ORIGINAL PAPER

Ligand Binding and G-protein Coupling of the Serotonin$_{1A}$ Receptor in Cholesterol-enriched Hippocampal Membranes

**Amitabha Chattopadhyay · Md. Jafurulla ·
Thomas J. Pucadyil**

Abstract   The serotonin$_{1A}$ receptor is the most extensively studied member of the family of seven transmembrane domain G-protein coupled serotonin receptors. Since a large portion of such transmembrane receptors remains in contact with the membrane lipid environment, lipid–protein interactions assume importance in the structure-function analysis of such receptors. We have earlier reported the requirement of cholesterol for serotonin$_{1A}$ receptor function in native hippocampal membranes by specific depletion of cholesterol using methyl-β-cyclodextrin. In this paper, we monitored the serotonin$_{1A}$ receptor function in membranes that are enriched in cholesterol using a complex prepared from cholesterol and methyl-β-cyclodextrin. Our results indicate that ligand binding and receptor/G-protein interaction of the serotonin$_{1A}$ receptor do not exhibit significant difference in native and cholesterol-enriched hippocampal membranes indicating that further enrichment of cholesterol has little functional consequence on the serotonin$_{1A}$ receptor function. These results therefore provide new information on the effect of cholesterol enrichment on the hippocampal serotonin$_{1A}$ receptor function.

Keywords   Serotonin$_{1A}$ receptor · cholesterol-enriched membranes · 8-OH-DPAT · p-MPPF · methyl-β-cyclodextrin · G-protein coupling

Abbreviations

BCA	bicinchoninic acid
DMPC	dimyristoyl-sn-glycero-3-phosphocholine
GPCR	G-protein coupled receptor
GTP-γ-S	guanosine-5′-O-(3-thiotriphosphate)
MβCD	methyl-β-cyclodextrin
5-HT	5-hydroxytryptamine (serotonin)
5-HT$_{1A}$ receptor	5-hydroxytryptamine-1A receptor
8-OH-DPAT	8-hydroxy-2(di-N-propylamino) tetralin

A. Chattopadhyay (✉) · Md. Jafurulla · T. J. Pucadyil
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India
E-mail: amit@ccmb.res.in

p-MPPI	4-(2′-methoxy)-phenyl-1-[2′-(N-2″-pyridinyl)-*p*-iodobenzamido] ethyl-piperazine
PMSF	phenylmethylsulfonyl fluoride
p-MPPF	4-(2′-methoxy)-phenyl-1-[2′-(N-2″-pyridinyl)-*p*-fluorobenzamido] ethyl-piperazine

Introduction

Lipid–protein interactions assume importance in the structure-function analysis of membrane proteins (Huber et al. 2004). Since a large portion of transmembrane receptors remains in contact with the membrane lipid environment, there exists the possibility that the membrane could be an important modulator of receptor structure and function (Lee 2004). Cholesterol is an essential component of eukaryotic membranes and is often found distributed non-randomly in domains in biological and model membranes (Schroeder et al. 1995; Mukherjee and Maxfield 2004). The effect of cholesterol on the structure and function of integral membrane proteins has been a subject of intense investigation (Burger et al. 2000). Cholesterol has been reported to modulate membrane protein function either through a specific molecular interaction with such proteins (Gimpl et al. 2002), or due to alterations in the membrane physical properties induced by the presence of cholesterol (Ohvo-Rekila et al. 2002; Lee 2004), or due to a combination of both factors. In view of the importance of cholesterol in the organization, dynamics and function of eukaryotic membranes (Schroeder et al. 1995; Mukherjee and Maxfield 2004), which regulate membrane protein function (Burger et al. 2000), the interaction of cholesterol with membrane proteins represents an important determinant in the functional studies of such proteins.

Serotonergic signaling plays a crucial 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 (Artigas et al. 1996; Ramboz et al. 1998; Rocha et al. 1998; Meneses 1999). Disruptions in 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 (Ramboz et al. 1998; Heisler et al. 1998; Parks et al. 1998; Sarnyai et al. 2000). Serotonergic signaling is initiated by the binding of serotonin to distinct cell surface receptors which have been classified into many groups (Hoyer et al. 2002). Among the 14 subtypes of serotonin receptors, the G-protein coupled serotonin$_{1A}$ (5-HT$_{1A}$) receptor subtype is the most extensively studied for a number of reasons that include its importance in neuronal physiology and the early availability of ligands that bind the receptor with high affinity and specificity that has resulted in extensive pharmacological characterization of this receptor (Pucadyil et al. 2005a). The 5-HT$_{1A}$ receptor has been implicated in neural development (del Olmo et al. 1998; Gross et al. 2002) and protection of stressed neuronal cells undergoing degeneration and apoptosis (Singh et al. 1996). Importantly, antagonists of the 5-HT$_{1A}$ receptor represent a major class of molecules with potential therapeutic effects in anxiety- or stress-related disorders (Griebel 1999). Furthermore, 5-HT$_{1A}$ receptor levels have been shown to be increased in schizophrenia (Sumiyoshi et al. 1996) and major depression (Fajardo et al. 2003). Interestingly, several epidemiological studies have correlated altered serum cholesterol concentration with major depressive disorder (MDD)-like symptoms and the prevalence of suicide in humans (Papakostas et al. 2004). Such behavioral symptoms have earlier been partially

attributed to disruptions in serotonergic signaling as a result of an alteration in serotonin receptor function (Engelberg 1992; Vevera et al. 2005). A more detailed analysis of the effects of modulating membrane cholesterol content on the function of such receptors could provide insight into the etiology of such disorders.

The role of cholesterol in modulating the hippocampal 5-HT$_{1A}$ receptor function has been explored earlier. Thus, depletion of cholesterol from native hippocampal membranes reduces 5-HT$_{1A}$ receptor functions such as ligand binding and G-protein coupling (Pucadyil and Chattopadhyay 2004a). In addition, replenishment of cholesterol into cholesterol-depleted membranes restored such functions to a significant extent thus pointing toward the specificity of the requirement of cholesterol in receptor function. If the ligand binding function of the 5-HT$_{1A}$ receptor is determined by specific interaction between cholesterol and the receptor, then such interaction would reach saturation at native levels of membrane cholesterol so that any further enrichment in cholesterol content may not affect ligand binding function of the receptor. In this paper, we tested this proposal by monitoring the effect of enrichment of hippocampal membranes with cholesterol on the ligand binding and G-protein coupling of 5-HT$_{1A}$ receptors in such membranes.

Materials and Methods

Materials

BCA, cholesterol, DMPC, MβCD, EDTA, EGTA, MgCl$_2$, MnCl$_2$, Na$_2$HPO$_4$, iodoacetamide, PMSF, 5-HT, p-MPPI, sucrose, polyethylenimine, sodium azide and Tris were obtained from Sigma Chemical Co. (St. Louis, MO, USA). GTP-γ-S was from Roche Applied Science (Mannheim, Germany). BCA reagent kit for protein estimation was from Pierce (Rockford, IL, USA). Amplex Red cholesterol assay kit was from Molecular Probes (Eugene, OR, USA). [^{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, 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. Fresh bovine brains were obtained from a local slaughterhouse within 10 min of death and the hippocampal region was carefully dissected out. The hippocampi were immediately flash frozen in liquid nitrogen and stored at −70°C till further use.

Preparation of Native Hippocampal Membranes

Native hippocampal membranes were prepared as described previously (Harikumar and Chattopadhyay 1998). Briefly, bovine hippocampal tissue (~100 g) was homogenized as 10% (w/v) in a polytron homogenizer in buffer A (2.5 mM Tris, 0.32 M sucrose, 5 mM EDTA, 5 mM EGTA, 0.02% sodium azide, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4). The homogenate was centrifuged at 900g for 10 min at 4°C. The resultant supernatant was filtered through four layers of cheesecloth and centrifuged at 50,000g for 20 min at 4°C. The pellet obtained was suspended in 10 vol. of buffer B (50 mM Tris, 1 mM EDTA, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4) using a hand-held Dounce homogenizer and centrifuged at 50,000g for 20 min at 4°C. This procedure was repeated until the supernatant was clear. The final pellet (native membranes) was suspended in a minimum volume of buffer C (50 mM Tris, pH 7.4), homogenized using a hand-held Dounce homogenizer, flash frozen in liquid nitrogen

and stored at −70°C. Protein concentration was assayed using the BCA assay kit (Smith et al. 1985).

Cholesterol Enrichment of Native Membranes

Native hippocampal membranes were enriched with cholesterol using a water soluble cholesterol-MβCD complex prepared as described previously (Pucadyil and Chattopadhyay 2004a), with a few modifications. Stock solutions of the cholesterol-MβCD complex (typically containing 2:20 and 4:30 mM cholesterol:MβCD) were prepared by dissolving the required amounts of cholesterol and MβCD in buffer C by constant shaking at room temperature (25°C). Stock solutions were freshly prepared before each experiment. Native membranes were incubated with the cholesterol-MβCD complex (final concentration being either 1:10 or 2:15 mM of cholesterol:MβCD) at a protein concentration of 2 mg/ml in buffer C for 1 h at room temperature (25°C) under constant shaking. Membranes were then spun down at 50,000g for 10 min at 4°C, washed once with buffer C and resuspended in the same buffer. Cholesterol content in membranes was estimated using the Amplex Red cholesterol assay kit (Amundson and Zhou 1999).

Estimation of Inorganic Phosphate

Concentration of lipid phosphate was determined subsequent to total digestion by perchloric acid (McClare 1971) using Na_2HPO_4 as standard. DMPC was used as an internal standard to assess lipid digestion. Samples without perchloric acid digestion produced negligible readings.

Radioligand Binding Assays

Receptor binding assays were carried out as described earlier (Kalipatnapu and Chattopadhyay 2004) with some modifications. Briefly, tubes in duplicate with 0.5 mg protein in a total volume of 1 ml of buffer D (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 E (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 being 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 (Bruns et al. 1983). Filters were then washed 3 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

Agonist binding assays were carried out as described above in the presence of varying concentrations of GTP-γ-S as described previously (Harikumar and Chattopadhyay 1999). 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 (Higashijima et al. 1987):

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

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.

Results and Discussion

Native hippocampal membranes represent a relatively enriched natural source for the G-protein coupled serotonin$_{1A}$ (5-HT$_{1A}$) receptor (Pucadyil et al. 2005a; Kalipatnapu and Chattopadhyay 2005). These membranes were enriched in cholesterol content using the water soluble cholesterol:MβCD complex. Such a complex has earlier been reported to efficiently catalyze cholesterol replenishment of cholesterol-depleted membranes (Pucadyil and Chattopadhyay 2004a) and enrichment of CHAPS-solubilized hippocampal membranes with cholesterol (Chattopadhyay et al. 2005). As shown in Fig. 1, treatment of native hippocampal membranes with increasing concentrations of cholesterol complexed with MβCD results in a progressive increase in the cholesterol content. Thus, treatment of membranes with 1:10 and 2:15 mM of cholesterol:MβCD complex increases the membrane cholesterol content by ~33 and 38%, respectively. Importantly, the phospholipid content of membranes remained unaltered after this treatment (Fig. 1). The cholesterol to phospholipid ratio we observe in native membranes is ~0.49 (mol/mol), similar to what we have reported earlier (Pucadyil and Chattopadhyay 2004b). Treatment of native membranes with 1:10 and 2:15 mM of cholesterol:MβCD complex increases this ratio to ~0.67 and 0.78, respectively.

We monitored the effect of such enrichment in the cholesterol content of hippocampal membranes on the ligand binding and G-protein coupling of 5-HT$_{1A}$ receptors. The agonist 8-OH-DPAT and antagonist p-MPPF ligands have earlier been well characterized to bind the 5-HT$_{1A}$ receptor with high affinity and specificity (Arvidsson et al. 1981; Gozlan et al. 1983; Kung et al. 1994; Barr and Manning 1997). Importantly, radioligand binding analyses with such ligands have earlier provided information on 5-HT$_{1A}$ receptor properties such as the

Fig. 1 Effect of increasing concentrations of cholesterol-MβCD complex on the cholesterol (shaded bars) and phospholipid (open bars) contents of hippocampal membranes. Membranes were treated with cholesterol-MβCD complex at a concentration of 1:10 and 2:15 mM of cholesterol:MβCD. The data represent means ± SEM of duplicate points from at least five independent experiments. See Materials and methods for other details
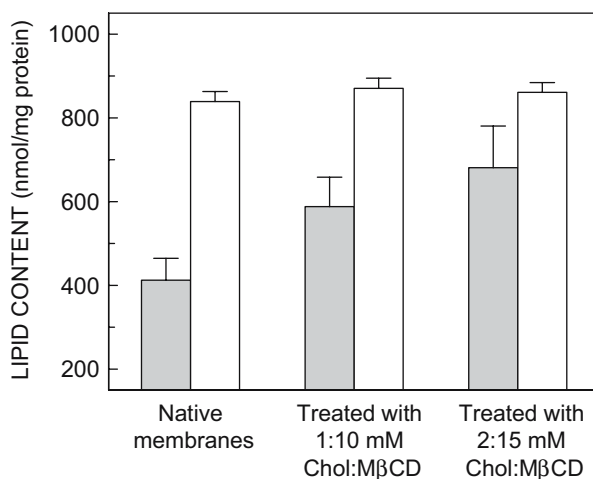
proportion of receptors coupled to G-proteins while it exists in a native membrane environment (Harikumar and Chattopadhyay 1999; Javadekar-Subhedar and Chattopadhyay 2004). Figure 2 shows the specific agonist [^{3}H]8-OH-DPAT and antagonist [^{3}H]p-MPPF binding to 5-HT$_{1A}$ receptors in cholesterol-enriched membranes. As is apparent from the figure, enrichment of cholesterol by ~38% results in a small (20%) reduction in specific binding of the agonist [^{3}H]8-OH-DPAT to the 5-HT$_{1A}$ receptor with no significant effect on the specific binding of the antagonist [^{3}H]p-MPPF.

The 5-HT$_{1A}$ receptor is negatively coupled to adenylate cyclase through pertussis toxin-sensitive G-proteins (Emerit et al. 1990). Sensitivity of specific agonist [^{3}H]8-OH-DPAT binding to guanine nucleotides and hence the G-protein coupling status of the receptor can be monitored by performing agonist binding assays in the presence of GTP-γ-S, a non-hydrolyzable analogue of GTP (Harikumar and Chattopadhyay 1999). Thus, the presence of GTP-γ-S induces transition of the receptor from a high affinity to a low affinity state. Figure 3 shows the inhibition of specific [^{3}H]8-OH-DPAT binding to the 5-HT$_{1A}$ receptor in presence of GTP-γ-S in a characteristic concentration-dependent manner in native and cholesterol-enriched membranes. The half maximal inhibition concentration (IC$_{50}$) value for inhibition of specific [^{3}H]8-OH-DPAT binding by GTP-γ-S is ~63 nM in native membranes, similar to what we reported earlier (Harikumar and Chattopadhyay 1999; Kalipatnapu and Chattopadhyay 2004; Pucadyil and Chattopadhyay 2004a). However, the inhibition curve for the cholesterol-enriched membranes exhibits no significant change in the IC$_{50}$ value (~45 nM). This indicates that the sensitivity of specific agonist binding to GTP-γ-S is similar in native and cholesterol-enriched membranes indicating that the G-protein coupling of the receptor is not affected upon cholesterol enrichment. It must be mentioned here that G-protein coupling of the 5-HT$_{1A}$ receptor is significantly compromised upon depletion of cholesterol from hippocampal membranes, with the GTP-γ-S sensitivity of agonist binding displaying a ~2.5 fold shift toward higher concentrations of GTP-γ-S in cholesterol-depleted membranes (Pucadyil and Chattopadhyay 2004a).

The lack of any significant effect of cholesterol enrichment on the 5-HT$_{1A}$ receptor function (ligand binding and G-protein coupling) is surprising considering earlier studies that have assessed the modulatory role of cholesterol on membrane protein function. Such studies have indicated that membrane proteins show optimal function in native membranes containing natural levels of cholesterol. Thus, the GABA$_A$ receptor functions most efficiently in native

Fig. 2 Effect of cholesterol enrichment on the specific binding of the agonist [^{3}H]8-OH-DPAT (open bars) and the antagonist [^{3}H]p-MPPF (shaded bars) to 5-HT$_{1A}$ receptors in hippocampal membranes. Cholesterol enrichment was carried out using 2:15 mM of cholesterol:MβCD complex. Values are expressed as percentage of specific radioligand binding in native membranes without any treatment. The data represent means ± SEM of duplicate points from at least five independent experiments. See Materials and methods for other details
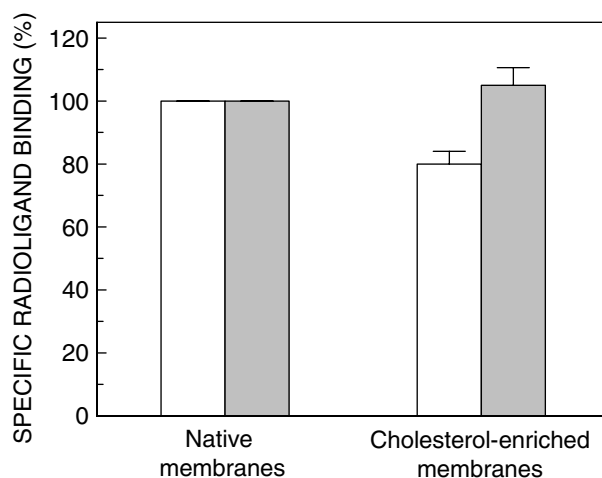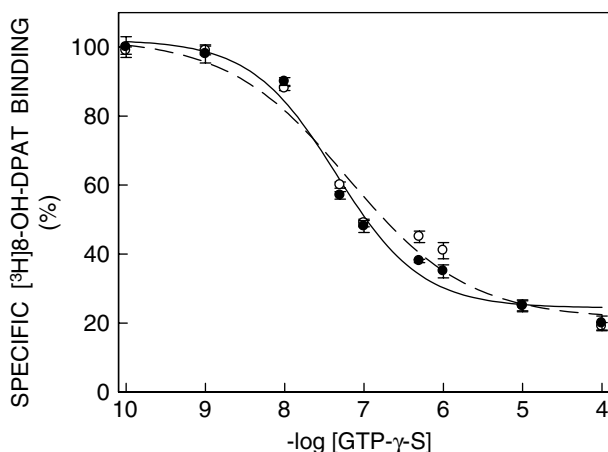
Fig. 3 Effect of increasing concentrations of GTP-γ-S on specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors from native (- - ○ - -) and cholesterol-enriched (—●—) membranes. Cholesterol enrichment was carried out using 2:15 mM of cholesterol:MβCD complex. Values are expressed as percentage of specific binding obtained in the absence of GTP-γ-S. The curves are non-linear regression fits to the experimental data using Eq. 1. Data represent means ± SEM of duplicate points from at least four independent experiments. See Materials and methods for other details

hippocampal membranes containing natural levels of cholesterol (Sooksawate and Simmonds 1998, 2001), with an increase or decrease in the cholesterol level leading to reduction in receptor function. These results have been interpreted to be due to a specific requirement of cholesterol at native levels to maintain optimal GABA$_A$ receptor function possibly through a specific cholesterol–receptor interaction, while the reduction in function due to cholesterol enrichment being due to an alteration in membrane physical properties.

Our results suggest that the 5-HT$_{1A}$ receptor ligand binding remains unaltered upon cholesterol enrichment of hippocampal membranes. Since the enrichment of cholesterol does not significantly affect the 5-HT$_{1A}$ receptor ligand binding whereas cholesterol depletion reduces receptor function (Pucadyil and Chattopadhyay 2004a), it appears that the concentration of cholesterol in native membranes is sufficient for proper receptor function. We have earlier shown that oxidation of membrane cholesterol to cholestenone results in a marked reduction in the 5-HT$_{1A}$ receptor ligand binding function (Pucadyil et al. 2005b). Interestingly, while cholesterol oxidation reduces the functionality of cholesterol, it does not dramatically perturb the bulk membrane physical properties as measured by fluorescence polarization of membrane-embedded probes. Thus, a reduction in the 5-HT$_{1A}$ receptor function observed under such conditions possibly suggests the presence of a specific cholesterol–receptor interaction that supports ligand binding activity. If the cholesterol concentration present in native membranes is sufficient to completely fulfill such a specific cholesterol–receptor interaction, then further enrichment of cholesterol would bear no functional consequence to receptor function. This could perhaps explain our results on the relative insensitivity of ligand binding of 5-HT$_{1A}$ receptors to cholesterol-enrichment. In summary, our present results provide new information on the effect of cholesterol enrichment on the 5-HT$_{1A}$ receptor function in hippocampal membranes. In a broader context, these results are relevant in understanding the role of the membrane lipid environment on the functioning of G-protein coupled transmembrane receptors in general.

References

Amundson DM, Zhou M (1999) Fluorometric method for the enzymatic determination of cholesterol. J Biochem Biophys Methods 38:43–52

Artigas F, Romero L, de Montigny C, Blier P (1996) Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT$_{1A}$ antagonists. Trends Neurosci 19:378–383

Arvidsson LE, Hacksell U, Nilsson JL, Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikstrom H (1981) 8-Hydroxy-2-(di-n-propylamino)tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. J Med Chem 24:921–923

Barr AJ, Manning DR (1997) Agonist-independent activation of Gz by the 5- hydroxytryptamine$_{1A}$ receptor co-expressed in *Spodoptera frugiperda* cells. Distinguishing inverse agonists from neutral antagonists. J Biol Chem 272:32979–32987

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

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

Chattopadhyay A, Jafurulla Md, Kalipatnapu S, Pucadyil TJ, Harikumar KG (2005) Role of cholesterol in ligand binding and G-protein coupling of serotonin$_{1A}$ receptors solubilized from bovine hippocampus. Biochem Biophys Res Commun 327:1036–1041

del Olmo E, López-Giménez JF, Vilaró MT, Mengod G, Palacios JM, Pazos A (1998) Early localization of mRNA coding for 5-HT$_{1A}$ receptors in human brain during development. Mol Brain Res 60:123–126

Emerit MB, el Mestikawy S, Gozlan H, Rouot B, Hamon M (1990) Physical evidence of the coupling of solubilized 5-HT$_{1A}$ binding sites with G regulatory proteins. Biochem Pharmacol 39:7–18

Engelberg H (1992) Low serum cholesterol and suicide. Lancet 339:727–729

Fajardo O, Galeno J, Urbina M, Carreira I, Lima L (2003) Serotonin, serotonin 5-HT$_{1A}$ receptors and dopamine in blood peripheral lymphocytes of major depression patients. Int Immunopharmacol 3:1345–1352

Gimpl G, Burger K, Fahrenholz F (2002) A closer look at the cholesterol sensor. Trends Biochem Sci 27:596–599

Gozlan H, el Mestikawy S, Pichat L, Glowinski J, Hamon M (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature 305:140–142

Griebel G (1999) 5-HT$_{1A}$ receptor blockers as potential drug candidates for the treatment of anxiety disorders. Drug News Perspective 12:484–490

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R (2002) Serotonin$_{1A}$ receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416:396–400

Harikumar KG, 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 KG, 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

Heisler LK, Chu H-M, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH (1998) Elevated anxiety and antidepressant-like responses in serotonin 5-HT$_{1A}$ receptor mutant mice. Proc Natl Acad Sci USA 95:15049–15054

Higashijima T, Ferguson KM, Sternweis PC, Smigel MD, Gilman AG (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 JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71:533–554

Huber T, Botelho AV, Beyer K, Brown MF (2004) Membrane model for the G-protein-coupled receptor rhodopsin: hydrophobic interface and dynamical structure. Biophys J 86:2078–2100

Javadekar-Subhedar V, Chattopadhyay A (2004) Temperature-dependent interaction of the bovine hippocampal serotonin$_{1A}$ receptor with G-proteins. Mol Membr Biol 21:119–123

Kalipatnapu S, Chattopadhyay A (2004) Interaction of serotonin$_{1A}$ receptors from bovine hippocampus with tertiary amine local anesthetics. Cell Mol Neurobiol 24:403–422

Kalipatnapu S, Chattopadhyay A (2005) Membrane protein solubilization: recent advances and challenges in the solubilization of serotonin$_{1A}$ receptors. IUBMB Life 57:505–512

Kung HF, Kung M-P, Clarke W, Maayani S, Zhuang Z-P (1994) A potential 5-HT$_{1A}$ receptor antagonist: p-MPPI. Life Sci 55:1459–1462

Lee AG (2004) How lipids affect the activities of integral membrane proteins. Biochim Biophys Acta 1666:62–87

McClare CWF (1971) An accurate and convenient organic phosphorus assay. Anal Biochem 39:527–530

Meneses A (1999) 5-HT system and cognition. Neurosci Biobehav Rev 23:1111–1125

Mukherjee S, Maxfield FR (2004) Membrane domains. Annu Rev Cell Dev Biol 20:839–866

Ohvo-Rekila H, Ramstedt B, Leppimaki P, Slotte JP (2002) Cholesterol interactions with phospholipids in membranes. Prog Lipid Res 41:66–97

Papakostas GI, Ongur D, Iosifescu DV, Mischoulon D, Fava M (2004) Cholesterol in mood and anxiety disorders: review of the literature and new hypothesis. Eur Neuropsychopharmacol 14:135–142

Parks CL, Robinson PS, Sibille E, Shenk T, Tóth M (1998) Increased anxiety of mice lacking the serotonin$_{1A}$ receptor. Proc Natl Acad Sci USA 95:10734–10739

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

Pucadyil TJ, Chattopadhyay A (2004b) Exploring detergent insolubility in bovine hippocampal membranes: a critical assessment of the requirement for cholesterol. Biochim Biophys Acta 1661:9–17

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

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

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA 95:14476–14481

Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, Hen R (1998) Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 393:175–178

Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Tóth M (2000) Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin$_{1A}$ receptors. Proc Natl Acad Sci USA 97:14731–14736

Schroeder F, Woodford JK, Kavecansky J, Wood WG, Joiner C (1995) Cholesterol domains in biological membranes. Mol Membr Biol 12:113–119

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

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85

Sooksawate T, Simmonds MA (1998) Increased membrane cholesterol reduces the potentiation of GABA$_A$ currents by neurosteroids in dissociated hippocampal neurones. Neuropharmacology 37:1103–1110

Sooksawate T, Simmonds MA (2001) Effects of membrane cholesterol on the sensitivity of the GABA$_A$ receptor to GABA in acutely dissociated rat hippocampal neurones. Neuropharmacology 40:178–184

Sumiyoshi T, Stockmeier CA, Overholser JC, Dilley GE, Meltzer HY (1996) Serotonin$_{1A}$ receptors are increased in postmortem prefrontal cortex in schizophrenia. Brain Res 708:209–214

Vevera J, Fisar Z, Kvasnicka T, Zdenek H, Starkova L, Ceska R, Papezova H (2005) Cholesterol-lowering therapy evokes time-limited changes in sertonergic transmission. Psychiatry Res 133:197–203