Cellular and Molecular Neurobiology, Vol. 26, Nos. 4–6, July/August 2006 (© 2006)
DOI: 10.1007/s10571-006-9098-9

The Human Serotonin$_{1A}$ Receptor Expressed in Neuronal Cells: Toward a Native Environment for Neuronal Receptors

Yamuna Devi Paila[1] and Amitabha Chattopadhyay[1,2,3]

Received July 12, 2005; accepted September 9, 2005; Published online: June 27, 2006

SUMMARY

1. The serotonin$_{1A}$ (5-HT$_{1A}$) receptor is an important representative of G-protein coupled family of receptors. It is the most extensively studied among the serotonin receptors, and appears to be involved in various behavioral and cognitive functions.

2. We report here the pharmacological and functional characterization of the human serotonin$_{1A}$ receptor stably expressed in HN2 cell line, which is a hybrid cell line between hippocampal cells and mouse neuroblastoma.

3. Our results show that serotonin$_{1A}$ receptors in HN2-5-HT$_{1A}$R cells display ligand-binding properties that closely mimic binding properties observed with native receptors. We further demonstrate that the differential discrimination of G-protein coupling by the specific agonist and antagonist, a hallmark of the native receptor, is maintained for the receptor in HN2-5-HT$_{1A}$R cells. Importantly, the serotonin$_{1A}$ receptor in HN2-5-HT$_{1A}$R cells shows efficient downstream signalling by reducing cellular cyclic AMP levels.

4. We conclude that serotonin$_{1A}$ receptors expressed in HN2-5-HT$_{1A}$R cells represent a useful model system to study serotonin$_{1A}$ receptor biology, and is a potential system for solubilization and purification of the receptor in native-like membrane environment.

KEY WORDS: serotonin$_{1A}$ receptor; 8-OH-DPAT; *p*-MPPF; GTP-γ-S; Cyclic AMP; Neuronal cells.

INTRODUCTION

Serotonin (5-hydroxytryptamine or 5-HT) is an intrinsically fluorescent (Chattopadhyay *et al.*, 1996) biogenic amine, which acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous systems. It exerts its diverse actions by binding to a class of transmembrane receptors termed serotonin receptors. These receptors represent one of the largest, evolutionarily ancient, and highly conserved families of G-protein-coupled receptors (GPCR) (Peroutka and Howell, 1994). Serotonin receptors appear to be involved in generation and modulation of various behavioral, cognitive, and

[1] Centre for Cellular and Molecular Biology, Hyderabad, India.
[2] Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India.
[3] To whom correspondence should be addressed; e-mail: amit@ccmb.res.in.

developmental functions. The serotonin receptors have been classified into many groups (at least 14 subtypes) on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization, and second-messenger-coupling pathways (Hoyer *et al*., 2002). The serotonin$_{1A}$ (5-HT$_{1A}$) receptors are important members of the superfamily of seven transmembrane domain G-protein-coupled receptors. The 5-HT$_{1A}$ receptor is an important representative of this large family of receptors and is the most extensively studied of the serotonin receptors for a number of reasons (Pucadyil *et al*., 2005a). The 5-HT$_{1A}$ receptor agonists (Blier *et al*., 1990) and antagonists (Griebel, 1999) have been shown to possess potential therapeutic effects in anxiety- or stress-related disorders. Interestingly, mutant (knockout) mice lacking the 5-HT$_{1A}$ receptor generated a few years back exhibit enhanced anxiety-related behavior, and these mice serve as excellent model systems to understand anxiety-related behavior in higher animals (Toth, 2003). In addition, the 5-HT$_{1A}$ receptor has recently been shown to have a role in neural development (Gaspar *et al*., 2003) and protection of stressed neuronal cells undergoing degeneration and apoptosis (Singh *et al*., 1996). Treatment using agonists for the 5-HT$_{1A}$ receptor constitutes a potentially useful approach in case of children with developmental disorders (Azmitia, 2001). We have earlier partially purified and solubilized 5-HT$_{1A}$ receptors from bovine hippocampus in a functionally active form (Harikumar and Chattopadhyay, 1998; Chattopadhyay *et al*., 2002). More importantly, we have recently shown by a variety of approaches utilizing selective depletion (Pucadyil and Chattopadhyay, 2004, 2005), oxidation (Pucadyil *et al*., 2005b), sequestering (Pucadyil *et al*., 2004a), and complexation (Paila *et al*., 2005) of cholesterol, that membrane cholesterol is essential for ligand binding and G-protein coupling of the hippocampal 5-HT$_{1A}$ receptor.

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 (Tate and Grisshammer, 1996). An important consideration in such expression systems is selecting a cell type, which is derived from the tissue of natural occurrence of the receptor. This is particularly true for receptors of neural origin since the membrane lipid composition of cells in the nervous system is unique. This unique membrane lipid composition has been correlated with increased complexity in the organization of the nervous system during evolution (Sastry, 1985). Organization and dynamics of cellular membranes in the nervous system therefore play a crucial role in the function of neuronal membrane receptors. Lipids found in neuronal membranes are often necessary for maintaining the structure and function of neuronal receptors. For example, it has recently been shown that gangliosides specifically interact with proteins localized in the exoplasmic leaflet of neuronal plasma membranes (Prioni *et al*., 2004). Moreover, spatiotemporal signaling in neuronal membranes is believed to be stringently controlled by membrane domains (such as "lipid rafts") formed by specific lipids and through lipid–protein interactions (Tsui-Pierchala *et al*., 2002; Fivaz and Meyer, 2003; Golub *et al*., 2004). Keeping this in mind, we report here the pharmacological and functional characterization of the human 5-HT$_{1A}$ receptor stably

expressed in HN2 cells, which are a hybrid cell line between hippocampal cells and mouse neuroblastoma (Lee *et al.*, 1990). Using the selective agonist 8-OH-DPAT (8-hydroxy-2(di-*N*-propylamino)tetralin), and antagonist 4-(2′-methoxy)-phenyl-1-[2′-(*N*-2″-pyridinyl)-*p*-fluorobenzamido]ethyl-piperazine (*p*-MPPF), we demonstrate that agonist and antagonist binding to the receptor exhibit differential sensitivity to the nonhydrolyzable GTP analogue, GTP-γ-S, as was observed earlier with the native receptor from bovine hippocampus. In addition, the 5-HT$_{1A}$ receptor expressed in these cells displays typical downstream signaling of the receptor as monitored by ligand-dependent changes in cyclic AMP (cAMP) levels. These results show that the human 5-HT$_{1A}$ receptor expressed in HN2 cells displays characteristic features found in the native receptor isolated from bovine hippocampus and represents a realistic model system for the receptor.

MATERIALS AND METHODS

Materials

EDTA, fetal calf serum, $MgCl_2$, $MnCl_2$, 8-OH-DPAT, *p*-MPPI, penicillin, streptomycin, gentamycin sulfate, polyethylenimine, PMSF (phenylmethylsulfonyl fluoride), 5-HT (serotonin), sodium bicarbonate, and Tris were obtained from Sigma Chemical Co. (St. Louis, MO). DMEM (Dulbecco's Modified Eagle Medium) and geneticin (G-418) were from Life Technologies (Grand Island, NY). 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). [^{3}H]8-OH-DPAT (sp. activity = 135.0 Ci/mmol) and [^{3}H]*p*-MPPF (sp. activity = 70.5 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA). The cyclic [^{3}H]AMP (TRK 432) assay kit was purchased from Amersham Biosciences (Buckinghamshire, U.K.). GF/B glass microfiber filters were from Whatman International (Kent, U.K.). All other chemicals used were of the highest purity available. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

Cells and Cell Culture

The intronless human genomic clone G-21 (Fargin *et al.*, 1988) which encodes the human serotonin$_{1A}$ receptor was used to generate stable transfectants in HN2 cells which is a hybrid cell line between hippocampal cells and mouse neuroblastoma (Lee *et al.*, 1990). These cells expressing the human serotonin$_{1A}$ receptor, originally referred to as HN2-5 (Banerjee *et al.*, 1993), were a generous gift from Dr. Probal Banerjee (College of Staten Island, City University of New York, U.S.A). These cells will be referred to as HN2-5-HT$_{1A}$R in this report. Cells were grown in DMEM supplemented with 3.7 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 (Chattopadhyay *et al.*, 2004). 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 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 postnuclear 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. Total protein concentration in membranes thus isolated was determined using the BCA assay kit (Smith *et al.*, 1985).

Radioligand Binding Assays

Receptor binding assays were carried out as described earlier (Pucadyil *et al.*, 2004b) with some modifications. Briefly, tubes in duplicate with ~60 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 25°C. Nonspecific 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) 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 (Bruns *et al.,* 1983). 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 for antagonist binding studies. The concentrations of GTP-γ-S leading to 50% inhibition of specific agonist binding (IC_{50}) were calculated by nonlinear regression fitting of the data to a four-parameter logistic function (Higashijima *et al.*, 1987):

$$B = [a/(1 + (x/I)^s)] + b \tag{1}$$

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. Nonspecific 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 (Pucadyil *et al*., 2004b). The concentration of the bound radioligand (RL*) was calculated from the equation

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

where B is the bound radioactivity in disintegrations per minute (dpm) (i.e., total dpm – nonspecific dpm), V is 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 nonlinear regression analysis of binding data using Graphpad Prism software version 4.00 (San Diego, CA). Data obtained after regression analysis were used to plot graphs with the GRAFIT program version 3.09b (Erithacus Software, Surrey, U.K.)

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 the presence of a range of concentrations (typically from 10^{-11} to 10^{-6} M) of the unlabeled competitor. The concentration of the bound radiolabeled ligand was calculated from Eq. (2). Data for the competition assays were analyzed using Eq. (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 (Akera and Cheng, 1977; DeBlasi *et al*., 1989):

$$K_d = \mathrm{IC}_{50} - L \tag{3}$$

$$B_{max} = B \times (\mathrm{IC}_{50}/L) \tag{4}$$

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 is expressed as the apparent dissociation constant (K_i) for the competing ligands, where K_i is calculated using the Cheng–Prusoff equation (Cheng and Prusoff, 1973):

$$K_i = \mathrm{IC}_{50}/[1 + (L/K_d)] \tag{5}$$

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.

Estimation of Cyclic AMP Levels in Cells

The ability of ligands to affect the forskolin-stimulated increase in cAMP levels in cells was assessed as described below. HN2-5-HT_{1A}R cells were plated at a density of 1×10^4 cells per well of a 24-well plate and grown for 4 days in culture under conditions as described earlier. Cells were rinsed with PBS and incubated with forskolin (20 μM) and phosphodiesterase inhibitor IBMX (50 μM) in the presence of increasing concentrations of 8-OH-DPAT at 37°C for 30 min in serum-free DMEM medium. The assay was terminated by adding lysis buffer (10 mM Tris, 5 mM EDTA, pH 7.4 buffer) to the cells. Cell lysates were boiled for 3 min and spun at 50,000 $\times$ *g* for 10 min to remove precipitated protein. The amount of cAMP in an aliquot of the supernatant was estimated using the cyclic [^{3}H]AMP assay system, which is based on the protein binding method described previously (Norstedt and Fredholm, 1990). Agonist-dependent dose-response curves were analyzed according to the 4-parameter logistic function [Eq. (1)].

RESULTS

Linearity of Radioligand Binding With Increasing Concentrations of Total Protein

We characterized the specific binding of the 5-HT_{1A} receptor agonist [^{3}H]8-OH-DPAT and antagonist [^{3}H]*p*-MPPF to membranes prepared from HN2 cells that stably express 5-HT_{1A} receptors (termed as HN2-5-HT_{1A}R cells). Figure 1 shows that the binding of the radiolabeled ligands is linear over a broad range of protein concentrations. Nonspecific binding defined with 10 μM serotonin for agonist binding and 10 μM *p*-MPPI for antagonist binding was $\sim$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 60 μg total protein), 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 (Hulme, 1990). 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.

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 HN2-5-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 Graphpad Prism software version 4.0 program and the binding parameters are shown in Table I. Our estimated K_d value ($\sim$2.20 nM) for [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors in HN2-5-HT_{1A}R membranes is in excellent agreement with the K_d value reported earlier for the native rat (Milligan *et al.*, 2001), and bovine (Harikumar and Chattopadhyay, 1998,

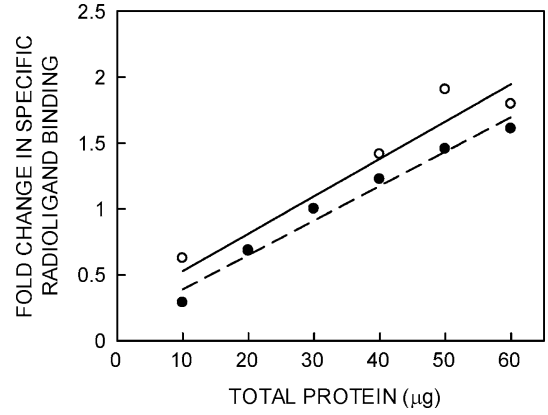

Fig. 1. Fold change in specific binding of the agonist [^{3}H]8-OH-DPAT (—○—) and antagonist [^{3}H]*p*-MPPF (—•—) to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes with increasing amounts of total membrane protein. Values have been normalized with respect to specific binding obtained with 30 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 section for other details.

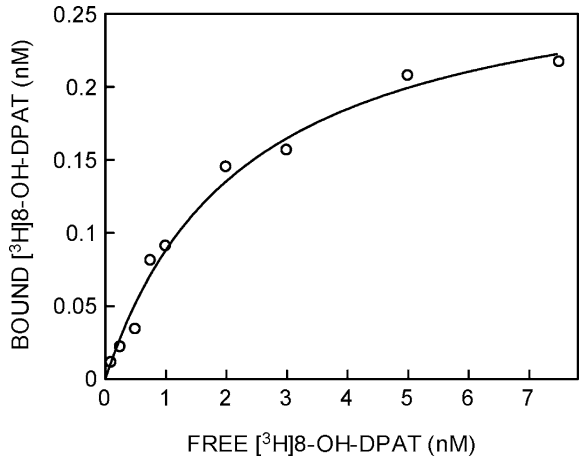

Fig. 2. Saturation binding analysis of specific [^{3}H]8 OH-DPAT binding to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. A representative plot is shown for specific [^{3}H]8-OH-DPAT binding with increasing concentrations (0.1–7.5-nM) of free [^{3}H]8-OH-DPAT. The curve is a nonlinear regression fit to the experimental data using the Graphpad Prism software version 4.00 program. See Materials and Methods section and Table I for other details.

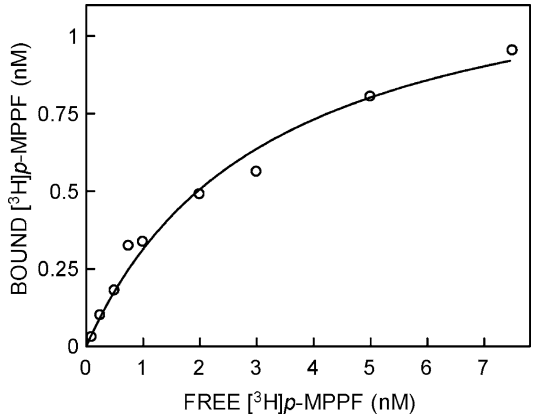

Fig. 3. Saturation binding analysis of specific [^{3}H]*p* MPPF binding to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. A representative plot is shown for specific [^{3}H]*p*-MPPF binding with increasing concentrations (0.1–7.5-nM) of free [^{3}H]*p*-MPPF. The curve is a nonlinear regression fit to the experimental data using the Graphpad Prism software version 4.00 program. See Materials and Methods section and Table I for other details.

1999) hippocampal 5-HT$_{1A}$ receptor. Table I also shows that the 5-HT$_{1A}$ receptors expressed in HN2-5-HT$_{1A}$R cells bind to [^{3}H]*p*-MPPF with a K_d of $\sim$2.70 nM, in good agreement with the affinity displayed by the native hippocampal receptor (Harikumar and Chattopadhyay, 2001).

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 the presence of unlabeled ligands, which act as competitors. Figures 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 HN2-5-HT$_{1A}$R membranes. The half-maximal inhibition

Table I. Binding Parameters[a] of the Agonist [^{3}H]8-OH-DPAT and Antagonist [^{3}H]*p*-MPPF Binding to 5-HT$_{1A}$ Receptors From HN2-5-HT$_{1A}$R Cells

Ligand	K_d (nM)	B_{max} (pmol/mg of protein)
[^{3}H]8-OH-DPAT	2.20 ± 0.05	2.08 ± 0.30
[^{3}H]*p*-MPPF	2.70 ± 0.39	9.20 ± 1.88

[a]Binding parameters were calculated by analyzing saturation binding isotherms with a range (0.1–7.5 nM) of both radioligands using the Graphpad Prism software version 4.00 program. The data shown in the table represent the means ± SE of three independent experiments. See Materials and Methods section for other details.

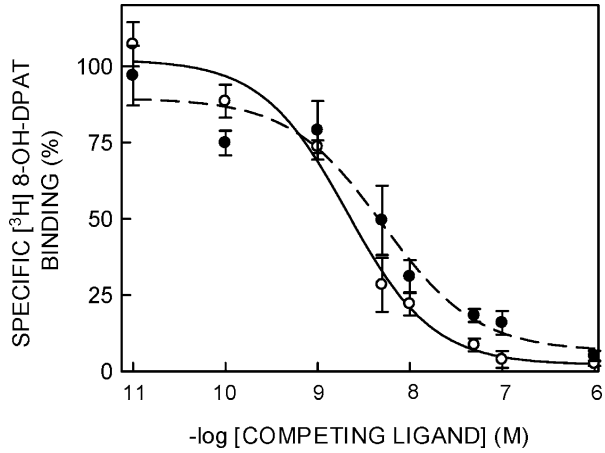

Fig. 4. Competition binding analysis of specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. Values are expressed as a percentage of specific binding obtained in the absence of the competing ligand. Radioligand binding assays were carried out with [^{3}H]8-OH-DPAT in the presence of a range of 8-OH-DPAT (—○—) and 5-HT (—•—) concentrations. The curves are nonlinear regression fits to the experimental data using Eq. (1). The data points represent means ± SE of duplicate points from three independent experiments. See Materials and Methods section and Table II for other details.

concentrations (IC_{50}) and the inhibition constants (K_i) for the competing ligands are shown in Table II.

Based on the formalism developed previously (Akera and Cheng, 1977; DeBlasi *et al.*, 1989), binding parameters obtained from saturation binding analysis (see Table I) 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 III. As shown in the table, these values are in good agreement with values reported in Table I.

Sensitivity of Ligand Binding to GTP-γ-S

Most of the seven transmembrane domain receptors are coupled to G-proteins (Clapham, 1996), and guanine nucleotides are known to regulate ligand binding. The 5-HT$_{1A}$ receptor agonists such as 5-HT or 8-OH-DPAT are known to specifically activate the G_i/G_o class of G-proteins (Emerit *et al.*, 1990; Clawges *et al.*, 1997). In contrast, antagonists do not catalyze the activation of G-proteins (Kung *et al.*, 1995). 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 nonhydrolyzable analog of GTP.

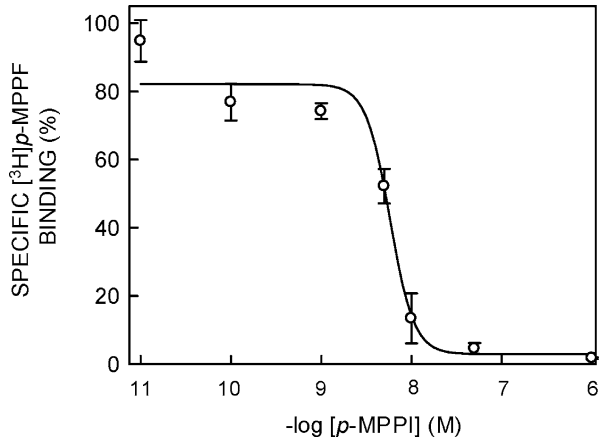

Fig. 5. Competition binding analysis of specific [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. Values are expressed as a percentage of specific binding obtained in the absence of the competing ligand. Radioligand binding assay was carried out with 0.5 nM [^{3}H]*p* MPPF in the presence of a range of *p*-MPPI concentrations. The curve is a nonlinear regression fit to the experimental data using Eq. (1). The data points represent means ± SE of duplicate points from three independent experiments. See Materials and Methods section and Table II for other details.

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 (Harikumar and Chattopadhyay, 1999; Javadekar-Subhedar and Chattopadhyay, 2004). Our results show that in the 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 (Harikumar and Chattopadhyay, 1999). In agreement with these results, Fig. 6 shows a characteristic reduction in binding of the agonist [^{3}H]8-OH-DPAT in the presence of a range of concentration of GTP-γ-S with an estimated

Table II. Competition Binding Analysis[a] of [^{3}H]8-OH-DPAT and [^{3}H]*p*-MPPF Binding to 5-HT$_{1A}$ Receptors From HN2-5-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	2.08 ± 0.40	1.65 ± 0.35	–	–
5-HT	5.10 ± 0.50	1.74 ± 0.46	–	–
p-MPPI	–	–	5.77 ± 0.09	4.47 ± 0.63

[a]Competition binding data were analyzed using Eq. (1) to determine IC$_{50}$ values. The K_i values were obtained using Eq. (5) for which the K_d values were obtained from Table I. 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 ± SE of three independent experiments. See Materials and Methods section for other details.

Table III. Binding Parameters[a] for [^{3}H]8-OH-DPAT and [^{3}H]*p*-MPPF Obtained From Competition Binding Experiments

	[^{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	1.57 ± 0.40	4.02 ± 0.80	–	–
p-MPPI	–	–	4.80 ± 0.74	9.72 ± 0.58

[a]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 II. The data represent the means ± SE of three independent experiments. See Materials and Methods section for other details.

IC_{50} of 90.4 ± 1.8 nM, which is in excellent agreement with our earlier results with the receptor from native hippocampal source (Kalipatnapu and Chattopadhyay, 2004; Pucadyil and Chattopadhyay, 2004). This indicates that the human 5-HT$_{1A}$ receptor is coupled to G-proteins when expressed in HN2 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 (Harikumar and Chattopadhyay, 1999; Javadekar-Subhedar and Chattopadhyay, 2004; Kalipatnapu *et al.*, 2004). Figure 6 shows that the specific [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cells remains invariant

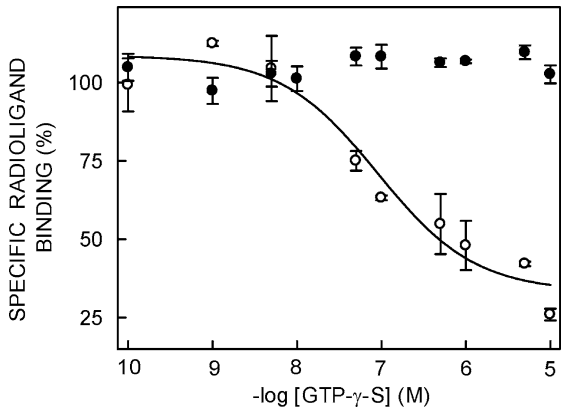

Fig. 6. Effect of increasing concentrations of GTP-γ-S on the specific binding of the agonist [^{3}H]8-OH-DPAT (○) and antagonist [^{3}H]*p*-MPPF (●) to 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. Values are expressed as a percentage of the specific binding obtained in the absence of GTP-γ-S. The curve associated with [^{3}H]8-OH-DPAT binding is a nonlinear regression fit to the experimental data using Eq. (1). The data points represent means ± SE of duplicate points from three independent experiments. See Materials and Methods section for other details.

over a large range of concentrations of GTP-γ-S, in a manner analogous to what is observed with the native receptor from 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 HN2-5-HT_{1A}R cells. Interestingly, the B_{max} values reported in Table I for 5-HT_{1A} receptors using the antagonist [^{3}H]*p*-MPPF are far greater ($\sim$4-fold higher) than that obtained using agonist [^{3}H]8-OH-DPAT. This has been previously shown for native systems such as rat hippocampus (Kung *et al.*, 1995) and bovine hippocampus (Harikumar and Chattopadhyay, 1999). 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 (Kenakin, 1997), 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 HN2-5-HT_{1A}R cells and native systems are in good agreement and therefore the pharmacological characteristics of the receptor appear to be preserved in HN2-5-HT_{1A}R cells.

Ligand-Dependent Downstream Signaling: Measurement of Cyclic AMP Levels

The primary function of 5-HT_{1A} receptors is to inhibit adenylate cyclase thereby reducing the levels of cyclic AMP (cAMP). While in some systems this reduction can be observed in the basal level of cAMP itself, in others the effect is made more dramatic by spiking the cAMP levels using forskolin, which independently stimulates adenylate cyclase (Pucadyil *et al.*, in press). We examined the signaling function of the 5-HT_{1A} receptor expressed in HN2 cells by monitoring its ability to catalyze downstream signal transduction processes upon stimulation with 5-HT_{1A} receptor ligands. The 5-HT_{1A} receptor agonists such as 5-HT and 8-OH-DPAT are known to specifically activate the G_i/G_o class of G-proteins (Raymond *et al.*, 1993), which subsequently reduce the cAMP levels. As shown in Fig. 7, the forskolin-stimulated increase in cAMP levels is efficiently inhibited by 8-OH-DPAT in a characteristic concentration-dependent manner with an IC_{50} value of 1.35 ± 0.07 nM in good agreement with the earlier reported value (Kellett *et al.*, 1999). This indicates that the normal function of these receptors to transduce signals via G-proteins, which inhibit adenylate cyclase, is maintained in HN2-5-HT_{1A}R cells. Interestingly, cAMP is known to regulate immune responses, and modifications in cAMP signaling are involved in the pathophysiology and treatment of depression. For example, patients suffering from depression have been reported to show lower adenylate cyclase activity due to altered serotonergic signaling (Mizrahi *et al.*, 2004).

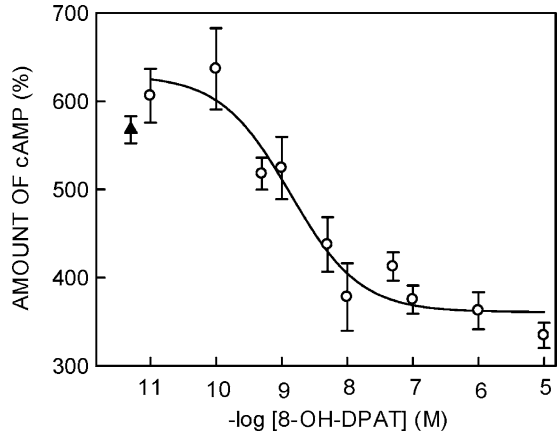

Fig. 7. Effect of increasing concentrations of 8-OH-DPAT on downstream signaling of 5-HT$_{1A}$ receptors in HN2-5-HT$_{1A}$R cells. The ability of the specific 5-HT$_{1A}$ receptor agonist 8-OH-DPAT to inhibit the forskolin-stimulated cAMP levels was assayed in HN2-5-HT$_{1A}$R cells. All data points are expressed as percentage of the cAMP levels in cells in the absence of forskolin and 8-OH-DPAT. The data for the increase in cAMP levels in the presence of forskolin in untreated (▲) cells is shown for comparison. Inhibition curves were analyzed by the 4-parameter logistic function [Eq. (1)]. The data points represent means ± SE of at least three independent experiments. See Materials and Methods section for other details.

DISCUSSION

The G-protein-coupled receptor superfamily comprises the largest class of molecules involved in signal transduction across the plasma membrane, thus providing a mechanism of communication between the exterior and the interior of the cell (Pierce *et al*., 2002) and represents ~1% of the mammalian genome (Hur and Kim, 2002). It is estimated that up to 50% of clinically prescribed drugs act as either agonists or antagonists at GPCRs which points out their immense therapeutic potential (Karnik *et al*., 2003). As mentioned earlier, the 5-HT$_{1A}$ receptors are important representative members of the superfamily of GPCRs.

A useful approach for performing pharmacological studies on GPCRs is to use a functional receptor system that converts receptor–ligand interaction into a cellular signal, which allows to monitor the relationship between concentration and response (Kenakin, 1997). With the advent of molecular biology, there have 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 often complicate interpretation of drug testing results in such systems, and can lead to receptors with characteristics different from native receptors. For example, it has earlier been reported that although the rat cortical

5-HT_{1A} receptor exists only in the high-affinity state in its native environment, it displays both high and low affinity when expressed in HEK293 cells (Watson *et al*., 2000). It is therefore judicious to develop recombinant expression systems in which the membrane lipid composition closely mimics the native lipid environment. Although 5-HT_{1A} receptors have previously been expressed in nonneuronal cell lines such as CHO (Newman-Tancredi *et al*., 1997) and HEK293 (Kellett *et al*., 1999), there have been very few attempts to express and characterize the receptor in neuronal cells. Our choice of HN2 cells as an expression system for characterizing the 5-HT_{1A} receptors is based on the observation that cell lines of neural origin represent realistic models for understanding signal transduction in neuronal cells (Lee *et al*., 1990).

In this paper, we report the pharmacological and functional characterization of the human serotonin$_{1A}$ receptor stably expressed in HN2 cells, which are derived from hippocampal cells and mouse neuroblastoma. Our results show that 5-HT_{1A} receptors expressed in HN2 cells display ligand-binding properties that are in good agreement to what is observed with native receptors such as rat and bovine hippocampal 5-HT_{1A} receptors. In addition, 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 HN2 cells. More importantly, 5-HT_{1A} receptors in HN2-5-HT_{1A}R cells can efficiently catalyze downstream signal transduction by reducing cAMP levels.

Lipid–protein interactions play a crucial role in maintaining the structure and function of integral membrane proteins and receptors (Lee, 2004). A hallmark of neuronal cells is the abundance of cholesterol in their membranes. The central nervous system which accounts for only 2% of the body mass contains $\sim$25% of free cholesterol present in the whole body. Cholesterol represents an important lipid in this context since it is known to regulate the function of neuronal receptors (Burger *et al*., 2000; Pucadyil and Chattopadhyay, 2004) thereby affecting neurotransmission and could give rise to mood and anxiety disorders (Papakostas *et al*., 2004). Cholesterol organization, traffic, and dynamics in the brain are stringently controlled since the input of cholesterol into the central nervous system is almost exclusively from *in situ* synthesis as there is no evidence for the transfer of cholesterol from blood plasma to brain (Dietschy and Turley, 2001). As a result, a number of neurological diseases share a common etiology of defective cholesterol metabolism in the brain (Porter, 2002). In the Smith–Lemli–Opitz syndrome, for example, the marked abnormalities in brain development and function leading to serious neurological and mental dysfunctions have their origin in the fact that the major input of brain cholesterol comes from *in situ* synthesis and such synthesis is defective in this syndrome (Waterham and Wanders, 2000). Interestingly, we have previously shown using a variety of approaches that the function of hippocampal 5-HT_{1A} receptor displays a great degree of sensitivity to membrane cholesterol (Pucadyil and Chattopadhyay, 2004, 2005; Pucadyil *et al*., 2004a; Pucadyil *et al*., 2005; Paila *et al*., 2005). Expression of this receptor in a cell line of neuronal origin therefore assumes greater relevance in this context, and provides a convenient cellular system to address these issues.

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. It is in this context that membrane receptors expressed in a cell line with native-like membrane lipid environment gain significance. The levels of receptors expressed this way are often much higher than that found in native tissues making these systems amenable to solubilization and purification of the given receptor. 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. Our future efforts will focus on effective solubilization and purification of functional 5-HT$_{1A}$ receptors from HN2-5-HT$_{1A}$R cells. This system therefore should provide a useful model system to understand 5-HT$_{1A}$ receptor biology.

ACKNOWLEDGMENTS

This work was supported by the Council of Scientific and Industrial Research, Government of India. Y. D. P. thanks the Council of Scientific and Industrial Research for the award of a Senior Research Fellowship. A. C. is an Honorary Faculty Member of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. We gratefully acknowledge Dr. Probal Banerjee for the kind gift of HN2-5 cells and for sharing useful information, and Dr. K. G. Harikumar for preliminary experiments. We thank members of our laboratory for critically reading the manuscript.

REFERENCES

Akera, T., and Cheng, V. J. K. (1977). A simple method for the determination of affinity and binding site concentration in receptor binding studies. *Biochim. Biophys. Acta* **470:**412–423.

Azmitia, E. C. (2001). Neuronal instability: Implications for Rett's syndrome. *Brain Dev.* **23**(Suppl. 1):S1–S10.

Banerjee, P., Berry-Kravis, E., Bonafede-Chhabra, D., and Dawson, G. (1993). Heterologous expression of the serotonin 5-HT$_{1A}$ receptor in neural and non-neural cell lines. *Biochem. Biophys. Res. Commun.* **192:**104–110.

Blier, P., de Montigny, C., and Chaput, Y. (1990). A role for the serotonin system in the mechanism of action of antidepressant treatments: Preclinical evidence. *J. Clin. Psychiatry* **51:**14–20.

Bruns, R. F., Lawson-Wendling, K., and Pugsley, T. A. (1983). A rapid filtration assay for soluble receptors using polyethylenimine-treated filters. *Anal. Biochem.* **132:**74–81.

Burger, K., Gimpl, G., and Fahrenholz, F. (2000). Regulation of receptor function by cholesterol. *Cell. Mol. Life Sci.* **57:**1577–1592.

Chattopadhyay, A., Harikumar, K. G., and Kalipatnapu, S. (2002). Solubilization of high affinity G-protein coupled serotonin$_{1A}$ receptors from bovine hippocampus using pre-micellar CHAPS at low concentration. *Mol. Membr. Biol.* **19:**211–220.

Chattopadhyay, A., Jafurulla, Md., and Kalipatnapu, S. (2004). Solubilization of serotonin$_{1A}$ receptors heterologously expressed in Chinese hamster ovary cells. *Cell. Mol. Neurobiol.* **24:**293–300.

Chattopadhyay, A., Rukmini, R., and Mukherjee, S. (1996). Photophysics of a neurotransmitter: Ionization and spectroscopic properties of serotonin. *Biophys. J.* **71:**1952–1960.

Cheng, Y. C., and Prusoff, W. H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **22:**3099–3108.

Clapham, D. E. (1996). The G-protein nanomachine. *Nature* **379:**297–299.
Clawges, H. M., Depree, K. M., Parker, E. M., and Graber, S. G. (1997). Human 5-HT_1 receptor subtypes exhibit distinct G protein coupling behaviors in membranes from Sf9 cells. *Biochemistry* **36:**12930–12938.
DeBlasi, A., O'Reilly, K., and Motulsky, H. J. (1989). Calculating receptor number from binding experiments using same compound as radioligand and competitor. *Trends Pharmacol. Sci.* **10:**227–229.
Dietschy, J. M., and Turley, S. D. (2001). Cholesterol metabolism in the brain. *Curr. Opin. Lipidol.* **12:**105–112.
Emerit, M. B., El Mestikawy, S., Gozlan, H., Rouot, B., and Hamon, M. (1990). Physical evidence of the coupling of solubilized 5-HT_{1A} binding sites with G regulatory proteins. *Biochem. Pharmacol.* **39:**7–18.
Fargin, A., Raymond, J. R., Lohse, M. J., Kobilka, B. K., and Lefkowitz, R. J. (1988). The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. *Nature* **335:**358–360.
Fivaz, M., and Meyer, T. (2003). Specific localization and timing in neuronal signal transduction mediated by protein–lipid interactions. *Neuron* **40:**319–330.
Gaspar, P., Cases, O., and Maroteaux, L. (2003). The developmental role of serotonin: News from mouse molecular genetics. *Nat. Rev. Neurosci.* **4:**1002–1012.
Golub, T., Wacha, S., and Caroni, P. (2004). Spatial and temporal control of signaling through lipid rafts. *Curr. Opin. Neurobiol.* **14:**542–550.
Griebel, G. (1999). 5-HT_{1A} receptor blockers as potential drug candidates for the treatment of anxiety disorders. *Drug News Perspect.* **12:**484–490.
Harikumar, K. G., and Chattopadhyay, A. (1998). Metal ion and guanine nucleotide modulations of agonist interaction in G-protein-coupled serotonin$_{1A}$ receptors from bovine hippocampus. *Cell. Mol. Neurobiol.* **18:**535-–553.
Harikumar, K. G., and Chattopadhyay, A. (1999). Differential discrimination of G-protein coupling of serotonin$_{1A}$ receptors from bovine hippocampus by an agonist and an antagonist. *FEBS Lett.* **457:**389–392.
Harikumar, K. G., and Chattopadhyay, A. (2001). Modulation of antagonist binding to serotonin$_{1A}$ receptors from bovine hippocampus by metal ions. *Cell. Mol. Neurobiol.* **21:**453–464.
Higashijima, T., Ferguson, K. M., Sternweis, P. C., Smigel, M. D., and Gilman, A. G. (1987). Effects of Mg^{2+} and the $\beta\gamma$-subunit complex on the interactions of guanine nucleotides with G proteins. *J. Biol. Chem.* **262:**762–766.
Hoyer, D., Hannon, J. P., and Martin, G. R. (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **71:**533–554.
Hulme, E. C. (1990). Receptor binding studies, a brief outline. In Hulme, E. C. (ed.), *Receptor–Effector Coupling: A Practical Approach*, IRL, New York, pp. 203–215.
Hur, E. M., and Kim, K.-T. (2002). G protein-coupled receptor signalling and cross-talk: Achieving rapidity and specificity. *Cell. Signal.* **14:**397–405.
Javadekar-Subhedar, V., and Chattopadhyay, A. (2004). Temperature-dependent interaction of the bovine hippocampal serotonin$_{1A}$ receptor with G-proteins. *Mol. Membr. Biol.* **21:**119–123.
Kalipatnapu, S., and Chattopadhyay, A. (2004). Interaction of serotonin$_{1A}$ receptors from bovine hippocampus with tertiary amine local anesthetics. *Cell. Mol. Neurobiol.* **24:**403–422.
Kalipatnapu, S., Jafurulla, Md., Chandrasekaran, N., and Chattopadhyay, A. (2004). Effect of Mg^{2+} on guanine nucleotide sensitivity of ligand binding to serotonin$_{1A}$ receptors from bovine hippocampus. *Biochem. Biophys. Res. Commun.* **323:**372–376.
Karnik, S. S., Gogonea, S., Patil, S., Saad, Y., and Takezako, T. (2003). Activation of G-protein-coupled receptors: A common molecular mechanism. *Trends Endocrinol. Metab.* **14:**431–437.
Kellett, E., Carr, I. C., and Milligan, G. (1999). Regulation of G protein activation and effector modulation by fusion proteins between the human 5-hydroxytryptamine$_{1A}$ receptor and the α subunit of $G_{i1}\alpha$: Differences in receptor-constitutive activity imparted by single amino acid substitutions in $G_{i1}\alpha$. *Mol. Pharmacol.* **56:**684–692.
Kenakin, T. (1997). Differences between natural and recombinant G protein-coupled receptor systems with varying receptor/G protein stoichiometry. *Trends Pharmacol. Sci.* **18:**456–464.
Kung, M.-P., Frederick, D., Mu, M., Zhuang, Z.-P., and Kung, H. F. (1995). 4-(2′-Methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-iodobenzamido]-ethyl-piperazine ([^{125}I]*p*-MPPI) as a new selective radioligand of serotonin$_{1A}$ sites in rat brain: In vitro binding and autoradiographic studies. *J. Pharmacol. Exp. Ther.* **272:**429–437.
Lee, A. G. (2004). How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta* **1666:**62–87.

Lee, H. J., Hammond, D. N., Large, T. H., Roback, J. D., Sim, J. A., Brown, D. A., Otten, U. H., and Wainer, B. H. (1990). Neuronal properties and trophic activities of immortalized hippocampal cells from embryonic and young adult mice. *J. Neurosci.* **10:**1779–1787.

Milligan, G., Kellet, E., Dacquet, C., Dubreuil, V., Jacoby, E., Millan, M. J., Lavielle, G., and Spedding, M. (2001). S 14506: Novel receptor coupling at 5-HT$_{1A}$ receptors. *Neuropharmacology* **40:**334–344.

Mizrahi, C., Stojanovic, A., Urbina, M., Carreira, I., and Lima, L. (2004). Differential cAMP levels and serotonin effects in blood peripheral mononuclear cells and lymphocytes from major depression patients. *Int. Immunopharmacol.* **4:**1125–1133.

Newman-Tancredi, A., Conte, C., Chaput, C., Verrièle, L., and Millan, M. J. (1997). Agonist and inverse agonist efficacy at human recombinant serotonin 5-HT$_{1A}$ receptors as a function of receptor:G-protein stoichiometry. *Neuropharmacology* **36:**451–459.

Norstedt, C., and Fredholm, B. B. (1990). A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. *Anal. Biochem.* **189:**231–234.

Paila, Y. D., Pucadyil, T. J., and Chattopadhyay, A. (2005). The cholesterol-complexing agent digitonin modulates ligand binding of the bovine hippocampal serotonin$_{1A}$ receptor. *Mol. Membr. Biol.* **22:**241–249.

Papakostas, G. I., Öngür, D., Iosifescu, D. V., Mischoulon, D., and Fava, M. (2004). Cholesterol in mood and anxiety disorders: Review of the literature and new hypotheses. *Eur. Neuropsychopharmacol.* **14:**135–142.

Peroutka, S. J., and Howell, T. A. (1994). The molecular evolution of G protein-coupled receptors: Focus on 5-hydroxytryptamine receptors. *Neuropharmacology* **33:**319–324.

Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002). Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **3:**639–650.

Porter, F. D. (2002). Malformation syndromes due to inborn errors of cholesterol synthesis. *J. Clin. Invest.* **110:**715–724.

Prioni, S., Mauri, L., Loberto, N., Casellato, R., Chigorno, V., Karagogeos, D., Prinetti, A., and Sonnino, S. (2004). Interactions between gangliosides and proteins in the exoplasmic leaflet of neuronal plasma membranes: A study performed with a tritium-labeled GM1 derivative containing a photoactivable group linked to the oligosaccharide chain. *Glycoconj. J.* **21:**461–470.

Pucadyil, T. J., and Chattopadhyay, A. (2004). Cholesterol modulates ligand binding and G-protein coupling to serotonin$_{1A}$ receptors from bovine hippocampus. *Biochim. Biophys. Acta* **1663:**188–200.

Pucadyil, T. J., and Chattopadhyay, A. (2005). Cholesterol modulates the antagonist-binding function of hippocampal serotonin$_{1A}$ receptors. *Biochim. Biophys. Acta* **1714:**35–42.

Pucadyil, T. J., Kalipatnapu, S., and Chattopadhyay, A. (2005a). The serotonin$_{1A}$ receptor: A representative member of the serotonin receptor family. *Cell. Mol. Neurobiol* **25:**553–580.

Pucadyil, T. J., Kalipatnapu, S., Harikumar, K. G., Rangaraj, N., Karnik, S. S., and Chattopadhyay, A. (2004a). G-protein-dependent cell surface dynamics of the human serotonin$_{1A}$ receptor tagged to yellow fluorescent protein. *Biochemistry* **43:**15852–15862.

Pucadyil, T. J., Shrivastava, S., and Chattopadhyay, A. (2004b). The sterol-binding antibiotic nystatin differentially modulates ligand binding of the bovine hippocampal serotonin$_{1A}$ receptor. *Biochem. Biophys. Res. Commun.* **320:**557–562.

Pucadyil, T. J., Shrivastava, S., and Chattopadhyay, A. (2005b). Membrane cholesterol oxidation inhibits ligand binding function of hippocampal serotonin$_{1A}$ receptors. *Biochem. Biophys. Res. Commun.* **331:**422–427.

Raymond, J. R., Olsen, C. L., and Gettys, T. W. (1993). Cell-specific physical and functional coupling of human 5-HT$_{1A}$ receptors to inhibitory G protein α-subunits and lack of coupling to Gsα. *Biochemistry* **32:**11064–11073.

Sastry, P. S. (1985). Lipids of nervous tissue: Composition and metabolism. *Prog. Lipid Res.* **24:**69–176.

Singh, J. K., Chromy, B. A., Boyers, M. J., Dawson, G., and Banerjee, P. (1996). Induction of the serotonin$_{1A}$ receptor in neuronal cells during prolonged stress and degeneration. *J. Neurochem.* **66:**2361–2372.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150:**76–85.

Tate, C. G., and Grisshammer, R. (1996). Heterologous expression of G-protein-coupled receptors. *Trends Biotechnol.* **14:**426–430.

Toth, M. (2003). 5-HT$_{1A}$ receptor knockout mouse as a genetic model of anxiety. *Eur. J. Pharmacol.* **463:**177–184.

Tsui-Pierchala, B. A., Encinas, M., Milbrandt, J., and Johnson, E. M. (2002). Lipid rafts in neuronal signaling and function. *Trends Neurosci.* **25:**412–417.

Waterham, H. R., and Wanders, R. J. A. (2000). Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith–Lemli–Opitz syndrome. *Biochim. Biophys. Acta* **1529:**340–356.

Watson, J., Collin, L., Ho, M., Riley, G., Scott, C., Selkirk, J. V., and Price, G. W. (2000). 5-HT_{1A} receptor agonist–antagonist binding affinity difference as a measure of intrinsic activity in recombinant and native tissue systems. *Br. J. Pharmacol.* **130:**1108–1114.