiochem. Affi-Gel 401, $NaDodSO_4$, electrophoresis-grade urea, *N,N,N′,N′*-tetramethylethylenediamine, ammonium persulfate, Coomassie brilliant blue R-250, and the kit for silver staining were purchased from Bio-Rad. Marker proteins used for molecular weight calibrations were purchased from Pharmacia. The ^{125}I radioiodination system for protein iodination was purchased from New England Nuclear.

Solubilization of Opiate Receptors from Brain Neuronal Cells. Solubilization of brain opiate receptors was carried out as described (10). P_2 (mitochondrial/synaptosomal) membranes were prepared from the brains, minus the cerebellum, of male Sprague–Dawley rats (150–200 g). The P_2 pellet was suspended in 2 vol of cold 10 mM Tris·HCl buffer (pH 7.5). CHAPS was added to a final concentration of 10 mM and the suspension was homogenized on ice in a ground-glass homogenizer (10 strokes). After incubation on ice for 15 min,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; hybromet, 7α-{1(*R*)-hydroxy-1-methyl-3-*p*-[4-(3′-bromomercuri-2′-methoxy-propoxy)-phenyl]-propyl}-6,14-*endo*-ethenotetrahydrothebaine; PEG, polyethylene glycol; DADLE, [D-Ala^2, D-Leu^5]enkephalin; EKC, ethylketocyclazocine.

†To whom reprint requests should be addressed.

the suspension was centrifuged at 105,000 × *g* for 60 min at 4°C. The resulting clear supernatant (CHAPS extract) was applied immediately to the affinity column or stored at −70°C. The CHAPS extract prepared in this way was assayed for opiate binding activity by using the PEG method (10) after a 1:10 dilution into Tris·HCl buffer (pH 7.5), as described in the legend to Table 2.

Synthesis of 7α-{(1*R*)-Hydroxy-1-Methyl-3-*p*-[4-(3′-Bromomercuri-2′-Methoxy-Propoxy)-Phenyl]-Propyl}-6,14-*Endo*ethenotetrahydrothebaine (Hybromet). The synthesis of hybromet **4** was carried out as shown in Fig. 1. *p*-Hydroxyphenylethyl alcohol was condensed with allyl bromide to give *p*-allyloxyphenylethyl alcohol. This alcohol was converted to a crystalline tosylate (mp, 39°C), which, on refluxing with sodium iodide in acetone solution, afforded the corresponding iodide. The latter was converted to the Grignard reagent **2**, which was allowed to react with 7α-acetyl-6,14-endoethenothebaine **6**, obtained by condensing thebaine with methyl vinyl ketone as described by Bentley *et al.* (15, 16) to give the crystalline adduct **3** (mp, 160–162°C). Mercuration in chloroform/methanol solution with mercuric acetate followed by treatment with potassium bromide gave the desired bromomercuri compound (mp, 117–113°C). The NMR and IR spectra as well as the elemental analyses of the new compounds agree with the structural assignments.

Purification of Active Opiate Receptors Using Affinity Chromatography. The CHAPS-solubilized receptors have been purified by specific adsorption on an affinity column consisting of Affi-Gel 401 (a sulfhydryl derivative of agarose) to which the opiate analog (hybromet) is attached. Specific elution of μ receptors was achieved by using the selective μ opioid normorphine (1 μM). For preparation of the column, preswollen Affi-Gel 401 was washed with 0.1 M $NaHCO_3$ on a glass fiber filter, suspended in 250 ml of 0.5 M NaCl, and filtered. The gel was resuspended in 15 ml of cold 0.1 M $NaHCO_3$; 40 mg of the synthetic ligand, hybromet, was added, and the suspension was shaken gently at 4°C for 16 hr. After incubation with the ligand, the gel was allowed to settle, excess buffer was removed, and the gel was taken up in 15 ml of fresh 0.1 M $NaHCO_3$. The gel was then poured into a column (1.5 × 3.5 cm; total volume, 6 ml) and equilibrated with 300 ml of 10 mM Tris·HCl buffer (pH 7.5) before use.

For purification of the receptors by affinity chromatography, 40–50 mg of CHAPS extract was incubated at 37°C for 30 min to facilitate the dissociation of endogenous opioid ligands. The sample was then applied to the column and eluted with 10 mM Tris·HCl buffer (pH 7.5) containing 1 mM

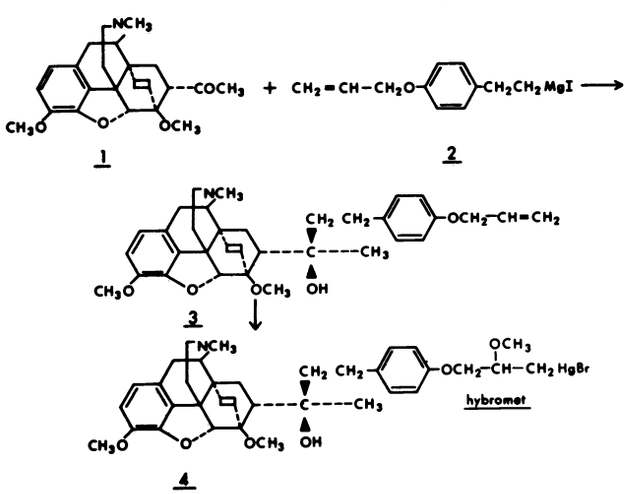

FIG. 1. Synthesis of hybromet.

CHAPS. Five-milliliter fractions were collected and the absorbance was monitored at 225 nm. When the absorbance approached zero, the opiate receptor was eluted from the column with 1 μM normorphine in 10 mM Tris·HCl (pH 7.5). The protein elution profile was monitored by absorbance at 225 nm. Opiate binding to the purified receptor and other fractions was measured by the PEG method (10). Details of this method are described in the legend to Table 2. Fractions exhibiting [^{3}H]-labeled opiate binding activity were pooled, concentrated, and dialyzed against 10 mM Tris·HCl (pH 7.5) (in order to remove normorphine) in collodion bags for 24 hr at 4°C. Protein concentrations were determined by the method of Lowry *et al.* (17) using bovine serum albumin as standard or were monitored in column eluates by measuring absorbance at 225 nm.

Polyacrylamide Gel Electrophoresis (PAGE). The purified receptor preparations were examined under nondenaturing conditions by PAGE using a 5% resolving gel in the presence of 1 mM CHAPS at 4°C according to the method of Davis (18). Samples (≈40 μg) were analyzed under denaturing conditions by $NaDodSO_4$/PAGE, using a 10% polyacrylamide resolving gel and a 5% stacking gel at room temperature according to the method of Laemmli (19). Proteins were visualized by silver staining (20). In a separate experiment, the purified receptor preparation was iodinated with ^{125}I using the radioiodination system from New England Nuclear and analyzed by $NaDodSO_4$/PAGE as before. The slab gel was stained with the silver stain, dried under vacuum, and exposed to Kodak X-Omat AR film for 4 days at −70°C to visualize the ^{125}I-containing subunits.

RESULTS

Purification of Opiate Receptors from Rat Brain. Opiate receptors were solubilized in an active state from rat neural membranes using the zwitterionic detergent CHAPS. The solubilized extract was purified by affinity chromatography using Affi-Gel 401 (sulfhydryl derivative of agarose) to which hybromet (step 4 of Fig. 1), an opioid ligand with high affinity for the μ receptor, had been attached. Elution was achieved by using 12 column vol of 10 mM Tris·HCl buffer (pH 7.5) containing 1 mM CHAPS at 4°C, followed by buffer to which 1 μM normorphine had been added. Normorphine was chosen for specific elution of the μ opioid receptor because of its high selectivity for that receptor (2). The elution profile is shown in Fig. 2. Opiate receptor activity, as measured by the stereospecific binding of [^{3}H]etorphine, migrated as two peaks. The first, the minor peak of receptor activity, was eluted with buffer alone and migrated just following the major protein peak; this activity peak most likely represents unretarded receptor protein that did not adhere to the affinity column. The second and major peak of [^{3}H]etorphine binding activity appeared following addition of normorphine (1 μM) to the elution buffer. Fractions corresponding to this second peak were pooled, concentrated, and dialyzed to remove the normorphine.

[^{3}H]Etorphine binding to the partially purified opiate receptors was studied as a function of radioligand concentration. Half-maximal binding was observed at ≈1 nM radioligand. Scatchard analysis (Fig. 3) of the binding data revealed a class of high-affinity sites. An apparent K_d = 1.4 nM and B_{max} = 2800 fmol/mg of protein were calculated by using computer-assisted linear regression analysis. In two independent experiments, the κ receptor binding activity {defined as [^{3}H]ethylketocyclazocine (EKC) binding in the presence of 1 μM normorphine} was <10% of the [^{3}H]etorphine binding activity. The opioid peptide, [D-Ala^2, D-Leu^5]enkephalin (DADLE, a putative δ ligand) did not inhibit specific [^{3}H]etorphine binding to the affinity-purified fraction, suggesting the absence of δ receptor activity under

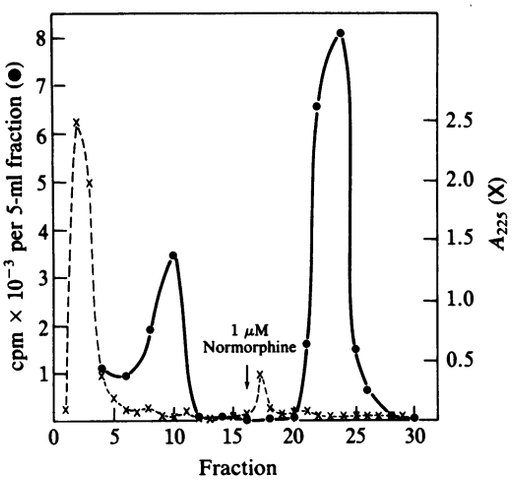

FIG. 2. Elution profile from affinity column. Forty milligrams of CHAPS extract was applied to the column and eluted with 12 column vol of 10 mM Tris·HCl buffer (pH 7.5) containing 1 mM CHAPS, followed by 1 μM normorphine in 10 mM Tris·HCl buffer (pH 7.5). Five-milliliter fractions were collected. Absorbance at 225 nm (X) was monitored, and [^{3}H]etorphine binding activity (●) was measured.

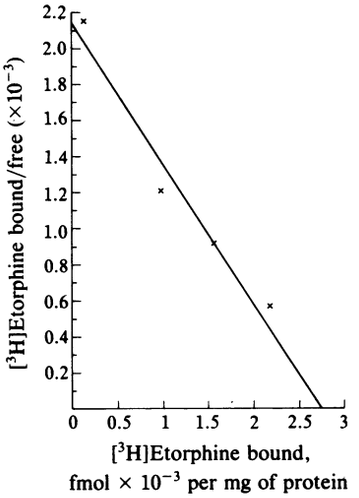

FIG. 3. Scatchard plot for specific binding of [^{3}H]etorphine to pooled and concentrated affinity column active fractions. Receptor binding assays were performed by the PEG method (10) as described in the legend to Table 2. Values are means of triplicates. K_d = 1.3 nM; β_{max} = 2800 fmol/mg of protein; Y = 0.97.

these conditions. Even under conditions (10) selective for δ receptor binding (3 mM $MnCl_2$/10 μM GTP/100 mM NaCl), δ binding was not detectable. The purification of opiate binding components from rat brain membranes is summarized in Table 1. The crude CHAPS extract containing 27 mg of protein afforded 150 μg of the partially purified protein. A yield of 0.6% was obtained. The fold purification relative to the crude solubilized extract stored at 4°C during the 5-day purification is 500.

Properties of Partially Purified Opiate Receptors. Table 2 summarizes the effects of protein-modifying reagents on the purified receptor preparation. Many of the properties of the purified receptors are similar to those of the membrane-associated receptors. Opiate binding activity was markedly inhibited by heat (60°C for 15 min) and by trypsin (10 μg/ml for 15 min at 30°C), findings which suggest that the sites are proteinaceous. Incubation with proteinase K (15 min, 30°C) also resulted in a significant inhibition (>90%) relative to control binding. Pretreatment of the purified fraction with the sulfhydryl reagent *N*-ethylmaleimide (0.5 mM) for 15 min at 30°C followed by quenching with dithiothreitol (2.5 mM) reduced specific binding by 70%. Opiate binding activity was also inhibited by the guanyl nucleotide GTP (25 μM GTP produced 87% inhibition relative to control binding at 4°C, and at 50 μM GTP, the inhibition was >90%). This result suggests that the solubilized, purified receptors may retain coupling to an inhibitory guanyl nucleotide binding protein (N_i).

Effects of Protease Inhibitors on the Stability of the Solubilized Receptors. Fig. 4 shows the opiate receptor activity levels of the CHAPS extract as a function of time. At specified times, samples stored at −20°C were thawed and assayed for opiate binding by using the PEG method. In the absence of added reagents, activity levels were observed to decrease markedly in the first 24 hr to <10% of the activity observed immediately after solubilization. Addition of protease inhibitors Trasylol (200 units/ml), leupeptin (50 μg/ml), and EDTA (10 mM) to the diluted CHAPS extract immediately after the ultracentrifugation step prevented this loss of activity. CHAPS extract to which the inhibitors had been added exhibited 100% of the original binding activity 5 days after solubilization. In the case in which receptors were purified in the absence of protease inhibitors and the inhibitors were added after the affinity chromatography step, no improvement in stability was observed. [^{3}H]Etorphine binding to affinity chromatography-purified opiate receptors in the presence of the inhibitor mixture was inhibited ≈50% relative to control samples in the absence of added reagents.

Preliminary Characterization of the Purified Receptors. Electrophoresis of the purified receptors under native conditions revealed a broad band at a position consistent with an

Table 1. Summary of opiate receptor purification from rat brain

Fraction	Stereospecifically bound [^{3}H]etorphine		Protein		Purification, fold	% yield (protein)
	cpm/ml	fmol/mg of protein	mg/ml	Total mg		
CHAPS extract*	3383	10.4	9	27	—	—
CHAPS extract†	626	1.6	9	27	—	—
Affinity column fractions†						
Pool I (1–3)	—	—	1.5	7.5	—	27
Pool II (10–20)	—	—	0.2	1	—	3.7
Pool III (23–33)	96	20	0.2	1	7	3.7
Pool IV (34–39)	390	812	0.03	0.15	506‡	0.6

[^{3}H]Etorphine concentration = 1 nM.
*Assayed the same day that solubilization was carried out (day 1).
†Assayed on day 5 following solubilization.
‡The fold purification is reported relative to the activity of the CHAPS extract assayed on day 5 following solubilization.

Table 2. Effects of protein modifying agents and GTP on [^{3}H]etorphine binding to purified brain opiate receptors

Reagent	Concentration	[^{3}H]Etorphine binding, % (relative to control)
Control		100
Heat (60°C, 15 min)		2
Trypsin	10 μg/ml	1
GTP	25 μM	13
	50 μM	3
Proteinase K	1 mg/ml	1
N-Ethylmaleimide + dithiothreitol	0.5 mM, 5 mM	31

Specific binding of [^{3}H]etorphine to the purified affinity column active fractions was measured by the PEG method (10). Tubes in triplicate containing 2 nM tritiated ligand, purified receptor, and 10 nM Tris·HCl (pH 7.4) were incubated in the presence and absence of unlabeled ligand (levorphanol) at 37°C for 20 min. At the end of the incubation, 40 μl of 1% gamma globulin were added to each tube, followed by 1 ml of 23% PEG 6000 in 100 mM KOAc (pH 5.6). Samples were incubated an additional 10 min at 4°C. Free ligand was separated from the protein-bound ligand by filtration under reduced pressure through Whatman GF/B filters and the filters were washed twice with 5 ml of buffer containing 7% PEG. Bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was measured in the presence of a large excess of unlabeled ligand.

approximate M_r of 300,000–350,000, in confirmation of earlier reports based on molecular exclusion chromatography (6, 7). On exposure to NaDodSO$_4$ and reducing conditions, this band resolved into a major subunit of M_r ≈94,000 as well as faint bands at positions corresponding to M_r 44,000 and M_r 35,000 (Fig. 5). Identical results were obtained whether the protein bands were visualized by silver staining (Fig. 5, lane 4) or by radioautography after ^{125}I incorporation (Fig. 5, lane 5). This experiment was replicated in five different preparations. In a preliminary experiment, the affinity-purified receptor preparation was applied to a hydroxylapatite column; elution with a phosphate buffer gradient afforded an active receptor species. When this hydroxylapatite-purified receptor was analyzed on NaDodSO$_4$/PAGE only the M_r 94,000 subunit was obtained (Fig. 5, lane 6), a result which suggests that this subunit may be responsible for ligand binding. NaDodSO$_4$/PAGE performed on the crude extract, CHAPS extract, and various pools of the affinity column fractions clearly showed the effective removal of protein by affinity chromatography (Fig. 5).

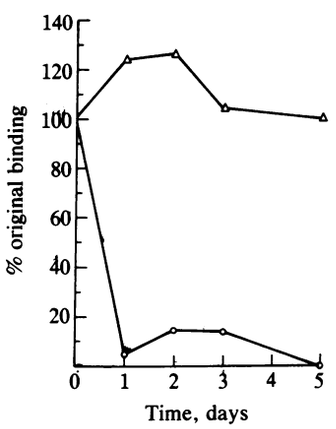

FIG. 4. Effect of added protease inhibitors on stability of CHAPS-solubilized extract of rat brain stored at −20°C. The stabilizing effects of the protease inhibitors (△) were calculated as percent of the control (○) value obtained on day 1. Protease inhibitor mixture: Trasylol (200 units/ml), leupeptin (50 μg/ml), and EDTA (10 mM) (final concentrations).

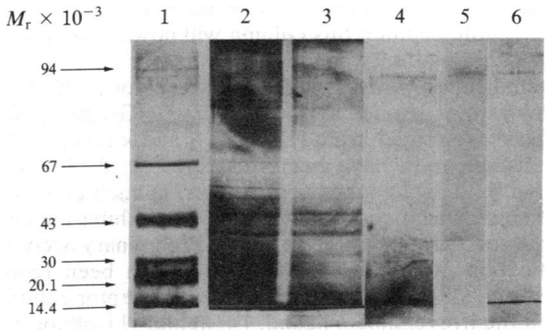

FIG. 5. NaDodSO$_4$/PAGE of affinity column fractions. Electrophoresis was carried out on 10% gels; protein was visualized by silver staining (lanes 1–4 and 6) or by radioautography (lane 5). Lane 1, molecular weight markers; lane 2, the crude homogenate of rat brain; lane 3, the CHAPS extract (CHEX) applied to the affinity column; lane 4, the pooled and concentrated active fractions eluted from the affinity column with 1 μM normorphine, as visualized by silver staining; lane 5, radioautography of an iodinated preparation of the affinity-purified active fraction; lane 6, the pooled and concentrated active fraction from the hydroxylapatite column, as visualized by silver staining.

Finally, in a preliminary study, antibodies were raised to the affinity-purified receptor in female rabbits. Immunochemical titrations showed that increasing amounts of antiserum could effectively displace increasing amounts of specific [^{3}H]etorphine binding to rat brain homogenates (25 μl of a 1:10 diluted antiserum displaced ≈60% of [^{3}H]etorphine binding). The antiserum was also effective against the binding of [^{3}H]dihydromorphine (μ-selective opioid) but not against the binding of [^{3}H]EKC (κ opioid), [^{3}H]DADLE (δ opioid), and [^{3}H]phencyclidine (σ ligand).

DISCUSSION

We have purified μ opiate receptors from rat brain ≈500-fold using an affinity column in which hybromet, an opioid ligand which binds to the μ receptor with high affinity, has been coupled to a sulfhydryl derivative of agarose beads. A single sharp peak of opiate binding activity was observed following normorphine elution. Although the specificity of hybromet has not been assessed, specificity of the purified receptor was achieved by elution with normorphine. Normorphine has been shown to be a highly selective μ ligand (2) with very low affinity for δ receptors and no detectable affinity for κ receptors. K receptor binding activity was <10% of the μ receptor activity. The pooled, concentrated fractions exhibited high affinity (K_d = 1.4 nM). The density of opiate receptors in rat brain membranes (as measured by using the nonselective opioid [^{3}H]etorphine) is ≈200 fmol/mg of protein. Using radiolabeled enkephalin analogs to label opiate receptors, we had estimated a M_r of 300,000–400,000 (6, 7). Assuming this molecular weight and one binding site per receptor molecule, then 100 mg of protein (or the tissue from one adult rat brain) would be expected to contain ≈10 μg of receptor. Thus, a much greater purification than has been achieved (on the basis of receptor activity) is necessary to achieve a homogenous receptor preparation. We have found that during the 6-day purification, concentration, and dialysis procedure, >90% of the original opiate receptor activity is lost (at 4°C) in the CHAPS extract. Thus, the actual fold purification of the receptor molecules may have been greatly underestimated. Assuming a correction for the loss of receptor activity, we calculate that the fold purification would be increased by a factor of at least 10 or even 100, which would be sufficient to account for a homogeneous preparation of the receptor. In preliminary experiments, we have achieved

a 1300-fold purification of the receptor using an hydroxylapatite column; this column will now be used sequentially with the affinity column step.

Solubilization of δ opiate receptors from the neuroblastoma-glioma hybrid cell line NG-108-15 (12), κ receptors from toad brain (11), and μ and κ receptors from mammalian brain have been described (4, 8, 10). Opiate binding to the solubilized species was, in each case, shown to be stereospecific and saturable and to exhibit the characteristics of a protein of high affinity. Preliminary accounts of purification of opiate receptors have also been reported. Klee *et al.* (13) purified an inactivated δ receptor complex to which the irreversible δ ligand, [^{3}H]fentanyl isothiocyanate, had been attached; the M_r under denaturing conditions was shown to be 58,000. Simon and co-workers (14) achieved a 30-fold purification using a wheat germ agglutinin-agarose column. Abood's group (9), using an affinity column in which 14-β-bromacetamidomorphine had been linked to ω-aminohexyl-Sepharose, achieved considerable purification of opiate binding activity, although background binding was high. The uniqueness of our preparation resides in the high signal-to-noise or percent specific binding (>75%) and high fold purification.

Many of these properties of the purified μ receptors were found to be similar to those of the membrane-associated receptors, including (*i*) sensitivity to protein-modifying agents, (*ii*) modulation by guanyl nucleotides, and (*iii*) affinity for opioid ligands. Activity was markedly inhibited by heat and trypsin, findings which suggest that the sites are proteinaceous. Opiate binding activity was also inhibited by the guanyl nucleotide GTP. The findings of (*a*) a lack of δ receptor activity and (*b*) a very low level of κ receptor activity in affinity-purified fractions indicate that the purified receptor is μ-specific. The possibility does exist that under other ionic conditions (10), δ receptor activity might be unmasked. Electrophoresis of the affinity-purified opiate receptors under denaturing conditions revealed three bands corresponding to M_r of 94,000, 44,000, and 35,000. Our findings of M_r 35,000 and M_r 44,000 subunits are consistent with those of Bidlack *et al.* (9), although we were unable to visualize the bands with Coomassie blue staining. The finding of a M_r 35,000 subunit is also consistent with our previous finding that a M_r 35,000 species was covalently labeled by relatively μ-selective [^{3}H]enkephalin derivatives (7). The exact subunit structure for the μ receptor remains to be determined.

It may seem surprising to obtain such apparent homogeneity after such a low fold purification. A major reason for this low specific activity may be the progressive loss of activity observed throughout the purification procedure, as a result of proteolytic digestion. There are similar reports in the literature where such inactivation occurs (21, 22). The inclusion of protease inhibitors markedly retarded this activity loss. Unfortunately, these inhibitors also inhibited opiate binding to the purified receptor and significantly inhibited the specific adsorption of the receptor to the affinity column resulting in a lowered column efficiency. Another factor contributing to the overall low yields may be the inability of 1 μM normorphine to elute all of the bound receptor from the affinity column. In this regard, higher concentration of normorphine improved the yield modestly. It is also possible that one or more of the bands may be contaminating proteins or degradation products of the active molecule. As mentioned in the *Results*, only the M_r 94,000 subunit was obtained on NaDodSO$_4$/PAGE of the purified active fraction after further purification on a hydroxylapatite column (preliminary data). Thus, it is intriguing to speculate that the M_r 94,000 species represents the μ opioid recognition component and that the M_r 44,000 and M_r 35,000 species represent the subunits of the inhibitory guanyl nucleotide binding protein (N_i) implicated in opiate action (23–25).

In summary, opiate receptors from rat brain have been partially purified by affinity chromatography. The resulting PAGE analysis and sensitivity to guanyl nucleotides are consistent with a large heteroligomer, which may contain, in part, the N_i protein.

Note Added in Proof. After submission of this manuscript, Gioannini *et al.* (26) reported a 300-fold purification of opioid binding using CH-Sepharose modified with a new naltrexone derivative, β-naltrexyl-6-ethylene-diamine, and elution with naloxone.

We thank Hoffman–La Roche for their gift of levorphanol, Sterling Winthrop Research Laboratories for cyclazocine, The National Institute on Drug Abuse for etorphine and normorphine, Endo Laboratories for naloxone, and Ms. Melissa Fitz-Sage for excellent technical assistance. This work was supported by National Institutes of Health Grants DA 01843 (to R.S.Z.) and DA 01674 (to S.A.). R.S.Z. is recipient of a Research Career Development Award (DA-00069) from the National Institute on Drug Abuse.

1. Chang, K. J. & Cuatrecasas, P. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 2729–2734.
2. Zukin, R. S. & Zukin S. R. (1981) *Life Sci.* **29,** 2681–2690.
3. Pasternak, G. W. & Snyder, S. M. (1974) *Mol. Pharmacol.* **11,** 478–484.
4. Howells, R., Gioannini, T. M., Hiller, J. & Simon, E. J. (1982) *J. Pharmacol. Exp. Ther.* **222,** 629–633.
5. Lin, M. K. & Simon, E. J. (1978) *Nature (London)* **271,** 383–384.
6. Simon, E. J., Hiller, J. M. & Edelman, I. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1947–1949.
7. Zukin, R. S. & Kream, R. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1593–1597.
8. Bidlack, J. M. & Abood, L. G. (1980) *Life Sci.* **27,** 331–340.
9. Bidlack, J. M., Abood, L. G. & Archer, S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 636–639.
10. Chow, T. & Zukin, R. S. (1983) *Mol. Pharmacol.* **24,** 203–212.
11. Ruegg, U. T., Miller, J. M. & Simon, E. J. (1980) *Eur. J. Pharmacol.* **64,** 367–368.
12. Simonds, W. F., Koshi, G., Streaty, R. A., Hjelmeland, L. M. & Klee, W. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4623–4627.
13. Klee, W. A., Simonds, W. F., Sweat, F. W., Burke, T. R., Jacobson, A. E. & Rice, K. C. (1982) *FEBS Lett.* **150,** 125–128.
14. Gioannini, T., Foucard, B., Hiller, J. M., Hatten, M. E. & Simon, E. J. (1982) *Biochem. Biophys. Res. Commun.* **105,** 1120–1125.
15. Bentley, K. W., Hardy, D. G. & Meek, B. D. (1967) *J. Am. Chem. Soc.* **89,** 3273–3280.
16. Bentley, K. W. & Hardy, D. G. (1967) *J. Am. Chem. Soc.* **89,** 3267–3273.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
18. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121,** 404–427.
19. Laemmli, V. (1970) *Nature (London)* **227,** 680–685.
20. Merril, C. R., Goldman, D., Sedman, S. A. & Eberi, M. H. (1981) *Science* **211,** 1437–1438.
21. Agnew, W. S. & Raftery, M. A. (1979) *Biochemistry* **18,** 1912–1919.
22. Maneckjee, R. & Baylin, S. B. (1983) *Biochemistry* **22,** 6058–6083.
23. Bokoch, G. M., Katada, T., Northup, J. K., Newlett, E. L. & Gilman, A. G. (1983) *J. Biol. Chem.* **258,** 2072–2075.
24. Murayama, T. & Ui, M. (1983) *J. Biol. Chem.* **258,** 3319–3326.
25. Codina, J., Hildebrandt, J., Iyengar, R., Birnbaumer, L., Sekura, R. D. & Manclark, C. R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4276–4280.
26. Gioannini, T. L., Howard, A., Hiller, J. M. & Simon, E. J. (1984) *Biochem. Biophys. Res. Commun.* **119,** 624–629.