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Interaction of Agonist Peptide [^{3}H]Tyr-D-Ala-Phe-Phe-NH_2 with μ-Opioid Receptor in Rat Brain and CHO-μ/1 Cell Line

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SPETEA, M., F. ÖTVÖS, G. TÓTH, T. M.-D. NGUYEN, P. W. SCHILLER AND A. BORSODI. *Interaction of agonist peptide [^{3}H]Tyr-D-Ala-Phe-Phe-NH_2 with μ-opioid receptor in rat brain and CHO-μ/1 cell line.* PEPTIDES **19**(6) 1091–1098, 1998.—Opioid receptor binding properties of [^{3}H]Tyr-D-Ala-Phe-Phe-NH_2 (TAPP) were characterized in rat brain and Chinese hamster ovary (CHO) cells expressing the rat μ-receptor. In rat brain, [^{3}H]TAPP labeled a single class of opioid sites with a dissociation constant (K_d) of 0.31 nM and maximal number of binding sites (B_{max}) of 119 fmol/mg protein. In CHO-μ/1 cell membranes, the K_d and B_{max} values were 0.78 nM and 1806 fmol/mg protein, respectively. Binding to rat brain was demonstrated to be pharmacologically identical to that obtained with CHO-μ/1 cell membranes and modulated by Na^+ ions and guanine nucleotides. The high affinity and selectivity of [^{3}H]TAPP together with its low non-specific binding make this radioligand a useful tool for labeling the native and cloned μ-opioid receptor. © 1998 Elsevier Science Inc.

Opioid receptors μ-Opioid agonist peptides Rat brain CHO-μ/1 cell line [^{3}H]TAPP Radioligand binding

AMONG the three main opioid receptor types, referred to as μ, δ and κ, μ-opioid receptors are perhaps of the greatest clinical importance (22). They bind morphine with high affinity and are associated with drug addiction and modulation of pain perception. The presence of multiple μ-opioid receptor subtypes has also been suggested (16,23,38). In addition to the opioid alkaloids, endogenous opioid peptides are also known to bind to μ-receptors. Recently, two tetrapeptides have been isolated from bovine frontal cortex and were named endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) (41). These endogenous peptides were found to exhibit the highest specificity and affinity for the μ-receptor among all endogenous opioid compounds discovered so far in the mammalian nervous system.

During the past two decades, one of the aims of opioid pharmacology has been to design drugs with high selectivity for each of the three receptor types (4,30,35). Some of them are already used as therapeutic agents because they permit improved treatment for pain, but their use is complicated by many other effects. Since the naturally occurring enkephalins (Tyr-Gly-Gly-Phe-X, where X is Met or Leu) are rapidly degraded by various peptidases, initial efforts were aimed at making the peptide molecules more resistant to

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enzymatic degradation, and this goal was achieved through introduction of D-Ala in position 2 of the peptide sequence and through amidation of the C-terminal carboxyl group. The D-amino acid in the second position was found to be vital for receptor binding and biologic activity (18). In contrast to mammalian opioid peptides, those isolated from amphibian skin, such as dermorphin and dermorphin-related peptides, contain a D-Ala residue in the second position of the peptide sequence and carboxyamide group at the C-terminal end. The dermorphins were shown to be highly potent and selective μ-opioid agonists (17,21,28). A number of linear analogs of enkephalins, β-casomorphins, and dermorphins with selectivity for the μ-opioid receptors have been developed, some of them also in radiolabeled form. Among them are: DAMGO ([D-Ala^2, Me-Phe^4, Gly-ol^{5}enkephalin; 12); PL017 (Tyr-Pro-NMePhe-D-Pro-NH_2; 3,7); TRIMU 5 (Tyr-D-Ala-Gly-NH$(CH_2)_2$-CH$(CH_3)_2$; 11); and DALDA (Tyr-D-Arg-Phe-Lys-NH_2; 31). In the search for more potent μ-selective ligands, a dermorphin tetrapeptide analog containing a D-Ala residue in position 2 and two Phe residues in positions 3 and 4 has been synthesized (31). The prototype ligand Tyr-D-Ala-Phe-Phe-NH_2 (TAPP) showed high μ-receptor affinity and μ-selectivity.

Recent cDNA cloning studies have revealed primary structures of μ-, δ- and κ-opioid receptors. The cloning efforts have clearly identified these receptors as members of the seven-transmembrane domains G-protein-coupled receptors superfamily (14). The availability of the cloned opioid receptors allowed studies of individual receptor types with regard to pharmacological profile, structure-function analysis, cellular effector coupling, and regulation of expression. Manipulation of opioid receptors by site-directed mutagenesis, deletions, and chimera constructions was providing information protein domains that may be important for ligand binding and receptor function (13,42). Transfection of an individual opioid receptor in various heterologous host cells can now be used to provide a pure, homogenous population of receptors. Several cell lines expressing cloned mouse, rat, or human μ-opioid receptors are good models for determining binding affinities and selectivities of new opioid drug candidates. The cloned μ-opioid receptors have been expressed in epithelial cells like COS (monkey fibroblast; 39) and CHO (Chinese hamster ovary; 2,6,10,27). Yeast cells (36) and excitable cells, such as the pituitary cell line GH_3 (25) have also been used to investigate pharmacological properties of cloned μ-opioid receptors.

In the present study we describe the synthesis of the new opioid agonist peptide radioligand [^{3}H]TAPP showing high specific radioactivity (56.8 Ci/mmol). Furthermore, we report the binding characteristics of this radioligand in membrane preparations from rat brain and from a CHO cell line expressing the rat μ-opioid receptor, as determined by using direct in vitro ligand binding assays.

METHOD

Materials

TAPP was synthesized in the Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Quebec, Canada, as described (31). DAMGO and [D-Ser^2, Leu^5, Thr^6]enkephalin (DSLET) were purchased from Bachem Feinbiochemica, Bubendorf, Switzerland. U69,593 was obtained from Upjohn Company (Kalamazoo, MI). Ile^{5,6}deltorphin II (29), TIPP (Tyr-Tic-Phe-Phe-OH) (32), and dihydromorphine were synthesized in our Isotope Laboratory. Cyprodime was synthesized by Dr. H. Schmidhammer (Institute of Organic and Pharmaceutical Chemistry, University of Innsbruck, Innsbruck, Austria; 33). Naltrindole (26) was a gift from Dr. S. Hosztafi (Alkaloida Chemical Company Ltd., Tiszavasvári, Hungary). DPDPE ([D-Pen ^{2}, D-Pen^5]enkephalin (19) was kindly provided by Dr. K. Medzihradszky (Central Research Institute for Chemistry, Budapest, Hungary). Dextrorphan and levorphanol were obtained from Hoffmann-La Roche (Nutley, NJ). Naloxone hydrochloride, bovine serum albumin (BSA), sodium chloride (NaCl), 5′-guanylyl-imidodiphosphate (Gpp(NH)p), Dulbecco's modified Eagle's medium (DMEM), and phosphate-buffered saline (PBS) were purchased from Sigma Chemicals (St. Louis, MO). Fetal calf serum (FCS) was obtained from Jaques BOYS (Remise, France), and geneticin (G418) was obtained from Life Technologies (Grand Island, NY). All other reagents used in this study were of analytical grade.

Synthesis of [3,5-^{3}H]Tyr-D-Ala-Phe-Phe-NH_2

The diiodinated precursor of TAPP, [3,5-I_2]Tyr-D-Ala-Phe-Phe-NH_2, was prepared by solid-phase synthesis according to the protocol described elsewhere (31). [^{3}H]TAPP was prepared by catalytic dehalogenation of the diiodinated precursor using Pd/$BaSO_4$ as catalyst and tritium gas in a tritium manifold (37). The precursor (4.2 mg) was dissolved in 1.2 ml of dimethylformamide and exposed to 555 GBq (15 Ci; Technobexport, Russia) of tritium gas in the presence of 11 mg PdO/$BaSO_4$ (Merck) and 5 μl of triethylamine for 60 min at room temperature. The catalyst was removed by filtration through Whatman GF/C glass filters and washed several times with ethanol. Traces of labile tritium were removed by repeatedly evaporating with an ethanol–water (1:1) solution of the radiolabeled product. The radioactivity of the crude peptide was 8.88 GBq (240 mCi). The crude tritiated peptide was purified by thin-layer chromatography on a Kieselgel 60 F_{254} plate (Merck) using acetonitrile-methanol-water (4:1:1) as the solvent system. The purity of the radiolabeled peptide was assessed by thin-layer chromatography in the following three different systems: acetonitrile—methanol–water (4:1:1), R_f = 0.46 (0.67); butanol-acetic acid-water (4:1:1), R_f = 0.58 (0.64); and butanol–acetic acid–ethylacetate–water (5:1:4:1), R_f =

0.46 (0.57); where R_f values in parentheses refer to the precursor diiodo derivative. The purity was also checked by high-performance liquid chromatography on a Vydac 218TP54 (250 × 4 mm) column, starting with 20% acetonitrile/0.1% trifluoroacetic acid in water as eluent during 5 min, then using a gradient of the organic modifier (1.5% increase per min). The purity of the radioligand was greater than 95%, and its total activity was 2.75 GBq (74.2 mCi). The amount of pure material was determined by UV spectroscopy at a wavelength of 275 nm using unlabeled peptide as standard. The specific radioactivity of the purified [^{3}H]TAPP was 2.10 TBq/mmol (56.8 Ci/mmol).

Membrane Preparations

A crude membrane fraction was isolated from Wistar rat brains according to the method previously described (34). Rat brains (minus cerebella) were homogenized on ice in 50 mM Tris-HCl buffer (pH 7.4) using a Teflon glass homogenizer. The homogenate was centrifuged at 40,000 × g for 20 min at 4°. The resulting pellets were resuspended in fresh Tris-HCl buffer, incubated at 37°C for 30 min, and recentrifuged. The final pellets were suspended in 50 mM Tris-HCl (pH 7.4) containing 0.32 M sucrose and stored at -70°C until use.

CHO-μ/1 cells expressing the rat μ-opioid receptor were maintained in DMEM supplemented with 10% FCS and 400 μg/ml geneticin (G418) in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37°C. Membranes from CHO-μ/1 cells were prepared according to the published procedure (10). Cell monolayers were washed twice with PBS, harvested with a rubber policeman, and centrifuged at 1,000 × g for 10 min. Pellets were suspended in 50 mM Tris-HCl buffer (pH 7.4) and homogenized using a Teflon glass homogenizer. The cell homogenates were centrifuged at 20,000 × g for 25 min at 4°C, and the final pellets were resuspended in fresh Tris-HCl buffer. Aliquots of this preparation were stored at -70°C for further use in binding assays. Protein concentrations were determined by the method of Bradford (5), using BSA as a standard.

Receptor Binding Assays

All binding experiments were performed in 50 mM Tris-HCl buffer (pH 7.4) in a final volume of 1 ml, containing 300–500 μg protein in the case of the rat brain membrane homogenates, and containing 100–250 μg protein in the case of the CHO-μ/1 cell membrane preparations. Incubations of the membrane suspensions with the radioligand were carried out at 25°C and stopped by rapid filtration through Whatman GF/C filters, using a Brandel Cell Harvester. The filters were washed with 3 × 5 ml of ice-cold Tris-HCl buffer (pH 7.4). The bound radioactivity was measured in a toluene-based scintillation cocktail using a Beckman scintillation counter. Non-specific binding was defined as the bound radioactivity in the presence of 10 μM unlabeled naloxone.

The time course for association of 0.5 nM [^{3}H]TAPP was determined by incubation of the membrane preparation at 25°C for various periods of time in the absence or presence of 10 μM unlabeled naloxone to define total and non-specific binding, respectively. The reactions were terminated by immediate filtration at the specified times. The specific binding was calculated by subtraction of the non-specific binding from the total binding. Determinations in duplicate were made for each time point. In the dissociation experiments, the radioligand was incubated with the membrane preparation, and its dissociation was initiated by the addition of an excess concentration of unlabeled naloxone (10 μM) once the steady-state had been reached. Saturation

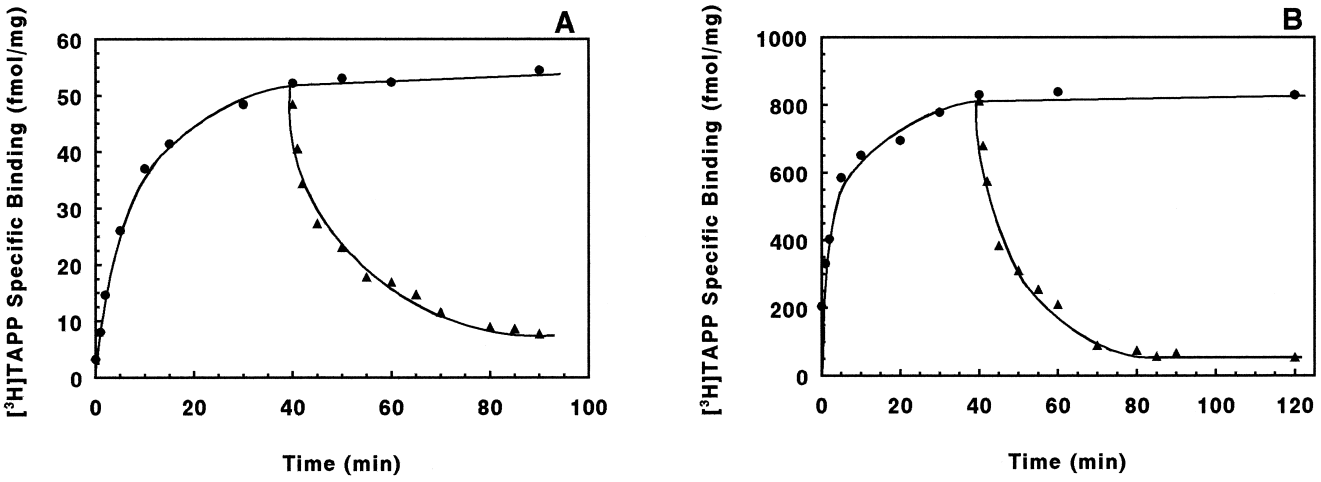

FIG. 1. Association and dissociation kinetics of [^{3}H]TAPP binding. Membranes from rat brain (A) or CHO-μ/1 cell (B) were incubated with 0.5 nM [^{3}H]TAPP for various periods of time at 25°C. Dissociation was initiated by addition of 10 μM naloxone at the steady state.

TABLE 1

COMPARISON OF BINDING PARAMETERS FOR [^{3}H]TAPP IN RAT BRAIN AND CHO-μ/l CELL MEMBRANES

	Rat Brain	CHO-μ/l Cells
Kinetic parameters		
k_{+1} (M^{-1} sec^{-1}) ($\times 10^6$)	1.96 ± 0.66	2.34 ± 1.03
k_{-1} (sec^{-1}) ($\times 10^{-4}$)	9.29 ± 2.49	17.03 ± 5.30
K_d (nM)*	0.47	0.73
Equilibrium parameters		
K_d (nM)	0.31 ± 0.02	0.78 ± 0.09
B_{max} (fmol/mg)	119.1 ± 8.2	1806 ± 138
n_H	1.01 ± 0.02	1.04 ± 0.07

* The equilibrium dissociated constants, K_d, were derived from the averaged kinetic values as follows: $K_d = k_{-1}/k_{+1}$.

All values represent the mean ± SEM of three or four experiments.

binding experiments were performed by incubating the membranes with increasing concentrations of [^{3}H]TAPP from 0.01 to 3.5 nM. Competition binding studies were carried out by incubation of protein with 0.5 nM [^{3}H]TAPP in the presence of different concentrations of unlabeled ligands. All assays were carried out in duplicate and repeated at least three times, and the given values are the mean ± SEM. Kinetic data are analyzed according to the method described by Weiland and Molinoff (40). Competition inhibition constants (K_i) were calculated with the LIGAND program utilizing a non-linear least squares fitting algorithm (20)

RESULTS

To determine appropriate conditions for performing equilibrium binding studies of [^{3}H]TAPP, the effect of incubation temperature on binding and the kinetics of association and dissociation were investigated.

The effect of incubation temperature on specific [^{3}H]TAPP binding was initially examined using the rat brain membranes. Since specific binding of [^{3}H]TAPP at 0°C and 35°C was much less than at 25°C (data not shown), all subsequent binding experiments were performed at 25°C.

Kinetic studies revealed rapid, monophasic association and dissociation of the labeled tetrapeptide from the opioid receptors in the membrane preparations used (Fig. 1). The specific binding of [^{3}H]TAPP to rat brain and CHO-μ/1 cell membranes reached the steady-state after 40 min of incubation at 25°C. Dissociation of specifically bound radioligand was initiated by the addition of 10 μM unlabeled naloxone at the steady-state and showed that the binding process was reversible. The association (k_{+1}) and dissociation rate constants (k_{-1}) for [^{3}H]TAPP binding were calculated, and their values are given in Table 1. From these rate constants, the kinetically derived equilibrium dissociation constants (K_d) were calculated and resulted to be 0.47 and 0.73 nM for binding of [^{3}H]TAPP to rat brain and CHO-μ/1 cell membrane preparations, respectively.

Binding of [^{3}H]TAPP at 25°C was saturable and of high affinity in both membrane preparations used. The results of representative saturation binding experiments are shown in Figure 2. The K_d and the maximal number of binding sites (B_{max}) were calculated by linear regression analysis of the saturation isotherms from Scatchard plots (Table 1). In rat brain membranes, [^{3}H]TAPP bound specifically to a single class of opioid binding sites with a K_d value of 0.31 nM and maximal binding of 119 fmol/mg protein (Fig. 2A). Similarly, saturation studies with CHO cells expressing the μ-opioid receptor indicated that [^{3}H]TAPP bound with a K_d value of 0.78 nM and a B_{max} of 1806 fmol/mg protein (Fig. 2B). The Hill coefficients (n_H) were calculated and found to

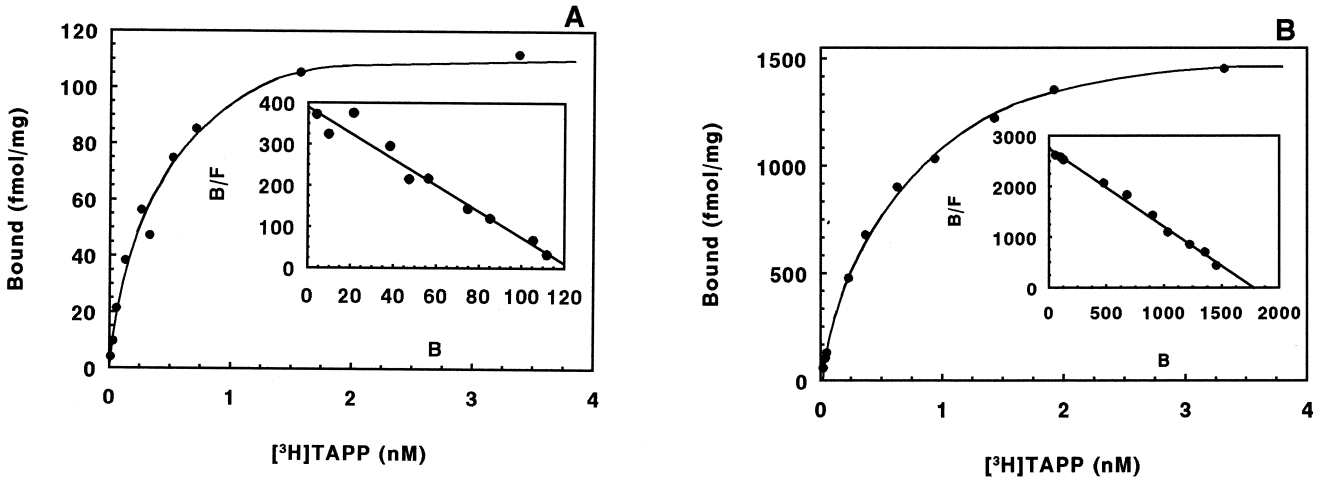

FIG. 2. Saturation of [^{3}H]TAPP binding. Membranes from rat brain (A) or CHO-μ/1 cell (B) were incubated with increasing concentrations of [^{3}H]TAPP in the presence and absence of 10 μM naloxone for 45 min at 25°C. Insert: Scatchard plots.

TABLE 2

[^{3}H]TAPP BINDING COMPETITION EXPERIMENTS WITH VARIOUS TYPE-SELECTIVE OPIOID LIGANDS USING RAT BRAIN AND CHO-μ/l CELL MEMBRANES

Ligand	Type	K_i (nM)*	
		Rat Brain	CHO-μ/l Cells
Dihydromorphine	μ	0.28 ± 0.02	0.63 ± 0.11
DAMGO	μ	1.16 ± 0.29	1.29 ± 0.28
Cyprodime	μ	7.23 ± 1.36	4.59 ± 1.60
U69,593	κ	591.3 ± 23.4	252.8 ± 37.2
DSLET	δ	8.71 ± 1.63	7.34 ± 0.72
Naltrindole	δ	25.7 ± 1.4	10.8 ± 0.8
DPDPE	δ	707.1 ± 124.9	1013 ± 187
TIPP	δ	1705 ± 333	9153 ± 765
Ile^{5,6} deltorphin II	δ	2518 ± 832	1717 ± 142
Levorphanol		0.51 ± 0.13	0.38 ± 0.06
Dextrorphan		778.8 ± 123.1	538.6 ± 216.8

Membranes were incubated with 0.5 nM [^{3}H]TAPP in the presence of increasing concentrations of unlabeled opioid ligands for 45 min at 25°C.

* K_i values were determined by LIGAND analysis. Each value represents the mean ± SEM of three or four experiments.

be 1.01 and 1.04 for rat brain and CHO-μ/1 cell membranes, respectively. The obtained B_{max} value, 119 fmol/mg protein, for [^{3}H]TAPP binding to rat brain membranes is in agreement with those reported for other μ-opioid peptide radioligands, such as [^{3}H]dermorphin (1), the enkephalin analog [^{3}H]DAMGO (9) and the β-casomorphin analog [^{3}H]PL017 (3). In membranes from transfected CHO-μ/1 cells, the calculated B_{max} value, 1806 fmol/mg protein, is similar to that observed for [^{3}H]DAMGO binding to the same type of cells (2,6).

Non-specific binding of [^{3}H]TAPP to rat brain preparations was under 30% of total binding at a radioligand concentration equal to the K_d value, whereas in CHO-μ/1 cell membranes it was much lower, about 10% of total binding under the same conditions.

The stereoselectivity of [^{3}H]TAPP binding to rat brain and CHO-μ/1 cell membranes was indicated by the high affinity of the opiate agonist, levorphanol, and the low affinity of a pharmacologically inactive enantiomer, dextrorphan, determined in [^{3}H]TAPP binding displacement experiments (Table 2 and Fig. 3).

Several μ-receptor selective ligands, including dihydromorphine, DAMGO, levorphanol, and cyprodime, competed for [^{3}H]TAPP binding sites in both rat brain and CHO-μ/1 cells with much higher affinities than the κ-specific U69,593, and δ-selective peptide ligands DPDPE, TIPP, and Ile^{5,6}deltorphin II. The data represented as binding inhibition constants (K_i) are shown in Table 2. The K_i values determined with CHO-μ/1 cell membrane preparation are similar to those obtained with rat brain membranes. In rat brain homogenates, the μ-selective ligands display high affinities with K_i values between 0.3–7.2 nM. About the same range of affinities was observed in CHO-μ/1 cell membranes. The relatively low K_i values observed with the δ-selective ligands, DSLET (11) and naltrindole (26), is in agreement with the previously established fact that they are not among the most selective δ-ligands.

The specific binding of [^{3}H]TAPP to rat brain and CHO-μ/1 cell membranes was substantially reduced in the presence of Na^+ ions (Fig. 4) or the exogenous guanine nucleotides, such as Gpp(NH)p (Fig. 5). NaCl greatly decreased the specific binding of [^{3}H]TAPP to μ-opioid receptors with half maximal inhibition at 10–20 mM salt concentration. In rat brain membranes, [^{3}H]TAPP specific binding was almost completely abolished in the presence of 50 mM NaCl.

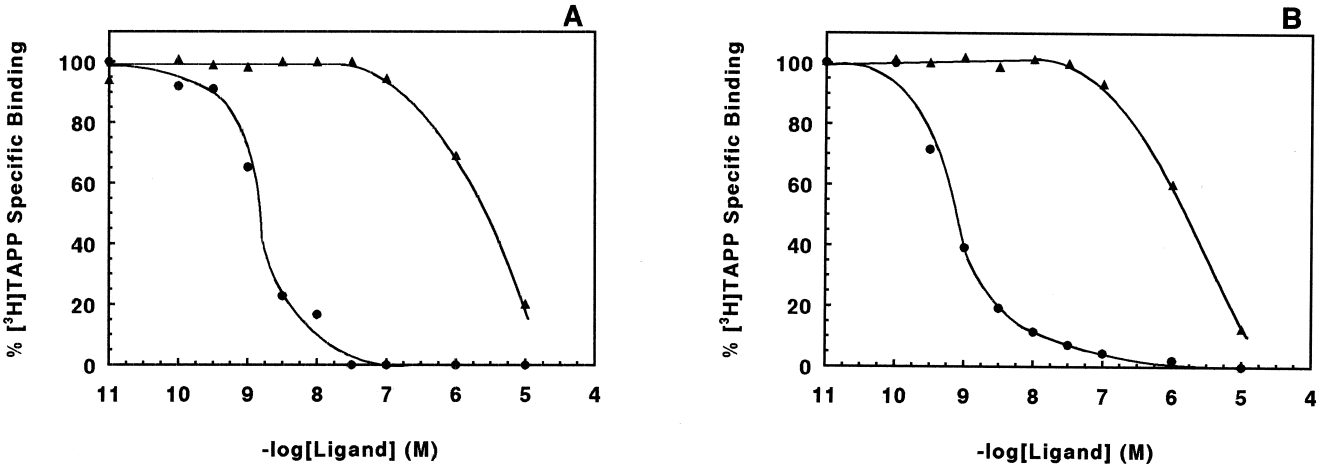

FIG. 3. Stereoselectivity of [^{3}H]TAPP binding. Membranes from rat brain (A) or CHO-μ/1 cell (B) were incubated with 0.5 nM radioligand in the presence of increasing concentrations of levorphanol (●) and dextrorphan (▲) for 45 min at 25°C, as described in the Method section.

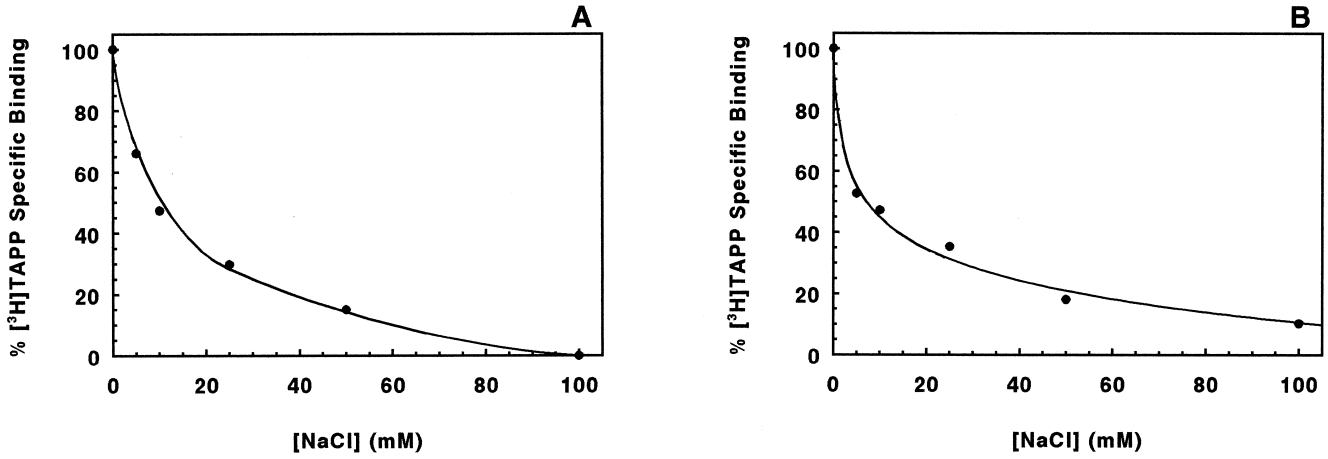

FIG. 4. Effect of NaCl on [^{3}H]TAPP specific binding. Membranes from rat brain (A) or CHO-μ/1 cells (B) were incubated with 0.5 nM [^{3}H]TAPP in the presence of increasing concentrations of NaCl for 45 min at 25°C.

The non-hydrolysable GTP analog, Gpp(NH), produced about an 80% reduction in specific [^{3}H]TAPP binding at a concentration of 100 μM.

DISCUSSION

In the present study, the binding properties of a new radiolabeled tetrapeptide opioid analog, [^{3}H]TAPP, were determined in membrane preparations from rat brain and from a CHO-μ/1 cell line stably transfected with the rat μ-opioid receptor.

The kinetic studies showed that [^{3}H]TAPP associated and dissociated rapidly, in a monophasic manner, from specific opioid receptors in the two membrane preparations. Saturation binding experiments revealed that only a single class of opioid binding sites is labeled by this radioligand. The affinity for this site agreed well with the kinetically determined values for [^{3}H]TAPP binding to both membrane preparations. The Hill slopes for [^{3}H]TAPP binding were close to 1.0, also suggesting ligand binding to a single population of opioid receptors and the non-cooperative nature of the binding process. The higher μ-receptor affinity observed in rat brain may be due to differences in the membrane environment of CHO-μ/1 cells versus brain homogenates. [^{3}H]TAPP showed high affinity in both preparations (K_d values under nanomolar range), but the number of binding sites labeled in transfected CHO-μ/1 cell membranes was significantly higher than that in rat brain homogenates, indicating a higher μ-receptor expression in the recombinant cells. Besides, it is well established that the brain contains multiple opioid receptors, whereas CHO-μ/1 cells contain a homogenous population of μ-opioid receptors (2,6).

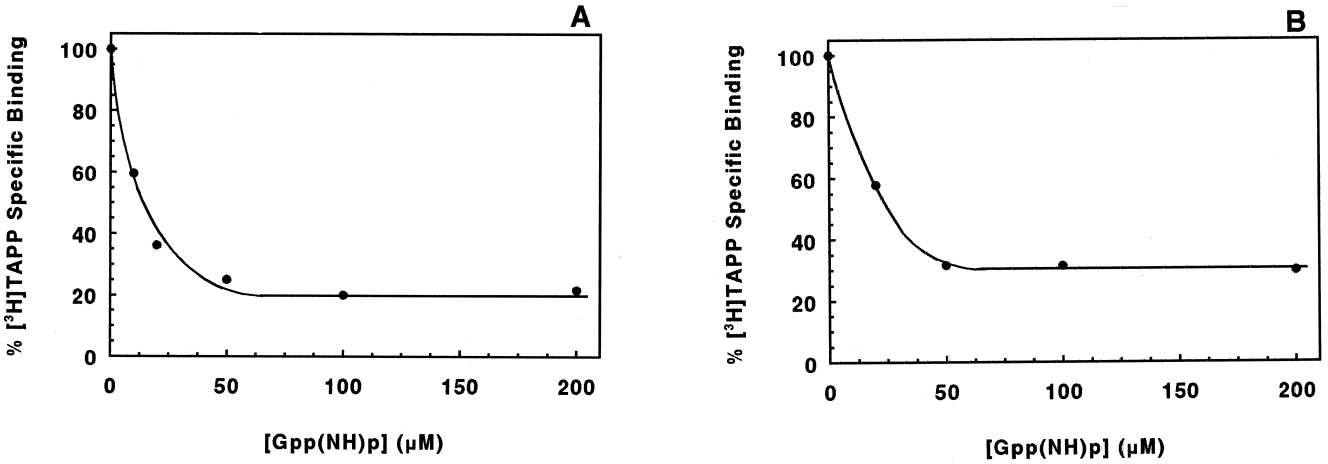

FIG. 5. Effect of Gpp(NH)p on [^{3}H]TAPP specific binding. Membranes from rat brain (A) or CHO-μ/1 cells (B) were incubated with 0.5 nM [^{3}H]TAPP in the presence of increasing concentrations of Gpp(NH)p for 45 min at 25°C.

To further characterize the properties of [^{3}H]TAPP binding, the abilities of several type-selective opioid ligands to displace its binding from rat brain and transfected CHO-μ/1 cell membranes was examined. Both the affinity and rank order of inhibition by opioid ligands of [^{3}H]TAPP binding in rat brain were similar to those observed in membranes from CHO-μ/1 cells. These results indicate that in brain membranes, [^{3}H]TAPP labeled a receptor site with pharmacological properties identical to those exhibited by the μ-opioid receptors heterologously expressed in CHO cell membranes.

The interaction of [^{3}H]TAPP with the native and cloned μ-opioid receptor has also been characterized in terms of its modulation by Na^+ ions and guanine nucleotides. The effect of Na^+ ions and Gpp(NH)p on [^{3}H]TAPP specific binding is in agreement with the agonist character of this peptide. As it is well established, agonists have a lower affinity for opioid receptors in the presence of Na^+ ions, whereas antagonist binding is less affected (24). It has been hypothesized that the binding of Na^+ ions induces a conformational change which renders the opioid receptor site less likely to bind agonists and more likely to bind antagonists (24). Mutagenesis studies on the cloned opioid receptors suggested that the site for Na^+ ions modulation of ligand binding is a conserved Asp^{95} in the second transmembrane region of the protein molecule (15), but the molecular mechanism of this action remains to be elucidated. The reduction of specific binding of [^{3}H]TAPP in the presence of exogenous nucleotide, such as Gpp(NH)p, also indicated the functional coupling of the μ-opioid receptor to a G-protein regulated signal transduction system in both rat brain and transfected CHO-μ/1 cells (8).

The results obtained in the present study revealed that the new radiolabeled peptide analog [^{3}H]TAPP interacted with high affinity with μ-opioid receptor in the two selected membrane preparations. A very good correlation was observed between [^{3}H]TAPP binding in rat brain homogenates versus that in CHO cells transfected with the μ-opioid receptor. It was demonstrated that in rat brain membranes, [^{3}H]TAPP specifically labeled an opioid receptor site with pharmacological properties similar to those exhibited by the cloned rat μ-receptor expressed in CHO cells. Importantly, this radioligand fulfilled the criteria of stereoselectivity, saturability, reversibility, and low non-specific binding necessary for useful radioligands. Furthermore, the binding of [^{3}H]TAPP was found to be modulated by Na^+ ions and guanine nucleotides, in agreement with the agonist nature of this peptide. This compound exhibits a higher μ-receptor affinity than other well-known μ-specific peptide agonists, including dermorphin (1), DAMGO (12), and PL017 (3). These properties of [^{3}H]TAPP, together with its high stability and high specific radioactivity (56.8 Ci/mmol), make it a very promising tool for analyzing the properties and function of the μ-opioid receptor in native tissue preparations and transfected cells. The use of this radioligand should promote a further understanding of the opioid system at the molecular level.

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