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The Serotonin$_{1A}$ Receptor: A Representative Member of the Serotonin Receptor Family

Thomas J. Pucadyil,[1] Shanti Kalipatnapu,[1] and Amitabha Chattopadhyay[1,2]

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SUMMARY

1. Serotonin is an intrinsically fluorescent biogenic amine that acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous system. Serotonergic signaling appears to play a key role in the generation and modulation of various cognitive and behavioral functions.

2. Serotonin exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups. The serotonin$_{1A}$ (5-HT$_{1A}$) receptor is the most extensively studied of the serotonin receptors and belongs to the large family of seven transmembrane domain G-protein coupled receptors.

3. The tissue and sub-cellular distribution, structural characteristics, signaling of the serotonin$_{1A}$ receptor and its interaction with G-proteins are discussed.

4. The pharmacology of serotonin$_{1A}$ receptors is reviewed in terms of binding of agonists and antagonists and sensitivity of their binding to guanine nucleotides.

5. Membrane biology of 5-HT$_{1A}$ receptors is presented using the bovine hippocampal serotonin$_{1A}$ receptor as a model system. The ligand binding activity and G-protein coupling of the receptor is modulated by membrane cholesterol thereby indicating the requirement of cholesterol in maintaining the receptor organization and function. This, along with the reported detergent resistance characteristics of the receptor, raises important questions on the role of membrane lipids and domains in the function of this receptor.

KEY WORDS: serotonin; serotonin$_{1A}$ (5-HT$_{1A}$) receptor; bovine hippocampus; fluorescence; 8-OH-DPAT; cholesterol.

THE DISCOVERY OF SEROTONIN

The discovery of serotonin (5-hydroxytryptamine or 5-HT) was rather serendipitous (for a comprehensive account of the discovery of serotonin, see Whitaker-Azmitia, 1999). It was known to physiologists since the middle of the nineteenth century that after blood clots, the serum possesses a substance which constricts vascular smooth muscle so as to increase vascular tone. This vasoconstrictor substance was often referred to as "vasotonin." Around the turn of the twentieth century, platelets

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

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were identified as the source of this substance. Later on in the late 1940's, Page and coworkers isolated and characterized this "tonic" substance in "serum" and named it "serotonin" (Rapport *et al.*, 1948). The chemical structure of serotonin was found to be 5-hydroxytryptamine (5-HT). Independent of these developments, Italian scientists were studying a substance found in high concentrations in enterochromaffin cells of the intestinal mucosa which was also eventually found to be serotonin (Erspamer and Asero, 1952). The identification of serotonin in the central nervous system was made by Twarog and Page (1953) and is considered to be one of the most important discoveries in neuroscience (Whitaker-Azmitia, 1999).

SEROTONIN: AN INTRINSICALLY FLUORESCENT NEUROTRANSMITTER

Serotonin is a biogenic amine present in a variety of organisms ranging from worms to humans (Hen, 1992). It acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous system (Jacobs and Azmitia, 1992). Serotonin is a derivative of the naturally occuring amino acid tryptophan which is intrinsically fluorescent (Eftink, 1991). It is interesting to note that the intrinsic fluorescence of serotonin was detected and reported even when its definite physiological function was not known (Bowman *et al.*, 1955; Udenfriend *et al.*, 1955). Further, it was observed that serotonin fluorescence was dependent on its ionization state (Chen, 1968). The intrinsic fluorescence properties of serotonin and its modulation by ionization and polarity of the environment were comprehensively characterized a few years back (Tan *et al.*, 1995; Chattopadhyay *et al.*, 1996). Very recently, it has been possible to visualize serotonin distribution in living cells using three-photon nonlinear excitation microscopy (Maiti *et al.*, 1997) and this has opened up new opportunities in the area of serotonin biology.

SEROTONIN RECEPTORS: THE SEROTONIN$_{1A}$ (5-HT$_{1A}$) RECEPTOR AS A KEY COMPONENT OF SEROTONERGIC SIGNALING

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 (Griebel, 1995; Artigas *et al.*, 1996; Graeff *et al.*, 1996; Ramboz *et al.*, 1998; Rocha *et al.*, 1998; Meneses, 1999). Novel roles for serotonin in heart disease (Nebigil and Maroteaux, 2001), asthma (Barnes *et al.*, 1998) and phagocytosis (Freire-Garabal *et al.*, 2003) have been recently reported. In addition, the serotonin pathway plays a crucial role in brain development processes such as neurogenesis and axonal branching during various stages of development (del Olmo *et al.*, 1998; Gross *et al.*, 2002; Gaspar *et al.*, 2003). 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 (Rocha *et al.*, 1998; Ramboz *et al.*, 1998; Heisler *et al.*, 1998; Parks *et al.*, 1998; Murphy *et al.*, 1999; Parsons *et al.*, 2001).

Serotonin exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups 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 (Barnes and Sharp, 1999; Hoyer *et al.*, 2002). Based on an analysis of the extent of amino acid homology between various species, it has been estimated that the primordial serotonin receptor must have evolved more than 800 million years ago (Peroutka and Howell, 1994). The development of pharmacological ligands with enhanced specificity along with the molecular cloning of several of these receptors and subsequent heterologous expression have unambiguously confirmed the existence of at least 14 subtypes of serotonin receptors (Hoyer *et al.*, 2002). Most of the serotonin receptors, except the 5-HT$_3$ receptor, belong to the large family of seven transmembrane domain G-protein coupled receptors (Pierce *et al.*, 2002), that couple to and transduce signals via guanine nucleotide binding regulatory proteins (G-proteins) (Clapham, 1996; Helmreich and Hofmann, 1996). The serotonin$_{1A}$ (5-HT$_{1A}$) receptor is an important member of this large family of receptors and is estimated to have differentiated ~650 million years ago from the 5-HT$_1$ receptor subfamily in the time period during which vertebrates diverged from invertebrates (Peroutka and Howell, 1994). The 5-HT$_{1A}$ receptor is the most extensively studied of the serotonin receptors for a number of reasons (Chattopadhyay *et al.*, 2002). One of the main reasons is the availability of a selective ligand 8-OH-DPAT (8-hydroxy-2-(di-*N*-propylamino)tetralin) that allows extensive biochemical, physiological, and pharmacological characterization of the receptor (Arvidsson *et al.*, 1981; Gozlan *et al.*, 1983). The 5-HT$_{1A}$ receptor was the first among all the serotonin receptors to be cloned and sequenced (Kobilka *et al.*, 1987; Fargin *et al.*, 1988; Albert *et al.*, 1990). The human, rat and mouse 5-HT$_{1A}$ receptors have been cloned, and their amino acid sequences deduced (Fargin *et al.*, 1988; Albert *et al.*, 1990; Charest *et al.*, 1993). The cloning of the 5-HT$_{1A}$ receptor gene has shown that it belongs to the superfamily of G-protein-coupled receptors, with 50% amino acid homology with the β_2-adrenergic receptor in the transmembrane domain (Kobilka *et al.*, 1987). More importantly, the receptor has been stably expressed in a number of neural and non-neural cell lines (Banerjee *et al.*, 1993; Chattopadhyay *et al.*, 2004). Furthermore, it was the first serotonin receptor for which polyclonal antibodies were obtained (Fargin *et al.*, 1988; El Mestikawy *et al.*, 1990; Azmitia *et al.*, 1996; Kia *et al.*, 1996) allowing their visualization at the subcellular level in various regions of the brain.

The 5-HT$_{1A}$ receptor has recently been shown to have a role 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). Treatment using agonists for the 5-HT$_{1A}$ receptor constitutes a potentially useful approach in case of children with developmental disorders (Azmitia, 2001). The 5-HT$_{1A}$ receptor agonists (Blier *et al.*, 1990) and antagonists (Griebel, 1999) represent a major class of molecules with potential therapeutic effects in anxiety- or stress-related disorders. As a result, the 5-HT$_{1A}$ receptor serves as an important target in the development

of therapeutic agents to treat neuropsychiatric disorders such as anxiety and depression. On the clinical front, 5-HT$_{1A}$ receptor levels have been shown to be altered in schizophrenia (Sumiyoshi *et al.*, 1996), and in patients suffering from major depression (Fajardo *et al.*, 2003). Interestingly, a recent observation has associated genetic polymorphisms at the upstream repressor region of the 5-HT$_{1A}$ receptor gene to major depression and suicide in humans (Lemonde *et al.*, 2003) linking its expression status to these clinical syndromes. A recent report describes the attenuation of 5-HT$_{1A}$ receptor antagonist binding and signaling in brains of suicide victims (Hsiung *et al.*, 2003). Besides, the 5-HT$_{1A}$ receptors are implicated in regulation of blood pressure, feeding, temperature regulation (Dourish *et al.*, 1987), and regulation of working memory (Ohno and Watanabe, 1996). The 5-HT$_{1A}$ receptor gene has also been implicated in Tourette's syndrome, a common hereditary motor and vocal tic disorder (Lam *et al.*, 1996) and in Huntington's disease (Yohrling *et al.*, 2002). Interestingly, mutant (knockout) mice lacking the 5-HT$_{1A}$ receptor generated a few years back exhibit enhanced anxiety-related behavior (Ramboz *et al.*, 1998; Heisler *et al.*, 1998; Parks *et al.*, 1998). These represent important examples of how a single gene mutation can alter complex behavior and provide important animal models of genetic vulnerability to conditions such as anxiety disorders and aggression (Gingrich and Hen, 2001). This review is focused on the organization and signaling of the 5-HT$_{1A}$ receptor with a special emphasis on the role of the membrane lipid environment in functioning of the receptor.

TISSUE AND SUB-CELLULAR DISTRIBUTION OF SEROTONIN$_{1A}$ RECEPTORS

The presence of mRNA coding for the 5-HT$_{1A}$ receptor has been detected in the lymphatic tissue, gut, muscle, kidney and most notably in several regions of the brain (Kobilka *et al.*, 1987; Albert *et al.*, 1990; Chalmers and Watson, 1991). The distribution of 5-HT$_{1A}$ receptors in the central nervous system assumes significance in light of the functions associated with the receptor. Tissue distribution of receptors in the brain has been analyzed using (i) radiolabeled ligands that specifically bind to 5-HT$_{1A}$ receptors, and (ii) antibodies raised against peptides corresponding to regions of the receptor that are unique in sequence. Autoradiography of brain slices incubated with the 5-HT$_{1A}$ specific agonist [^{3}H]8-OH-DPAT and antagonist [^{123}I]*p*-MPPI reveals highest label density in the limbic forebrain regions such as the hippocampus, raphe nuclei, amygdala, hypothalamus, and cortex, whereas the lowest density is found in the extrapyrimidal areas such as the basal ganglia, substantia nigra and in the adult cerebellum (Palacios *et al.*, 1990; Kung *et al.*, 1994a). This distribution mirrors the mRNA densities for the receptor in these regions (Chalmers and Watson, 1991; Pompeiano *et al.*, 1992; Burnet *et al.*, 1995). These regions of the central nervous system, where the labeling density is high, are known to regulate various physiological functions such as thermoregulation, cardiovascular function, aggressive and sexual behavior, mood, appetite, and sleep–wake cycle. The localization of 5-HT$_{1A}$ receptors in these regions correlates well with symptoms that appear upon disruption of serotonergic signaling in these regions of the central nervous system

(Ramboz *et al.*, 1998; Heisler *et al.*, 1998; Parks *et al.*, 1998; Sarnyai *et al.*, 2000). In addition, regional distribution analyses of the 5-HT$_{1A}$ receptor using sequence specific antibodies have revealed similar patterns as that seen with radiolabeled ligands. Autoradiograms obtained from brain sections exposed to 5-HT$_{1A}$ receptor specific antibodies and labeled with [^{35}S]-labeled secondary antibodies show an overall similar distribution as that found using [^{3}H]8-OH-DPAT (El Mestikawy *et al.*, 1990).

At the sub-cellular level, the receptor has been found to be localized to the somatodendritic region of neurons isolated from the raphe nuclei which acts as an autoreceptor and inhibits cell firing. The hippocampus, on the other hand, has receptors primarily located in the postsynaptic regions which act as heteroreceptors (Palacios *et al.*, 1990; Ghavami *et al.*, 1999; Riad *et al.*, 2000). A cell type specific protein sorting mechanism would explain this difference in localization of the receptor in different regions of the brain. Efforts to elucidate such a mechanism have relied on expression of the receptor in polarized epithelial cells. On account of the postulated similarities in protein sorting mechanisms between epithelial cells and neurons (Dotti and Simons, 1990; Ikonen and Simons, 1998), the basolateral and apical membranes of epithelial cells are proposed to correspond to the somatodendritic and axonal regions in neurons, respectively. The 5-HT$_{1A}$ receptors expressed in Eli Lilly and company canine kidney (LLC-PK1) cells display a predominantly basolateral membrane localization, analogous to the somatodendritic localization found in certain parts of the central nervous system (Langlois *et al.*, 1996). Expression in Madin-Darby canine kidney (MDCK-II) epithelial cells, however, shows no specific targeting to the basolateral membrane (Ghavami *et al.*, 1999). The apparent differences in localization of 5-HT$_{1A}$ receptors in these cells indicate that a specific somatodendritic/basolateral membrane-sorting motif may not be the sole determinant in targeting these receptors to appropriate sub-cellular regions.

Interestingly, the observed heterogeneity in the distribution of 5-HT$_{1A}$ receptors has been associated with diversity in pharmacology and signal transduction characteristics of the receptor. For example, 5-HT$_{1A}$ receptors in the raphe nuclei, where its localization is somatodendritic, are not linked to the inhibition of adenylyl cyclase as they are in the hippocampus, where the localization is postsynaptic (Yocca and Maayani, 1990). In light of differences in the lipid composition of membranes in the somatodendritic/basolateral vs. the axonal/apical regions (Simons and van Meer, 1988; Ikonen and Simons, 1998; Holthuis *et al.*, 2001), it is possible that the diversity in signal transduction characteristics of receptors localized in these domains is on account of the differences in the lipid composition. Thus 5-HT$_{1A}$ receptors expressed in these tissues represent an interesting natural system for exploring lipid–protein interactions as a function of localization of this receptor.

MOLECULAR AND STRUCTURAL CHARACTERISTICS OF SEROTONIN$_{1A}$ RECEPTORS

The 5-HT$_{1A}$ receptor belongs to the G-protein coupled receptor (GPCR) superfamily which 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 (Hall *et al.*, 1999; Shanti and Chattopadhyay, 2000; Pierce *et al.*, 2002). These receptors can be activated by ligands as chemically diverse as biogenic amines, peptides, glycoproteins, lipids, nucleotides and even photons, thus mediating diverse physiological processes such as neurotransmission, cellular metabolism, secretion, cellular differentiation and growth, and inflammatory and immune responses. GPCRs therefore represent major targets for the development of novel drug candidates in all clinical areas (Bikker *et al.*, 1998). It is estimated that 50% of clinically prescribed drugs act as either agonists or antagonists at GPCRs which points out their immense therapeutic potential (Shanti and Chattopadhyay, 2000; Karnik *et al.* 2003).

The 5-HT_{1A} receptor is expressed very early in development (del Olmo *et al.*, 1998) and is one of the first G-protein coupled receptors (GPCR) for which the gene was cloned (Kobilka *et al.*, 1987; Fargin *et al.*, 1988; Albert *et al.*, 1990; Fujiwara *et al.*, 1990; Stam *et al.*, 1992; Chanda *et al.*, 1993). The gene is intronless and its mRNA is expressed mainly in the brain, spleen, neonatal kidney and gut (Kobilka *et al.*, 1987; Fargin *et al.*, 1988; Albert *et al.*, 1990; Kirchgessner *et al.*, 1993; Raymond *et al.*, 1993a). The receptor gene is located on chromosome 5 in humans (Kobilka *et al.*, 1987) and chromosome 13 in mice (Oakey *et al.*, 1991). The human gene encodes a predicted protein of 422 amino acids. Although no high resolution structure is available for the 5-HT_{1A} receptor, hydropathy plots of the amino acid sequences predict the presence of seven putative transmembrane domains, each of ∼25 residues in length, which are thought to represent membrane-spanning α-helices (Raymond *et al.*, 1999). Based on the presence of three consensus *N*-linked glycosylation residues in the amino terminus and on its homology to the β_2-adrenoceptor, the receptor is predicted to have a topology where the amino terminus is oriented facing the extracellular space. According to this topology, the hydrophilic sequences connecting the transmembrane helices would form three intracellular (IC) and extracellular (EC) loops in the protein with respect to the plasma membrane. This topology, with the amino terminus facing the extracellular space and the carboxy terminus facing the cytoplasmic space, places the 5-HT_{1A} receptor in family A (see Fig. 1) of GPCRs (Gether, 2000).

The related GPCRs of this family include receptors that (i) bind to other biogenic amines such as adrenaline, dopamine, acetylcholine, and histamine, (ii) bind peptides such as cholecystokinin, neurotensin, and bombesin, and (iii) belong to the opsin family such as rhodopsin. The overall homology among these receptors is low and restricted to a few key residues. The high degree of conservation of these residues suggests that they have an essential role in maintaining either structure or function of these receptors. The only residue that is conserved among these proteins is the Arg in the Asp–Arg–Tyr (DRY) motif (see Fig. 1) at the initial segment of the second intracellular loop (IC2) (Probst *et al.*, 1992). This motif is intimately involved in activating G-proteins but not binding to them. The specificity of interaction with G-proteins, for which the possible combinations are rather large, is thought to arise from other residues present in the second and third intracellular loops (IC2 and IC3) (Bourne, 1997) (see Fig. 2). However, the presence of these residues might not be the sole determinant in the specificity of interactions with G-proteins. Other factors such as relative availability of activating ligands, receptors and G-proteins, in addition to

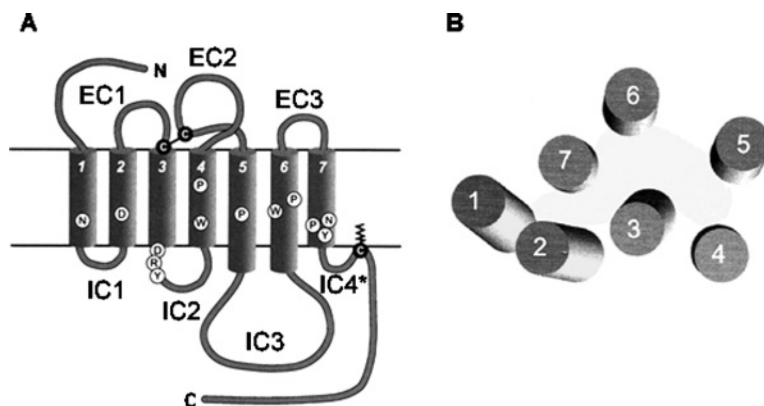

Fig. 1. A schematic representation of the family A class of G-protein coupled receptors. These receptors are characterized by a series of highly conserved key residues (black letter in white circles) shown in Panel A. In most family A receptors, a disulfide bridge connects the first (EC1) and second extracellular loop (EC2). In addition, a majority of the receptors have one or two palmitoylated cysteine in the carboxy-terminal tail (also see Fig. 2) causing the formation of a putative fourth intracellular loop which is referred to as IC4*. The conserved DRY motif at the bottom of the third transmembrane domain (TM 3) serves to activate G-proteins. Panel B shows the arrangement of the transmembrane helices in the membrane of a model family A type of G-protein coupled receptor, rhodopsin, as seen from the extracellular side with each helix represented by a cylinder. The orientation and position of the helices are according to the projection maps of frog rhodopsin as decribed in Unger *et al.* (1997). The helices are organized sequentially in a counter-clockwise fashion with helix 3 being almost in the center of the molecule. Adapted and modified from Gether (2000).

the specific subtype of G-proteins that are expressed in a particular cell type, also determine the specificity of interaction or lack of it (Horn *et al.*, 2000).

Receptors that bind to biogenic amines (such as the 5-HT$_{1A}$ receptor) are known to possess binding sites at the transmembrane helix-lined pocket within the membrane (see Fig. 2) (Bourne, 1997). Ligands that act as agonists, bind to this pocket and induce a conformational change in the transmembrane helices. This change acts as a switch to activate G-proteins bound to the second and third intracellular loops (IC2 and IC3) of the receptor. Other structural features include a putative fourth cytoplasmic loop (referred to as IC4*, see Fig. 2) that is formed on account of post-translational palmitoylation at the two conserved cysteine residues at the carboxy terminus of the receptor. Interestingly, a recent report highlights the importance of this post-translational modification, on the ability of the mouse 5-HT$_{1A}$ receptor to couple to and transduce signals via G-proteins (Papoucheva *et al.*, 2004). This study reports that the 5-HT$_{1A}$ is stably palmitoylated at Cys^{417} and Cys^{420} and palmitoylation deficient mutants, i.e., those that lack the two conserved cysteine residues, are unable to interact with G-proteins and transduce signals upon binding to activating ligands. The Cys^{187} in the putative second extracellular domain is thought to form a disulfide bond with Cys^{109} (see Fig. 2). In comparison with the β_2-adrenoceptor (Dohlman *et al.*, 1990; Fraser, 1989), this disulfide bond may stabilize receptor conformation and possibly explain the action of disulfide reducing and modifying

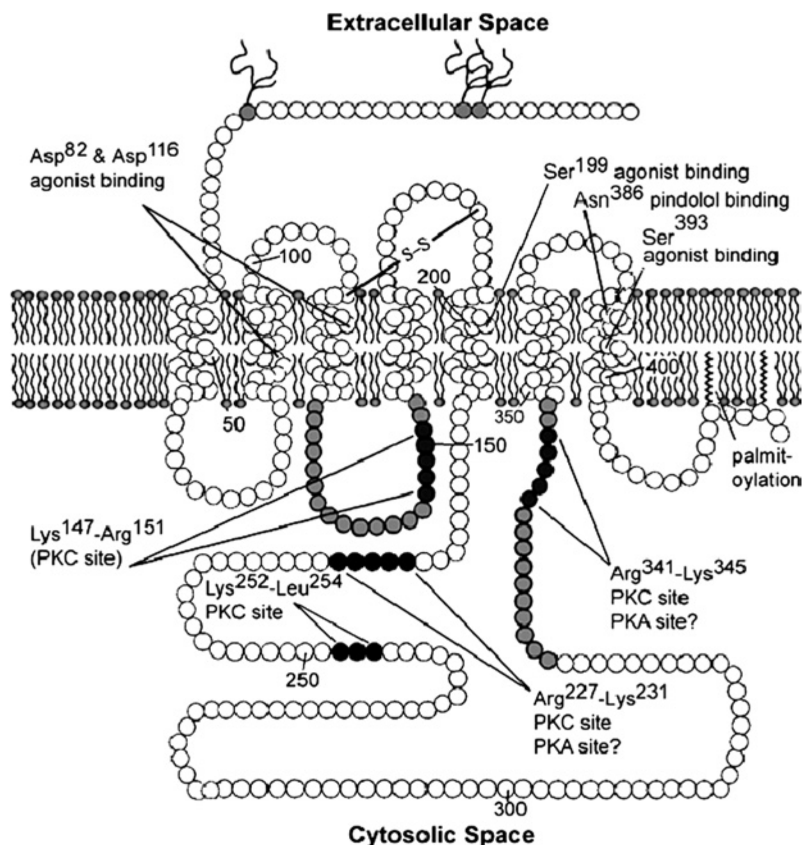

Fig. 2. A schematic representation of the membrane-embedded human 5-HT$_{1A}$ receptor showing its topological and other structural features. The membrane is shown as a bilayer of two leaflets of lipids. The amino acids in the receptor sequence are shown as circles and are marked after every 50 residues for convenience. Seven transmembrane stretches, each composed of 20–26 amino acids, are depicted as α-helices. There are three potential sites of *N*-linked glycosylation on the amino terminus (depicted as branching trees). A disulfide bond putatively exists between Cys^{109} and Cys^{187}. Transmembrane (TM) domains contain residues (which are marked) that are important for ligand binding (see text for further details). Putative palmitoylation sites are Cys^{417} and/or Cys^{420}. Light gray circles represent contact sites for G-proteins. Black circles represent sites for protein kinase mediated phosphorylation. Adapted and modified from Raymond *et al.* (1999).

agents that inhibit ligand binding to the 5-HT$_{1A}$ receptor (Emerit *et al.*, 1991; Harikumar *et al.*, 2000).

RECEPTOR ACTIVATION AND CORRELATION TO SIGNAL TRANSDUCTION

Although the 5-HT$_{1A}$ receptor is among the first GPCRs to be cloned, there are relatively few detailed structure–function analyses in relation to ligand binding and

activation of this receptor. Several insights have however been obtained from site-directed mutagenesis approach. A distinct pharmacological characteristic feature of the 5-HT$_{1A}$ receptor is its affinity for the class of drugs called β-blockers. While they normally are high affinity ligands for the β-adrenergic receptors, drugs such as pindolol also bind to and antagonize 5-HT$_{1A}$ receptors with moderate affinity (apparent dissociation constant $K_i = \sim 19$ nM) (Zifa and Fillion, 1992; Griebel, 1995). Interestingly, a single residue that is conserved between 5-HT$_{1A}$ and β-adrenergic receptors, Asn^{386} (see Fig. 2), has been identified to be involved in recognizing pindolol as a ligand. (Guan *et al.*, 1992). When this residue is replaced with Val, 5-HT$_{1A}$ receptors displayed a much lower affinity ($\sim$100 fold reduction) for pindolol without any effect on the affinity for other ligands. Various point mutations made in the transmembrane domains of the receptor have identified residues required for the binding of the natural ligand 5-HT to the receptor. Three substitutions, Asp^{82} to Asn, Asp^{116} to Asn and Ser^{199} to Ala (see Fig. 2), resulted in a 60–100 fold decreased affinity for 5-HT whereas the binding characteristics of the antagonist pindolol remained unaffected (Ho *et al.*, 1992). Interestingly, these mutations did not alter the ability of the receptor to induce GTP catalysis upon excess serotonin addition indicating that the mutants were competent in terms of their interaction with G-proteins. Hence, these residues appear to be essential for binding to 5-HT and possibly other agonists, but not antagonists such as pindolol. In comparison with β-adrenoceptors, the carboxylate group on Asp^{82} and/or Asp^{116} probably acts as a counter ion to the amine group of 5-HT thus stabilizing its interaction (Strader *et al.*, 1989). While the Asp^{82} is conserved in all cloned GPCRs, Asp^{116} is found only in those that bind to biogenic amines such as dopamine, acetylcholine and serotonin. Other residues that are required for interaction with the agonist also appear to be located within the transmembrane domains. Site-directed mutagenesis studies have indicated the critical role of Asn^{396} and Ser^{391} in the transmembrane domains (TM7) for binding the widely used specific agonist 8-OH-DPAT to the receptor. Substitutions of the Asn^{396} with Ala, Val or Phe reduced the binding of 8-OH-DPAT (Chanda *et al.*, 1993).

G-protein coupled receptors are known to activate G-proteins upon binding to agonists. The ternary complex model and its extensions best describe this phenomenon (De Lean *et al.*, 1980; Kent *et al.*, 1980; Samama *et al.*, 1993; Christopoulos and Kenakin, 2002). This model proposes that at equilibrium, the receptor can independently exist in two conformations. One is the ground state (R) and the other is the partially activated state (R*). It is only the R* state that is able to couple to G-proteins. The agonist has preferential affinity for and stabilizes the R* state and subsequently promotes G-protein coupling to the receptor to form the ternary complex, i.e., the agonist-activated receptor-G-protein (AR*G) complex. Ligands that stabilize the resting state (R) and therefore decrease the number of spontaneously active receptors (R*), act as inverse agonists, whereas antagonists display similar affinities for both R and R*. As a consequence, these models predict that the affinity and potency of ligands acting on receptors is dependent on the basal R/R* ratio. In the ternary complex which represents the activated state of the receptor, GDP bound to the G-protein is exchanged for GTP, releasing the α and $\beta\gamma$ subunits of the G protein that can then alter the activity of effector molecules such as adenylyl cyclase.

As mentioned earlier, the DRY motif present in the second intracellular loop (IC2) (see Fig. 1) is considered to be the switch that activates G-proteins upon agonist binding to the receptor. Other regions in the intracellular loops that possess contact sites for G-proteins with 5-HT$_{1A}$ receptors have been identified by using synthetic peptides corresponding to limited stretches of these loops. These peptides have been used to independently assess their ability to modulate the function of G-proteins, in this case the G$\alpha_{i/o}$ class, which are known to preferentially interact with the 5-HT$_{1A}$ receptor over other classes of G-proteins (see later). Peptides corresponding to the entire second intracellular loop (IC2 from Asp^{133}–Arg^{153}) and residues from the third intracellular loop (IC3 from Ala^{331}–Leu^{347}) of the 5-HT$_{1A}$ receptor could stimulate binding of a non-hydrolyzable radiolabeled analogue of GTP, [^{35}S]GTP-γ-S, to purified G-proteins. In addition, they inhibit forskolin stimulated adenylyl cyclase activity, the natural downstream consequence of activated 5-HT$_{1A}$ receptors (Varrault *et al.*, 1994). The Thr residues in these peptides are considered to play a crucial role in acting as potential contact sites of the receptor with G-proteins, particularly with the $\beta\gamma$ subunit (Lembo and Albert, 1995; Lembo *et al.*, 1997; Albert *et al.*, 1998). These studies also support a model where stretches of the second and third intracellular loops (IC2 and IC3) form amphipathic α-helices that promote binding of G-proteins to the receptor. Phosphorylation of these Thr residues by protein kinase C (see Fig. 2) is thought to desensitize the receptor by reducing the extent of G-protein coupling upon subsequent addition of the agonist. This reduces the intracellular response generated from the receptor upon persistent activation with agonists.

SPECIFICITY OF G-PROTEIN INTERACTION

The presence of three G-protein subunits, α, β, and γ, and with each of these subunits having multiple isoforms, generates an astounding number of possible receptor/G-protein combinations. However, there appears to be some structural determinant in the receptor that restricts the promiscuity of interactions and confer specificity to downstream signal transduction. It would understandably require a great deal of effort to comprehensively elucidate the cognate G-protein that interacts with 5-HT$_{1A}$ receptors. In addition, other complications such as different cell types possessing various isoforms and stoichiometries of G-protein subunits make this task more daunting. In light of these facts, it is remarkable that a good amount of literature already exists describing the molecular specificity of the 5-HT$_{1A}$ receptor and G-protein interaction. Co-expression of the 5-HT$_{1A}$ receptor along with mammalian G-protein subunits in *Spodoptera frugiperda* (Sf9) insect cells, which have low levels of endogenous G-proteins themselves, indicates that the receptor interacts with Gα subunits of different kinds namely, α_{i1}, α_{i2}, α_{i3}, α_o and α_z. (Mulheron *et al.*, 1994; Butkerait *et al.*, 1995; Barr *et al.*, 1997). There is however no detectable interaction with α_{12}, α_{13}, α_s, and α_q in this system. Receptors expressed in *E. coli* appear to interact with higher affinity to α_{i3} than to α_{i1} or α_{i2} (Bertin *et al.*, 1992). Thus the rank order of interaction preferences of the 5-HT$_{1A}$ receptor with various Gα subunits is found to be $\alpha_{i3} > \alpha_{i2} > \alpha_{i1} >> \alpha_o >> \alpha_s$. Experiments carried out with receptors expressed in mammalian cells reveal a rank order of interaction preferences of

$\alpha_{i3} > \alpha_{i2} \sim \alpha_{i1} \sim \alpha_o > \alpha_z >> \alpha_s$ (Raymond *et al.*, 1993b; Garnovskaya *et al.*, 1997). While these studies point out an apparent discrepancy in the ability of 5-HT$_{1A}$ receptors to distinguish between isoforms of the $\alpha_{i/o/z}$ subunits, they clearly indicate that the receptor does not interact with α_s subunits. Studies in mammalian cells using methods of selective inactivation of G-protein α subunits, either using antibodies against specific Gα subunits to neutralize their function, or using the antisense approach to inactivate Gα subunits have revealed similar results although the relative affinity of the receptor for α_{i3} and α_{i2} is not yet resolved (Fargin *et al.*, 1991; Gettys *et al.*, 1994; Liu *et al.*, 1994). These studies scored the ability of 5-HT$_{1A}$ receptors to interact with Gα subunits based on the efficacy of the subsequent downstream signal transduction processes such as agonist-stimulated inhibition of adenylyl cyclase. Interestingly, it has been reported that the inhibition of adenylyl cyclase activity occurs through its interaction with Gα_{i3} subunits on the apical surface and with Gα_{i2} subunits on the basolateral surface in polarized LLC-PK1 cells (Langlois *et al.*, 1996). In summary, the coupling of 5-HT$_{1A}$ receptors to the Gα_i class of proteins is fairly well established in natural as well as heterologous expression systems. However, it must be mentioned that this hierarchy of interaction of the receptor to different Gα subunits may not always be so well defined and may depend on the type of tissue where such interactions are studied. This is apparent from the recent observation that while several *in vivo* studies suggest that physiological effects of 5-HT$_{1A}$ receptor activation are mediated by G$\alpha_{i/o}$ proteins in the hippocampus and dorsal raphe (Clarke *et al.*, 1987; Innis and Aghajanian, 1987; Blier *et al.*, 1993; Romero *et al.*, 1994), these receptors are found to couple to Gα_z proteins in the hypothalamus (Serres *et al.*, 2000). It is even more surprising since this study shows that the levels of expression of Gα_{i1}, Gα_{i2} and Gα_z are nearly similar in the hypothalamus.

The primary function of 5-HT$_{1A}$ receptors is to inhibit adenylyl cyclase thereby reducing the levels of cyclic AMP (cAMP) in cells. 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 adenylyl cyclase. This function of 5-HT$_{1A}$ receptors has been documented in several systems, both native (the hippocampus, striatum and cortex (De Vivo and Maayani, 1986; Weiss *et al.*, 1986; Bockaert *et al.*, 1987; Oksenberg and Peroutka, 1988; Schoeffter and Hoyer, 1988; Dumuis *et al.*, 1988)), and non-native systems (HN2, P11, NCB-20, F11, Chinese hamster ovary (CHO), HeLa, human embryonic kidney (HEK 293), NIH 3T3, COS-7, GH$_4$C$_1$ from pituitary glands, and LLC-PK1 cells (reviewed in Raymond *et al.*, 2001)). The inhibition in adenylyl cyclase is pertussis toxin sensitive again indicating that the receptor interacts with the G$\alpha_{i/o}$ class of proteins. In fact, the C terminus of the Gα_i family of G-proteins is proposed to be important for 5-HT$_{1A}$ receptor–G-protein interaction (Kowal *et al.*, 2002).

The list of downstream signal transduction pathways emanating from activated G-proteins is quite extensive for any system. This is also true when G-proteins are activated by 5-HT$_{1A}$ receptors. Apart from the primary signal transduction pathway where Gα subunits mediate inhibition of adenylyl cyclase, activated $\beta\gamma$ subunits initiate additional signal transduction pathways such as activation of phospholipase C and protein kinase C, and modulation of inwardly rectified K^+ channels, high conductance anion channels, CFTR Cl^- channels, and Ca^{2+} channels (reviewed in

Raymond *et al.*, 1999, 2001). Importantly, unlike the primary downstream event of adenylyl cyclase inhibition, the efficacy of these additional signal transduction processes appears to be highly cell type dependent and therefore limits their use to study receptor activation (Raymond *et al.*, 2001).

PHARMACOLOGY OF THE SEROTONIN$_{1A}$ RECEPTOR

As mentioned earlier, the availability of the selective ligand 8-OH-DPAT makes the 5-HT$_{1A}$ receptor the most studied receptor amongst the serotonin receptors (Arvidsson *et al.*, 1981; Gozlan *et al.*, 1983). This highly selective prototypical agonist displays high potency *in vivo* and continues to be the most preferred agonist for 5-HT$_{1A}$ receptors. It displays high affinity ($K_d = 0.3$–1.8 nM) for the receptor isolated from various sources (Zifa and Fillion, 1992; Harikumar and Chattopadhyay, 1998a, 1999; Harikumar *et al.*, 2000; Kalipatnapu *et al.*, 2004) and displays a typical sensitivity to GTP-γ-S, the non-hydrolyzable analogue of GTP (Harikumar and Chattopadhyay, 1999; Javadekar-Subhedar and Chattopadhyay, 2004) indicating the requirement of G-proteins to interact with the receptor for this ligand to bind. Other ligands acting as agonists in recombinant systems but as partial agonists in animal models include drugs such as the buspirone, ipsaspirone, gepirone, tandospirone, BMY 7378, and NAN-190 which are of potential therapeutic value, especially for the treatment of anxiety and depression (Zifa and Fillion, 1992).

Although selective 5-HT$_{1A}$ agonists such as 8-OH-DPAT have been discovered a number of years back, the development of selective 5-HT$_{1A}$ antagonists has been relatively slow and less successful. The selective antagonist for the 5-HT$_{1A}$ receptor *p*-MPPI, and its fluorinated analogue *p*-MPPF, have been introduced later (Kung *et al.*, 1994a,b, 1995; Thielen and Frazer, 1995). These compounds bind specifically to the 5-HT$_{1A}$ receptor with high affinity (Kung *et al.*, 1994a,b; Harikumar and Chattopadhyay, 1998b, 2001; Kalipatnapu *et al.*, 2004). Moreover, binding of this ligand remains unaffected in presence of GTP-γ-S indicating that they belong to the category of neutral antagonists, i.e., their binding does not require G-proteins to interact with the receptors (Harikumar and Chattopadhyay, 1999). Furthermore, co-expression of the 5-HT$_{1A}$ receptor with Gα_z class of G-proteins in *Spodoptera frugiperda* (Sf9) cells has unequivocally confirmed that *p*-MPPF acts as a neutral antagonist (Barr and Manning, 1997). Interestingly, the same study has categorized *p*-MPPI as an inverse agonist, which according to the ternary complex model would tend to stabilize the G-protein uncoupled form of the receptor. In addition to *p*-MPPF, WAY-100135 is a neutral antagonist of the 5-HT$_{1A}$ receptor (Cliffe *et al.*, 1993). Spiperone is a ligand that has been proposed to have a higher affinity for the G-protein uncoupled form of the receptor. Studies carried out in heterologously expressed 5-HT$_{1A}$ receptors in Chinese hamster ovary (CHO) cells have demonstrated an increase in the number of binding sites recognized by spiperone when cell membranes were treated with GTP to uncouple G-proteins from receptors (Sundaram *et al.*, 1993). Spiperone has a moderate affinity ($K_i \sim 48$ nM) for the receptor (Hall *et al.*, 1985), which has limited its use in membrane preparations with low receptor expression. However, along with the classical agonist 8-OH-DPAT, spiperone can

provide insight to decipher mechanisms governing receptor and G-protein interactions in recombinant systems.

A recent report (Milligan *et al.*, 2001), has described the synthesis and pharmacological characterization of a ligand, S 14506, which in many ways has further refined our understanding of certain aspects of receptor activation and G-protein coupling. S 14506 is a prototypical antagonist for 5-HT$_{1A}$ receptors. It binds to the receptor with high affinity ($K_d = 0.13$–0.79 nM). Receptor binding is not inhibited in presence of the non-hydrolyzable analogue GppNHp. Moreover, the number of binding sites labeled with this ligand is always more than those detected using the agonist 8-OH-DPAT. All these characteristics would classify this ligand as a neutral antagonist. However, S 14506 can stimulate the high affinity GTPase activity in G-proteins upon binding to the receptor, a characteristic feature of agonists, at rates that surpass those found with the natural ligand serotonin. Molecular modeling studies have indicated that the ligand spans the site which is thought to bind to serotonin and the DRY switch (see Fig. 2) thus activating G-proteins. This report therefore hints at possibilities to synthesize ligands of G-protein coupled receptors which are highly potent in activating G-proteins through the receptor and yet behave as classical antagonists.

MEMBRANE BIOLOGY OF SEROTONIN$_{1A}$ RECEPTORS: RECEPTOR–CHOLESTEROL INTERACTION

Since the structure, organization and function of integral membrane proteins crucially depend on the membrane lipid composition and environment, native membranes prepared from bovine hippocampus represent an ideal natural source for the 5-HT$_{1A}$ receptor. The receptor is relatively abundant in this tissue. Studies carried out using this system have led to characterization of the receptor and more importantly, have provided important novel information and insight on the interaction of the receptor with its surrounding membrane lipids in its native environment. A brief overview of these studies is provided below.

Metal ion modulation of ligand binding is a characteristic feature of G-protein coupled receptors (Yabaluri and Medzihradsky, 1997). The interaction of physiologically relevant ions with certain charged residues in the receptor could in principle alter the ligand recognition by the receptor. In fact, in the case of α_2-adrenergic receptor, Asp^{79} is shown to be involved in the interaction of Na^+ with the receptor (Horstman *et al.*, 1990) and a conserved aspartate (Asp^{82}) in a similar region of the 5-HT$_{1A}$ receptor is shown to be essential for agonist binding (Ho *et al.*, 1992). Thus the nature and concentration of ions present in the environment could be an important parameter determining the ligand binding characteristics of the 5-HT$_{1A}$ receptor. The agonist 8-OH-DPAT binding to the 5-HT$_{1A}$ receptor is inhibited by monovalent cations such as Na^+, K^+ and Li^+ in a concentration-dependent manner whereas divalent cations such as Ca^{2+}, Mg^{2+} and Mn^{2+} induce an enhancement of the agonist binding at certain concentrations (Harikumar and Chattopadhyay, 1998a). The interaction of these ions with the 5-HT$_{1A}$ receptor is characterized by an altered agonist binding affinity and a reduction in number of binding sites (DeVinney

and Wang 1995; Harikumar and Chattopadhyay, 1998a). The antagonist binding to 5-HT_{1A} receptors from bovine hippocampus is characterized by reduced affinity in presence of both monovalent and divalent cations (Harikumar and Chattopadhyay, 2001). The mechanism of inhibition of agonist and antagonist binding by metal ions could be different considering the proposal that agonist and antagonist binding sites could be overlapping but not identical in the bovine hippocampal 5-HT_{1A} receptor. The agonist and antagonist binding activity in hippocampal 5-HT_{1A} receptors are therefore very well regulated by the ionic environment. Multiple affinity states of the 5-HT_{1A} receptor induced by metal ions could be physiologically significant. For example, effect of Na^+ on 5-HT_{1A} receptor affinity states may be relevant in hypertension since excess dietary Na^+ may exert its pressor effect in part by potentiating 5-HT_{1A} receptor function (Insel and Motulsky, 1984).

Serotonergic signaling has been shown to play an important role in the regulation of alcohol intake, preference and dependence. A number of studies have indicated the involvement of serotonergic neurotransmission in alcohol tolerance and dependence (Crabbe *et al.*, 1996; Pandey *et al.*, 1996). The direct effect of various alcohols on ligand binding and G-protein coupling of the bovine hippocampal 5-HT_{1A} receptor has been examined by Harikumar and Chattopadhyay (1998b, 2000). The results show that alcohols inhibit the specific binding of the agonist 8-OH-DPAT (except in case of ethanol) and the antagonist *p*-MPPF to 5-HT_{1A} receptors in a concentration dependent manner (Harikumar and Chattopadhyay, 1998b). These results further show that the action of alcohols on the hippocampal 5-HT_{1A} receptor could be modulated by guanine nucleotides (Harikumar and Chattopadhyay, 2000). Experiments involving effects of ethanol (Harikumar and Chattopadhyay, 1998b, 2000), and modifications of disulfide and sulfhydryl groups by agents that differ in their hydrophobicity (Harikumar *et al.*, 2000) suggest that the antagonist binding site in the hippocampal 5-HT_{1A} receptors is localized in a more polar environment (perhaps shallower in relation to the membrane) than the agonist binding site, which is known to be formed by residues present in the transmembrane domains in the receptor (see above).

G-protein coupled receptors represent strong candidates for the action of local anesthetics since anesthetics have been demonstrated to affect G-protein signal transduction pathways (Hollmann *et al.*, 2001; Ishizawa *et al.*, 2002). Interestingly, tertiary amine local anesthetics were shown to interact with the 5-HT_{1A} receptor by inhibiting specific agonist and antagonist binding when used at clinically relevant concentrations (Kalipatnapu and Chattopadhyay, 2004). In addition, the local anesthetics reduce the extent of interaction of the receptor with G-proteins. These results, along with fluorescence polarization studies with probes located at different depths in the membrane and ligand binding carried out after a significant alteration in the lipid composition of the membranes (i.e., depletion of $\sim$85% of membrane cholesterol), indicate a direct interaction between the receptor and the local anesthetics as the predominant mechanism of receptor–anesthetics interaction.

Since agonists bind to receptors coupled to G-proteins (Sundaram *et al.*, 1993; Harikumar and Chattopadhyay, 1998a) whereas antagonist binds to both G-protein coupled and uncoupled forms of the receptor (Kung *et al.*, 1995; Harikumar and Chattopadhyay, 1999), their relative binding abilities can be used to differentially

discriminate the extent of interaction between the receptor and G-proteins. This feature has been proposed to explain the striking differences in agonist and antagonist binding to the 5-HT$_{1A}$ receptors from bovine hippocampal membranes upon exposure to high temperatures (Javadekar-Subhedar and Chattopadhyay, 2004). Incubation of bovine hippocampal membranes to high temperatures irreversibly affects agonist binding to 5-HT$_{1A}$ receptors. However, the antagonist binding remains relatively unaffected. Since integral membrane proteins are considered to possess high thermal stability (Haltia and Freire, 1995), these results indicate inactivation of the peripheral G-proteins at high temperature thus making the agonist binding more sensitive to such treatments.

Membrane protein purification represents an area of considerable challenge in contemporary molecular biology. Studies carried out on purified and reconstituted membrane receptors have considerably advanced our knowledge of the molecular aspects of receptor function (Gether, 2000). It is noteworthy that none of the subtypes of G-protein coupled serotonin receptors have yet been purified to homogeneity. An essential criterion for purification of an integral membrane protein is that the protein must be carefully removed from the native membrane and individually dispersed in solution. This process is known as solubilization and is most effectively accomplished using amphiphilic detergents (Silvius, 1992; Banerjee, 1999). Solubilization of a membrane protein is a process in which the proteins and lipids that are held together in the native membrane are suitably dissociated in a buffered detergent solution. The controlled dissociation of the membrane results in the formation of small protein and lipid clusters that remain dissolved in the aqueous solution. Effective solubilization and purification of G-protein-coupled receptors in a functionally active form represent an important step in understanding structure–function relationship and pharmacological characterization of a specific receptor. Yet, solubilization of a membrane protein with retention of activity poses a formidable challenge since many detergents irreversibly denature membrane proteins (Garavito and Ferguson-Miller, 2001). This is the reason for the rather short list of membrane proteins which have been solubilized with retention of function.

Critical factors affecting solubilization include appropriate choice of detergent and the concentration at which it is used. Detergents self associate to form non-covalent aggregates (micelles) above a narrow range of concentration referred to as the critical micelle concentration (CMC). While detergents can be most effective when used beyond their CMC, loss of function of the protein of interest could occur at such high concentrations. However, the phenomenon of reduction in the CMC of a charged detergent upon addition of salts can be exploited to achieve functional solubilization of membrane proteins. The resultant 'effective CMC' of the detergent takes into account contributions from other components in the system (such as lipids, proteins, ionic strength, pH, temperature) and its determination can be useful in optimizing solubilization conditions (Chattopadhyay and Harikumar, 1996). A low ('pre-micellar') concentration of the mild and non-denaturing, zwitterionic detergent CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) has been used for solubilizing the 5-HT$_{1A}$ receptors in presence of salt followed by polyethylene glycol precipitation to remove the salt (Chattopadhyay and Harikumar, 1996; Chattopadhyay *et al.*, 2002; Chattopadhyay *et al.*, 2004). This results in efficient

solubilization of 5-HT_{1A} receptors with a high ligand binding affinity and ability to couple to G-proteins. As high concentrations of CHAPS is known to cause dissociation of G-protein subunits from the membrane (Jones and Garrison, 1999; Bayewitch *et al.*, 2000), the use of salt to effectively lower the concentrations required to achieve optimal solubilization of the 5-HT_{1A} receptor thus represents an elegant approach. Efficient solubilization of the receptor from the native source with high ligand binding affinity and intact signal transduction components may constitute the first step in the molecular characterization of this G-protein-coupled receptor.

The choice of the detergent CHAPS and its ability to solubilize 5-HT_{1A} receptors from bovine hippocampal membranes, which is not achieved optimally using other detergents (Harikumar and Chattopadhyay, unpublished observations), brings to light the importance of membrane lipids in maintaining the function of membrane proteins. In fact, it has earlier been shown that different classes of detergents used for solubilization of membrane receptors result in differential solubilization of lipids and proteins since some detergents even extract some of the 'annular' lipids necessary for preserving the function of the receptor (Banerjee *et al.*, 1995). This could result in non-functional solubilized receptor. The importance of the immediate lipid environment of the membrane protein therefore has to be kept in mind while choosing the appropriate detergent for optimal solubilization with retention of function.

Lipid–protein interactions play a crucial role in maintaining the structure and function of biological membranes (Lee, 2003). A possible role of lipids in a variety of neurological disorders is well documented (Waterham and Wanders, 2000). Effects on membrane function are presumed to be mediated by membrane proteins and for this reason, monitoring lipid–protein interactions assumes significance. A large portion of any given transmembrane receptor remains in contact with the membrane lipid environment. This raises the obvious possibility that the membrane could be an important modulator of receptor structure and function (Burger *et al.*, 2000). A study of such lipid–receptor interactions is of particular significance because a cell has the ability of varying the lipid composition of its membrane in response to a variety of stress and stimuli, thus changing the environment and the activity of the receptors in its membrane.

It is well known that membrane cholesterol is crucial to support a range of membrane related functions (Yeagle, 1985). Cholesterol is an essential component of eukaryotic membranes and plays a crucial role in membrane organization, dynamics, function and sorting (Simons and Ikonen, 2000). It is often found distributed nonrandomly in domains or pools in biological and model membranes (Liscum and Underwood, 1995; Mukherjee and Chattopadhyay, 1996; Simons and Ikonen, 1997, 2000; Rukmini *et al.*, 2001). These domains are believed to be important for the maintenance of membrane structure and function. In view of the importance of cholesterol in relation to membrane domains, the interaction of cholesterol with membrane proteins (Epand *et al.*, 2001) and receptors (Burger *et al.*, 2000) represents an important determinant in functional studies of such proteins and receptors, especially in the nervous system. Interestingly, the central nervous system which accounts for only 2% of the body mass contains ~25% of free cholesterol present in the whole body (Dietschy and Turley, 2001). Although the brain is an organ that is highly enriched in cholesterol, the organization and dynamics of brain cholesterol

is still poorly understood (Wood *et al.*, 1999). Brain cholesterol is synthesized *in situ* (Kabara, 1973) and is developmentally regulated (Turley *et al.*, 1998) Cholesterol organization, traffic, and dynamics in the brain is stringently controlled since the input of cholesterol into the central nervous system is almost exclusively from *in situ* synthesis as there is no available evidence for the transfer of cholesterol from blood stream to brain (Dietschy and Turley, 2001). As a result of this, 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 the *in situ* synthesis and such synthesis is defective in this syndrome (Waterham and Wanders, 2000). Some of these diseases show symptoms that are similar to those which appear upon disruption of serotonergic signaling (Papakostas *et al.*, 2004). The interaction between cholesterol and other molecular components (such as receptors) in the brain therefore assumes relevance for a comprehensive understanding of brain function.

The modulatory role of cholesterol on the ligand binding activity and G-protein coupling of the bovine hippocampal 5-HT$_{1A}$ receptor has recently been shown by depleting cholesterol from native membranes using methyl-β-cyclodextrin (Pucadyil and Chattopadhyay, 2004a). Removal of cholesterol from hippocampal membranes using various concentrations of methyl-β-cyclodextrin resulted in a concentration-dependent reduction in specific binding of the agonist 8-OH-DPAT to 5-HT$_{1A}$ receptors. This is accompanied by alterations in binding affinity and sites obtained from analysis of binding data. Importantly, cholesterol depletion affected G-protein-coupling of the receptor as monitored by the GTP-γ-S assay. The concomitant changes in membrane order were reported by changes in fluorescence polarization of membrane probes which are incorporated at different locations (depths) in the membrane. Replenishment of membranes with cholesterol led to recovery of ligand binding activity as well as membrane order to a considerable extent. These results provide evidence, for the first time, that cholesterol is necessary for ligand binding and G-protein coupling of this important neurotransmitter receptor. More importantly, these results could have significant implications in understanding the influence of the membrane lipid environment on the activity and signal transduction of other G-protein coupled transmembrane receptors. The clinical significance of membrane cholesterol levels resulting in receptor dysfunction has been aptly exemplified in the case of cholecystokinin (CCK) receptors (Xiao *et al.*, 1999, 2000). Thus, agonist binding is reduced and G-protein coupling affected for CCK receptors isolated from muscle tissues in human gallbladders with cholesterol stones. These effects are reversed upon treatment with cholesterol-free liposomes.

The most important aspect of these results is that manipulations of membrane cholesterol content can induce significant changes in the activity and G-protein coupling of the 5-HT$_{1A}$ receptor. Whether such manipulations in membrane cholesterol content could be induced *in vivo* represents a challenging question. The turnover of brain cholesterol is very low, with a half-life of ~6 months (Dietschy and Turley, 2001). As a result, the cerebrospinal fluid levels of cholesterol are ~40–50 fold lower than the plasma cholesterol (Sooksawate and Simmonds, 1998). Due to the presence

of the blood brain barrier, alterations in serum levels of cholesterol are believed not to affect the total cholesterol level in the central nervous system. However, under such conditions, the neuronal plasma membrane fractions have not been studied adequately. In addition, regions in the central nervous system (such as the hypothalamic area) that are somewhat weakly protected by the blood brain barrier may be sensitive to plasma cholesterol fluctuations. Interestingly, a recent report has suggested that chronic *in vivo* administration of cholesterol lowering drugs like statins specifically reduce brain cholesterol levels leaving the serum cholesterol levels unaffected (Kirsch *et al.*, 2003). Importantly, it has been shown that treating humans with such cholesterol lowering drugs significantly decreases the incidence of Alzheimer's disease (Wolozin *et al.*, 2000; Jick *et al.*, 2000). It is possible that the more severe deficiency in cholesterol levels in the brain occurs on account of the lower turnover of cholesterol in this tissue. Interestingly, low serum cholesterol concentration has been correlated with an increase in the prevalence of suicide in humans (Steegmans *et al.*, 1996) and is partly attributed to an altered serotonin metabolism (Zureik *et al.*, 1996). In addition, a recent report describes the attenuation of 5-HT_{1A} receptor antagonist binding and signaling in brains of suicide victims (Hsiung *et al.*, 2003). In light of the requirement of membrane cholesterol to influence the 5-HT_{1A} receptor function (Pucadyil and Chattopadhyay, 2004a), the role of brain membrane cholesterol in the etiology of psychological disorders that are correlated with an altered cholesterol metabolism needs further investigation.

As mentioned earlier, cholesterol is thought to maintain domain-like organization in biological membranes. Many of these domains are believed to be important for the maintenance of membrane structure and function on account of their unique lipid–protein composition and their potential to sequester receptors and signaling molecules in the plane of the membrane. Recent observations suggest that cholesterol exerts many of its actions by maintaining a specialized type of membrane domain, termed "lipid raft," in a functional state (Simons and Ikonen, 1997; Edidin, 2003). Although the existence of lipid rafts in membranes in general and brain membranes in particular, has not been unequivocally shown (Munro, 2003), they are thought of as lateral organizations on the plane of the membrane that are enriched in cholesterol and sphingolipids and specific proteins that are implicated in cell signaling and traffic. The integrity of the raft regions of the membrane is thought to be crucial to regulate signal transduction events (Simons and Toomre, 2000) and entry of pathogens into the cell (van der Goot and Harder, 2001; Pucadyil *et al.*, 2004a).

In spite of the important role played by membrane domains in cellular signaling, their significance in the organization and function of 5-HT_{1A} receptors is still not known. This issue has been recently addressed through a novel approach utilizing the fluorescence of the enhanced yellow fluorescence protein (EYFP) tagged to the 5-HT_{1A} receptor stably expressed in CHO cells (Kalipatnapu *et al.*, 2003a,b). The ligand binding properties of the EYFP tagged 5-HT_{1A} receptor were found to be unaltered upon EYFP fusion (Pucadyil and Chattopadhyay, 2003). Insolubility of membrane constituents in non-ionic detergents has proved to be a useful tool to characterize membrane domains (Brown and Rose, 1992; Shogomori and Brown, 2003). These domains have been operationally defined using the criterion of their insolubility in non-ionic detergents such as Triton X-100 at 4°C. Detergent insolubility

of 5-HT$_{1A}$ receptors has been assessed by treatment of cells in culture with cold Triton X-100 followed by quantitation of the residual fluorescence of the receptor (Kalipatnapu *et al.*, 2003a,b). These results show that detergent treatment results in significant retention of EYFP fluorescence. The amount of residual fluorescence was found to be dependent on the concentration of the detergent used. This approach was validated by experiments performed under similar conditions in cells labeled with membrane domain-specific fluorescent lipid probes, DiIC$_{16}$ and *FAST* DiI (Mukherjee *et al.*, 1999), or with the detergent soluble protein marker, transferrin receptor (Mayor and Maxfield, 1995). This represents the first demonstration of the localization of 5-HT$_{1A}$ receptors in detergent resistant membrane domains. The status of these domains during signaling and receptor activation and upon alteration in membrane lipid composition opens up new areas in receptor signaling and membrane domain organization of 5-HT$_{1A}$ receptors and is presently under investigation (Kalipatnapu and Chattopadhyay, unpublished observations). Interestingly, it has recently been shown that the detergent insolubility of bovine hippocampal membranes is not critically dependent on the membrane cholesterol content (Pucadyil and Chattopadhyay, 2004b).

These results bring out several interesting possibilities on the function and organization of 5-HT$_{1A}$ receptors in the general context of lipid–protein interactions. Understanding lipid–protein interactions of this important G-protein coupled receptor in membranes represents an interesting area in serotonin receptor biology. These studies assume greater importance on account of the enormous implications of 5-HT$_{1A}$ receptor function in human health and the observation that several diagnosed brain diseases are attributed to an altered lipid–protein interaction.

CONCLUSION AND FUTURE PERSPECTIVES

Present research on the 5-HT$_{1A}$ receptor has reached an interesting point. The early development of the specific agonist 8-OH-DPAT, the cloning of its gene and production of knockout mice have generated important biochemical, pharmacological and genetic leads. Along with this, novel information on the lipid requirement (for example, cholesterol) in maintaining the organization, ligand binding and G-protein coupling of the receptor (Pucadyil and Chattopadhyay, 2004a) has proved to be essential in developing a comprehensive understanding of the receptor function against the backdrop of the membrane lipid environment in which the receptor is embedded. In parallel, the question of whether this representative member of the G-protein coupled serotonin receptor family resides in detergent resistant membrane domains is being addresed (Kalipatnapu *et al.*, 2003a). These advances in understanding the receptor structure and function are noteworthy especially considering the fact that inputs from structural biology (crystallography and NMR) has yet been missing for this important receptor. Correlating the altered function of the receptor with changes in lipid composition of membranes due to physiological stress (or, with alterations in receptor sequence due to mutations) would lead to a better understanding of the receptor structure and function in healthy and diseased states.

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