Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 327 (2005) 1036–1041
BBRC
www.elsevier.com/locate/ybbrc

Role of cholesterol in ligand binding and G-protein coupling of serotonin$_{1A}$ receptors solubilized from bovine hippocampus☆

Amitabha Chattopadhyay*, Md. Jafurulla, Shanti Kalipatnapu, Thomas J. Pucadyil, Kaleeckal G. Harikumar[1]

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

Received 8 December 2004
Available online 29 December 2004

Abstract

The serotonin$_{1A}$ (5-HT$_{1A}$) receptor is an important member of the superfamily of seven transmembrane domain G-protein-coupled receptors. We report here that solubilization of the hippocampal 5-HT$_{1A}$ receptor by the zwitterionic detergent CHAPS is accompanied by loss of membrane cholesterol which results in a reduction in specific agonist binding activity and extent of G-protein coupling. Importantly, replenishment of cholesterol to solubilized membranes using MβCD–cholesterol complex restores the cholesterol content of the membrane and significantly enhances the specific agonist binding activity and G-protein coupling. These novel results provide useful information on the role of cholesterol in solubilization of G-protein-coupled receptors, an important step for molecular characterization of these receptors.

Keywords: Serotonin$_{1A}$ receptor; Solubilization; Cholesterol; CHAPS; G-protein coupling

Serotonin receptors represent one of the largest, evolutionarily ancient, and highly conserved families of G-protein-coupled receptors [1]. Serotonin (5-hydroxytryptamine or 5-HT) is an intrinsically fluorescent [2], biogenic amine which acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous system [3]. Serotonergic signaling appears to play a key role in the generation and modulation of various cognitive and behavioral functions such as sleep, mood, pain, addiction, locomotion, sexual activity, depression, anxiety, alcohol abuse, aggression, and learning [4–6]. 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 [4,7–9]. Serotonin exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups [10]. Serotonin receptors are members of a superfamily of seven transmembrane domain receptors [11] that couple to and transduce signals via GTP-binding regulatory proteins (G-proteins) [12]. Among the 14 subtypes of serotonin receptors, the G-protein-coupled 5-HT$_{1A}$ receptor is the best characterized for a variety of reasons [13], and is implicated in several anxiety-related disorders. This is supported by the fact that the 5-HT$_{1A}$ receptor knockout mice are an excellent model system to understand anxiety-related behavior in higher animals [7–9].

☆ *Abbreviations:* 5-HT, 5-hydroxytryptamine; 5-HT$_{1A}$ receptor, 5-hydroxytryptamine-1A receptor; BCA, bicinchoninic acid; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; DMPC, dimyristoyl-*sn*-glycero-3-phosphocholine; 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; GTP-γ-S, guanosine-5′-*O*-(3-thiotriphosphate); MβCD, methyl-β-cyclodextrin; PEG, polyethylene glycol; PMSF, phenylmethylsulfonyl fluoride.

* Corresponding author. Fax: +91 40 2716 0311.
E-mail address: amit@ccmb.res.in (A. Chattopadhyay).
[1] Present address: Cancer Center and Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Scottsdale, AZ 85259, USA.

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.102

An essential criterion for purification of an integral membrane protein is that the protein must be carefully removed from its native membrane and dispersed in solution. This process, usually achieved using amphiphilic detergents, is termed as solubilization [14,15]. We have earlier partially purified and solubilized the 5-HT_{1A} receptor from bovine hippocampus in a functionally active form [16,17] using CHAPS which is one of the most commonly used detergents in membrane biochemistry and is a mild, non-denaturing, zwitterionic detergent. We have also reported the solubilization of 5-HT_{1A} receptors stably expressed in Chinese hamster ovary (CHO) cells using the same detergent [18].

A common problem associated with most solubilization experiments is delipidation, i.e., loss of lipids. This often leads to considerable loss of activity of the solubilized protein or receptor since lipid–protein interactions play a crucial role in maintaining the structure and function of integral membrane proteins and receptors [19]. This makes the solubilization process less effective. For example, displacement of lipids from the receptor was shown to be an integral feature of detergent-induced inactivation in case of the nicotinic acetylcholine receptor [20]. The phenomenon of delipidation and its consequences on the activity of solubilized membrane proteins have been utilized to gain useful insight into the lipid requirements of membrane proteins [20,21].

It has been previously reported that solubilization by CHAPS leads to loss of cholesterol, an essential component of eukaryotic membranes, from the solubilized membranes [22,23]. Interestingly, we have recently shown the requirement of membrane cholesterol in modulating ligand binding activity of the 5-HT_{1A} receptor from the bovine hippocampus [24]. This was achieved by the use of methyl-β-cyclodextrin (MβCD) which physically depletes cholesterol from membranes. Treatment of bovine hippocampal membranes with MβCD resulted in specific removal of membrane cholesterol without any change in phospholipid content. Removal of cholesterol from bovine hippocampal membranes in this manner resulted in a reduction in ligand binding to the 5-HT_{1A} receptor [24]. If cholesterol is necessary for ligand binding of the 5-HT_{1A} receptor [24] and solubilization by CHAPS leads to its removal from the solubilized membranes [22,23], the apparent loss observed in the activity of the 5-HT_{1A} receptor upon solubilization could be due to the loss of cholesterol. In this report, we have tested this proposal by assaying the cholesterol content of native and solubilized membranes containing the 5-HT_{1A} receptor from bovine hippocampus. Our results show that there is considerable loss of cholesterol (~60%) upon solubilization which is accompanied by reduced ligand binding of the receptor. More importantly, we show here, for the first time, that a significant fraction of the receptor activity could be restored upon replenishment of the solubilized membranes with cholesterol.

Materials and methods

Materials. 5-HT, CHAPS, cholesterol, EDTA, EGTA, iodoacetamide, MβCD, $MgCl_2$, $MnCl_2$, NaCl, PMSF, PEG, polyethylenimine, sodium azide, sucrose, and Tris were obtained from Sigma Chemical (St. Louis, MO, USA). GTP-γ-S was purchased from Roche Applied Science (Mannheim, Germany). [^{3}H]8-OH-DPAT (sp. activity 135.0 Ci/mmol) was from DuPont New England Nuclear (Boston, MA, USA). BCA reagent kit for protein estimation was obtained from Pierce (Rockford, IL, USA). Amplex Red cholesterol assay kit was from Molecular Probes (Eugene, OR, USA). All other chemicals used were of the highest purity available. GF/B glass microfiber filters were from Whatman International (Kent, UK). 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 until further use.

Preparation of native hippocampal membranes. Native hippocampal membranes were prepared as described earlier [17]. Bovine hippocampal tissue (~100 g) was homogenized as 10% (w/v) with 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, and 10 mM iodoacetamide, pH 7.4). The homogenate was centrifuged at 900*g* for 10 min at 4 °C. The supernatant was filtered through four layers of cheesecloth and the pellet was discarded. The supernatant was further centrifuged at 50,000*g* for 20 min at 4 °C. The resulting pellet was suspended in 10 vol of buffer B (50 mM Tris, 1 mM EDTA, 0.24 mM PMSF, and 10 mM iodoacetamide, pH 7.4) using a hand-held Dounce homogenizer and centrifuged at 50,000*g* for 20 min at 4 °C. This procedure was repeated until the supernatant was clear. The final pellet (native membrane) was resuspended 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 determined using the BCA reagent [25].

Solubilization of native membranes. Native hippocampal membranes were solubilized as described earlier with some modifications [17]. Membranes were incubated with 5 mM CHAPS and 1 M NaCl in buffer D (50 mM Tris, 1 mM EDTA, and 10 mM $MgCl_2$, pH 7.4) at a final protein concentration of ~3 mg/ml for 30 min at 4 °C with occasional shaking. The membranes were briefly sonicated (5 s) using a Branson model 250 sonifier at the beginning of the incubation period, and mildly homogenized using a hand-held Dounce homogenizer (five times) at the beginning and the end of the incubation period. After incubation for 30 min, the contents were centrifuged at 100,000*g* for 1 h at 4 °C. The clear supernatant was carefully removed from the pellet and used for PEG precipitation. PEG precipitation of the crude CHAPS-solubilized membrane was performed to remove NaCl from the solubilized extract, since the agonist binding of the 5-HT_{1A} receptor is inhibited by NaCl [26]. This procedure is believed to remove the detergent and salt [27]. PEG precipitation was carried out by diluting the extract with equal volume of 40% (w/w) PEG-8000 in buffer D. Following vigorous vortexing and incubation for 10 min on ice, the samples were centrifuged at 15,000*g* for 10 min at 4 °C. The pellet was carefully rinsed twice with buffer C, resuspended in the same buffer, and either used immediately for radioligand binding assays or for cholesterol replenishment.

Cholesterol replenishment of PEG-precipitated solubilized membranes using MβCD–cholesterol complex. PEG-precipitated solubilized membranes were replenished with cholesterol using cholesterol–MβCD complex as described earlier [24] with a few modifications. The complex was prepared by dissolving the required amounts of cholesterol and MβCD in a ratio of 1:10 (mol/mol), respectively, in buffer C by

constant shaking at room temperature (25 °C). Stock solutions (typically 2:20 mM cholesterol:MβCD) of this complex were freshly prepared before each experiment. Cholesterol replenishment was carried out at a protein concentration of ~1.3 mg/ml by incubating the PEG-precipitated solubilized membranes with the cholesterol–MβCD complex for 30 min in buffer C at room temperature (25 °C) under constant shaking. Membranes were then spun down at 100,000*g* for 1 h at 4 °C, suspended in buffer C, and used for radioligand binding assays. Cholesterol content of membranes was estimated using the Amplex Red cholesterol assay kit [28]. Values are expressed as the percentage of cholesterol content in native hippocampal membranes and shown in Fig. 1A.

Receptor binding assays. Receptor binding assays were carried out as described earlier [24]. Briefly, tubes in duplicate containing ~0.5 mg total protein in a total volume of 1 ml of buffer E (50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, and 5 mM $MnCl_2$, pH 7.4) were used. Tubes were incubated with the radiolabeled agonist [^{3}H]8-OH-DPAT (final concentration in the assay tube 0.29 nM) for 1 h at room temperature. Non-specific binding was determined by performing the assay in the presence of 10 μM unlabeled 5-HT. The incubation was terminated by rapid filtration under vacuum in a Millipore multiport filtration apparatus through Whatman GF/B 2.5 cm diameter (1.0 μm pore size) glass microfiber filters which were presoaked in 0.15% (w/v) polyethylenimine for 3 h [29]. The filters were then washed three times with 3 ml of ice-cold water, dried, and the retained radioactivity was measured in a Packard Tri-Carb 1500 scintillation counter using 5 ml of scintillation fluid.

Sensitivity of agonist binding to GTP-γ-S. In experiments with GTP-γ-S, agonist binding assays were carried out as described above in the presence of varying concentrations of GTP-γ-S as described previously [30]. 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 [31]:

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

where B is the specific binding of the agonist normalized to control binding (in absence of GTP-γ-S), x denotes the 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. Data were analyzed for statistical significance using one-way ANOVA (Microcal Origin Version 5.0, Microcal Software, USA).

Estimation of phospholipid content of membranes. Concentration of lipid phosphate of membranes was determined subsequent to total digestion by perchloric acid [32] 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.

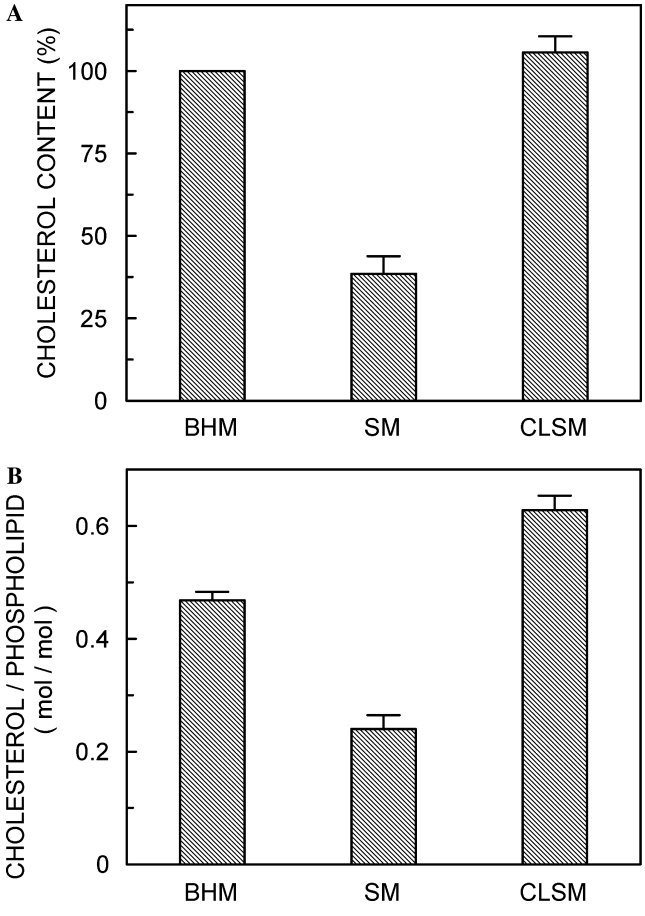

Fig. 1. (A) Cholesterol contents and (B) cholesterol to phospholipid ratios (mol/mol) of native bovine hippocampal membranes (BHM), PEG-precipitated solubilized membranes (SM), and PEG-precipitated solubilized membranes enriched with cholesterol (CLSM). Cholesterol and phospholipid contents were assayed as described in Materials and methods. Data represent means ± standard errors of at least three independent experiments. See Materials and methods for other details.

Results

Fig. 1 shows the change in lipid composition due to solubilization of native hippocampal membranes by CHAPS. Fig. 1A shows the relative cholesterol contents of native bovine hippocampal membranes (BHM) and PEG-precipitated solubilized membranes (SM). There is ~60% reduction in the cholesterol content of the hippocampal membranes upon solubilization. Treatment of solubilized membranes with MβCD–cholesterol complex results in replenishment of cholesterol. Thus, the PEG-precipitated solubilized membranes enriched with cholesterol (CLSM) were found to contain ~105% of the original cholesterol content (Fig. 1A). The corresponding cholesterol to phospholipid ratios are shown in Fig. 1B.

The specific agonist ([^{3}H]8-OH-DPAT) binding activities of these membranes are shown in Fig. 2. The specific [^{3}H]8-OH-DPAT binding of the 5-HT_{1A} receptor shows a reduction upon solubilization by CHAPS. Thus, the specific binding of the agonist reduces upon solubilization to ~67% of that obtained with native membranes. We attribute this to the loss of lipids accompanying solubilization (see Fig. 1). Interestingly, we have previously reported that removal of cholesterol from hippocampal membranes due to treatment with the cholesterol complexing agent MβCD results in a decrease in specific agonist activity [24]. In order to explore whether the observed loss in specific agonist binding is due to loss of cholesterol during solubilization, we replenished the cholesterol content of the solubilized membranes using MβCD–cholesterol complex.

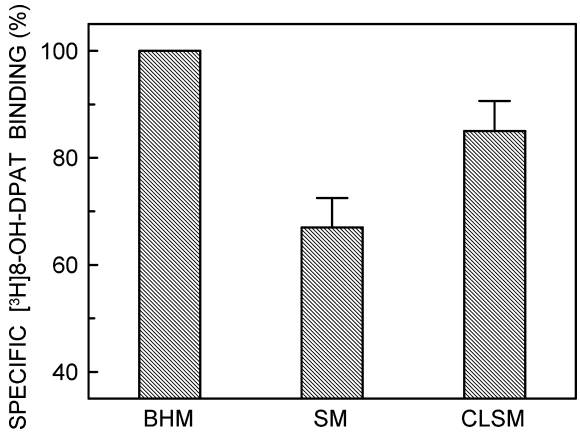

Fig. 2. Effect of enrichment of cholesterol in PEG-precipitated solubilized bovine hippocampal membranes on the specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors. Bovine hippocampal membranes are denoted as BHM, PEG-precipitated solubilized membranes as SM, and PEG-precipitated solubilized membranes enriched with cholesterol as CLSM. Values are expressed as a percentage of the specific binding obtained with native membranes. The data points shown are the means ± standard errors of at least four independent experiments. See Materials and methods for other details.

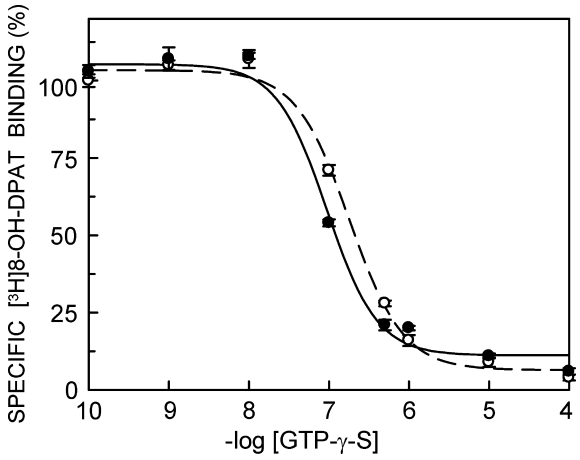

Fig. 3. Effect of increasing concentrations of GTP-γ-S on the specific binding of the agonist [^{3}H]8-OH-DPAT to 5-HT$_{1A}$ receptors from SM (–○–) and CLSM (–●–) membranes. Values are expressed as a percentage of the specific binding obtained in the absence of GTP-γ-S. The curves are non-linear regression fits to the experimental data using the four parameter logistic function [31]. The data points represent means ± standard errors of duplicate points from at least three independent experiments. The data for native bovine hippocampal membranes are not shown for clarity. See Materials and methods and Table 1 for other details.

This resulted in an increase of specific agonist binding to ~85% of the native membranes, possibly due to an increase in cholesterol content of the membrane (shown in Fig. 1A).

The hippocampal 5-HT$_{1A}$ receptor is negatively coupled to adenylate cyclase through G$_i$-proteins [33]. Sensitivity of agonist binding to guanine nucleotides can be monitored by performing the agonist binding assay in the presence of GTP-γ-S, a non-hydrolyzable analogue of GTP [30]. In order to assess the status of receptor–G-protein interaction for the 5-HT$_{1A}$ receptor in these membranes, we monitored the specific agonist binding of the receptor in the presence of increasing concentrations of GTP-γ-S. These results are shown in Fig. 3 and Table 1. Fig. 3 shows the inhibition of specific agonist binding to the 5-HT$_{1A}$ receptor by GTP-γ-S in a characteristic concentration-dependent manner. The corresponding values of the half maximal inhibition concentrations (IC$_{50}$) are shown in Table 1. As seen from the table, the IC$_{50}$ value for BHM is ~78 nM and is increased to ~181 nM upon solubilization using CHAPS. This implies that the G-protein coupling of the CHAPS-solubilized receptors is considerably reduced. Upon replenishment of cholesterol to PEG-precipitated solubilized membranes, the IC$_{50}$ value shows a significant decrease (~96 nM). This shows that the extent of G-protein coupling is enhanced when cholesterol is loaded back into the solubilized membranes. This confirms our earlier conclusion that membrane cholesterol is required for efficient G-protein coupling of the hippocampal 5-HT$_{1A}$ receptor [24].

Table 1
Effect of cholesterol replenishment in PEG-precipitated solubilized bovine hippocampal membranes on sensitivity of [^{3}H]8-OH-DPAT binding to GTP-γ-S[a]

Condition	IC$_{50}$ (nM)
BHM	78 ± 4
SM	181 ± 6
CLSM	96 ± 5

[a] The IC$_{50}$ values shown in the table represent means ± standard errors from at least three independent experiments. Bovine hippocampal native membranes are denoted as BHM, PEG-precipitated solubilized membranes as SM, and PEG-precipitated solubilized membranes enriched with cholesterol as CLSM. See Materials and methods for other details.

Discussion

Solubilization of biological membranes is often accompanied by selective or differential solubilization of membrane lipids [22,23]. Thus, the lipid environment around a protein of interest assumes significance since the extent of solubilization as well as the function of the solubilized protein could depend on it. Interestingly, the possibility of membranes being organized into domains consisting of certain classes of lipids and proteins which are resistant to detergent extraction has generated a lot of interest. In fact, detergent insolubility of membrane components is commonly used as a biochemical tool to monitor domain organization of membranes [34–36].

We report here that solubilization of the hippocampal 5-HT_{1A} receptor by CHAPS is accompanied by a loss of membrane cholesterol which results in a reduction in specific agonist binding activity and extent of G-protein coupling. Replenishment of cholesterol to solubilized membranes using MβCD–cholesterol complex restores the cholesterol content of the membrane and significantly enhances specific agonist binding activity and G-protein coupling. Since other membrane lipids besides cholesterol could be lost during solubilization, it is possible that further enhancement of ligand binding and G-protein coupling could be achieved by appropriate incorporation of any other lipid lost.

The 5-HT_{1A} receptor belongs to the family of G-protein-coupled receptors. These receptors constitute a superfamily of proteins whose function is to transmit information across a cell membrane from the extracellular environment to the interior of the cell thus providing a mechanism of communication between the exterior and the interior of the cell [11]. G-protein-coupled receptors represent the single largest family of cell surface receptors involved in signal transduction. Our results are relevant for optimal solubilization of G-protein-coupled receptors in general, especially considering the fact that very few G-protein-coupled receptors have been successfully solubilized. Efficient solubilization of receptors from their native source with high ligand binding activity and intact signal transduction components may constitute the first step in the molecular characterization of the G-protein-coupled receptors.

Acknowledgments

This work was supported by the Council of Scientific and Industrial Research, Government of India. S.K. and T.J.P. thank the Council of Scientific and Industrial Research for the award of Senior Research Fellowships. K.G.H. thanks the Council of Scientific and Industrial Research for the award of a Research Associateship. Some of the preliminary experiments were performed by Gargi Ray. We thank S. Rajanna for help with the tissue collection and members of our laboratory for critically reading the manuscript.

References

[1] S.J. Peroutka, T.A. Howell, The molecular evolution of G-protein coupled receptors: focus on 5-hydroxytryptamine receptors, Neuropharmacology 33 (1994) 319–324.
[2] A. Chattopadhyay, R. Rukmini, S. Mukherjee, Photophysics of a neurotransmitter: ionization and spectroscopic properties of serotonin, Biophys. J. 71 (1996) 1952–1960.
[3] B.L. Jacobs, E.C. Azmitia, Structure and function of the brain serotonin system, Physiol. Rev. 72 (1992) 165–229.
[4] M.J.S. Heath, R. Hen, Genetic insights into serotonin function, Curr. Biol. 5 (1995) 997–999.
[5] F. Artigas, L. Romero, C. de Montigny, P. Blier, Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists, Trends Neurosci. 19 (1996) 378–383.
[6] D. Julius, Serotonin receptor knockouts: a moody subject, Proc. Natl. Acad. Sci. USA 95 (1998) 15153–15154.
[7] S. Ramboz, R. Oosting, D.A. Amara, H.F. Kung, P. Blier, M. Mendelsohn, J.J. Mann, D. Brunner, R. Hen, Serotonin receptor 1A knockout: an animal model of anxiety-related disorder, Proc. Natl. Acad. Sci. USA 95 (1998) 14476–14481.
[8] L.K. Heisler, H.-M. Chu, T.J. Brennan, J.A. Danao, P. Bajwa, L.H. Parsons, L.H. Tecott, Elevated anxiety and antidepressant-like responses in serotonin 5-HT_{1A} receptor mutant mice, Proc. Natl. Acad. Sci. USA 95 (1998) 15049–15054.
[9] C.L. Parks, P.S. Robinson, E. Sibille, T. Shenk, M. Toth, Increased anxiety of mice lacking the serotonin$_{1A}$ receptor, Proc. Natl. Acad. Sci. USA 95 (1998) 10734–10739.
[10] D. Hoyer, J.P. Hannon, G.R. Martin, Molecular, pharmacological and functional diversity of 5-HT receptors, Pharmacol. Biochem. Behav. 71 (2002) 533–554.
[11] K.L. Pierce, R.T. Premont, R.J. Lefkowitz, Seven transmembrane receptors, Nat. Rev. Mol. Cell Biol. 3 (2002) 639–650.
[12] D.E. Clapham, The G-protein nanomachine, Nature 379 (1996) 297–299.
[13] T.J. Pucadyil, S. Kalipatnapu, A. Chattopadhyay, The serotonin$_{1A}$ receptor: a representative member of the serotonin receptor family, Cell. Mol. Neurobiol. (2004) in press.
[14] L.M. Hjelmeland, A. Chrambach, Solubilization of functional membrane proteins, Methods Enzymol. 104 (1984) 305–318.
[15] A.M. Seddon, P. Curnow, P.J. Booth, Membrane proteins, lipids and detergents: not just a soap opera, Biochim. Biophys. Acta 1666 (2004) 105–117.
[16] A. Chattopadhyay, K.G. Harikumar, Dependence of critical micelle concentration of a zwitterionic detergent on ionic strength: implications in receptor solubilization, FEBS Lett. 391 (1996) 199–202.
[17] A. Chattopadhyay, K.G. Harikumar, S. Kalipatnapu, Solubilization of high affinity G-protein-coupled serotonin$_{1A}$ receptors from bovine hippocampus using pre-micellar CHAPS at low concentration, Mol. Membr. Biol. 19 (2002) 211–220.
[18] A. Chattopadhyay, Md. Jafurulla, S. Kalipatnapu, Solubilization of serotonin$_{1A}$ receptors heterologously expressed in chinese hamster ovary cells, Cell. Mol. Neurobiol. 24 (2004) 293–300.
[19] A.G. Lee, Lipid–protein interaction in biological membranes: a structural perspective, Biochim. Biophys. Acta 1612 (2003) 1–40.
[20] O.T. Jones, J.H. Eubanks, J.P. Earnest, M.G. McNamee, A minimum number of lipids are required to support the functional properties of the nicotinic acetylcholine receptor, Biochemistry 27 (1988) 3733–3742.
[21] J. Kirilovsky, M. Schramm, Delipidation of a β-adrenergic receptor preparation and reconstitution by specific lipids, J. Biol. Chem. 258 (1983) 6841–6849.
[22] P. Banerjee, J.T. Buse, G. Dawson, Asymmetric extraction of membrane lipids by CHAPS, Biochim. Biophys. Acta 1044 (1990) 305–314.
[23] P. Banerjee, J.B. Joo, J.T. Buse, G. Dawson, Differential solubilization of lipids along with membrane proteins by different classes of detergents, Chem. Phys. Lipids 77 (1995) 65–78.
[24] T.J. Pucadyil, A. Chattopadhyay, Cholesterol modulates ligand binding and G-protein coupling to serotonin$_{1A}$ receptors from bovine hippocampus, Biochim. Biophys. Acta 1663 (2004) 188–200.
[25] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1985) 76–85.

[26] K.G. Harikumar, A. Chattopadhyay, Metal ion and guanine nucleotide modulations of agonist interaction in G-protein-coupled serotonin$_{1A}$ receptors from bovine hippocampus, Cell. Mol. Neurobiol. 18 (1998) 535–553.
[27] D. Ofri, A.M. Ritter, Y.F. Liu, T.L. Gioannini, J.M. Hiller, E.J. Simon, Characterization of solubilized opioid receptors: reconstitution and uncoupling of guanine nucleotide-sensitive agonist binding, J. Neurochem. 58 (1992) 628–635.
[28] D.M. Amundson, M. Zhou, Fluorometric method for the enzymatic determination of cholesterol, J. Biochem. Biophys. Methods 38 (1999) 43–52.
[29] R.F. Bruns, K. Lawson-Wendling, T.A. Pugsley, A rapid filtration assay for soluble receptors using polyethylenimine-treated filters, Anal. Biochem. 132 (1983) 74–81.
[30] K.G. Harikumar, A. Chattopadhyay, Differential discrimination of G-protein coupling of serotonin$_{1A}$ receptors from bovine hippocampus by an agonist and an antagonist, FEBS Lett. 457 (1999) 389–392.
[31] T. Higashijima, K.M. Ferguson, P.C. Sternweis, M.D. Smigel, A.G. Gilman, Effects of Mg^{2+} and the βγ-subunit complex on the interactions of guanine nucleotides with G proteins, J. Biol. Chem. 262 (1987) 762–766.
[32] C.W.F. McClare, An accurate and convenient organic phosphorus assay, Anal. Biochem. 39 (1971) 527–530.
[33] M.B. Emerit, S. el Mestikawy, H. Gozlan, B. Rouot, M. Hamon, Physical evidence of the coupling of solubilized 5-HT1A binding sites with G regulatory proteins, Biochem. Pharmacol. 39 (1990) 7–18.
[34] D.A. Brown, J.K. Rose, Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface, Cell 68 (1992) 533–544.
[35] L.H. Chamberlain, Detergents as tools for the purification and classification of lipid rafts, FEBS Lett. 559 (2004) 1–5.
[36] S. Kalipatnapu, A. Chattopadhyay, A GFP fluorescence-based approach to determine detergent insolubility of the human serotonin$_{1A}$ receptor, FEBS Lett. 576 (2004) 455–460.