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Ligand Binding Characteristics of the Human Serotonin$_{1A}$ Receptor Heterologously Expressed in CHO Cells

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The serotonin$_{1A}$ (5-HT$_{1A}$) receptors are important members of the superfamily of seven transmembrane domain G-protein coupled receptors. They appear to be involved in various behavioral, cognitive and developmental functions. Mammalian cells in culture heterologously expressing membrane receptors represent convenient systems to address problems in receptor biology. We report here the pharmacological characterization of one of the first isolated clones of CHO cells stably expressing the human 5-HT$_{1A}$ receptor using the selective agonist 8-OH-DPAT and antagonist *p*-MPPF. In addition, we demonstrate that agonist and antagonist binding to the receptor exhibit differential sensitivity to the non-hydrolyzable GTP analogue, GTP-γ-S, as was observed earlier with the native receptor from bovine hippocampus. These results show that the human 5-HT$_{1A}$ receptor expressed in CHO cells displays characteristic features found in the native receptor isolated from bovine hippocampus and promises to be a potentially useful system for future studies of the receptor.

KEY WORDS: Serotonin$_{1A}$ receptor, 8-OH-DPAT, *p*-MPPF, G-protein coupling, GTP-γ-S, heterologous expression.

ABBREVIATIONS: GPCR, G-protein coupled receptor; 5-HT$_{1A}$ receptor, 5-hydroxytryptamine-1A receptor; 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; GTP-γ-S, guanosine-5′-*O*-(3-thiotriphosphate); *p*-MPPI, 4-(2′-methoxy)-phenyl-1-[2′-(N-2″-pyridinyl)-*p*-iodobenzamido]ethyl-piperazine; PMSF, phenylmethylsulfonyl fluoride; 5-HT, serotonin; *p*-MPPF, 4-(2′-methoxy)-phenyl-1-[2′-(N-2″-pyridinyl)-*p*-fluorobenzamido]ethyl-piperazine.

INTRODUCTION

Serotonin (5-hydroxytryptamine or 5-HT) is an intrinsically fluorescent [1], biogenic amine which acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous systems [2]. Serotonergic signaling appears to play a key role in the generation and modulation of various cognitive and behavioral functions such as sleep, mood, pain, addiction, locomotion, sexual activity, depression, anxiety, alcohol abuse, aggression and learning [3–7]. Disruptions in

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serotonergic systems have been implicated in the etiology of mental disorders such as schizophrenia, migraine, depression, suicidal behavior, infantile autism, eating disorders, and obsessive–compulsive disorder [4, 8–10]. In addition, recent studies indicate that serotonin receptors play a crucial role in brain development processes such as neurogenesis and axonal branching during various stages of development [7, 11].

Serotonin exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups [12, 13]. Serotonin receptors are members of a superfamily of seven transmembrane domain receptors [14] that couple to GTP-binding regulatory proteins (G-proteins) [15]. Among the 14 subtypes of serotonin receptors, the G-protein coupled 5-HT_{1A} receptor subtype is the most extensively studied for a number of reasons. One of the main reasons for this is the availability of a selective ligand 8-hydroxy-2(di-*N*-propyl)tetralin (8-OH-DPAT) that allows extensive biochemical, physiological, and pharmacological characterization of the receptor [16]. The 5-HT_{1A} receptor was the first among all the serotonin receptors to be cloned and sequenced [17–19]. The human, rat, and mouse 5-HT_{1A} receptors have been cloned, and their amino acid sequences deduced [18–20]. Sequence analysis of the 5-HT_{1A} receptor gene has shown that it belongs to the superfamily of G-protein-coupled receptors, with 50% amino acid homology in the transmembrane domain with the β_2-adrenergic receptor. Furthermore, it was the first serotonin receptor for which polyclonal antibodies were obtained [18, 21–23] allowing their visualization at the subcellular level in various regions of the brain. On the clinical front, 5-HT_{1A} receptor levels have been shown to be increased in schizophrenia [24, 25]. Interestingly, a recent observation has associated genetic polymorphisms exhibited at the upstream repressor region of the 5-HT_{1A} receptor gene to major depression and suicide in humans [26]. The 5-HT_{1A} receptor has recently been shown to have a role in neural development [27] and protection of stressed neuronal cells undergoing degeneration and apoptosis [28, 29]. In addition, the 5-HT_{1A} receptor antagonists represent a major class of molecules with potential therapeutic effects in anxiety- or stress-related disorders [30]. We have earlier solubilized and partially purified the 5-HT_{1A} receptor from bovine hippocampus [31, 32] and have shown modulation of ligand binding to the bovine hippocampal 5-HT_{1A} receptor by metal ions, guanine nucleotides, alcohols, local anesthetics, membrane cholesterol, and covalent modifications of the disulfides and sulfhydryl groups [33–40].

Mammalian cells in culture heterologously expressing membrane receptors represent convenient systems to address problems in receptor biology due to higher expression levels of the receptors [41]. Keeping this in mind, we recently solubilized the human 5-HT_{1A} receptor stably expressed in CHO cells in a functionally active form [42]. In light of the potential anomalies associated with heterologous receptor expression systems such as multiple affinity states [43] and variations between experimental data from different laboratories on account of different protocols for creation and isolation of stable expression clones, we report here the pharmacological characterization of one of the first isolated [44] clones of CHO cells stably expressing the human 5-HT_{1A} receptor using the selective agonist 8-OH-DPAT and antagonist 4-(2′-methoxy)-phenyl-1-[2′-(*N*-2″-pyridinyl)-*p*-fluorobenzamido]ethylpiperazine (*p*-MPPF). In addition, we demonstrate that agonist and antagonist

binding to the receptor exhibit differential sensitivity to the non-hydrolyzable GTP analogue, GTP-γ-S, as was observed earlier with the native receptor from bovine hippocampus. These results show that the human 5-HT_{1A} receptor expressed in CHO cells displays characteristic features found in the native receptor isolated from bovine hippocampus.

MATERIALS AND METHODS

Materials

EDTA, fetal calf serum, $MgCl_2$, $MnCl_2$, 8-OH-DPAT, *p*-MPPI, penicillin, streptomycin, gentamycin sulfate, PMSF (phenylmethylsulfonyl fluoride), 5-HT (serotonin), sodium bicarbonate, polyethylenimine, and Tris were obtained from Sigma Chemical Co. (St. Louis, MO, USA). D-MEM/F-12 (Dulbecco's Modified Eagle Medium: nutrient mixture F-12 (Ham) (1:1)) and geneticin (G 418) were from Life Technologies (Grand Island, NY, USA). GTP-γ-S (guanosine-5′-*O*-(3-thiotriphosphate)) was from Roche Applied Science (Mannheim, Germany). BCA (bicinchoninic acid) reagent kit for protein estimation was from Pierce (Rockford, IL, USA). [^{3}H]8-OHDPAT (sp. activity=123.0 Ci/mmol) and [^{3}H]*p*-MPPF (sp. activity = 70.5 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA, USA). GF/B glass microfiber filters were from Whatman International (Kent, UK). All other chemicals used were of the highest purity available. Water was purified through a Millipore (Bedford, MA, USA) Milli-Q system and used throughout.

Cells and Cell Culture

The intronless human genomic clone G-21 [18] which encodes the human serotonin$_{1A}$ receptor was used to generate stable transfectants in CHO cells. These cells heterologously expressing the human serotonin$_{1A}$ receptor, originally referred to as T-CHO [44], were a generous gift from Dr. Probal Banerjee (College of Staten Island, City University of New York, USA). These cells will be referred to as CHO-5-HT_{1A}R in this report. Cells were grown in D-MEM/F-12 (1:1) supplemented with 2.4 g/l of sodium bicarbonate, 10% fetal calf serum, 60 μg/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml gentamycin sulfate and 200 μg/ml geneticin in a humidified atmosphere with 5% CO_2 at 37 °C.

Preparation of Cell Membranes

Cell membranes were prepared as described earlier [42]. Confluent cells were harvested by treatment with ice-cold buffer containing 10 mM Tris, 5 mM EDTA, 0.1 mM PMSF, pH 7.4. Cells were then homogenized for 10 s at 4 °C at maximum speed with a Polytron homogenizer. The cell lysate was centrifuged at 500 × *g* for 10 min at 4 °C and the resulting post-nuclear supernatant was centrifuged at 40,000 × *g* for 30 min at 4 °C. The pellet thus obtained was suspended in 50 mM Tris buffer, pH 7.4, flash frozen in liquid nitrogen and stored at −70 °C till further use. Total protein concentration in membranes thus isolated was determined using the BCA assay kit [45].

Radioligand Binding Assays

Receptor binding assays were carried out as described earlier with some modifications [33]. Briefly, tubes in duplicate with 40 μg total protein in a volume of 1 ml of buffer A (50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM $MnCl_2$, pH 7.4) for agonist binding studies, or in 1 ml of buffer B (50 mM Tris, 1 mM EDTA, pH 7.4) for antagonist binding assays were used. Tubes were incubated with the radiolabeled agonist [^{3}H]8-OH-DPAT (final concentration in assay tube being 0.29 nM) or antagonist [^{3}H]*p*-MPPF (final concentration in assay tube was 0.5 nM) for 1 h at room temperature (25 °C). Non-specific binding was determined by performing the assay either in the presence of 10 μM serotonin (for agonist binding assays) or in the presence of 10 μM *p*-MPPI (for antagonist binding assays). The binding reaction was terminated by rapid filtration under vacuum in a Brandel cell harvester (Gaithersburg, MD, USA) through Whatman GF/B 2.5 cm diameter glass microfiber filters (1.0 μm pore size) which were presoaked in 0.15% (w/v) polyethylenimine for 1 h [46]. The filters were then washed three times with 3 ml of cold water (4 °C), dried and the retained radioactivity was measured in a Packard Tri-Carb 1500 liquid scintillation counter using 5 ml of scintillation fluid.

GTP-γ-S Sensitivity Assay

For experiments in which GTP-γ-S was used, ligand binding assays were performed in the presence of varying concentrations of GTP-γ-S in buffer A for agonist binding and buffer B with 2 mM $MgCl_2$ for antagonist binding studies. The concentrations of GTP-γ-S leading to 50% inhibition of specific agonist binding (IC_{50}) were calculated by non-linear regression fitting of the data to a four parameter logistic function [47]:

$$B = \frac{a}{1 + (x/I)^S} + b, \tag{1}$$

where B is the specific binding of the agonist normalized to control binding (in the absence of GTP-γ-S), x denotes concentration of GTP-γ-S, a is the range ($y_{max} - y_{min}$) of the fitted curve on the ordinate (y-axis), I is the IC_{50} concentration, b is the background of the fitted curve (y_{min}) and S is the slope factor.

Saturation Radioligand Binding Assays

Saturation binding assays were carried out with varying concentrations (0.1–7.5 nM) of the radiolabeled agonist [^{3}H]8-OH-DPAT and antagonist [^{3}H]*p*-MPPF under conditions as described above. Non-specific binding was measured in the presence of 10 μM serotonin for agonist and 10 μM *p*-MPPI for antagonist binding. Binding data were analyzed as described previously [38]. The concentration of the bound radioligand (RL*) was calculated from the equation:

$$RL^* = 10^{-9} \times B/(V \times SA \times 2220)M, \tag{2}$$

where B is the bound radioactivity in disintegrations per minute (dpm) (i.e., total dpm - non-specific dpm), V the assay volume in ml, and SA is the specific activity of the radioligand. The data could be fitted best to a one site ligand binding equation. The dissociation constant (K_d) and maximum binding sites (B_{max}) were calculated by non-linear regression analysis of binding data using the HOT module of the LIGAND program (Biosoft, Cambridge, UK) [48,49]. Data obtained after regression analysis were used to plot graphs with the GRAFIT program version 3.09b (Erithacus Software, Surrey, UK).

Competition Binding Assays

Competition binding assays against the radiolabeled agonist [^{3}H]8-OH-DPAT (0.29 nM) and antagonist [^{3}H]*p*-MPPF (0.5 nM) were carried out in presence of a range of concentrations (typically from 10^{-12} to 10^{-5} M) of the unlabeled competitor. The concentration of the bound radiolabeled ligand was calculated from equation 2. Data for the competition assays were analyzed using equation 1 to obtain the IC_{50} concentrations of the unlabeled competitor ligand. Binding parameters, namely dissociation constant (K_d) and maximum binding sites (B_{max}), were calculated from the following equations as previously described [50, 51]:

$$K_d = IC_{50} - L, \tag{3}$$

$$B_{max} = B \times (IC_{50}/L), \tag{4}$$

where L is the concentration of the radiolabeled ligand, (0.29 nM for the agonist and 0.5 nM for the antagonist) used in the assay and B is the concentration of the bound ligand in the absence of the competitor. The affinity of the displacing ligands are expressed as the apparent dissociation constant (K_i) for the competing ligands, where K_i is calculated using the Cheng–Prusoff equation [52]:

$$K_i = IC_{50}/[1 + ([L]/K_d)], \tag{5}$$

where IC_{50} is the concentration of the competing ligand leading to 50% inhibition of specific binding and [L] and K_d are the concentration and dissociation constant of the labeled ligand. K_d values are those determined from saturation binding assays for the respective radioligand.

RESULTS

Linearity of Radioligand Binding with Increasing Concentrations of Total Protein

The development of selective 5-HT_{1A} antagonists has been relatively slow and less successful although selective 5-HT_{1A} agonists such as 8-OH-DPAT were discovered long back [16]. This is reflected in the relatively few reports describing specific antagonist binding properties in heterologously expressed 5-HT_{1A} receptors. A few years back, two specific antagonists for the 5-HT_{1A} receptor, *p*-MPPI and

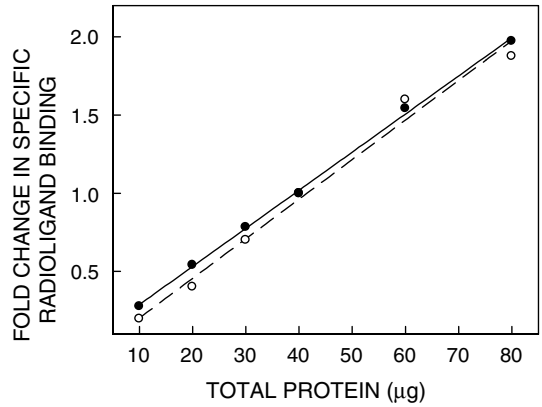

Fig. 1. Fold change in specific binding of the agonist [^{3}H]8-OH-DPAT (---○---) and antagonist [^{3}H]*p*-MPPF (—•—) to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cell membranes with increasing amounts of total membrane protein. Values have been normalized with respect to specific binding obtained with 40 μg total protein in the assay. Concentrations of [^{3}H]8-OH-DPAT (0.29 nM) and [^{3}H]*p*-MPPF (0.5 nM) were kept constant in the assay. Data shown are means of duplicate points from a representative experiment. See Materials and Methods for other details.

p-MPPF, have been introduced [53–55]. These compounds bind specifically to 5-HT$_{1A}$ receptors with high affinity.

We have pharmacologically characterized binding of the selective 5-HT$_{1A}$ receptor agonist [^{3}H]8-OH-DPAT and antagonist [^{3}H]*p*-MPPF to cell membranes prepared from CHO cells that stably express 5-HT$_{1A}$ receptors. Figure 1 shows that the binding of the radiolabeled ligands is linear over a broad range of protein concentrations. Non-specific binding defined with 10 μM serotonin for agonist binding and 10 μM *p*-MPPI for antagonist binding was ~10% or less than the total binding. These results suggest that under the conditions of the assay (i.e., with 0.29 nM of [^{3}H]8-OH-DPAT or 0.5 nM of [^{3}H]*p*-MPPF, and using 40 μg total protein for ligand binding assays as described later), there is no significant depletion of the radiolabel during the course of the assay. In other words, these conditions are appropriate for analyzing binding parameters of the receptor using the radiolabled agonist and antagonist [56]. In addition, these results suggest that the incubation time of 1 h for the assay is sufficient for radioligand binding to have reached equilibrium conditions. As a control, we checked specific binding of [^{3}H]8-OH-DPAT and [^{3}H]*p*-MPPF to membranes prepared from untransfected CHO cells. There was no detectable binding observed with these membranes.

Saturation Binding Analysis of Radiolabeled Agonist and Antagonist

The saturation binding analyses of the specific agonist [^{3}H]8-OH-DPAT and antagonist [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R membranes were carried out using a range of concentration (0.1–7.5 nM) of the radiolabeled ligands and the binding plots are shown in Figs. 2 and 3. The data for saturation binding were analyzed using the LIGAND program and the binding parameters are shown in Table 1. Importantly, our estimated K_d value (~0.38 nM) for [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in CHO-5-HT$_{1A}$R membranes is

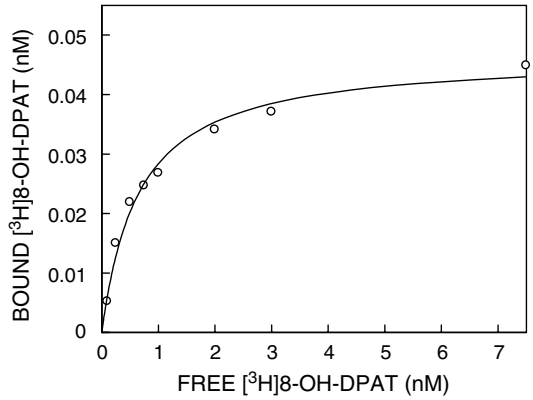

Fig. 2. Saturation binding analysis of specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cell membranes. A representative plot is shown for specific [^{3}H]8-OH-DPAT binding with increasing concentrations (0.1-7.5 nM) of free [^{3}H]8-OH-DPAT. The curve is a non-linear regression fit to the experimental data using the LIGAND program. See Materials and Methods and Table 1 for other details.

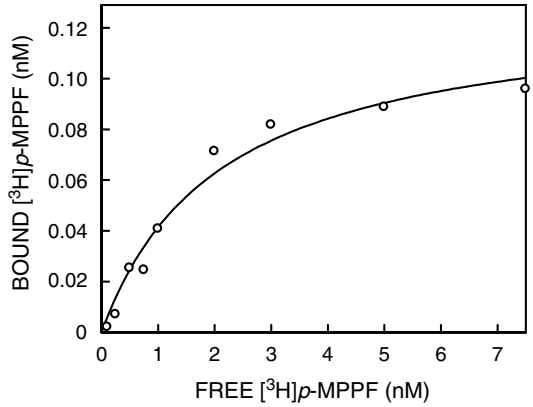

Fig. 3. Saturation binding analysis of specific [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cell membranes. A representative plot is shown for specific [^{3}H]*p*-MPPF binding with increasing concentrations (0.1–7.5 nM) of free [^{3}H]*p*-MPPF. The curve is a non-linear regression fit to the experimental data using the LIGAND program. See Materials and Methods and Table 1 for other details.

Table 1. Binding parameters[a] of the agonist [^{3}H]8-OH-DPAT and antagonist [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cells.

Ligand	K_d (nM)	B_{max} (pmol/mg of protein)
[^{3}H]8-OH-DPAT	0.38 ± 0.13	1.24 ± 0.09
[^{3}H]*p*-MPPF	3.51 ± 0.48	3.93 ± 0.39

[a]Binding parameters were calculated by analyzing saturation binding isotherms with a range (0.1–7.5 nM) of both radioligands using the LIGAND program. The data shown in the table represent the means ± SEM of three independent experiments. See Materials and Methods for other details.

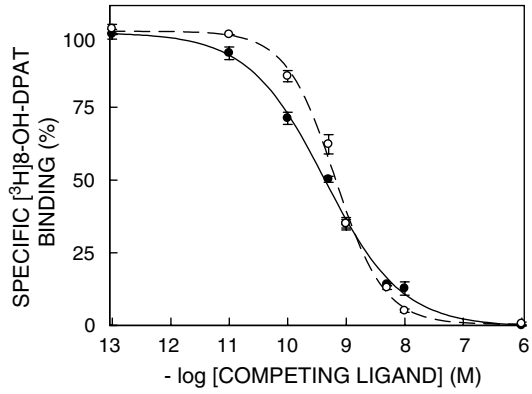

Fig. 4. Competition binding analysis of specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cell membranes. Values are expressed as a percentage of specific binding obtained in the absence of the competing ligand. Radioligand binding assays were carried out with [^{3}H]8-OH-DPAT in presence of a range of 8-OH-DPAT (- - -○- - -) and 5-HT (—•—) concentrations. The curves are non-linear regression fits to the experimental data using equation 1. The data points represent means ± SEM of duplicate points from three independent experiments. See Materials and Methods and Table 2 for other details.

in excellent agreement with the K_d value reported earlier by us for the native bovine hippocampal 5-HT$_{1A}$ receptor [33, 35, 37–39]. Moreover, this is also in agreement with the K_d values for recombinant 5-HT$_{1A}$ receptors reported by other groups [57, 58]. Table 1 also shows that the 5-HT$_{1A}$ receptors expressed in CHO- 5-HT$_{1A}$R cells bind to [^{3}H]*p*-MPPF with a K_d of ~3.51 nM, in good agreement with the affinity displayed by the native hippocampal receptor [35, 37, 38] and with earlier reported value for [^{3}H]*p*-MPPF binding to heterologously expressed 5-HT$_{1A}$ receptors [59].

Competition Binding Analysis of Radiolabeled Agonist and Antagonist

Further pharmacological characterization of the specific agonist and antagonist binding was carried out by performing competition binding experiments in presence of unlabeled ligands which act as competitors. Figs. 4 and 5 show the competition displacement curves of specific agonist [^{3}H]8-OH-DPAT by the competing ligands 8-OH-DPAT and 5-HT, and of the antagonist [^{3}H]*p*-MPPF by *p*-MPPI for 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R membranes. The half maximal inhibition concentrations (IC_{50}) and the inhibition constants (K_i) for the competing ligands are shown in Table 2.

Based on the formalism developed earlier [50,51], binding parameters obtained from saturation binding analysis (see Table 1) were compared with those obtained from competition binding analysis with similar ligands but in their unlabeled form acting as competitors. The binding parameters, namely K_d and B_{max}, thus obtained are reported in Table 3. As shown in the table, these values are in good agreement with values reported in Table 1. This self-consistency in the binding parameters irrespective of the method of analysis lends further reliability to the parameters reported by us.

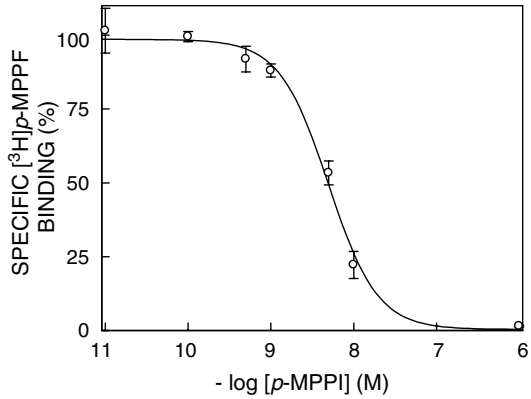

Fig. 5. Competition binding analysis of specific [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cell membranes. Values are expressed as a percentage of specific binding obtained in the absence of the competing ligand. Radioligand binding assay was carried out with 0.5 nM [^{3}H]*p*-MPPF in presence of a range of *p*-MPPI concentrations. The curve is a non-linear regression fit to the experimental data using equation 1. The data points represent means ± SEM of duplicate points from three independent experiments. See Materials and Methods and Table 2 for other details.

Table 2. Competition binding analysis[b] of [^{3}H]8-OH-DPAT and [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from CHO-5-HT$_{1A}$R cells.

	[^{3}H]8-OH-DPAT		[^{3}H]*p*-MPPF	
Competing ligand	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)
8-OH-DPAT	0.64 ± 0.02	0.36 ± 0.01	–	–
5-HT	0.36 ± 0.04	0.21 ± 0.02	–	–
p-MPPI	–	–	5.03 ± 0.61	4.4 ± 0.53

[b]Competition binding data were analyzed using equation 1 to determine IC_{50} values. The K_i values were obtained using equation 5 for which the K_d values were obtained from Table 1. Binding of [^{3}H]8-OH-DPAT (0.29 nM) and [^{3}H]*p*-MPPF (0.5 nM) was competed out with a range of concentrations of the unlabeled ligands. The data represent the means ± SEM of three independent experiments. See Materials and Methods for other details.

Table 3. Binding parameters[c] for [^{3}H]8-OH-DPAT and [^{3}H]*p*-MPPF obtained from competition binding experiments from CHO-5-HT$_{1A}$R cells.

	[^{3}H]8-OH-DPAT		[^{3}H]*p*-MPPF	
Competing ligand	K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)
8-OH-DPAT	0.35 ± 0.02	1.67 ± 0.08	–	–
p-MPPI	–	–	4.53 ± 0.61	8.36 ± 1.38

[c]Competition binding data were analyzed with a range of concentrations of unlabeled 8-OH-DPAT against [^{3}H]8-OH-DPAT (0.29 nM) and with unlabeled *p*-MPPI against [^{3}H]*p*-MPPF (0.5 nM). Binding parameters were calculated using eqs. 3 and 4 from the IC_{50} values reported in Table 2. The data represent the means ± SEM of three independent experiments. See Materials and Methods for other details.

Sensitivity of Ligand Binding to GTP-γ-S

Most of the seven transmembrane domain receptors are coupled to G-proteins [15], and guanine nucleotides are known to regulate ligand binding. The 5-HT_{1A} receptor agonists such as 8-OH-DPAT are known to specifically activate the G_i/G_o class of G-proteins [60, 61]. In contrast, antagonists do not catalyze the activation of G-proteins [53]. Therefore, agonist binding to such receptors displays sensitivity to agents that uncouple the normal cycle of guanine nucleotide exchange at the G-protein alpha subunit caused by activation of the receptor. Sensitivity of agonist binding to guanine nucleotides can be monitored by performing ligand binding assays in the presence of GTP-γ-S, a non-hydrolyzable analogue of GTP.

We have previously shown that the specific binding of the agonist [^{3}H] 8-OH-DPAT to bovine hippocampal 5-HT_{1A} receptors is sensitive to guanine nucleotides and is inhibited with increasing concentrations of GTP-γ-S [35, 62]. Our results showed that in presence of GTP-γ-S, the 5-HT_{1A} receptor undergoes an affinity transition, from a high affinity G-protein coupled to a low affinity G-protein uncoupled state [35]. In agreement with these results, Figure 6 shows a characteristic reduction in binding of the agonist [^{3}H]8-OH-DPAT in presence of a range of concentration of GTP-γ-S with an estimated IC_{50} of 3.7 ± 0.9 nM. This indicates that the human 5-HT_{1A} receptor is coupled to G-proteins when heterologously expressed in CHO cells and exhibits typical sensitivity to GTP-γ-S, a characteristic feature of the native hippocampal receptor.

In contrast to the agonist binding, antagonist [^{3}H]*p*-MPPF binding to 5-HT_{1A} receptors from the bovine hippocampus has previously been shown to be insensitive to GTP-γ-S [35, 38]. Figure 6 shows that the specific [^{3}H]*p*-MPPF binding to 5-HT_{1A} receptors from CHO cells remains invariant over a large range of concentrations of GTP-γ-S, in a manner analogous to what is observed with the native receptor from

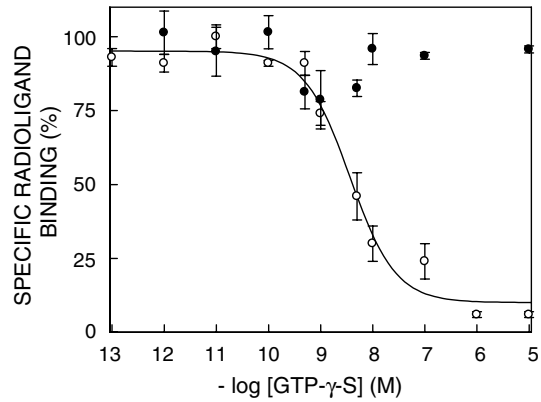

Fig. 6. Effect of increasing concentrations of GTP-γ-S on the specific binding of the agonist [^{3}H]8-OH-DPAT (o) and antagonist [^{3}H]*p*-MPPF (•) to 5-HT_{1A} receptors from CHO-5-HT_{1A}R cell membranes. Values are expressed as a percentage of the specific binding obtained in the absence of GTP-γ-S. The curve associated with [^{3}H]8-OH-DPAT binding is a non-linear regression fit to the experimental data using equation 1. The data points represent means ± SEM of duplicate points from three independent experiments. See Materials and Methods for other details.

bovine hippocampus. This implies that the agonist 8-OH-DPAT and the antagonist *p*-MPPF binding can be used to differentially discriminate G-protein coupling of the 5-HT$_{1A}$ receptor in CHO cells. Interestingly, the B_{max} values reported in Table 1 for 5-HT$_{1A}$ receptors using the antagonist [^{3}H]*p*-MPPF are far greater (~3 fold higher) than that obtained using agonist [^{3}H]8-OH-DPAT. This has been previously shown for native systems such as rat hippocampus [53] and bovine hippocampus [35, 38]. Since the binding of the antagonist [^{3}H]*p*-MPPF is unaffected by GTP-γ-S (Fig. 6) it indicates that [^{3}H]*p*-MPPF binds to all available populations of the receptor, those coupled to G-proteins and free (not coupled to G-proteins) receptors. The B_{max} value for the antagonist [^{3}H]*p*-MPPF therefore is greater than the corresponding value for the agonist [^{3}H]8-OH-DPAT, which would predominantly bind to G-protein coupled form of the receptor. Since endogenous G-proteins could be in limiting amounts compared to heterologously expressed receptors in such expression systems [63], the B_{max} values of the agonist and antagonist may tend to display greater differences in such systems compared to native systems. However, the ligand binding affinities of the 5-HT$_{1A}$ receptor from CHO cells and native systems are in good agreement and therefore the pharmacological characteristics of the receptor appear to be preserved in CHO-5-HT$_{1A}$R cells.

DISCUSSION

G-protein-coupled receptors (GPCRs) constitute a superfamily of proteins whose function is to transmit information across a cell membrane from the extracellular environment to the interior of the cell thus providing a mechanism of communication between the exterior and the interior of the cell [64, 14]. Such a process requires that the signal transduction be specific to the initiating stimulus and have well defined intracellular sequence of events. GPCRs represent the single largest family of cell surface receptors involved in signal transduction. This receptor superfamily includes over 2000 receptors which respond to a variety of molecules such as neurotransmitters, hormones, taste and odorant molecules, and even photons, thus mediating a multitude of functions. These transmembrane receptors act as key players in diverse physiological processes such as neurotransmission, cellular metabolism, secretion, cellular differentiation and growth, and inflammatory and immune responses. GCPRs therefore represent major targets for the development of novel drug candidates in all clinical areas [65]. It is estimated that $>50\%$ of the drugs in the market act as either surrogate activators or inhibitors of GPCRs that have defined native ligands which points out the immense therapeutic potential of these receptors [66]. As mentioned earlier, the 5-HT$_{1A}$ receptors are important representative members of the superfamily of GPCRs.

One of the approaches for performing pharmacological studies on GPCRs is to use a functional receptor system that converts ligand interaction with the receptor into a cellular signal which allows to monitor the relationship between concentration and response [63]. With the advent of molecular biology, there has been an increasing number of genetically engineered recombinant receptor systems for the study of drug–receptor interactions. This has led to a corresponding increase in the testing of new drugs in recombinant receptor systems. However, differences in host membrane lipid composition and the relative stoichiometry of the receptor to other cellular compo-

nents from that found in the natural system may complicate interpretation of drug testing results in such systems. It is therefore judicious to monitor receptor–ligand interactions in heterologous systems with the goal of critically assessing how closely they reflect the pharmacological characteristics of the native system.

We report here the pharmacological characterization of one of the first stable human serotonin$_{1A}$ receptor expression systems in CHO cells using the selective agonist [^{3}H]8-OH-DPAT and the antagonist [^{3}H]*p*-MPPF. Our results show that 5-HT$_{1A}$ receptors heterologously expressed in CHO cells display ligand binding properties that are in good agreement to what is observed with native receptors such as the bovine hippocampal 5-HT$_{1A}$ receptors. More importantly, we demonstrate that the differential discrimination of G-protein coupling by the agonist 8-OH-DPAT and the antagonist *p*-MPPF, a hallmark of the native receptor, is preserved for the receptor expressed in CHO cells.

Since native tissues (of neuronal origin in particular) often have very low quantities of a specific type of receptor, solubilization and purification of neuronal receptors from native sources continue to be challenging issues in contemporary membrane biology. Effective solubilization and purification of membrane receptors with optimum ligand binding activity and intact signal transduction components represent important steps in understanding structure–function relationship and pharmacological characterization of a specific receptor, and may constitute the first step in the detailed molecular characterization of GPCRs. It is in this context that heterologously expressed membrane receptors assume significance. Although the 5-HT$_{1A}$ receptor has been heterologously and stably expressed in fibroblast cells earlier [44, 57], no attempts have so far been made to solubilize the heterologously expressed receptor in a functional form. We have recently reported effective solubilization of 5-HT$_{1A}$ receptors in a functionally active form from CHO-5-HT$_{1A}$R cells using the mild zwitterionic detergent CHAPS [42]. This system therefore should provide a useful model system to understand 5-HT$_{1A}$ receptor biology.

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