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The Serotonin$_{1A}$ Receptor and its Interaction with the Membrane Environment

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Serotonin is a neurotransmitter which signals across the plasma membrane 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. Although the pharmacological and signaling aspects of the functioning of the 5-HT$_{1A}$ receptor have been well explored, the role of the membrane environment in its function is only beginning to be addressed. This review describes various aspects of the membrane biology of 5-HT$_{1A}$ receptors such as modulation of ligand binding to 5-HT$_{1A}$ receptors under a variety of conditions, and functional solubilization of 5-HT$_{1A}$ receptors using the bovine hippocampal serotonin$_{1A}$ receptor as the model system. Recent developments in membrane organization of the 5-HT$_{1A}$ receptor using the phenomenon of detergent insolubility, and the role of membrane cholesterol in ligand binding and G-protein coupling of the 5-HT$_{1A}$ receptor are summarized.

Key Words : Serotonin, Serotonin$_{1A}$ receptor, G-protein coupling, Solubilization, Membrane lipid environment, Cholesterol, Detergent insolubility

Serotonin: An Important Neurotransmitter

Serotonin is one of the best known among the class of small molecules constituting classical neurotransmitters that are involved in several functions in the central and peripheral nervous systems. Serotonin is a biogenic amine synthesized from the naturally occurring amino acid tryptophan. Presence of significant amounts of serotonin in the mammalian central nervous system, originally described by Twarog and Page (1953), led to the proposal that serotonin could function as a neurotransmitter. This finding is considered to be one of the important discoveries in neuroscience. A comprehensive account of the rather serendipitous discovery of serotonin is given by Whitaker-Azmitia (1999). Serotonin (5-hydroxytryptamine or 5-HT; figure 1) is present in a variety of organisms ranging from worms to humans (Hen 1992). Serotonin is a derivative of 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). The fluorescence

Abbreviations: *5-HT*, 5-hydroxytryptamine; *5-HT$_{1A}$ receptor*, 5-hydroxytryptamine-1A receptor; *8-OH-DPAT*, 8-hydroxy-2(di-N-propylamino)tetralin; *CHAPS*, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; *CMC*, critical micelleconcentration; *DRM*, detergent resistant membranes; *DiI*, dialkylindocarbocyanine; *DiIC$_{16}$*, 1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; *FAST DiI*, 1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; *GPCR*, G-protein coupled receptor; *GTP-γ-S*, guanosine-5'-*O*-(3-thiotriphosphate); *MβCD*, methyl-β-cyclodextrin; *p-MPPF*, 4-(2'-methoxy)-phenyl-1-[2'-(N-2''-pyridinyl)-*p*-fluorobenzamido]ethyl-piperazine; *p-MPPI*, 4-(2'-methoxy)-phenyl-1-[2'-(N-2''-pyridinyl)-*p*-iodobenzamido]ethyl-piperazine; *WAY 100635*,(N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide.

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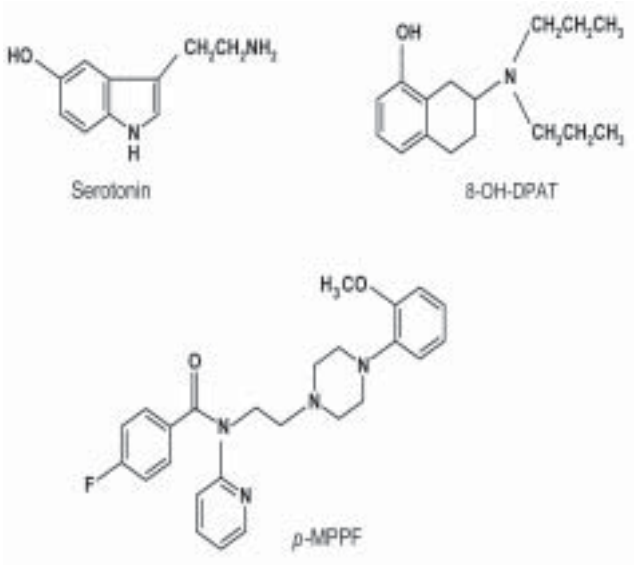

Figure 1. Chemical structures of ligands that bind to the serotonin$_{1A}$ receptor.

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). It has recently 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.

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 (Zifa & Fillion 1992, Julius 1998, Barnes & Sharp 1999). Disruptions in serotonergic systems have been implicated in the etiology of mental disorders such as schizophrenia, migraine, depression, suicidal behavior, infantile autism, eating disorders, and obsessive compulsive disorder (Hen 1992, Julius 1998, Pucadyil et al. 2005). Novel roles for serotonin in heart disease (Nebigil & Maroteaux 2001), asthma (Barnes et al. 1998) and phagocytosis (Freire-Garabal et al. 2003) have been recently identified. A recent report implicates a serotonin receptor in the infection of human glial cells by the human polyoma virus which causes a fatal demyelinating disease (Elphick et al. 2004). 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, Gaspar et al. 2003). In fact, serotonin was the first neurotransmitter for which a developmental role was proposed (Gaspar et al. 2003).

Serotonin exerts its diverse actions by binding to distinct cell surface receptors which are 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 (Hoyer et al. 2002). In addition to the nervous system, serotonin and its receptors are found in non-neuronal tissues such as blood, cardiovascular system and gut. 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). All 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).

The Serotonin$_{1A}$ Receptor: A Key Component of Serotonergic Signaling

The serotonin$_{1A}$ (5-HT$_{1A}$) receptor is an important member of the large family of serotonin receptors. It is the first among all the serotonin receptors to be cloned as an intronless genomic clone (G-21) of the human genome which cross-hybridized with a full length β-adrenergic receptor probe at reduced stringency (Kobilka et al. 1987). Sequence analysis of this genomic clone (to be later identified as the 5-HT$_{1A}$ receptor gene) indicated 43% amino acid homology with the β_2-adrenergic receptor in the transmembrane domain. While the gene was shown to be localized in chromosome 5 of the human genome and speculated to code for a potential member of the GPCR superfamily (Kobilka et al. 1987), its identity as a serotonin receptor was discovered only later (Fargin et al. 1988). Membranes prepared from COS-1 cells transiently transfected with G-21 showed typical ligand binding characteristics of the 5-HT$_{1A}$ receptor. Subsequently, genes for the rat and mouse 5-HT$_{1A}$ receptors have been cloned, and their amino acid sequences deduced (Albert et al. 1990; Charest et al. 1993). These developments facilitated stable expression of the receptor in a number of neural and non-neural cell lines (Banerjee et al. 1993, Newman-Tancredi et al. 1997, Kalipatnapu et al. 2004). Furthermore, it was the first serotonin receptor for which polyclonal antibodies were obtained (Fargin et al. 1988, Pucadyil et al. 2005) allowing their visualization at the subcellular level in various regions of the brain.

In addition, the availability of a selective ligand 8-OH-DPAT(8-hydroxy-2-(di-*N*-propylamino) tetralin) (Arvidsson et al. 1981, Gozlan et al. 1983), which acts

as an agonist for the 5-HT$_{1A}$ receptor, allowed extensive characterization of the 5-HT$_{1A}$ receptor. 8-OH-DPAT (see figure 1 for chemical structure) displays high affinity (K_d = 0.3-1.8 nM) for the 5-HT$_{1A}$ receptor isolated from various sources and displays a typical sensitivity to GTP-γ-S, a nonhydrolyzable analogue of GTP indicating that this ligand binds to the subpopulation of receptors which are coupled to G-proteins (see Pucadyil et al. 2005). Selective antagonists for the 5-HT$_{1A}$ receptor such as *p*-MPPI and WAY-100635 have been developed over the past few years which display several fold selectivity for the 5-HT$_{1A}$ receptor over other neurotransmitter receptors. The selective antagonist for the 5-HT$_{1A}$ receptor, *p*-MPPI, and its fluorinated analogue *p*-MPPF (see figure 1) introduced a few years back (Kung et al. 1994, 1995) bind specifically to the 5-HT$_{1A}$ receptor with high affinity (Kung et al. 1994, Harikumar & Chattopadhyay 1998b, 2001, Kalipatnapu et al. 2004). Moreover, binding of *p*-MPPF 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 & Chattopadhyay 1999).

The human 5-HT$_{1A}$ receptor is composed of 422 amino acids with a core molecular weight of ~46,000 (Raymond et al. 1999, Pucadyil et al. 2005). Considering the presence of three consensus sequences for *N*-linked glycosylation on the amino terminus, and the homology of the receptor with β-adrenergic receptor, it is predicted that the receptor is oriented in the plasma membrane with the amino terminus facing the extracellular region and the carboxy terminus facing the intracellular cytoplasmic region (Raymond et al. 1999, Pucadyil et al. 2005, see figure 2). The transmembrane domains (TM1-TM7) of the receptor are connected by hydrophilic sequences of three extracellular loops (EC1, EC2, EC3) and three intracellular loops (IC1, IC2, IC3). Such an arrangement is typical of the G-protein coupled receptor superfamily (Gether & Kobilka 1998). Although the structure of the 5-HT$_{1A}$ receptor has not yet been experimentally determined, mutagenesis studies have helped in identifying amino acid residues important for ligand binding and G-protein coupling of the 5-HT$_{1A}$ receptor (discussed in Pucadyil et al. 2005). Among the predicted structural features of the 5-HT$_{1A}$ receptor, palmitoylation status of the receptor has been confirmed in a recent report (Papoucheva et al. 2004). Palmitoylation of Cys-417 and Cys-420 of the heterologously expressed rat 5-HT$_{1A}$ receptor, and its requirement in G-protein coupling and signaling of the 5-HT$_{1A}$ receptor have been demonstrated in this report. An interesting aspect of this study is that palmitoylation of the 5-HT$_{1A}$ receptor was found to be stable and independent of stimulation by the agonist. This is unusual for GPCRs which undergo repeated cycles of palmitoylation and depalmitoylation (Milligan et al. 1995). It has therefore been proposed that stable palmitoylation of the receptor could play an important role in maintaining the receptor structure (Papoucheva et al. 2004).

The 5-HT$_{1A}$ receptor has recently been shown to have a role in neural development (del Olmo et al. 1998), 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 and antagonists represent a major class of molecules with potential therapeutic effects in anxiety- or stress-related disorders (Pucadyil et al. 2005). As a result, the 5-HT$_{1A}$ receptor serves as an important target in the

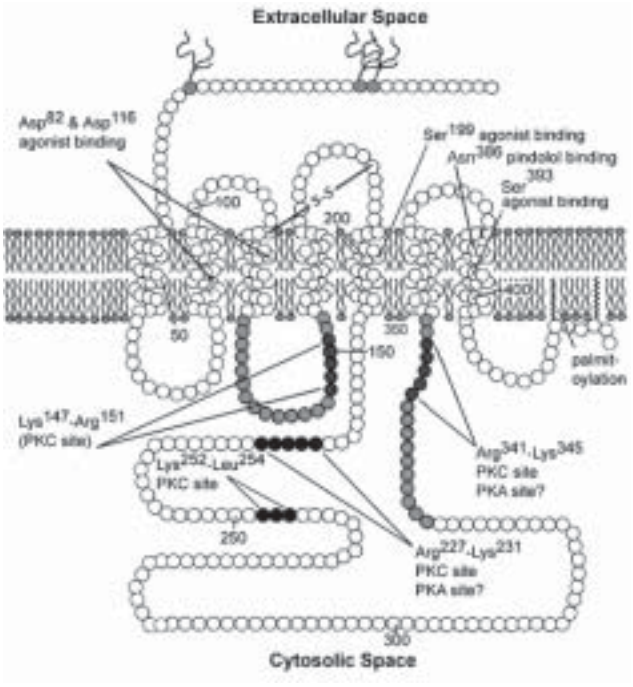

Figure 2. A schematic representation of the membrane embedded human 5-HT$_{1A}$ receptor showing its predicted topological and other structural features. The membrane is shown as a bilayer of two leaflets of lipids. The amino acids in the receptor sequence are shown as circles and are marked after every 50 residues for convenience. Seven transmembrane regions, each composed of 20-26 amino acids, are depicted as ±-helices. There are three potential sites of *N*-linked glycosylation on the amino terminus (depicted as branching trees). A putative disulfide bond between Cys-109 and Cys-187 is shown. Transmembrane (TM) domains contain residues (which are marked) that are important for ligand binding. 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 from Pucadyil et al. 2005.

development of therapeutic agents for neuropsychiatric disorders such as anxiety and depression. Interestingly, mutant (knockout) mice lacking the 5-HT_{1A} receptor generated a few years back exhibit enhanced anxiety-related behavior (Julius 1998), and therefore the 5-HT_{1A} receptor knockout mouse serves as an excellent model system to understand anxiety-related behavior in higher animals (Toth 2003).

On the clinical front, 5-HT_{1A} receptor levels have been shown to be altered in schizophrenia, and in patients suffering from major depression (Pucadyil et al. 2005). 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. The selective 5-HT_{1A} receptor agonist 8-OH-DPAT has recently been shown to inhibit growth of *Plasmodium falciparum* (reviewed in Chattopadhyay and Kalipatnapu 2004) opening novel possibilities in antimalarial drug research. Besides, the 5-HT_{1A} receptors are implicated in feeding, regulation of blood pressure, temperature, and working memory (Pucadyil et al. 2005). Taken together, the serotonin$_{1A}$ receptor is a central player in a multitude of physiological processes, and an important drug target.

Membrane Biology of Serotonin$_{1A}$ Receptors

The 5-HT_{1A} receptor is relatively abundant in the hippocampus of the brain. 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. Studies carried out using this system have led to characterization of ligand binding and G-protein coupling of the 5-HT_{1A} receptor, and more importantly, have provided important novel information on the interaction of the receptor with its surrounding membrane lipids in its native environment. A brief overview of these studies is provided below.

(a) Modulation of Ligand Binding

Modulation of ligand binding by metal ions is a characteristic feature of G-protein coupled receptors (Yabaluri & 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 (Pucadyil et al. 2005). 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 & 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 & Wang 1995, Harikumar & 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 & Chattopadhyay 2001). 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-HT1A receptor function (Insel & Motulsky 1984). Modulation 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 (Harikumar & Chattopadhyay 1999). This aspect is also apparent from effects of ethanol on agonist and antagonist binding of the 5-HT_{1A} receptor (Harikumar & Chattopadhyay 1998b, 2000) and modifications of disulfide and sulfhydryl groups by agents that differ in their hydrophobicity (Harikumar et al. 2000). Results from these experiments suggest that the antagonist binding site in the hippocampal 5-HT_{1A} receptor is localized in a more polar environment (perhaps at a shallower location in the membrane) than the agonist binding site, which is known to be formed by residues present in the transmembrane domains in the receptor.

The effects of alcohol on ligand binding and G-protein coupling of the 5-HT_{1A} receptor are significant in the overall context of the role of serotonergic signaling 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 previously examined (Harikumar & 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 & 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 & Chattopadhyay 2000).

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). Utilizing the 5-HT$_{1A}$ receptor as a model G-protein coupled receptor, the clinically relevant issue of the role of G-protein coupled receptors in the action of local anesthetics has been examined. In addition, since local anesthetics are known to cause membrane perturbation, these experiments are relevant in the context of analyzing the response of the 5-HT$_{1A}$ receptor to a possible modulation in its membrane environment. Taking these aspects into consideration, ligand binding characteristics and G-protein coupling of the 5-HT$_{1A}$ receptor have been monitored in the presence of the tertiary amine local anesthetics used at clinically relevant concentrations. Interestingly, tertiary amine local anesthetics were shown to inhibit specific agonist and antagonist binding of the 5-HT$_{1A}$ receptor (Kalipatnapu & Chattopadhyay 2004a). In addition, the local anesthetics were found to 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 ~85% of membrane cholesterol), suggest interaction between the receptor and the local anesthetics as a probable mechanism of receptoranesthetics interaction.

Since agonists bind to receptors coupled to G-proteins (Sundaram et al. 1993, Harikumar & Chattopadhyay 1999) whereas antagonist binds to both G-protein coupled and uncoupled forms of the receptor (Kung et al. 1995, Harikumar & 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 & 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 & Freire 1995), these results indicate inactivation of the peripheral G-proteins at high temperature. This could make the agonist binding more sensitive to such treatments.

(b) Functional Solubilization of Serotonin$_{1A}$ Receptors

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 from natural sources. 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 (Helenius & Simons 1975, Garavito & Ferguson-Miller 2001, Kalipatnapu & Chattopadhyay 2005). 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 important steps 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 & Ferguson-Miller 2001). This is the main reason for the rather modest list of membrane proteins which have been solubilized with retention of function, although ~30% of all cellular proteins are estimated to be integral membrane proteins (Liu et al. 2002).

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 & Harikumar 1996). A low ('premicellar') 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 (see figure 3; Chattopadhyay & Harikumar 1996, Chattopadhyay et al. 2002, Chattopadhyay et al. 2004). This has resulted 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 & Garrison 1999, Kalipatnapu & Chattopadhyay 2005), 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.

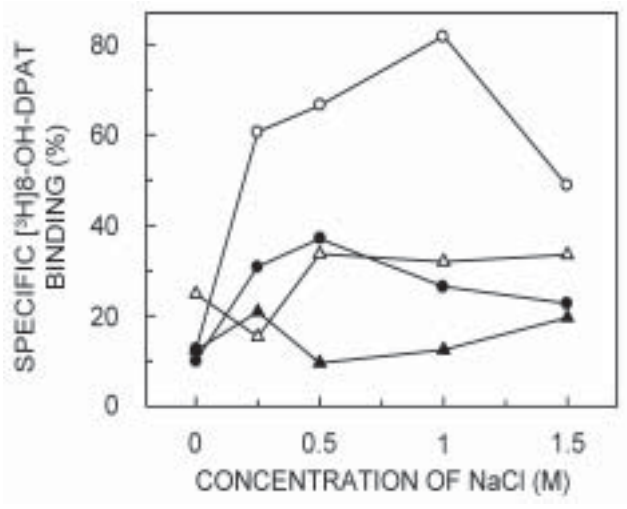

Figure 3. Solubilization of 5-HT_{1A} receptors from bovine hippocampal membranes with different concentrations of the zwitterionic detergent CHAPS and NaCl. Efficient solubilization of 5-HT_{1A} receptors has been achieved at a low concentration (5 mM) of the detergent in the presence of salt. Values are expressed as percentage of specific binding of the agonist [^{3}H]8-OH-DPAT obtained (expressed as femtomoles/mg of total protein) for native membranes without solubilization. The concentrations of CHAPS used were 5 (O), 7.5 (●), 10 (Δ), and 15 (s) mM. Data taken from Chattopadhyay et al. 2002.

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 & 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 (Jones et al. 1988, 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.

(c) Membrane Organization and Receptor-Cholesterol Interaction

The fluid mosaic model for cell membranes (Singer & Nicolson 1972) visualized a largely fluid membrane bilayer in which proteins are embedded. This model proposed a dynamic bilayer with free translational diffusion of lipids and proteins and possible interactions between them, and a restricted movement of the membrane components across the bilayer which would preserve asymmetry of the bilayer. Some of the tenets set by this model were later modified with results from several laboratories (Jacobson et al. 1995, Edidin 2003) favoring non-random organization of lipids and proteins, *i.e.*, heterogeneities (domains) in the membrane. Current understanding of membranes involves membrane domains with defined lipid and protein compositions, although the spatiotemporal resolution of these domains is not yet clear (Mukherjee & Maxfield 2004). These domains, sometimes referred to as 'rafts', are believed to serve as platforms for signaling by concentrating certain lipids (such as cholesterol and sphingolipids) and proteins while excluding others (Simons & Ikonen 1997, Edidin 2003, Mukherjee & Maxfield 2004). Organization of membranes into domains could play a key role in a number of processes such as membrane trafficking, sorting, signal transduction, and pathogen entry (Simons & Toomre 2000, van der Goot & Harder 2001, Mukherjee & Maxfield 2004, Pucadyil et al. 2004a).

The implication of membrane organization on the signaling functions of membrane proteins in general, and on G-protein coupled receptors in particular,

represents an interesting aspect. The classical view of receptor-G-protein function in cells proposes free diffusion of molecules on the cell surface and that their interaction would depend on random collisions, though the actual sites of interaction are specific (Neubig 1994). The specific and rapid signaling responses characteristic of GPCR activation appear to be difficult to explain based on uniform distribution of the receptors, G-proteins, and effectors – one or more of which could even be low in abundance on the cell surface (Huang et al. 1997, Ostrom & Insel 2004). This leads to the possibility that receptor-G-protein interactions may be dependent on their organization in membranes and not solely on the binding sites present on the interacting proteins. Thus, spatiotemporal organization and dynamic confinement of receptors and effector molecules on the plasma membrane microdomains is now believed to be an important determinant in GPCR signaling (Neubig 1994, Hur & Kim 2002).

The role of membrane domains in the organization and function of the G-protein coupled 5-HT$_{1A}$ receptor assumes relevance against this backdrop. This issue has been recently addressed employing the biochemical criterion of detergent insolubility. Resistance to solubilization by mild non-ionic detergents such as Triton X-100 at low temperature has emerged as an extensively used biochemical tool to identify, isolate and characterize certain types of membrane domains (Brown & Rose 1992, Brown & London 1998, Chamberlain 2004). The tight acyl chain packing of sphingolipids and saturated lipids is thought to confer detergent resistance to membrane regions enriched in these lipids and to the proteins which reside in them. Thus, insolubility in cold Triton X-100 has been increasingly used as a hallmark of the presence of 'rafts', the class of membrane domains enriched in sphingolipids and cholesterol (Brown & London 1998, Chamberlain 2004). Several GPI-anchored proteins, few transmembrane proteins and certain G-proteins have been found to reside in detergent resistant membrane domains, popularly referred to as DRMs (Brown & Rose 1992, Brown & London 1998, Chamberlain 2004).

Detergent insolubility of the 5-HT$_{1A}$ receptor has been monitored using 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 & Chattopadhyay 2004b). The ligand binding properties of the EYFP tagged 5-HT$_{1A}$ receptor were found to be unaltered upon EYFP fusion (Pucadyil et al. 2004b). 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 & Chattopadhyay 2004b). These results show that detergent treatment results in significant retention of EYFP fluorescence. In order to validate this fluorescence microscopic approach toward determination of detergent insolubility of membrane components, specific lipid (phasesensitive dialkylindocarbocyanine (DiI) probes) and protein (transferrin receptor) markers were used whose organization in membranes and ability to be extracted by cold non-ionic detergents have been well documented (Mayor & Maxfield 1995, Mukherjee et al. 1998). Results obtained from these experiments showed that this method is capable of distinguishing ordered domains labeled by DiIC$_{16}$ (1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) from the fluid regions of the membrane characterized by *FAST* DiI (1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) labeling (Kalipatnapu & Chattopadhyay 2004b). These results, along with the observation of low detergent insolubility of transferrin receptor, validated the novel observation of detergent insolubility of the 5-HT$_{1A}$ receptor in particular and GFP fluorescence-based approach in general (Kalipatnapu & Chattopadhyay 2004b). These experiments represent one of the first attempts to address membrane organization of the 5-HT$_{1A}$ receptor, and the fluorescencebased approach to monitor detergent insolubility can be potentially useful in exploring membrane organization of other G-protein coupled receptors. 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. Interestingly, it has recently been shown that the detergent insolubility of bovine hippocampal membranes is not critically dependent on the membrane cholesterol content (Pucadyil & Chattopadhyay 2004a).

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). In view of this proposal, and the significance of lipid-protein interaction in the assembly, stability and function of membrane proteins (Lee 2004, Palsdottir & Hunte 2004), understanding organization of membranes and its relation to membrane protein function assumes significance. Monitoring lipid-protein interactions, and determining specific lipid requirements of a given membrane protein represent challenging

tasks since very few membrane proteins have been purified to homogeneity. As a result, specific lipid requirements for membrane protein function have been reported in very few cases. The enzyme β-hydroxybutyrate dehydrogenase represents an important example for a membrane protein with an absolute requirement for a specific phospholipid. The choline headgroup of phophatidylcholine has been shown to be required for the proper activity of this enzyme (Isaacson et al. 1979). Examples of other membrane proteins whose function is shown to be affected by specific lipids are the P-glycoprotein for lipids such as PC and PE, and the Ca^{2+}-ATPase for PE and cholesterol (Opekarova and Tanner 2003). Further, neutral and anionic phospholipids have been shown to modulate the nicotinic acetylcholine receptor activity (Barrantes 2004).

In comparison to limited reports on specific lipid-protein interactions in purified systems, relatively greater information is beginning to be available for modulation of receptor function by membrane lipids in natural membranes (Burger et al. 2000). In particular, the role of cholesterol, an essential lipid of eukaryotic membranes, in the functioning of several membrane proteins and receptors from native and heterologous systems has been well addressed (Burger et al. 2000, Pucadyil & Chattopadhyay 2004b). Cholesterol plays a crucial role in membrane organization, dynamics, function and sorting (Simons & Ikonen 2000). It is often found distributed nonrandomly in domains or pools in biological and model membranes (Liscum & Underwood 1995, Simons & Ikonen 1997 2000, Rukmini et al. 2001). 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.

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 & Chattopadhyay 2004b). 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 (see figure 4a). This is accompanied by alterations in binding sites and affinity obtained from analysis of binding data. In addition, cholesterol depletion was found to affect G-protein coupling of the receptor. Importantly, replenishment of membranes with cholesterol led to recovery of ligand binding activity (figure 4b). These results provide evidence, for the first time, that cholesterol is necessary for ligand binding and G-protein coupling of this important neurotransmitter receptor (Pucadyil & Chattopadhyay 2004b). The importance of receptor-cholesterol interaction in the functioning of the 5-HT_{1A} receptor is further emphasized by the

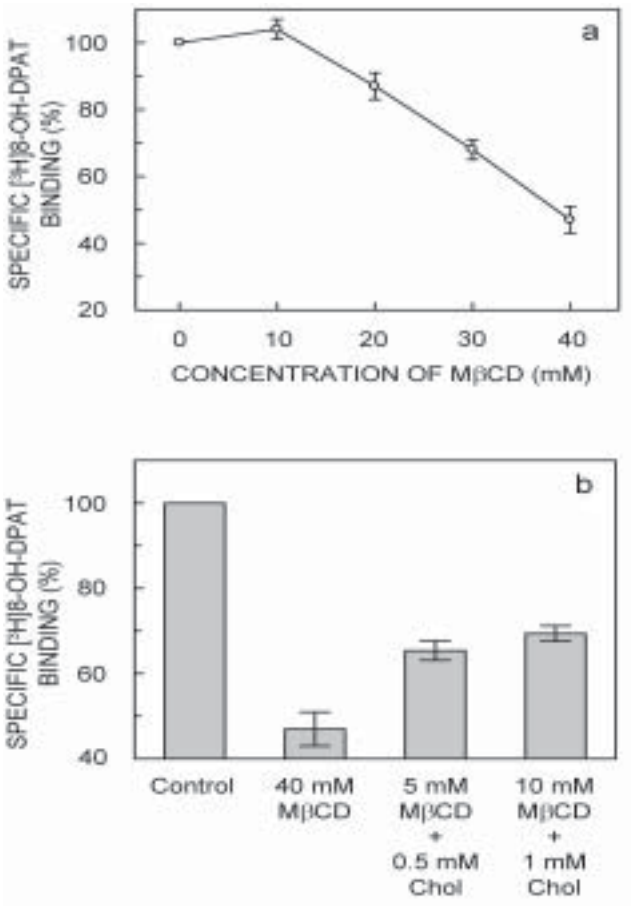

Figure 4. Cholesterol is required for the specific binding of the agonist [3H]8-OH-DPAT to hippocampal 5-HT_{1A} receptors. (a) The specific [3H]8-OH-DPAT binding is reduced upon treatment of bovine hippocampal membranes with increasing concentrations of MβCD. This indicates loss in agonist binding of 5-HT_{1A} receptors upon reduction in membrane cholesterol levels. Values are expressed as a percentage of specific binding for native membranes without M²CD treatment. Data taken from Pucadyil and Chattopadhyay 2004b. (b) Cholesterol replenishment into bovine hippocampal membranes treated with MβCD and its correlation with specific [^{3}H]8-OH-DPAT binding activity of the hippocampal 5-HT1A receptor. Cholesterol depletion was carried out by incubating bovine hippocampal membranes with 40 mM MβCD for 1 hr. This treatment leads to ~50% reduction in specific [^{3}H]8-OH-DPAT binding. Upon replenishment of membrane cholesterol in cholesteroldepleted hippocampal membranes using cholesterol-MβCD complex (at a final concentration of 0.5:5 mM and 1:10 mM (mol/mol of cholesterol-MβCD)), a significant recovery in the specific [^{3}H]8-OH-DPAT binding is observed. Values are expressed as a percentage of specific radiolabeled agonist binding in native membranes without any treatment. Data taken from Pucadyil & Chattopadhyay 2004b.

observation that ligand binding function of the 5-HT$_{1A}$ receptor could be modulated even by sequestering membrane cholesterol with agents such as digitonin (Paila et al. 2005) or nystatin (Pucadyil et al. 2004c). Thus, making the membrane cholesterol unavailable to the receptor is found to affect the functioning of the 5-HT$_{1A}$ receptor, further emphasizing the requirement of cholesterol in 5-HT$_{1A}$ receptor function.

One of the basic demonstrations of the importance of membrane environment in membrane protein function is the decrease in membrane protein activity upon delipidation of membranes (Jones et al. 1988; Chattopadhyay et al. 2005), a common consequence of the process of solubilization. Considering the significance of lipid-protein interactions in maintaining the structure and function of biological membranes (Lee 2004, Palsdottir & Hunte 2004), it is conceivable that replacement of a specific lipid environment with detergent or detergent-lipid during solubilization could affect the function of a membrane protein. For example, displacement of lipids from the receptor has been shown to be an integral feature of detergent-induced inactivation in case of the nicotinic acetylcholine receptor (Jones et al. 1988). The phenomenon of delipidation and its consequences on activity of solubilized membrane proteins have previously been utilized to gain insight into the specific lipid requirements of membrane proteins (Jones et al. 1988, Kalipatnapu & Chattopadhyay 2005). It is possible that the ability of a detergent to solubilize a membrane protein in its functional state depends on cosolubilization of certain membrane lipids. While CHAPS can efficiently solubilize 5-HT$_{1A}$ receptors from bovine hippocampus in a functionally active form (Chattopadhyay & Harikumar 1996, Chattopadhyay et al. 2002), a fraction of functional receptors is lost during solubilization. This could either be due to inability of the detergent to solubilize those receptors or could be a consequence of delipidation of the receptor. Solubilization of the hippocampal 5-HT$_{1A}$ receptors by CHAPS has been shown to be accompanied by loss of membrane cholesterol (Banerjee et al. 1995, Chattopadhyay et al. 2005). Since the role of cholesterol in modulation of ligand binding and G-protein coupling of the hippocampal 5-HT$_{1A}$ receptor has been demonstrated earlier (Pucadyil and Chattopadhyay 2004b, 2004c, Paila et al. 2005), it is possible that the apparent loss in activity of the solubilized receptor could be due to loss of cholesterol. This proposal has recently been tested by incorporating cholesterol in bovine hippocampal membranes solubilized in presence of CHAPS and NaCl. Interestingly, replenishment of membrane cholesterol to solubilized bovine hippocampal membranes resulted in an increase in ligand binding of the 5-HT$_{1A}$ receptor (Chattopadhyay et al. 2005). This reinforces the importance of the membrane lipid environment in function of membrane proteins.

These results on the role of cholesterol in the 5-HT$_{1A}$ receptor function 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. 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. 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 & 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 neuronal membranes such as the bovine hippocampal membranes therefore assumes relevance for a comprehensive understanding of brain function.

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 (Julius 1998), and the observation that several diagnosed brain diseases are attributed to altered lipid-protein interactions (Pavlidis et al. 1994).

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