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Purification of the opiate receptor of NG108-15 neuroblastoma–glioma hybrid cells

(affinity labeling/δ opiate receptor/glycoprotein)

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ABSTRACT **Opiate receptors from NG108-15 neuroblastoma–glioma hybrid cell membranes were purified to apparent homogeneity in a form covalently labeled with the opiate affinity ligand 3-methylfentanylisothiocyanate (super-FIT). The purification procedure consists of five steps: (*i*) quantitative labeling of opiate receptors in membranes with super-FIT; (*ii*) solubilization in a lubrol/3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate mixture; (*iii*) adsorption to and elution from a wheat germ agglutinin-Sepharose column; (*iv*) immunoaffinity chromatography on columns of immobilized anti-fentanyl; (*v*) preparative gel electrophoresis in the presence of NaDodSO$_4$. The protein was purified ≈30,000-fold in 3% overall yield to a state in which there is 1 mol of super-FIT bound per mol of protein, corresponding to 21,000 pmol per mg of protein, the theoretically expected specific activity. The protein is glycosylated and migrates on NaDodSO$_4$ gel electrophoresis with a M_r near 58,000. It has a strong tendency to dimerize, even in the presence of denaturing detergents, and it exists primarily as an oligomer in nondenaturing detergents.**

Opiate receptors in the nerve cell membrane are the first link in a biochemical coupling mechanism that allows opioid ligands outside the cell to modify enzyme function within the cell. In neuroblastoma–glioma NG108-15 hybrid cells, a particularly rich source, opiate receptors are coupled as stimulators of low K_m GTPase (1) and, consequently, as inhibitors of adenylate cyclase (2). A similar mechanism of action has been found in brain as well (3). Understanding of the coupling mechanism will require the isolation, purification, and reconstitution of each necessary component. In contrast to the plurality of opiate receptors believed to exist in brain (4), NG108-15 cells carry only a single type of opiate receptor, with δ opiate receptor characteristics (5). The development of the δ-opiate receptor selective affinity reagent [^{3}H]fentanylisothiocyanate ([^{3}H]FIT) (6) allowed the identification and partial purification of a M_r 58,000 glycoprotein subunit of the opiate receptor of NG108-15 cells (7). Recently, the μ opioid receptor subunit of rat brain membranes has also been shown to have a M_r of 58,000 (8). We report here the purification to homogeneity of the opiate receptor subunit from NG108-15 cells by using the more potent δ opiate receptor-selective acylating agent 3-[^{3}H]methylfentanylisothiocyanate ([^{3}H]super-FIT) (9).

MATERIALS AND METHODS

Chemicals. [^{3}H]Super-FIT (13 Ci/mmol; 1 Ci = 37 GBq) (9) and [^{3}H]FIT (27 Ci/mmol) (6) were synthesized from the corresponding amines (tritiated by New England Nuclear) by reaction with thiophosgene in a two-phase system of saturated aqueous $NaHCO_3$ and chloroform, and they were purified by TLC on silica gel in chloroform/methanol (20:1) (6).

3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), bovine serum albumin, and Pansorbin were from Calbiochem. Tris (ultra pure) was from Bethesda Research Laboratories. Soybean trypsin inhibitor was from Worthington. EGTA, leupeptin, N^{α} acetyl-L-lysine, dithiothreitol, phenylmethylsulfonyl fluoride, polyoxyethylene 9-lauryl ether ($C_{12}E_9$ lubrol), and *N*-acetyl-D-glucosamine were from Sigma. MSI Cameo 5μm HPLC nylon filters were from Fisher. Wheat germ agglutinin (WGA)-agarose beads (3–5 mg per ml of settled beads) were from E-Y Laboratories (San Mateo, CA). Cyanogen bromide-activated Sepharose 4B and bovine thyroglobulin were from Pharmacia. Guanidine·HCl (ultrapure) was from Schwarz/Mann. Propionic acid (Gold Label) was from Aldrich. NaDodSO$_4$ was from Bio-Rad.

Cells. NG108-15 neuroblastoma–glioma hybrid cells were grown as described (10) or were purchased from Meloy Laboratories (Springfield, VA), and were stored as cell pastes at −70°C.

Membrane Preparation and Extraction. NG108-15 cell pastes (40 g) were thawed and diluted to 200 ml with 10 mM potassium phosphate/1 mM EGTA/0.1 mM phenylmethylsulfonyl fluoride/1 μM leupeptin/10 μM soybean trypsin inhibitor, pH 8.0 (buffer A). The cell suspension was broken in a cell disruption bomb (Parr Instruments, Malina, IL) on ice after standing under nitrogen at 550 psi for 25 min (1 psi = 6.895 × 10^3 Pa). The broken cell preparation was centrifuged at 160,000 × *g* for 30 min (in a Beckman 60 Ti rotor), and the supernatant fluid was discarded. The crude membrane pellet was suspended to 800 ml with buffer A (1–2 mg of protein per ml).

[^{3}H]Super-FIT was added to a final concentration of 20 nM, and the membrane suspension was incubated at 37°C with agitation for 45 min, then chilled on ice. After addition of 200 ml of 5% (wt/vol) $C_{12}E_9$ lubrol/0.1 M CHAPS/0.75 M NaCl/1 mM EGTA/0.1 mM phenylmethylsulfonyl fluoride/1 μM leupeptin/10 μM soybean trypsin inhibitor, the suspension was mixed and centrifuged at 11,000 × *g* for 30 min at 4°C. The supernatant extract contained 72–97% of the protein and 91–96% of the radioactivity of the membranes.

WGA-Agarose Adsorption. A column of WGA-agarose (2.5 × 5.2 cm) was washed with 125 ml of 1% (wt/vol) $C_{12}E_9$ lubrol/20 mM CHAPS/0.15 M NaCl/10 mM Tris·HCl, pH 8.0 (4°C)/1 mM EGTA/0.1 mM phenylmethylsulfonyl fluoride/1 μM leupeptin (buffer B) with 10 μM soybean trypsin inhibitor. The detergent extract was applied at 150–200 ml/hr at 4°C. The column was washed with 250 ml of buffer B, eluted

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Abbreviations: FIT, fentanylisothiocyanate; super-FIT, methylfentanylisothiocyanate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; WGA, wheat germ agglutinin.

at 5 ml/hr with 0.5 M *N*-acetyl-D-glucosamine in buffer B, and the fractions containing [^{3}H]super-FIT-labeled receptor were pooled (15–30 ml). The eluate contained 6–16% of the applied radioactivity, all of which migrates with a M_r of 58,000 on electrophoresis and is the receptor, as previously shown (7). The bulk of the radioactivity, not bound to WGA-agarose, does not contain a M_r 58,000 component.

Anti-FIT IgG Affinity Chromatography. The pooled WGA-agarose eluate was applied at 0.5–2 ml/hr to a column of anti-FIT IgG-Sepharose (1.5 × 3 cm) equilibrated with buffer B. After washing overnight with buffer B, a Cameo nylon filter containing 75-μl bed volume of WGA-agarose was attached to the column outlet, and elution with 100 μM FIT/lysine in buffer B began at 1 ml/hr. After 15–28 hr, the Cameo filter was removed and attached to the Luer fitting of a 5-ml polypropylene syringe barrel. The WGA-agarose was washed with 3 × 0.5 ml of 20 mM CHAPS/0.15 M NaCl/10 mM Tris·HCl, pH 8.0 (buffer C) and then removed from the syringe. The filter was placed in an Eppendorf polypropylene microtube and the interstitial buffer was removed by centrifugation for 3 min in an Eppendorf microcentrifuge. The column was eluted with 75-μl portions of 0.5 M *N*-acetyl-D-glucosamine in buffer C by centrifugation, and the [^{3}H]super-FIT-labeled receptor protein was determined by liquid scintillation counting. Most of the protein was contained in the first 150 μl of eluate. After the anti-FIT IgG column had been eluted with FIT/lysine, it was eluted with 1 M propionic acid/1% $C_{12}E_9$ lubrol/20 mM CHAPS/0.15 M NaCl at 1 ml/hr. The fractions were immediately neutralized with 2 M Tris free base, and the peak fractions were pooled and concentrated on WGA-agarose in a Cameo filter as described above.

NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Slab gels were prepared by using a gradient of 5–10% polyacrylamide by a modification of the method of Laemmli (11), and they were allowed to polymerize for 5 hr or overnight (for preparative gels). The concentrated receptor from the anti-FIT agarose step was diluted with 1–3 vol of 5% NaDodSO$_4$/5 M urea/6% (wt/vol) dithiothreitol/50 mM Tris·HCl, pH 8.0, at room temperature, and applied to a spacing gel of 3% polyacrylamide. After electrophoresis overnight at 50 V with bromophenol blue tracking dye, the gel was stained with 0.1% (wt/vol) Coomassie blue/7% acetic acid/21% 2-propanol for 4 hr at room temperature, then destained overnight in 10% acetic acid/10% 2-propanol at 4°C. Silver staining was performed on analytical gels after Coomassie blue staining according to the method of Merril *et al.* (12). In preparative experiments (100–500 pmol of receptor per 0.6-cm lane), the M_r 58,000 band was excised from the slab gel with a razor blade and eluted as described by Hunkapiller *et al.* (13).

Preparation of Anti-FIT IgG Affinity Column. *Preparation of FIT–protein conjugates.* Bovine thyroglobulin or bovine serum albumin (20 mg) was dissolved in 1 ml of 0.1 M potassium borate (pH 9.0). To the stirring protein solution, 300 μl of methanol containing 17 μmol of [^{3}H]FIT was added. After 24 hr at 37°C, 10 ml of acetone was added and the precipitated protein was collected by centrifugation at 1200 × *g* for 10 min. The pellet was washed with acetone, then dissolved in H_2O. Thyroglobulin conjugate (14 mg) was recovered with a FIT substitution of 140 nmol per mg of protein. Serum albumin conjugate (10 mg) was recovered with a FIT substitution of 1700 nmol per mg of protein.

*Preparation of FIT/α-*N*-acetyl-*L*-lysine.* α-*N*-Acetyl-L-lysine (1.2 ml, 1 M) in H_2O containing 1.2 mmol of triethylamine was added to a rapidly stirring suspension of FIT hydrochloride (280 mg, 0.6 mmol) in 6 ml of methanol, which immediately clarified. The reaction mixture was stirred at room temperature for 1 hr, then at 37°C overnight. The product, FIT/α-*N*-acetyllysine (FIT/lysine) migrated as one major spot on thin-layer chromatography in *n*-butanol/acetic acid/H_2O (4:1:1) (R_f, 0.41). [^{3}H]FIT/α-*N*-acetyllysine was prepared similarly but on a smaller scale.

Preparation of antiserum. New Zealand White rabbits (Dutchland Laboratories, Denver, PA) of either sex were injected subcutaneously with 840 μg of thyroglobulin-conjugated FIT in phosphate-buffered saline as a 1:1 emulsion with Freund's complete adjuvant. After 2 weeks and every 4–8 weeks thereafter, the rabbits were given booster injections of 250 μg of bovine serum albumin-conjugated FIT in emulsion with Freund's incomplete adjuvant. Whole blood was collected from outer marginal ear veins. Antiserum was decanted after overnight incubation at 4°C and was stored at −70°C.

Characterization of antiserum. Anti-FIT antibody was assayed by incubation of antiserum dilutions with 1–100 nM [^{3}H]FIT/lysine in 0.1% (wt/vol) ovalbumin/1% (wt/vol) $C_{12}E_9$ lubrol/20 mM CHAPS/0.15 M NaCl/10 mM Tris·HCl, pH 8.0, overnight at 4°C. Immunoglobulins were precipitated by incubation with 250 μg of Pansorbin per μg of IgG for 30 min, and centrifugation at 1800 × *g* for 10 min. Radioactivity in the pellet was determined after resuspension in 1% (wt/vol) NaDodSO$_4$ by liquid scintillation counting. Nonspecific binding was defined as radioactivity bound in the presence of 10 μM unlabeled FIT/lysine.

Preparation of FIT-agarose. Biogel-102 (30 ml) in methanol was treated with 51 mg of FIT (free base) at 37°C for 5 hr with gentle agitation. The resin was washed with methanol/water, suspended in an equal volume of 10% sodium acetate and treated with 2 ml of acetic anhydride at room temperature overnight. The modified and blocked resin was washed exhaustively with water and stored in 0.02% sodium azide at 4°C.

Affinity purification of anti-FIT antibodies. Crude antiserum diluted 1:3 with 50 mM Tris chloride (pH 8.0) (4°C) was applied to a 10-ml bed volume of FIT-agarose at 10 ml/hr at 4°C. After extensive washing with 1 M guanidine·HCl/0.15 M NaCl/50 mM Tris chloride, pH 8.0, the column was eluted with 1 M propionic acid/0.1 M NaCl. The fractions were neutralized with 2 M Tris free base, and the protein was determined by measuring OD_{280}. Peak fractions were pooled and dialyzed against phosphate-buffered saline. Recovery of [^{3}H]FIT-lysine binding activity was 10–20%. The purified antibodies are of the IgG class, because they are recognized by goat anti-rabbit IgG and are bound completely to protein A.

Coupling of anti-FIT IgG to Sepharose 4B. Affinity-purified IgG was coupled to cyanogen-bromide-activated Sepharose 4B following manufacturer's instructions with 21 mg of protein per 7 ml of settled resin in a total volume of 40 ml. Direct binding studies on the coupled resin indicated a concentration of [^{3}H]FIT/lysine binding sites of 10 μM in the settled gel.

Amino Acid Analysis. A portion (10 pmol) of electrophoretically homogeneous [^{3}H]super-FIT-labeled receptor protein was mixed with 500 pmol of norleucine and lyophilized. The lyophilizate was dissolved in 50 μl of 6 M constant boiling HCl with one crystal of phenol added, then sealed under nitrogen. The sample was hydrolyzed at 110°C for 22 hr, lyophilized, and then dissolved in 200 μl of buffer (pH 2.2). One hundred fifty microliters was used for analysis in an automated Waters amino acid analyzer. Values obtained were adjusted for norleucine recovery.

Protein Determination. Protein was determined by the Peterson modification (14) of the method of Lowry (15), by [^{3}H]dinitrofluorobenzene labeling (16), or from amino acid analysis as indicated in the *Results*.

Radioactivity. Tritium content was determined by liquid scintillation counting in 7.5% aqueous Aquasol (New England Nuclear) at 45% efficiency.

RESULTS

The purification paradigm used here depends on the specific covalent modification of opiate receptors with FIT or super-FIT and the subsequent recognition of the bound affinity ligand by an antibody. We prepared such an antibody by immunization of rabbits with FIT covalently attached to each of two proteins. The affinity of the antibody for FIT/lysine was found to be 8 nM (Fig. 1). Super-FIT is recognized by the antibody with the slightly reduced affinity of 30 nM (Fig. 1). When super-FIT is bound to the receptor, however, the apparent affinity of the antibody, measured by binding experiments analogous to those shown in Fig. 1, is 1.1 μM. Therefore, high concentrations of antibody are needed for efficient binding of the labeled receptor.

Previous studies of NG108-15 opiate receptors showed complete inactivation by 20 nM super-FIT (9). For purification of labeled receptor protein, therefore, acylation with [^{3}H]super-FIT was performed at 20 nM. As in previous experiments with [^{3}H]FIT (7), the label was incorporated into several membrane components, as detected by autoradiography of polyacrylamide gels containing proteins electrophoretically separated in the presence of 0.1% (wt/vol) NaDodSO$_4$. The major band detected on autoradiograms (M_r, 58,000) was labeled only in the absence of competing opiate or in the presence of the inactive opiate enantiomer dextrorphan and is therefore specific labeling (7). Other bands are labeled both in the presence and absence of competing opiates and are thus nonspecifically labeled. After the WGA-agarose purification step, the only tritiated species present is the specifically labeled M_r 58,000 protein (7).

Because of significant loss of receptors upon repeated centrifugation, solubilization was performed with unwashed membranes. Quantitative solubilization of labeled proteins from the membranes was achieved with a combination of

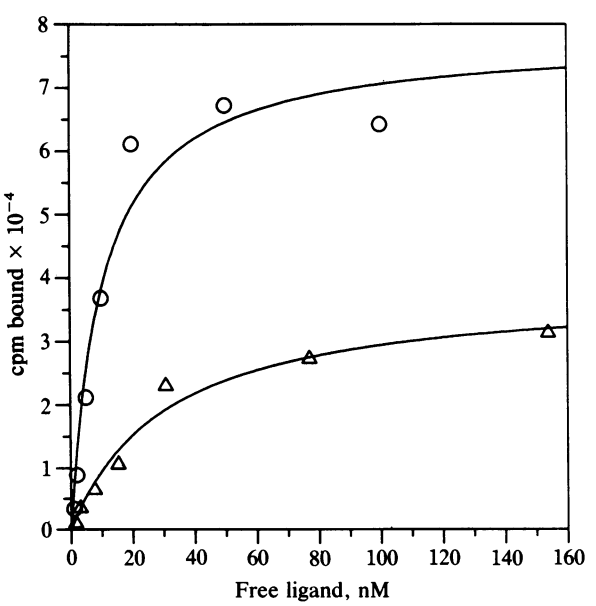

FIG. 1. Specific binding of [^{3}H]FIT/lysine and [^{3}H]super-FIT to anti-FIT IgG; 2.3 μg of the IgG fraction of FIT antiserum was incubated overnight at 4°C with increasing amounts of [^{3}H]FIT/lysine (27 Ci/mmol) (○) or [^{3}H]super-FIT (13 Ci/mmol) (△). Immunoglobulins were precipitated with Pansorbin and bound radioactivity in the pellet was determined. Nonspecific binding was defined as radioactivity bound in the presence of 10 μM unlabeled FIT/lysine, and was subtracted from the total. The curves represent a nonlinear least-squares regression analysis of the data according to the Adair equation in the form $y = (a \cdot x/k)/(1+x/k)$; y is the amount of bound ligand, a is the maximum amount of bound ligand, x is the concentration of free ligand, and k is the dissociation constant.

$C_{12}E_9$ lubrol (1% wt/vol) and CHAPS (20 mM). This mixture of detergents solubilized >90% of the radioactivity in the membranes.

The receptor is a glycoprotein that binds to WGA-agarose as had previously been observed (7). A 30-fold purification of labeled receptor on WGA-agarose from the crude detergent extract was achieved on elution with 0.5 M *N*-acetylglucosamine. Over 90% of the specifically labeled M_r 58,000 protein binds on a single passage through WGA-agarose and is eluted with the sugar. All of the nonspecifically labeled proteins wash through such a column. The receptor does not bind to concanavalin A or to lentil lectin, and it binds only weakly to *Ricinus communis* agglutinin II (data not shown). Although protease inhibitors were routinely included in the purification, the labeled receptor at this stage of purity was unchanged in mobility on NaDodSO$_4$ gels after storage on ice for up to 1 week in the absence of such inhibitors (data not shown). Omission of protease inhibitors from the buffers throughout the purification procedure was without effect on receptor size.

After WGA-agarose chromatography, the receptors were further purified by passage over a column of affinity-purified anti-FIT IgG coupled to agarose. This IgG shows significant cross-reactivity with fentanyl, super-FIT, and protein–FIT conjugates. This step results in a 200-fold purification with 25% recovery. No more than 50% of the applied receptor binds to the anti-FIT IgG column on a single passage, even at slow flow rates (0.5–2 ml/hr). This limiting value may reflect random availability of the super-FIT moiety on the outside (aqueous surface) of detergent micelles. Of the bound receptor, 50% can be recovered by elution with 100 μM FIT/lysine followed by elution with 1 M propionic acid. The eluate from this column is concentrated on a small (75 μl) WGA column without prior isolation, a step that also eliminates contaminating IgG leaking from the antibody column. The specific activity of the [^{3}H]super-FIT-labeled M_r 58,000 protein after anti-FIT IgG purification and subsequent WGA concentration can be as high as 6000 pmol per mg of protein.

Final purification is achieved by preparative NaDodSO$_4$/polyacrylamide gel electrophoresis. The M_r 58,000 band, identified by Coomassie blue staining, is excised with a razor blade. The protein is recovered from the gel by electroelution into a dialysis chamber (13). After this procedure, the receptor is homogeneous by analytical NaDodSO$_4$/PAGE (Fig. 2), although the recovery is low (10–30%).

The receptor displays a marked tendency to aggregate either with itself or with adventitious proteins. In particular, a minor labeled component is often seen at a M_r of 110,000. We believe this to represent a dimeric species, because reelectrophoresis of the eluted M_r 58,000 band shows the presence of small amounts of labeled material at this position (Fig. 2, lane D). One complete purification experiment is summarized in Table 1. The amino acid composition of the electroeluted receptor is shown in Table 2. There are appreciable amounts of amino acids in acid hydrolysates of electroeluted blank gel segments (17). Since the analyses summarized in Table 2 were performed with pmol amounts of protein, the contribution of the blank values is high. No attempt was made to estimate cysteine or tryptophan contents. These values and more precise amino acid compositions require analysis on a larger scale.

DISCUSSION

Our results document the purification to homogeneity of a M_r 58,000 glycoprotein subunit of the opiate receptor from NG108-15 cells. The receptor had been affinity-labeled by the δ opiate receptor-specific acylating reagent [^{3}H]super-FIT. It was purified ≈30,000-fold in 2–3% yield from crude membranes to a specific activity of 21,000 pmol per mg of protein.

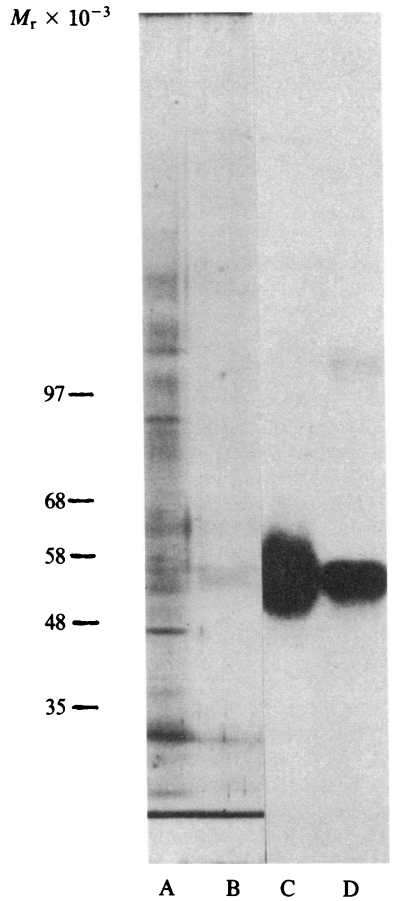

FIG. 2. $NaDodSO_4$ gel electrophoresis of purified [^{3}H]super-FIT-labeled opiate receptor fractions from NG108-15 neuroblastoma × glioma hybrid cells. Silver stained gel (lanes A and B) and corresponding autoradiogram (lanes C and D) after 10-day exposure. Lanes A and C, propionate eluate from anti-FIT IgG agarose containing 2 pmol of receptor; lanes B and D, 2 pmol of purified receptor after electroelution of M_r 58,000 band from preparative gel electrophoresis of anti-FIT IgG agarose eluate. The staining artifact at M_r 30,000 seen in lanes A and B is seen also in blank gel lanes. A trace of dimeric receptor is visible at M_r 110,000 in lane D.

If the molecular weight of the unglycosylated protein were 50,000, the theoretical specific activity for 1:1 binding would be 20,000 pmol per mg of protein. Thus, the preparation is not only electrophoretically homogeneous, it also has the specific activity expected of pure receptor.

The major purification step used is affinity chromatography on a column of anti-FIT IgG, which exploits the presence of the covalent affinity label [^{3}H]super-FIT on the receptor. After the WGA-agarose step, the receptor is the only protein so labeled, making the use of anti-FIT antibodies an effective strategy.

Table 2. Amino acid composition of NG108-15 opiate receptors

Amino acid	Residues per mol
Asp	32
Thr	29
Ser	37
Glu	12
Gly	39
Ala	40
Val	34
Met	11
Ile	23
Leu	41
Tyr	6
Phe	17
Lys	10
Arg	27

Samples were eluted from $NaDodSO_4$ gels and hydrolyzed. Equal-sized pieces of gel from the equivalent position in blank lanes were treated and analyzed similarly. The data shown have been corrected for these blank values.

The purification is selective for the affinity-labeled M_r 58,000 glycoprotein subunit, and we cannot, therefore, rule out the presence of other unlabeled subunits in the native receptor. In the 30% pure material (after the anti-FIT IgG affinity step), the relative proportions of the several contaminants and the M_r 58,000 band have varied among preparations. It is unlikely, therefore, that other protein subunits have been copurifying stoichiometrically with the M_r 58,000 protein. In particular, there is no evidence that Ni copurifies with the receptor in our experiments. In most experiments, the major contaminants present in the 30% pure preparations migrate with M_r values of 93,000, 45,000, 36,000, and 31,000. Proteins with similar mobilities make up the bulk of purified "receptor" preparations described by some investigators (18, 19). Partial purification of opiate receptors has also been reported by others (20, 21).

The receptor displays a strong tendency to aggregate. Thus, even in the presence of $NaDodSO_4$ and dithiothreitol, dimeric species can be observed in preparations that had been pure monomer. In both sucrose gradient centrifugation and gel filtration, the receptor migrates near a glycogen phosphorylase marker in the presence of the nondenaturing detergent CHAPS. These results strongly suggest that the receptor behaves as an oligomer under these conditions.

The super-FIT-acylated opiate receptor no longer binds opiates, as has been shown in membrane studies (9). Yet, super-FIT is an opiate agonist (9) and the super-FIT-labeled receptor may exhibit the properties required of an agonist–receptor complex for opiate stimulation of low K_m GTPase or

Table 1. Purification of [^{3}H]super-FIT-labeled opiate receptors

Step	Vol, ml	Protein,* μg	^{3}H, pmol	Specific activity: pmol per mg of protein	Specific activity: Range†	Recovery, %
Detergent extract	1000	1270×10^3	889	0.7	0.6–2.1 (n = 7)	100
WGA-agarose eluate	13.5	22×10^3	807	37	21–58 (n = 7)	91
Anti-FIT IgG-agarose						
FIT/lysine eluate	0.15	49	87	1790	1790–6480 (n = 6)	10
Propionate eluate	0.15	16.2	70	4320	2140–4320 (n = 2)	8
$NaDodSO_4$/PAGE eluate	0.17	0.385	8‡	20,800	20,800–22,700 (n = 2)	2

*Protein determinations were by a modification (14) of the method of Lowry *et al.* (15) or by [^{3}H]dinitrofluorobenzene labeling (16), using crystalline bovine serum albumin as a standard, except for the final $NaDodSO_4$/PAGE eluate, which was by amino acid analysis.
†Range of values obtained in purifications carried out to date.
‡Starting with 70 pmol of propionate eluate. In a second experiment, 385 pmol was applied and 73 pmol of purified receptor was recovered.

inhibition of adenylate cyclase activity. Reconstitution studies of purified [^{3}H]super-FIT-labeled receptor with purified bovine brain Ni (22) are necessary to test this possibility.

The purified receptor should also be useful for structural studies. Determination of amino acid sequence of a fragment labeled with super-FIT will allow characterization of the opiate binding site and provide information for synthesis of a DNA probe with which to clone the cDNA coding for the opiate receptor. Furthermore, amino acid sequence data will allow synthesis of peptide fragments for use as antigens to generate antibodies of predefined specificity (23). Ultimately, the receptor may be mapped into domains such as the ligand binding site and the site of Ni interaction, which could show structural homology with other receptors.

The successful use of anti-FIT antibodies in our studies augurs well for the purification of [^{3}H]super-FIT-labeled receptors from brain. After a preliminary purification sufficient to eliminate nonspecifically labeled proteins, the antibodies could be expected to provide the same degree of purification as with opiate receptors from NG108-15 hybrid cells. The purification of the δ opiate receptor subunit represents a powerful first step toward the eventual biochemical resolution of the mechanism of opiate effects on nerve cells.

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