Chapter 6
The human serotonin$_{1A}$ receptor expressed in neuronal cells: Toward a native environment for neuronal receptors.
Chapter 6. Serotonin$_{1A}$ receptors in neuronal cells

6.1. Introduction

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 previously 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; Gielen et al., 2006). Keeping this in mind, the pharmacological and functional characterization of the human serotonin$_{1A}$ receptor stably expressed in HN2 cells, which are a hybrid cell line between hippocampal cells and mouse neuroblastoma (Lee et al., 1990), are described in this chapter. Further, it is demonstrated that agonist and antagonist binding to the receptor exhibit differential sensitivity to the non-hydrolyzable GTP analogue, GTP-$\gamma$-S, as was observed earlier with the native receptor from bovine hippocampus. In addition, the serotonin$_{1A}$ receptor expressed in these cells displays typical downstream signaling of the receptor as monitored by ligand-dependent changes in cAMP levels. These results show that the human serotonin$_{1A}$ 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.

6.2. Materials and methods

Materials

EDTA, fetal calf serum, MgCl₂, MnCl₂, 8-OH-DPAT, p-MPPI, penicillin, streptomycin, gentamycin sulfate, polyethylenimine, PMSF, serotonin, sodium bicarbonate, and Tris were obtained from Sigma Chemical Co. (St. Louis, MO). DMEM and G-418 were from Life Technologies (Grand Island, NY). GTP-γ-S was from Roche Applied Science (Mannheim, Germany). BCA reagent for protein estimation was from Pierce (Rockford, IL). [³H]8-OH-DPAT (sp. activity = 135.0 Ci/mmol) and [³H]p-MPPF (sp. activity = 70.5 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA). The cyclic [³H]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 serotonin₁AR 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 serotonin₁AR 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 are referred to as HN2-5-HT₁AR
in this chapter. 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% CO₂ at 37 °C.

Preparation of cell membranes

Cell membranes were prepared as described earlier (Chattopadhyay et al., 2004; Kalipatnapu et al., 2004a; Paila and Chattopadhyay, 2006) and in section 3.2. and total protein concentration in membranes thus isolated was determined using the BCA assay (Smith et al., 1985).

Radioligand binding assays

Receptor binding assays were carried out as described in section 2.2 with ~60 µg total protein.

GTP-γ-S sensitivity assay

GTP-γ-S binding assays were carried out as described in section 2.2.

Saturation radioligand binding assays

Saturation radioligand binding assays were carried out as described in section 3.2.

Competition binding assays

Competition binding assays against the radiolabeled agonist [³H]8-OH-DPAT (0.29 nM) and antagonist [³H]p-MPPF (0.5 nM) were carried out in 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 equation 1 (section 2.2).
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\(_{\text{max}}\)), were calculated from the following equations as previously described (Akerma and Cheng, 1977; DeBlasi et al., 1989):

\[
K_d = \text{IC}_{50} - L \tag{13}
\]

\[
B_{\text{max}} = B \times (\text{IC}_{50}/L) \tag{14}
\]

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):

\[
K_i = \text{IC}_{50} / [1 + (L / K_d)] \tag{15}
\]

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 cAMP levels in cells**

cAMP levels in cells were estimated as described in section 3.2.

**6.3. Results**

**Linearity of radioligand binding with increasing concentrations of total protein**

The specific binding of the serotonin\(_{1A}\) receptor agonist \([^3\text{H}]8-\text{OH-DPAT}\) and antagonist \([^3\text{H}]\rho\text{-MPPF}\) to membranes prepared from HN2 cells that stably express
serotonin$_{1A}$ receptors (termed as HN2-5-HT$_{1A}$R cells) was characterized. Figure 6.1 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 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 radiolabeled 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

![Graph](image)

**Figure 6.2.** Saturation binding analysis of specific $[^3]$H$8$-OH-DPAT binding to serotonin$_{1A}$ receptors from HN2-5-HT$_{1AR}$ 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 Graphpad Prism software version 4.00 program. See section 6.2 and Table 6.1 for other details.

The saturation binding analyses of the specific agonist $[^3]$H$8$-OH-DPAT and antagonist $[^3]$H$p$-MPPF binding to serotonin$_{1A}$ receptors from HN2-5-HT$_{1AR}$ 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 Figures 6.2 and 6.3. Data for saturation binding were analyzed using Graphpad Prism software version 4.0 program and the binding parameters are shown in Table 6.1. The estimated $K_d$ value (~2.20 nM) for $[^3]$H$8$-OH-DPAT binding to serotonin$_{1A}$ receptors in HN2-5-HT$_{1AR}$ 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, 1999) hippocampal serotonin$_{1A}$ receptor. Table 6.1 also shows that the serotonin$_{1A}$ receptors expressed in HN2-5-HT$_{1AR}$ cells bind to $[^3]$H$p$-MPPF with a $K_d$ of ~2.70 nM, in good agreement with the affinity displayed by the native hippocampal receptor (Harikumar and Chattopadhyay, 2001).
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![Figure 6.3. Saturation binding analysis of specific $[^3H]p$-MPPF binding to serotonin$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. A representative plot is shown for specific $[^3H]p$-MPPF binding with increasing concentrations (0.1-7.5 nM) of free $[^3H]p$-MPPF. The curve is a non-linear regression fit to the experimental data using Graphpad Prism software version 4.00 program. See section 6.2 and Table 6.1 for other details.](image)

**Table 6.1**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_d$ (nM)</th>
<th>$B_{max}$ (pmol/mg of protein)</th>
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<tbody>
<tr>
<td>$[^3H]8$-OH-DPAT</td>
<td>2.20 ± 0.05</td>
<td>2.08 ± 0.30</td>
</tr>
<tr>
<td>$[^3H]p$-MPPF</td>
<td>2.70 ± 0.39</td>
<td>9.20 ± 1.88</td>
</tr>
</tbody>
</table>

*aBinding parameters were calculated by analyzing saturation binding isotherms with a range (0.1-7.5 nM) of both radioligands using Graphpad Prism software version 4.00 program. Data shown in the table represent the means ± SE of three independent experiments. See section 6.2 for other details.*
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Competition binding analysis of radiolabeled agonist and antagonist

![Graph showing competition binding analysis](image)

Figure 6.4. Competition binding analysis of specific $[^3H]8$-OH-DPAT binding to serotonin$_{1A}$ receptors from HN2-5-HT$_{1A}$R cell membranes. Values are expressed as percentages of specific binding obtained in the absence of the competing ligand. Radioligand binding assays were carried out with $[^3H]8$-OH-DPAT in the presence of a range of 8-OH-DPAT (---O---) and serotonin (----•----) concentrations. The curves are non-linear regression fits to the experimental data using equation 1 (section 2.2). Data points represent means ± SE of duplicate points from three independent experiments. See section 6.2 and Table 6.2 for other details.

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. Figures 6.4 and 6.5 show the competition displacement curves of specific agonist $[^3H]8$-OH-DPAT by the competing ligands 8-OH-DPAT and serotonin, and of the antagonist $[^3H]p$-MPPF by $p$-MPPF for serotonin$_{1A}$ receptors from HN2-5-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 6.2.
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Figure 6.5. Competition binding analysis of specific $[^3H]p$-MPPF binding to serotonin$_1A$ receptors from HN2-5-HT$_1A$R cell membranes. Values are expressed as percentages of specific binding obtained in the absence of the competing ligand. Radioligand binding assay was carried out with 0.5 nM $[^3H]p$-MPPF in the presence of a range of $p$-MPPI concentrations. The curve is a non-linear regression fit to the experimental data using equation 1 (section 2.2). Data points represent means ± SE of duplicate points from three independent experiments. See section 6.2 and Table 6.2 for other details.

Based on the formalism developed previously (Akera and Cheng, 1977; DeBlasi et al., 1989), binding parameters obtained from saturation binding analysis (see Table 6.1) 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 shown in Table 6.3. As shown in the table, these values are in good agreement with values in Table 6.1.

Sensitivity of ligand binding to GTP-$\gamma$-$S$

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,
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Table 6.2

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>IC$_{50}$ (nM)</td>
<td>K$_i$ (nM)</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>2.08 ± 0.40</td>
<td>1.65 ± 0.35</td>
</tr>
<tr>
<td>serotonin</td>
<td>5.10 ± 0.50</td>
<td>1.74 ± 0.46</td>
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<tr>
<td>$p$-MPPI</td>
<td>-</td>
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$[^3]$H8-OH-DPAT and $[^3]$H$p$-MPPF binding to serotonin$_{1A}$ receptors from HN2-5-HT$_{1A}$R cells

Competition binding data were analyzed using equation 1 (section 2.2) to determine IC$_{50}$ values. The K$_i$ values were obtained using equation 15 for which the K$_d$ values were obtained from Table 6.1. Binding of $[^3]$H8-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. Data represent the means ± SE of three independent experiments. See section 6.2 for other details.

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-$\gamma$-S, a non-hydrolyzable analogue of GTP. We have previously shown that the specific binding of the agonist $[^3]$H8-OH-DPAT to bovine hippocampal serotonin$_{1A}$ receptors is sensitive to guanine nucleotides and is inhibited with increasing concentrations of GTP-$\gamma$-S (Harikumar and Chattopadhyay, 1999; Javadekar-Subbedar and Chattopadhyay, 2004). Our results showed that in presence of GTP-$\gamma$-S, the serotonin$_{1A}$ receptor undergoes an affinity transition, from a high affinity...
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Table 6.3

| Competing ligand | \[^{3}\text{H}]8\text{-OH-DPAT\right|} | \[^{3}\text{H}]p\text{-MPPP|}
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<tr>
<td></td>
<td>(K_d) (nM)</td>
<td>(B_{\text{max}}) (pmol/mg protein)</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>1.57±0.40</td>
<td>4.02±0.80</td>
</tr>
<tr>
<td>(p)-MPPI</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^c\)Competition binding data were analyzed with a range of concentrations of unlabeled 8-OH-DPAT against \[^{3}\text{H}]8\text{-OH-DPAT (0.29 nM) and with unlabeled p-MPPI against [^{3}\text{H]}p-MPPP (0.5 nM). Binding parameters were calculated using equations 13 and 14 from the IC\textsubscript{50} values in Table 6.2. Data represent the means ± SE of three independent experiments. See section 6.2 for other details.

G-protein coupled to a low affinity G-protein uncoupled state (Harikumar and Chattopadhyay, 1999). In agreement with these results, Figure 6.6 shows a characteristic reduction in binding of the agonist \[^{3}\text{H}]8\text{-OH-DPAT\right|} in presence of a range of concentration of GTP-\(\gamma\)-S with an estimated IC\textsubscript{50} of 90.44±1.80 nM which is in excellent agreement with our earlier results with the receptor from native hippocampal source (Kalipatnapu and Chattopadhyay, 2004b; Pucadyil and Chattopadhyay, 2004a). This indicates that the human serotonin\textsubscript{1A} receptor is coupled to G-proteins when expressed in HN2 cells and exhibits typical sensitivity to GTP-\(\gamma\)-S, a characteristic feature of the native hippocampal receptor.
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Figure 6.6. Effect of increasing concentrations of GTP-γ-S on the specific binding of the agonist [3H]8-OH-DPAT (O) and antagonist [3H]p-MPPF (●) to serotonin1A receptors from HN2-5-HT1AR cell membranes. Values are expressed as percentages of the specific binding obtained in the absence of GTP-γ-S. The curve associated with [3H]8-OH-DPAT binding is a non-linear regression fit to the experimental data using equation 1 (section 2.2). Data points represent means ± SE of duplicate points from three independent experiments. See section 6.2 for other details.

In contrast to agonist binding, antagonist [3H]p-MPPF binding to serotonin1A 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., 2004b). Figure 6.6 shows that the specific [3H]p-MPPF binding to serotonin1A receptors from HN2-5-HT1AR 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 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 serotonin1A receptor in HN2-5-HT1AR cells. Interestingly, the $B_{max}$ values in Table 6.1 for serotonin1A receptors using the antagonist [3H]p-MPPF are far greater (~4 fold higher) than that obtained using agonist [3H]8-OH-DPAT. This has been previously shown for native systems such as rat hippocampus (Kung et al., 1995) and bovine hippocampus.
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(Harikumar and Chattopadhyay, 1999). Since the binding of the antagonist [³H]p-MPPF is unaffected by GTP-γ-S (Figure 6.6) it indicates that [³H]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 [³H]p-MPPF therefore is greater than the corresponding value for the agonist [³H]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 serotonin₁₄ receptor from HN2-5-HT₁₄R cells and native systems are in good agreement and therefore the pharmacological characteristics of the receptor appear to be preserved in HN2-5-HT₁₄R cells.

Ligand-dependent downstream signaling: measurement of cAMP levels

The primary function of serotonin₁₄ receptors is to inhibit adenylate cyclase thereby reducing the levels of 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., 2005a; Kalipatnapu and Chattopadhyay, 2007a). We examined the signaling function of the serotonin₁₄ receptor expressed in HN2 cells by monitoring its ability to catalyze downstream signal transduction processes upon stimulation with serotonin₁₄ receptor ligands. The serotonin₁₄ receptor agonists such as serotonin and 8-OH-DPAT are known to specifically activate the G_{i}/G_{o} class of G-proteins (Raymond et al., 1993) which
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Figure 6.7. Effect of increasing concentrations of 8-OH-DPAT on downstream signaling of serotonin_{1A} receptors in HN2-5-HT_{1AR} cells. The ability of the specific serotonin_{1A} receptor agonist 8-OH-DPAT to inhibit the forskolin-stimulated cAMP levels was assayed in HN2-5-HT_{1AR} cells. Data points are expressed as percentages of the cAMP levels in cells in the absence of forskolin and 8-OH-DPAT. 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 (equation 1; section 2.2). Data points represent means ± SE of at least three independent experiments. See section 6.2 for other details.

...subsequently reduce the cAMP levels. As shown in Figure 6.7, the forskolin-stimulated increase in cAMP levels is efficiently inhibited by 8-OH-DPAT in a characteristic concentration-dependent manner with an IC\textsubscript{50} value of 1.35 ± 0.07 nM in good agreement with 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_{1AR} 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).
6.4. Discussion

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 serotonin$_1$A 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 serotonin$_1$A receptors have previously been expressed in non-neuronal 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 serotonin$_1$A 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 chapter, the pharmacological and functional characterization of the human serotonin$_1$A receptor stably expressed in HN2 cells is described. Our results show that serotonin$_1$A 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 serotonin$_1$A receptors. In addition, it is demonstrated that the differential
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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, serotonin\textsubscript{1A} receptors in HN2-5-HT\textsubscript{1A}R cells can efficiently
catalyze downstream signal transduction by reducing cAMP levels.

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 \textit{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 \textit{in situ} synthesis
and such synthesis is defective in this syndrome (Waterham and Wanders, 2000).
Interestingly, it was previously shown using a variety of approaches that the function of
hippocampal serotonin\textsubscript{1A} receptor displays a great degree of sensitivity to membrane
cholesterol (Pucadyil and Chattopadhyay, 2004a, 2005; Pucadyil \textit{et al.}, 2004a; Pucadyil \textit{et al.},
2005b; Paila \textit{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
(Kalipatnapu and Chattopadhyay, 2005a). It is in this context that membrane receptors
expressed in a cell line with native-like membrane lipid environment gains significance.
The levels of receptors expressed this way are often much higher than 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.