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Cholesterol modulates the antagonist-binding function of hippocampal serotonin$_{1A}$ receptors

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Abstract

The serotonin$_{1A}$ receptor is the most extensively studied member of the family of seven transmembrane domain G-protein coupled serotonin receptors. 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. Since a significant portion of the protein lies embedded in the membrane and the ligand-binding pocket is defined by the transmembrane stretches in such receptors, membrane composition and organization represent a crucial parameter in the structure–function analysis of G-protein coupled receptors. In this paper, we have monitored the role of membrane cholesterol in the ligand-binding function of the hippocampal serotonin$_{1A}$ receptor. Our results demonstrate that the reduction of membrane cholesterol significantly attenuates the antagonist-binding function of the serotonin$_{1A}$ receptor. Based on prior pharmacological knowledge regarding the requirements for the antagonist to bind the receptor, our results indicate that membrane cholesterol modulates receptor function independently of its ability to interact with G-proteins. These effects on ligand-binding function of the receptor are predominantly reversed upon cholesterol-replenishment of cholesterol-depleted membranes. When viewed in the light of our earlier results on the effect of cholesterol depletion on the serotonin$_{1A}$ receptor/G-protein interaction, these results comprehensively demonstrate the importance of cholesterol in the serotonin$_{1A}$ receptor function and form the basis for understanding lipid–protein interactions involving this important neuronal receptor.

Keywords: 5-HT$_{1A}$ receptor; Hippocampus; 5-HT$_{1A}$ receptor antagonist; Cholesterol; Methyl-β-cyclodextrin

1. Introduction

Lipid–protein interactions play a crucial role in maintaining the structure and function of biological membranes [1]. Since most of the membrane functions are mediated by membrane proteins, 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. Cholesterol is an abundant and essential component of eukaryotic membranes, and is often found distributed non-randomly in domains in biological and model membranes [2,3]. Many of these domains are believed to be important for the maintenance of membrane structure and function. The effect of cholesterol on the function of integral membrane proteins has been a subject of intense investigation [4]. Additionally, cholesterol–phospholipid interactions have been shown to induce considerable effects on membrane physical properties [5,6]. In view of the importance of cholesterol in the organization, dynamics and function of eukaryotic membranes [2,7,8] which regulate membrane protein function [4], the interaction of cholesterol with membrane proteins represents an

Abbreviations: BCA, bicinchoninic acid; DMPC, dimyristoyl-*sn*-glycero-3-phosphocholine; 5-HT, 5-hydroxytryptamine; 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; PMSF, phenylmethylsulfonyl fluoride

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important determinant in the functional studies of such proteins.

The brain is abundant in membranous structures and is enriched in cholesterol. Thus, the central nervous system which accounts for only 2% of the body mass contains ~25% of free cholesterol present in the whole body [9,10]. However, the distribution and metabolism of brain cholesterol is still poorly understood [11]. Brain cholesterol is synthesized in situ with no available evidence for the transfer of cholesterol from blood plasma to brain [9,10]. The interaction between this exclusive pool of brain cholesterol and other molecular components (such as neuronal receptors) therefore assumes relevance for a comprehensive understanding of brain function.

Serotonin (5-hydroxytryptamine or 5-HT) is an intrinsically fluorescent [12] biogenic amine which acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous system [13]. 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 [14–17]. 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 [15,18–20]. Serotonin exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups [21]. Serotonin receptors are members of a superfamily of seven transmembrane domain receptors [22] that couple to GTP-binding regulatory proteins (G-proteins) [23]. Among the 14 subtypes of serotonin receptors, the G-protein coupled serotonin$_{1A}$ (5-HT$_{1A}$) receptor subtype is the most extensively studied for a number of reasons. These include the availability of specific ligands that bind the receptor with high affinity, successful cloning and heterologous expression of the receptor, availability of polyclonal antibodies, and most importantly, the significance of 5-HT$_{1A}$ receptor function to neuronal physiology ([24] and references therein). The 5-HT$_{1A}$ receptor has been shown to have a role in neural development [25,26] and protection of stressed neuronal cells undergoing degeneration and apoptosis [27]. Importantly, 5-HT$_{1A}$ receptor antagonists represent a major class of molecules with potential therapeutic effects in anxiety- or stress-related disorders [28]. Furthermore, 5-HT$_{1A}$ receptor levels have been shown to be increased in schizophrenia [29] and major depression [30]. Interestingly, several epidemiological studies have correlated low serum cholesterol concentration with an increase in major depressive disorder (MDD)-like symptoms and the prevalence of suicide in humans (reviewed in [31]). Such behavioral symptoms have earlier been partially attributed to disruptions in serotonergic signaling as a result of an alteration in serotonin receptor function [32]. A detailed analysis of the effects of modulating the membrane cholesterol content on the function of such receptors would provide strong and direct evidence to support this possibility.

We have previously explored the effects of cholesterol depletion on the function of 5-HT$_{1A}$ receptors endogenously present in native hippocampal membranes and demonstrated the crucial requirement of cholesterol for the agonist, 8-OH-DPAT, to bind the receptor [33]. Since the binding of the agonist 8-OH-DPAT requires a functional interaction between the receptor and G-protein [34–37], these results indicated that membrane cholesterol is crucial in sustaining this interaction. Unlike the agonist 8-OH-DPAT, the ability of the receptor to bind the neutral antagonist *p*-MPPF does not depend on the interaction between the receptor and G-protein [36,38–40]. In order to monitor whether cholesterol depletion would affect receptor function independent of its ability to interact with G-proteins, we report here the effect of cholesterol depletion on the antagonist *p*-MPPF binding to 5-HT$_{1A}$ receptors in native hippocampal membranes.

2. Materials and methods

2.1. Materials

BCA, cholesterol, MβCD, *p*-MPPI, iodoacetamide, PMSF and polyethylenimine were obtained from Sigma Chemical Co. (St. Louis, MO). Amplex Red cholesterol assay kit was from Molecular Probes (Eugene, OR). BCA reagent kit for protein estimation was from Pierce (Rockford, IL). The antagonist [^{3}H]*p*-MPPF (sp. activity = 70.5 Ci/mmol) was purchased from DuPont New England Nuclear (Boston, MA). GF/B glass microfiber filters were from Whatman International (Kent, UK). All other chemicals used were of the highest purity available. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout. Fresh bovine brains were obtained from a local slaughterhouse within 10 min of death and the hippocampal region was carefully dissected out. The hippocampi were immediately flash frozen in liquid nitrogen and stored at −70 °C till further use.

2.2. Methods

2.2.1. Preparation of native hippocampal membranes

Native hippocampal membranes were prepared as described previously [41]. Bovine hippocampal tissue (~100 g) was homogenized as 10% (w/v) in a polytron homogenizer in 2.5 mM Tris, 0.32 M sucrose, 5 mM EDTA, 5 mM EGTA, 0.02% sodium azide, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4 buffer. The homogenate was centrifuged at 900×*g* for 10 min at 4 °C. The resultant supernatant was filtered through four layers of cheesecloth and centrifuged at 50,000×*g* for 20 min at 4 °C. The pellet thus obtained was suspended in 10 vol. of 50 mM Tris, 1 mM EDTA, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4 buffer, using a hand-held Dounce homogenizer and centri-

fuged at 50,000×*g* for 20 min at 4 °C. This procedure was repeated until the supernatant was clear. The final pellet (native membranes) was suspended in a minimum volume of 50 mM Tris, pH 7.4 buffer, homogenized using a hand-held Dounce homogenizer, flash frozen in liquid nitrogen and stored at −70 °C. Protein concentration was assayed using the BCA assay kit [42].

2.2.2. Modulation of the cholesterol content of native membranes

Cholesterol content of native hippocampal membranes was modulated (depleted or replenished) using MβCD or MβCD–cholesterol complex as described previously [33]. Briefly, membranes resuspended at a protein concentration of 2 mg/ml were treated with different concentrations of MβCD in 50 mM Tris, pH 7.4 buffer, at room temperature (25 °C) with constant shaking for 1 h. Membranes were then spun down at 50,000×*g* for 5 min at 4 °C, washed and resuspended in the same buffer. Cholesterol-depleted hippocampal membranes were replenished with cholesterol using the water-soluble cholesterol–MβCD complex. The complex was prepared by dissolving the required amounts of cholesterol and MβCD in a ratio of 1:10 (mol/mol) respectively in 50 mM Tris, pH 7.4 buffer, by constant shaking at room temperature (25 °C). Stock solutions (typically 4:40 mM of cholesterol/MβCD) of this complex were freshly prepared before each experiment. Cholesterol replenishment was carried out at a protein concentration of 2 mg/ml by incubating the cholesterol-depleted membranes with various concentrations of the cholesterol–MβCD complex for 1 h in 50 mM Tris, pH 7.4 buffer, at room temperature (25 °C) under constant shaking. Membranes were then spun down at 50,000×*g* for 5 min at 4 °C, washed with 50 mM Tris pH 7.4 buffer and resuspended in the same buffer. Cholesterol was estimated using the Amplex Red cholesterol assay kit [43].

2.2.3. Estimation of inorganic phosphate

Concentration of lipid phosphate was determined subsequent to total digestion by perchloric acid [44] using Na_2HPO_4 as standard. DMPC was used as an internal standard to assess lipid digestion. Samples without perchloric acid digestion produced negligible readings.

2.2.4. Antagonist binding assays

Antagonist binding assays were carried out as described earlier with some modifications [36]. Tubes in duplicate with 0.5 mg protein in a total volume of 1 ml of 50 mM Tris, 1 mM EDTA, pH 7.4 buffer, were used for antagonist binding assay. Tubes were incubated with the radiolabeled antagonist [^{3}H]*p*-MPPF (final concentration in assay tube was 0.5 nM) for 1 h at room temperature (25 °C). Non-specific binding was determined by performing the assay in the presence of 10 μM *p*-MPPI. The binding reaction was terminated by rapid filtration under vacuum in a Brandel cell harvester (Gaithersburg, MD, USA) through Whatman GF/B 2.5 cm diameter glass microfiber filters (1.0 μm pore size) which were presoaked in 0.15% polyethylenimine for 1 h [45]. Filters were then washed 3 times with 3 ml of cold water (4 °C), dried and the retained radioactivity was measured in a Packard Tri-Carb 1500 liquid scintillation counter using 5 ml of scintillation fluid.

2.2.5. Competition binding assays with the antagonist

Competition binding assays against the radiolabeled antagonist [^{3}H]*p*-MPPF (0.5 nM) were carried out in the presence of a range of concentrations (10^{-12} to 10^{-5} M) of the unlabeled competitor *p*-MPPI. Non-specific binding was measured in the presence of 10 μM *p*-MPPI. The concentrations of *p*-MPPI leading to 50% inhibition of specific antagonist binding (IC_{50}) were calculated by non-linear regression fitting of the data to a four-parameter logistic function [46]:

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

where B is the specific binding (total-non-specific binding) of the antagonist (in fmol/mg of total membrane protein) and was calculated by independently measuring the counts per minute (cpm) of an aliquot of a standard stock solution of the radiolabeled antagonist [^{3}H]*p*-MPPF, x denotes concentration of *p*-MPPI, a is the range ($y_{max} - y_{min}$) of the fitted curve on the ordinate (y-axis), I is the IC_{50} concentration, b is the background of the fitted curve (y_{min}) and S is the slope factor. Binding parameters, namely dissociation constant (K_d) and maximum binding sites (B_{max}), were calculated from the following equations as previously described [47]:

$$K_d = IC_{50} - L \tag{2}$$

$$B_{max} = B \times (IC_{50}/L) \tag{3}$$

where L is the concentration of the radiolabeled antagonist (0.5 nM) used in the assay and B is the specific binding of the antagonist in absence of the competitor but in the presence of 0.5 nM radiolabeled antagonist. Statistical analysis was carried out using the Student's (two tailed and unpaired) *t*-test with Graphpad Prism software version 4.00 (San Diego, CA).

3. Results

3.1. Cholesterol depletion alters antagonist binding to serotonin$_{1A}$ receptors

Cholesterol depletion of hippocampal membranes was carried out using the water-soluble compound MβCD which has previously been shown to selectively and efficiently extract cholesterol from membranes [48]. Fig. 1A shows the effect of MβCD treatment on the cholesterol and phospho-

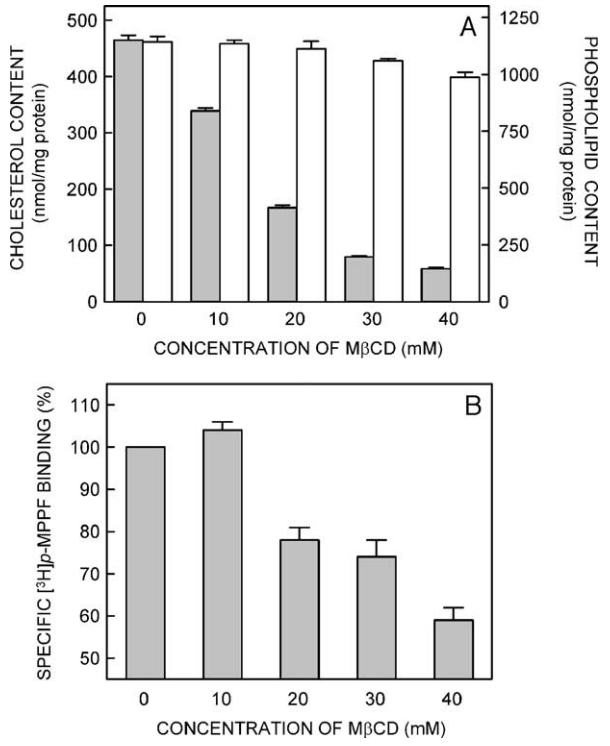

Fig. 1. Effect of increasing concentrations of MβCD on (A) cholesterol (gray bars) and phospholipid (white bars) contents of hippocampal membranes, and (B) specific [^{3}H]*p*-MPPF binding to 5-HT$_{1A}$ receptors in these membranes. Values in (B) are expressed as percentages of specific [^{3}H]*p*-MPPF binding for native membranes without MβCD treatment. All data represent means ± S.E. of at least three independent experiments. See Materials and methods for other details.

lipid concentrations in the membrane. Thus, treatment of membranes with 40 mM MβCD results in ~87% reduction in the cholesterol content. Importantly, the change in the phospholipid content under identical conditions is negligible even when 40 mM MβCD is used and in all cases, ~87% of phospholipids remained in the membrane after MβCD treatment. The cholesterol to phospholipid molar ratio in native membranes is 0.41 which reduces by ~85% to 0.06 in 40 mM MβCD-treated membranes. These results highlight the ability of MβCD in specifically catalyzing cholesterol efflux from membranes. We have earlier reported that the treatment of hippocampal membranes with a similar range of concentrations of MβCD (up to 40 mM) results in a decrease in the fluorescence polarization values of membrane-embedded probes which is proportional to the cholesterol content in these membranes [33]. Since fluorescence polarization is an overall indicator of the physical state of membranes, such linear changes in the polarization values of these probes in hippocampal membranes treated with MβCD at concentrations up to 40 mM ensure that the overall morphology of the membrane remains intact and would rule out the possibility of gross changes in membrane architecture.

Although selective 5-HT$_{1A}$ agonists such as 8-OH-DPAT have been discovered a number of years back [34,35], 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 analogue *p*-MPPF has been introduced later [38,49]. These compounds bind specifically to the 5-HT$_{1A}$ receptor with high affinity. Fig. 1B shows the reduction in the specific [^{3}H]*p*-MPPF binding with increasing concentrations of MβCD. This shows that removal of cholesterol from hippocampal membranes results in loss of specific binding of the antagonist [^{3}H]*p*-MPPF. Interestingly, the reduction in the antagonist binding does not show a linear dependence with the extent of cholesterol depletion (see Fig. 1A and B). Thus, a ~20% decrease in cholesterol content (using 10 mM MβCD) does not significantly affect the antagonist binding. However, reduction in the antagonist binding is significant when the cholesterol content is decreased further (using 20 mM MβCD or higher). The specific antagonist binding is reduced by ~41% of the original binding (observed without MβCD treatment) when 40 mM MβCD is used for cholesterol depletion.

We previously reported that depletion of membrane cholesterol reduces the specific 5-HT$_{1A}$ receptor agonist-binding function and correlated this effect to alterations in receptor/G-protein interaction as a consequence of cholesterol depletion [33]. It has been shown previously that the agonist 8-OH-DPAT and the antagonist *p*-MPPF display different sensitivities to guanine nucleotides and differentially discriminate the hippocampal 5-HT$_{1A}$ receptors based on its state of G-protein coupling [36,39]. Thus, while the agonist 8-OH-DPAT binds only to receptors coupled to G-proteins, the antagonist *p*-MPPF binds to both populations of the receptor (coupled and uncoupled to G-proteins). Our present results show that cholesterol depletion reduces the antagonist binding to 5-HT$_{1A}$ receptors although the extent of reduction is somewhat less than the corresponding reduction in agonist binding observed under similar conditions [33]. Taken together, these results point out that depletion of membrane cholesterol affects the 5-HT$_{1A}$ receptor function independent of its interaction with G-proteins.

We analyzed the nature and extent of reduction in antagonist binding to 5-HT$_{1A}$ receptors in cholesterol-depleted membranes by assessing ligand-binding parameters. Fig. 2 shows the competition binding analysis of specific [^{3}H]*p*-MPPF binding to the 5-HT$_{1A}$ receptor from bovine hippocampal membranes treated with MβCD. This method of analysis was preferred over a saturation binding analysis due to the high non-specific binding observed with cholesterol-depleted membranes when high concentrations of [^{3}H]*p*-MPPF are used in the assay. Importantly, we have previously observed a close agreement between the binding parameters for the specific agonist and antagonist binding to the 5-HT$_{1A}$ receptor obtained from competition binding and saturation binding assays [50]. The binding parameters obtained using the competition binding analysis are shown in Table 1. The table shows that when 40 mM MβCD is used for cholesterol depletion, there is a significant (~31%)

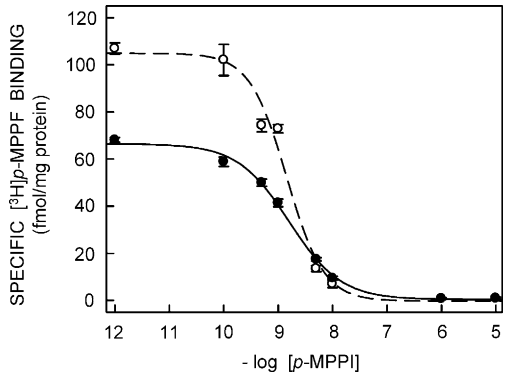

Fig. 2. Competition binding analysis of specific [^{3}H]*p*-MPPF binding to 5-HT_{1A} receptors in hippocampal membranes treated with MβCD. The plots correspond to the specific [^{3}H]*p*-MPPF binding with increasing concentrations of unlabeled competitor *p*-MPPI to control (- - - ○ - - -) and 40 mM MβCD treated (—●—) membranes and are the means±S.E. of four independent experiments. The curves are non-linear regression fits to the experimental data using Eq. (1). See Materials and methods and Table 1 for other details.

reduction in the maximum number of binding sites (B_{max}) with a minimal reduction in affinity (K_d) of the antagonist.

3.2. Cholesterol replenishment restores antagonist-binding activity of serotonin$_{1A}$ receptors

In order to confirm the reversibility of the changes induced by cholesterol depletion on the antagonist-binding function of 5-HT_{1A} receptors, cholesterol replenishment was carried out by incubating the depleted membranes with cholesterol–MβCD complex (see Materials and methods). As shown in Fig. 3A, this procedure is able to replenish bulk of the cholesterol to the depleted membranes as is evident from the cholesterol content of the replenished membranes. The efficiency of the cholesterol replenishment process (and also effect on the antagonist-binding activity, see Fig. 3B) is found to depend on the concentration of the cholesterol–MβCD complex used even when the ratio of cholesterol to MβCD is maintained constant at 1:10 (mol/mol). The optimum efficiency of loading cholesterol back to depleted membranes is found with 1 mM cholesterol complexed with 10 mM MβCD. Thus, ~72% of the original cholesterol content could be restored when 1 mM cholesterol complexed with 10 mM MβCD is used. Interestingly, use of higher concentrations of the complex (1.5:15 and 2:20 of cholesterol/MβCD) did not show a further increase in the efficiency of cholesterol replenishment (Fig. 3A) and antagonist-binding activity of 5-HT_{1A} receptors (Fig. 3B). It is possible that the high concentrations of MβCD (15 and 20 mM) present in the complex promotes further efflux of the replenished cholesterol so that the effective cholesterol content in the membrane does not exceed ~72% of the original cholesterol content. Importantly, under optimal conditions of cholesterol replenishment (using 1:10 mM cholesterol/MβCD complex), the antagonist binding activity recovers to ~81% of the original activity. These results show that the antagonist-binding function of hippocampal 5-HT_{1A} receptors is specifically and reversibly modulated by membrane cholesterol.

Table 1
Effect of cholesterol depletion on specific [^{3}H]*p*-MPPF binding parameters[a]

Treatment	K_d (nM)	B_{max} (fmol/mg protein)
Control	0.94±0.05	301.8±7.6*
40 mM MβCD	1.08±0.09	208.4±10*

[a] Binding parameters were calculated by analyzing competition binding experiments against the radiolabeled antagonist [^{3}H]*p*-MPPF in the presence of a range of concentrations (10^{-12} to 10^{-5} M) of the unlabeled competitor *p*-MPPI. The data represent the means±S.E. of four independent experiments. See Materials and methods and Fig. 2 for other details.

* These means are significantly ($P<0.05$) different.

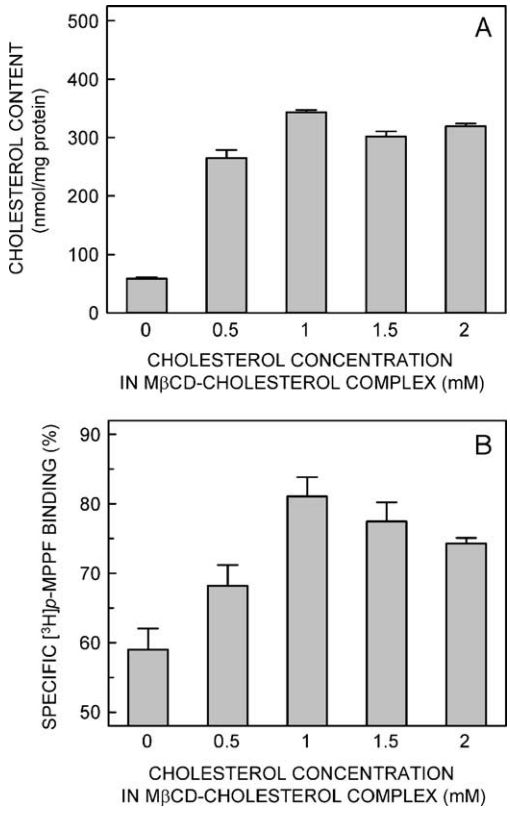

Fig. 3. Cholesterol replenishment of bovine hippocampal membranes treated with MβCD and its effect on (A) cholesterol content, and (B) specific [^{3}H]*p*-MPPF binding activity of hippocampal 5-HT_{1A} receptors. Panel A shows the cholesterol content when cholesterol-depleted membranes (using 40 mM MβCD) were treated with cholesterol–MβCD complex at various concentrations (indicated by the concentration of cholesterol in the complex). Panel B shows the accompanying effect of cholesterol replenishment on the specific [^{3}H]*p*-MPPF binding to membranes. Values are expressed as percentages of specific [^{3}H]*p*-MPPF binding in native membranes without any treatment. All data represent means±S.E. of at least three independent experiments. See Materials and methods for other details.

4. Discussion

The 5-HT_{1A} receptor belongs to the family of G-protein-coupled receptors. These receptors constitute the single largest family of cell surface molecules involved in the transmission of information across a cell membrane from the extracellular environment to the interior of the cell [22]. According to current estimates, about 1% of the mammalian genome codes for G-protein-coupled receptors [51]. These receptors are involved in the regulation of diverse physiological processes such as neurotransmission, cellular metabolism, secretion, cellular differentiation and growth and inflammatory and immune responses. G-protein-coupled receptors therefore represent major targets for the development of novel drug candidates in all clinical areas [52]. It is estimated that up to 50% of clinically prescribed drugs function as either agonists or antagonists of G-protein-coupled receptors which points to their immense therapeutic potential [53]. The membrane lipid composition and organization represents an important and necessary parameter in the structure–function analysis of G-protein coupled receptors [54]. This is particularly important with regard to G-protein coupled receptors such as the rhodopsin and the 5-HT_{1A} receptor since a large portion of the protein is embedded in the membrane [24,54,55] and the ligand binding pocket in these receptors is defined by the transmembrane portions of the protein (reviewed in [24]). While considerable amount of literature exists on lipid–protein interactions, specifically cholesterol–protein interaction, that modulates rhodopsin function [56], such literature for the 5-HT_{1A} receptor is sparse. Nevertheless, several clinical studies have established a correlation between symptoms arising due to alterations in the serum cholesterol content to those involving a disruption in the serotonergic signaling pathway [31].

We have explored the effects of specific and controlled modulation of the cholesterol content of native hippocampal membranes on the ligand-binding function of the 5-HT_{1A} receptor present endogenously in these membranes. We have earlier reported that the 5-HT_{1A} receptor exhibits a cholesterol-dependent interaction with G-proteins in the membrane [33]. This was based on prior knowledge regarding the requirement of receptor/G-protein interaction for agonist binding to the 5-HT_{1A} receptor [36], and analyzing the effects of cholesterol depletion on the agonist-binding property of the receptor. Our present results indicate that alterations in membrane cholesterol content can significantly affect the antagonist-binding activity of the hippocampal 5-HT_{1A} receptor. As mentioned earlier, the interaction between the receptor and G-proteins is not a prerequisite for the antagonist *p*-MPPF to bind the receptor [36]. The effects of cholesterol depletion on the 5-HT_{1A} receptor function, when assessed using the antagonist, therefore suggest an alteration in the structure/organization of the receptor in the membrane and not merely an alteration in the receptor/G-protein interaction.

Interestingly, the effect of cholesterol depletion on the agonist [33] and antagonist binding of 5-HT_{1A} receptors (see Fig. 1B) is not directly proportional to the extent of cholesterol depletion. Thus, while the use of higher concentrations of MβCD does not lead to proportional decreases in cholesterol concentrations, greater inhibition in ligand binding is observed with higher concentrations of MβCD. Importantly, cholesterol is often found non-randomly distributed in domains or pools in natural membranes and the process of cholesterol depletion by MβCD is reported to be asymmetric with respect to the amount extracted from these pools [57,58]. Thus, although the actual levels of cholesterol in membranes treated with higher concentrations of MβCD (such as 30 and 40 mM) may not be very different, the asymmetric extents to which cholesterol from such pools are depleted could lead to such marked effects on functional attributes (ligand binding) of the receptor.

The effect of cholesterol on the structure and function of integral membrane proteins has been a subject of intense investigation [4]. It has been proposed that such effects could occur either due to a specific local molecular interaction with membrane proteins [59], or due to alterations in the membrane physical properties induced by the presence of cholesterol [1,5,6], or due to a combination of both factors. For G-protein coupled receptors, previous literature indicates that cholesterol can modulate receptor function in a manner independent of its effects on receptor/G-protein interaction. In case of rhodopsin, the conversion of the photointermediates metarhodopsin I to metarhodopsin II upon exposure to light involves an expansion of the protein in the plane of the bilayer which occupies the available partial free volume from the surrounding bilayer. The presence of cholesterol in the membrane has been reported to inhibit the formation of metarhodopsin II due to its role in reducing the partial free volume in the membrane [60]. The requirement of cholesterol to support the oxytocin receptor ligand binding function provides another example [61]. Importantly, the decrease in ligand binding function of this receptor upon progressive cholesterol depletion is not correlated with a decrease in membrane order monitored through fluorescence polarization of membrane-embedded probes due to cholesterol depletion. Based on these and other results, a specific interaction between the oxytocin receptor and cholesterol in the membrane has been proposed to be necessary for receptor ligand binding function [61]. These results clearly demonstrate that receptor structure/conformation is modulated by the presence of cholesterol in the membrane. Whether the reduction in the antagonist-binding function of the 5-HT_{1A} receptor upon cholesterol depletion results in an alteration in receptor conformation due to a change in the membrane physical properties (such as for rhodopsin) or due to the loss of specific cholesterol–protein interaction (such as for oxytocin receptor) poses an interesting question.

Taken together, our results demonstrate that pharmacologically well-characterized ligands, capable of distinguishing alternate forms of the receptor (G-protein coupled and/or uncoupled), could be useful to delineate possible mechanisms by which membrane lipids modulate the function of the 5-HT_{1A} receptor. In a broader context, these results are relevant in understanding the role of the membrane lipid environment on the activity and signal transduction of other G-protein coupled transmembrane receptors.

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