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Antisera Against Peptides Derived from a Purified μ-Opioid Binding Protein Recognize the Protein as Well as μ-Opioid Receptors in Brain Regions and a Cell Line

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SUMMARY

Two peptides, which have no significant homology with known protein structures, were obtained by microsequencing of a μ-opioid binding protein purified to homogeneity from bovine striatal membranes. Polyclonal antibodies generated against portions of these peptides immunoprecipitated up to 65% of radiolabeled purified opioid binding protein. Sequential immunoprecipitations, using antibodies directed against portions of the two different peptides, confirmed that the peptides are derived from the same protein. Immunoblots of the protein with antipeptide antibodies revealed a protein band corresponding to the molecular weight of denatured reduced μ-opioid binding protein. The immunoresponse was blocked by the appropriate peptide and was not observed with irrelevant antisera. The antipeptide antibodies were used for immunoblots of sodium dodecyl sulfate extracts of tissues from bovine brain regions and of the μ receptor-containing cell line SK-N-SH. Affinity-purified antipeptide antibody detected an immunoreactive protein of molecular weight 65,000 in brain regions containing high levels of μ-opioid receptors (striatum, thalamus, hippocampus, and frontal cortex) and in the cell line SK-N-SH. Pons, which contains low levels of receptors, produced a a barely detectable signal, whereas white matter, HeLa cells, and C6 glioma cells, devoid of opioid binding activity, produced no detectable signal. The correlation between immunoreactivity and the presence of μ-opioid binding in brain regions and cell lines and the correspondence of the molecular weight of the immunoreactive protein to that of μ-opioid receptors provide strong evidence that the peptide antisera recognize μ receptors.

A number of laboratories have reported the purification of proteins capable of binding opioid ligands (for review, see Ref. 1). Recently, two laboratories (2, 3) have cloned and sequenced a receptor protein from NG108-15 cell cultures that has the pharmacological characteristics of the δ receptor. The structure of the cloned δ receptor, as determined by analysis of the primary amino acid sequence, confirms its membership in the family of G protein-coupled receptors, distinguished by the presence of seven hydrophobic putative membrane-spanning regions.

The purification to homogeneity by our laboratory (4) of a μ-OBP from bovine striatal membranes involved a combination of ligand and lectin affinity chromatography. Under reducing conditions, OBP migrates in SDS-PAGE to a position that corresponds to a molecular weight of 65,000. It binds opioid antagonists with high affinity and with nearly theoretical specific binding capacity. However, it binds agonists with low affinity (micromolar), presumably because it is not coupled to G protein.

Recently, we have succeeded in reconstituting purified OBP into liposomes containing G proteins (5). Reconstituted OBP was found to bind μ-opioid agonists selectively and with high affinity, close to that seen in membrane-bound μ receptors.

The structures of two peptides obtained from microsequencing of purified μ-OBP and the generation and properties of polyclonal antibodies against synthesized segments of the peptides are described here. The interaction of these antipeptide antibodies with purified OBP, as well as with extracts of various tissues and cell lines known to contain widely varying levels of μ-opioid receptors, is the main topic of this paper.

Experimental Procedures

Materials

Reagents used in electrophoresis and immunoblotting were purchased from Bio-Rad. Pansorbin cells used in immunoprecipitation

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ABBREVIATIONS: OBP, opioid binding protein; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin; PBS, phosphate-buffered saline; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; DAGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin.

assays were obtained from Calbiochem. Digitonin was purchased from ICN and processed before use, as described (6). Molecular weight rainbow markers and $Na^{125}I$ were purchased from Amersham. [^{3}H] Bremazocine (25 Ci/mmol) used in assaying purified OBP was from New England Nuclear-DuPont. Reagents used for peptide synthesis were supplied by Applied Biosystems. Brain regions were dissected from fresh bovine brains obtained from a slaughterhouse and delivered by Max Insel Cohen (Rahway, NJ). Crude cell membrane preparations were made as described (7).

Methods

Fragmentation and microsequencing of OBP. OBP was extracted from bovine striatal membranes and purified to homogeneity by the method published previously (4). The binding of purified OBP was evaluated with [^{3}H]bremazocine by the filtration binding assay described previously (8). Samples of OBP were then concentrated using Centriprep and/or Centricon-10 filters (Amicon). To reduce digitonin concentrations, after samples were concentrated to the desired volume they were diluted twice in 10 volumes of buffer and reconcentrated.

Fragmentation and microsequencing of OBP were carried out by Dr. C. Strader (Merck, Sharp & Dohme, Rahway, NJ), according to procedures previously used with purified β-adrenergic receptors (9). To facilitate assessment of the procedures, a small portion (5–10 pmol) of ^{125}I-labeled OBP (see procedure for iodination described below) was added to the sample of OBP (150–200 pmol) to be used for fragmentation. The OBP was subjected to SDS-PAGE, according to the method of Laemmli (10), through a 4.5% stacking gel and a 10% polyacrylamide running gel in the presence of 0.1% SDS. It was then transferred by electroblotting to nitrocellulose in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3, containing 0.01% SDS) for 1 hr at 100 V (11). An autoradiogram (1–3-hr exposure) of the nitrocellulose was obtained to determine the position of the protein band. The band was then excised from the blot and digested in a 13- × 100-mm glass tube with CNBr (100 mg/ml in 70% formic acid) in 5% acetonitrile for 20 hr at room temperature. The peptides generated from the fragmentation were separated by reverse phase high performance liquid chromatography on a Syncropak C4 column, using a gradient of 0–70% acetonitrile in trifluoroacetic acid buffer (20 mM). The amino acid sequences of the two peptides isolated were determined using a gas-phase sequenator.

Peptide synthesis. The peptide (I-1–12) used for the production of antisera Ab165 and Ab6639 was synthesized by Dr. David Schlesinger (New York Unversity School of Medicine). All others were made at the University of Michigan. A solid state synthesizer (Applied Biosystems model 431 A) was used according to procedures supplied by the manufacturer. Amino acid side chains were protected with standard blocking reagents. Protecting groups were removed and the peptide was cleaved from the resin with trifluoroacetic acid. Purification of peptides was carried out by high performance liquid chromatography using a C18 Porasil B column (Waters) with a 5–60% gradient of acetonitrile/0.5% trifluoroacetic acid. After removal of the acetonitrile by rotary evaporation, the aqueous solution was lyophilized to yield the desired peptide.

Antibody production. Antibodies were generated against synthetic peptides based on the sequences determined from the fragmentation of purified OBP. The immunogens were prepared by coupling the peptides to thyroglobulin, using either benzidine (12) or glutaraldehyde (13). New Zealand white rabbits received intradermal injections, once each week for 3 consecutive weeks, of 1 ml of conjugate (250 μg of peptide) diluted 1/1 in 1 ml of complete Freund's adjuvant. Animals were bled 3 weeks later. One week after the first bleed, the rabbits were boosted with 1 ml of conjugate (125 μg of peptide) diluted in 1 ml of complete Freund's adjuvant. They were bled 2 weeks later and boosted again the next week. Significant immunoreactivity was observed after the fourth bleed. A total of eight bleeds were taken from each rabbit.

Antibody purification. The antibody (Ab165) used in immunoblots with membrane extracts was purified by affinity chromatography. Affinity beads were prepared by incubating OBP peptide I-1–12 (2 mg) with 1 ml of Reacti-Gel 6X (Pierce) in 100 mM sodium borate, 0.9% NaCl, pH 8.5, at 4° for 42 hr. The gel was rinsed with 2 M Tris·HCl, pH 8.0, to block any unreacted sites and the gel was washed exhaustively with distilled water. The antibody (1 ml) was diluted with 12 ml of 150 mM sodium phosphate, 0.9% NaCl, 0.3% BSA, 0.1% bacitracin, pH 8.2, and incubated at 4° overnight with the aforementioned peptide-coupled gel. The gel was placed in a column, the flow-through fraction was collected, and the column was washed with 20–30 ml of 150 mM sodium phosphate buffer, pH 8.2. The antibody was eluted from the column with 5 ml of 100 mM glycine-HCl, pH 2.3, into 3 ml of 3 M Tris, pH 8.6, containing 0.1% BSA. The fraction containing the eluted antibody was dialyzed against 100 mM Tris, 2 M NaCl (2 liters). The dialyzing solution was changed every hour and gradually diluted until it contained 20 mM Tris and 0.4 M NaCl. The sample was then dialyzed overnight at 4° against PBS (100 mM sodium phosphate, 0.9% NaCl, pH 7.5). Finally the sample was concentrated by lyophilization to the original volume of antiserum.

Iodination procedure. OBP (100 ng) was iodinated according to the method of Greenwood *et al.* (14), using chloramine-T and $Na^{125}I$. Labeled protein was separated from free iodine on a Sephadex G25 column equilibrated with 50 mM Tris·HCl, 100 mM NaCl, 0.025% digitonin, pH 7.4. Samples of ^{125}I-OBP were precipitated with trichloroacetic acid to determine the amount of protein-bound radioactivity, which was generally between 75 and 85%.

Immunoprecipitation assays. Immunoprecipitation assays were set up in 1.5-ml microfuge tubes that had been prewashed with assay buffer, consisting of PBS containing 0.1% BSA and 0.5% Triton X-100. Final assay volume was 0.25 ml. Samples were incubated overnight at 4° with shaking. The next day the antibody-antigen complex was precipitated by incubation for 15 min at 4° with Pansorbin cells (25 μl) that had been washed and resuspended in assay buffer. The samples were centrifuged at 6000 × *g* for 2 min and the supernatant was removed. The pellets were washed by resuspension in assay buffer and centrifugation. The amount of radioactivity immunoprecipitated was determined by counting the pellets in an LKB BioGamma counter. Control samples were treated in the same manner using normal rabbit serum or preimmune serum from the same rabbit. Nonspecifically precipitated radioactivity was subtracted from all values for immunoprecipitation.

In experiments in which a sample of ^{125}I-OBP was treated sequentially with two antibodies, the supernatant resulting from immunoprecipitation with the first antibody was carefully removed. This supernatant was then treated with the second antibody according to the procedure described above.

For elution of the antigen off the antigen-antibody complex, the pellet from the immunoprecipitation was dissolved in SDS-PAGE sample buffer. The sample was centrifuged at 10,000 × *g* to remove insoluble residue. The supernatant was subjected to SDS-PAGE, as described below.

Procedure for immunoprecipitation of native opioid binding sites. Extracts of striatal membranes were prepared using the zwitterionic detergent CHAPS and bound as described by Ofri *et al.* (15). Solubilized material was incubated with antibody for 2 hr at 25°. Immunoprecipitation was carried out with Protein A-agarose beads according to the procedure described above for ^{125}I-OBP. Protein A-agarose beads did not inhibit the opioid binding activity of the supernatant in control samples. Opioid binding activity remaining in the supernatant after immunoprecipitation was then examined. The supernatant was removed, treated with an equal volume of 40% polyethylene glycol, vortexed, and centrifuged at 10,000 × *g* for 15 min (15). The resulting pellet was resuspended and bound with the radioactive opioid ligands. Samples were filtered through Whatman GF/B filters that had been presoaked in 0.3% polyethyleneimine (16). The amount of bound radioactivity was determined by scintillation counting in Ecoscint A (National Diagnostics).

Electrophoresis and immunoblotting. Samples of tissues or cell lines (30–50 μg of protein) were boiled for 1–2 min in sample buffer,

containing 100 mM dithiothreitol, 0.125 M Tris·HCl, pH 6.8, 4% SDS, 20% glycerol, and 0.01% bromophenol blue. Aliquots of OBP, which contained digitonin, were prepared for electrophoresis by incubation for 45–60 min at 37°, rather than boiling, with an equal volume of sample buffer. Samples were subjected to SDS-PAGE, and the protein was transferred to nitrocellulose according to the method of Towbin *et al.* (11). After transfer, nonspecific sites on nitrocellulose were blocked by incubation with PBS containing 3% BSA. Nonidet P-40 (0.56%) and EDTA (5.6 mM) were added, followed by antiserum at the desired dilution. After incubation overnight at 4°, the sheets were washed four times with PBS. The blot was then incubated for 1 hr at 25° in PBS, 3% BSA, 0.56% Nonidet P-40, 5.6 mM EDTA, containing goat anti-rabbit IgG conjugated to alkaline phosphatase (1/1000). The nitrocellulose was washed well and then exposed to a freshly prepared solution of the reagents 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium to produce color. The reaction was stopped by removing the substrate solution and rinsing the nitrocellulose with distilled water. For each blot, prestained molecular weight markers (Amersham) were run. Appropriate controls, such as nonimmune rabbit serum, were also run. For experiments designed to determine whether reaction with antiserum could be blocked by preabsorption with the appropriate peptide, the antiserum was preincubated with peptide solution (100–200 μM) for 2 hr at 25° or overnight at 4° before addition to nitrocellulose.

Results

Isolation and sequencing of peptides derived from purified μ-OBP. Direct amino acid sequencing of purified μ-OBP was unsuccessful, indicating that the amino terminus of OBP is blocked. After fragmentation with cyanogen bromide, two peptides with free amino termini were isolated. The two peptides, containing 20 (peptide I) and 13 (peptide II) amino acids, were sequenced as described. The amino acid sequences are not found in any database of known protein structures, nor do they exhibit a high degree of homology to sequences in any known protein, including the recently sequenced δ-opioid receptor. This is not surprising, because the amino acid sequences of the peptides indicate that they are from a highly hydrophilic and, presumably, poorly conserved region of the protein.

The identities of one amino acid in peptide I (residue 13) and one (residue 10) in peptide II are unknown. For the generation of antipeptide antibodies, therefore, we decided to synthesize the fragments on both sides of the unknown residue of peptide I (I-1–12 and I-14–20) and the first nine residues of peptide II (II-1–9). An amino-terminal tyrosine residue was added to the latter to permit iodination. Immunization of rabbits with thyroglobulin conjugates of these three peptides produced six different antibodies. The peptide fragments and the antibodies generated against them are listed in Table 1.

Immunoprecipitation of OBP with peptide antisera. All six antisera were found to recognize purified OBP. In an immunoprecipitation assay using ^{125}I-OBP as an antigen, the antibodies, at a dilution of 1/200, precipitated 40–65% of the labeled antigen added (Table 2). The ability of the antibodies to immunoprecipitate OBP was in the order Ab165 = Ab162 = Ab6639 > Ab163 > Ab166 > Ab161. Ab165 and Ab162 were potent enough to precipitate 30% of added antigen even at a 1/1000 dilution, whereas Ab161 precipitated <10% of added ^{125}I-OBP at this dilution. The radioactivity precipitated by normal rabbit serum or irrelevant antiserum accounted for 5–7% of the added labeled material at all dilutions tested. Elution of immunoprecipitated OBP from the antibody, followed by SDS-PAGE and autoradiography, produced a major radioactive band with an apparent molecular weight of 65,000. Preincubation of antiserum with the appropriate peptide greatly reduced immunoprecipitation of OBP. For example, peptide I-1–12 at a concentration of 100 μM inhibited the ability of Ab165 to immunoprecipitate ^{125}I-OBP by 80%, whereas irrelevant peptides had no effect.

Ab165 and Ab162 were prepared against sequences from peptide I and peptide II, respectively (Table 1). If both peptides are derived from the same protein, sequential treatment with the two antisera in either order should not produce additional immunoprecipitation by the second antibody added. The data in Table 3 show that sequential treatment of ^{125}I-OBP with the two antibodies precipitated ^{125}I-OBP to the same extent as treatment with one antibody only. The small additional amount of radioactivity precipitated by treatment with the second antibody was no more than that observed when the first and second antibodies were the same. This is strong evidence that the two peptides are derived from the same protein.

Inability of antisera to react with native opioid receptors. All of the immunoprecipitations discussed above were carried out with purified OBP that had been concentrated and radioiodinated with the oxidizing reagent chloramine-T. This

TABLE 1

Antipeptide antibodies generated against amino acid sequences derived from OBP

	Amino acid sequence[a]	Antibody
Peptide I-1-12	IRNLRQDRSKYY[X]	Ab165, Ab166, Ab6639
Peptide I-14-20	NFFYKRL	Ab163
Peptide II-1-9	Y*SNNVLFVSH[XFND]	Ab161, Ab162

[a] X, unknown amino acids; Y*, tyrosine added to permit iodination of peptide. Bracketed residues were not used for antibody production.

TABLE 2

Immunoprecipitation of purified ^{125}I-OBP by antisera generated against peptide sequences isolated from OBP

Purified ^{125}I-OBP added contained 25,000–40,000 cpm/sample. Immunoprecipitations were carried out using Pansorbin, as described in Experimental Procedures. Radioactivity precipitated nonspecifically by normal rabbit serum was subtracted from all values.

Antibody (1/200)	^{125}I-OBP immunoprecipitated
	%
Ab161	40 ± 2
Ab162	60 ± 2
Ab163	56 ± 2
Ab165	57 ± 3
Ab166	45 ± 1
Ab6639	63 ± 4

TABLE 3

Sequential immunoprecipitation of ^{125}I-OBP by antibodies generated against sequences from two different peptides isolated from OBP

Immunoprecipitations were carried out as described in Experimental Procedures, using antibodies at 1/200 dilution. After the first immunoprecipitation, the supernatant was removed and then incubated with a second antibody (1/200) as indicated. Radioactivity precipitated nonspecifically by normal rabbit serum was subtracted from all values.

First antibody	^{125}I-OBP precipitated	Second antibody	Additional ^{125}I precipitated	Total ^{125}I precipitated
	%		%	%
Ab162	62	Ab165	2	64
Ab162	60	Ab162	4	64
Ab165	56	Ab162	5	61
Ab165	54	Ab165	4	58
Normal rabbit serum	0	Ab165	62	62

treatment very likely denatures the protein at least partially. Attempts were made to immunoprecipitate active native opioid receptor. Crude CHAPS-solubilized active opioid binding sites were incubated with each of the six antibodies. The antigen-antibody complex was then adsorbed on Protein A-agarose. The degree of binding remaining in the supernatant was evaluated, using the following ligands: [^{3}H]bremazocine, [^{3}H] DAGO, and ^{125}I-β-endorphin. No consistent significant diminution of opioid binding was observed in supernatants from samples that had been treated with concentrations of antibody as high as 1/25. This suggests that the protein must be at least partially denatured for the antibodies to gain access to their epitopes.

Immunoblots of OBP with peptide antisera. The specificity of these antibodies was further confirmed by investigating their interaction with OBP in immunoblots. In all purified OBP preparations examined, a positive signal was detected corresponding to an immunoreactive protein that migrated through the SDS gel with a mobility indicative of a molecular weight of 65,000 under reducing conditions (Fig. 1). No signal was detected with nonimmune serum or an irrelevant antiserum. The strength of the signal varied with the antibody examined. Antibodies generated against peptide I-1–12 produced the strongest signal, whereas, for the same amount of OBP, higher concentrations (1/50 or 1/25 dilutions) were needed to produce a signal with antisera against peptides I-14–20 and II-1–9. The reaction with OBP could be totally blocked by preincubation of the antiserum with the appropriate peptide, whereas preabsorption with irrelevant peptides had no effect on the intensity of the signal.

Immunoblots of tissues and cell lines with antipeptide antibodies. Immunoblots of detergent-solubilized (CHAPS, digitonin, or SDS) bovine caudate membranes contained immunoreactive material that migrated to a position consistent with a molecular weight of 65,000, the previously reported molecular weight of μ-opioid receptors (17–19). This result prompted us to investigate the ability of the antibodies to detect the presence of μ receptors in different bovine brain regions as well as in the cell line SK-N-SH. Fig. 2 shows the immunoblots, with affinity-purified Ab165, of SDS extracts of tissues from various brain regions. Regions known to have moderate to high levels of μ-opioid receptors (20), such as striatum, frontal cortex, hippocampus, and thalamus, gave positive responses at an apparent molecular weight of 65,000 that were specifically blocked by preincubation of antiserum with peptide I-1–12. A barely detectable response was observed with pons, an area known to have low levels of receptors. White matter, a region devoid of opioid receptors, gave no detectable signal. Thus, the response to the antibody correlated well with the presence of μ-opioid receptors in these regions.

The cell line SK-N-SH, derived from a human neuroblastoma, contains mainly μ-type opioid receptors. SDS extracts of this cell line and of two cell lines, HeLa and C6 glioma, with no detectable opioid binding were examined in immunoblots with affinity-purified antipeptide serum Ab165. As depicted in Fig. 3, SK-N-SH cells contained an immunoreactive protein that migrated with the same mobility as the band observed with bovine brain regions and with purified OBP, whereas no signal could be detected with HeLa or C6 cells. Specificity of the signal from SK-N-SH cells was demonstrated by the fact that it was totally blocked when the antiserum was preincubated with 100 μM peptide I-1–12. It is of interest that the epitope appears to be conserved between species, in this case cow and human.

Most of the work described here was carried out with Ab165, but Ab161, which was generated against peptide II-1–9, produced identical results in immunoblots of tissues and cell lines.

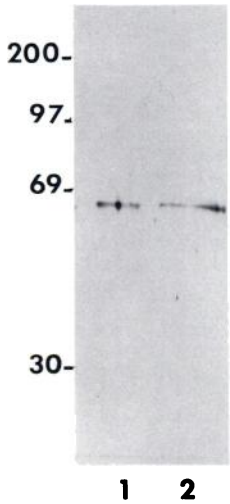

Fig. 1. Immunoblots of purified OBP with an antipeptide antibody. *Lanes 1* and *2*, signals produced by immunoblots of two typical preparations of purified OBP (approximately 30 ng). Samples were separated by SDS-PAGE, transferred to nitrocellulose, and incubated with Ab165 (1/100). The signal was produced by reaction of color substrate with alkaline phosphatase conjugated to goat anti-rabbit IgG (for details see Experimental Procedures).

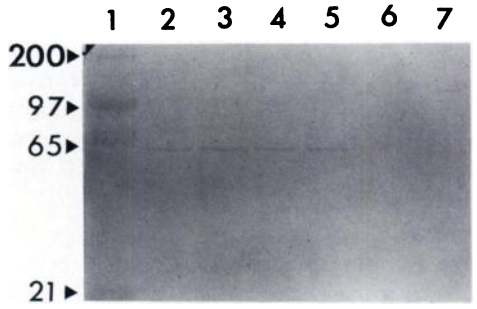

Fig. 2. Immunoblots of SDS extracts of various bovine brain regions with an affinity-purified antipeptide antibody. Tissues were solubilized and immunoblots with affinity-purified Ab165 (1/100) were carried out as described. *Lane 1*, molecular weight markers; *lane 2*, striatum; *lane 3*, frontal cortex; *lane 4*, hippocampus; *lane 5*, thalamus; *lane 6*, pons; *lane 7*, white matter.

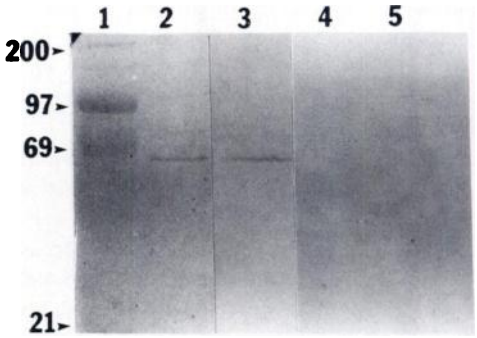

Fig. 3. Immunoblots of SDS extracts of cell lines with an antipeptide antibody. Samples were solubilized and immunoblots were prepared as described. *Lane 1*, molecular weight markers; *lane 2*, caudate; *lane 3*, SK-N-SH cells; *lane 4*, HeLa cells; *lane 5*, C6 glioma cells.

Discussion

In our original publication describing the purification of OBP, considerable evidence was presented that supported the identity of OBP as a μ binding protein (4), although none of it was absolutely conclusive. Conclusive evidence has now been provided by our recent success in reconstituting high affinity μ agonist binding to purified OBP in liposomes containing the necessary lipids and G proteins (5). The reconstituted OBP binds μ agonist ligands with affinities in the nanomolar range (e.g., for DAGO the K_d value is 1.5 nM, identical to that for membrane-bound μ receptors). The μ binding is highly selective, because δ and κ ligands bind with affinities 2–3 orders of magnitude lower. The binding is stereospecific, saturable, and totally inhibited by GTPγS.

In this paper we report the structures of two peptides isolated from purified OBP and sequenced. As stated, these peptides show no significant homology to any known protein, including the δ receptor from NG108–15 cells, in updated databases of protein structures examined recently. Oligonucleotides based on the structure of the peptides are currently being used to screen bovine brain cDNA and genomic libraries and to generate longer probes by polymerase chain reaction.

We have prepared polyclonal antisera against fragments of the two peptides and report here their recognition of purified OBP, as well as of an immunoreactive protein in SDS extracts of tissues and the cell line SK-N-SH. The antisera are able to precipitate a major portion of radioiodinated purified OBP and detect immunoreactive material of the correct size in immunoblots of purified OBP. These data indicate that the peptides to which the antibodies were produced are derived from this protein. Sequential immunoprecipitation experiments provide additional evidence that the two peptides are derived from the same protein. The system is evidently internally consistent, i.e., in numerous batches of purified OBP the antipeptide antisera recognize the major protein, which is the source of both peptides.

The antisera do not appear to recognize the native OBP, because our attempts to immunoprecipitate active native μ binding sites have been unsuccessful. The inability of antipeptide antibodies to recognize active membrane-bound or soluble receptors has been observed by other investigators working with antibodies to relatively short peptides derived from receptor proteins (21–24). The peptides against which the antibodies were generated may well occur in an internal position of the protein that is inaccessible to the antibodies when the protein is in its active conformation. The process of concentrating solutions of OBP, followed by iodination with chloramine-T, seems to denature OBP sufficiently to allow the antibody to interact with its epitope.

The antipeptide antisera recognize immunoreactive material with an apparent molecular weight of 65,000 in extracts of some bovine brain tissues, but not others, and in the cell line SK-N-SH, but not in HeLa or C6 cells. The results correlate well with the presence or absence of μ receptors in these materials. The molecular weight of 65,000 corresponds to that previously reported for purified OBP (4) and for μ-opioid binding sites cross-linked to ^{125}I-β-endorphin in bovine brain regions (17, 18) and in SK-N-SH cells (19).

Immunocytochemical studies with affinity-purified antipeptide antiserum Ab165 have recently been initiated.[1] The results show a distribution of immunoreactivity in rat brain and spinal cord very similar to that of μ-opioid binding observed in many laboratories, including our own, by autoradiography. It should be mentioned that the cerebellum gave a weak positive signal in immunoblots (data not shown) as well as in the immunocytochemical experiments. However, neither cerebellar signal was blocked by preabsorption of the antiserum with the appropriate peptide, I-1–12. A cross-reacting material appears to be responsible.

In summary, the polyclonal antisera we have generated against peptides sequenced from purified μ-OBP cross-react with the purified protein in immunoprecipitation and immunoblots. Evidence is presented indicating that the two peptides are derived from a single protein. The size of the band obtained in immunoblots of tissues and cell extracts and the correlation between the immunological response and the presence of μ receptors in various brain regions and cell lines indicate that the antibodies recognize μ-opioid receptors.

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