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*Brief Communication*

# Prolonged Treatment with Ligands Affects Ligand Binding to the Human Serotonin$_{1A}$ Receptor in Chinese Hamster Ovary Cells

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**SUMMARY**

1. The serotonin$_{1A}$ receptors are members of a superfamily of seven transmembrane domain receptors that couple to G-proteins, and appear to be involved in several behavioral and cognitive functions.

2. We monitored the effect of prolonged treatment of the human serotonin$_{1A}$ receptor expressed in Chinese hamster ovary (CHO) cells with pharmacologically well-characterized ligands on its binding to the agonist 8-hydroxy-2(di-*N*-propylamino)tetralin (8-OH-DPAT) and antagonist 4-(2′-methoxy)-phenyl-1-[2′-(*N*-2″-pyridinyl)-*p*-fluorodobenzamido]ethyl-piperazine (*p*-MPPF).

3. Our results indicate that prolonged treatment with the specific agonist (8-OH-DPAT) differentially affects subsequent binding of the agonist and antagonist to the receptor in a manner independent of receptor-G-protein coupling. Importantly, our results show that prolonged treatment with the commonly used antagonist *p*-MPPF, and its iodinated analogue 4-(2′-methoxy)-phenyl-1-[2′-(*N*-2″-pyridinyl)-*p*-iodobenzamido]ethyl-piperazine (*p*-MPPI), which have earlier been reported to display similar binding properties to serotonin$_{1A}$ receptors, induces significantly different effects on the ligand binding function of serotonin$_{1A}$ receptors.

**KEY WORDS:** serotonin$_{1A}$ receptor; agonist; antagonist; inverse agonist; G-protein coupling; desensitization.

## INTRODUCTION

Serotonin (5-hydroxytryptamine or 5-HT) acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous systems (Jacobs and Azmitia, 1992). Serotonergic signaling appears to play a key role in the generation and modulation of various cognitive and behavioral functions such as sleep, mood,

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pain, addiction, locomotion, sexual activity, depression, anxiety, alcohol abuse, aggression and learning (Artigas *et al*., 1996; Ramboz *et al*., 1998; Rocha *et al*., 1998; Meneses, 1999; Gross *et al*., 2002; Adayev *et al*., 2005). Disruptions in serotonergic systems have been implicated in the etiology of mental disorders such as schizophrenia, migraine, depression, suicidal behavior, infantile autism, eating disorders, and obsessive-compulsive disorder (Ramboz *et al*., 1998; Heisler *et al*., 1998; Parks *et al*., 1998; Sarnyai *et al*., 2000). The G-protein coupled serotonin$_{1A}$ receptor subtype represents the most well studied member among the subtypes of serotonin receptors (Raymond *et al*., 1999; Pucadyil *et al*., 2005), and its functional significance is apparent from the enhanced anxiety-related behavior exhibited by knockout mice lacking the receptor (Ramboz *et al*., 1998; Heisler *et al*., 1998; Parks *et al*., 1998). As a result, these mice represent important animal models for the analysis of complex traits such as anxiety and aggression in higher organisms (Gingrich and Hen, 2001).

Desensitization is a phenomenon whereby receptors exhibit reduction or loss of response upon prolonged activation by ligands, thereby acting as a feedback mechanism in terminating the cellular response generated by a prolonged stimulus (Ochoa *et al*., 1989; Lohse, 1993; Gainetdinov *et al*., 2004). The desensitization of serotonin$_{1A}$ receptors assumes significance due to the role of this receptor in neuronal physiology, and its involvement in the action of a variety of clinically prescribed drugs. Long-term treatment with serotonin$_{1A}$ receptor agonists and antagonists constitutes a powerful therapeutic approach in anxiety- or stress-related disorders (Blier *et al*., 1990; Griebel, 1999), and in developmental disorders (Azmitia, 2001). Further, several antidepressants increase the concentration of serotonin present in synapses by blocking serotonin transporters and autoreceptors leading to prolonged exposure of the receptor to serotonin (Hensler, 2003). The analysis of the effect of prolonged ligand exposure on the serotonin$_{1A}$ receptor function therefore assumes importance. This forms the basis of earlier reports which have analyzed the effect of long-term treatment with agonist and antagonist on the serotonin$_{1A}$ receptor ligand binding and signaling (Harrington *et al*., 1994; Hensler *et al*., 1996; Cowen *et al*., 1997; Hensler and Durgam, 2001; Hensler, 2003). While these studies have provided valuable insight into the possible mechanisms on the desensitization of the serotonin$_{1A}$ receptor upon long-term treatment with ligands, analysis of the relative binding ability of the serotonin$_{1A}$ receptors in the same cell type after prolonged ligand treatment to specific agonist and antagonist remains unexplored.

In this paper, we monitored the effect of prolonged treatment of the human serotonin$_{1A}$ receptor heterologously expressed in Chinese hamster ovary (CHO) cells to pharmacologically well-characterized agonist and antagonists specific for the serotonin$_{1A}$ receptor. Importantly, our results indicate that treatment with the specific agonist (8-OH-DPAT) differentially affects subsequent binding of the agonist 8-OH-DPAT and antagonist *p*-MPPF to the receptor in a manner independent of receptor-G-protein coupling. More importantly, our results show that prolonged treatment with the commonly used antagonist *p*-MPPF, and its iodinated analogue *p*-MPPI, which have earlier been reported to display similar binding properties (Thielen *et al*., 1996; Barr and Manning, 1997; Kalipatnapu *et al*., 2004) and antagonize the action of serotonin$_{1A}$ receptor specific agonists in animal models (Thielen and Frazer, 1995; Thielen *et al*., 1996), induces significantly different effects on ligand binding to serotonin$_{1A}$ receptors.

## MATERIALS AND METHODS

### Materials

Fetal calf serum, 8-OH-DPAT, *p*-MPPI, *p*-MPPF, penicillin, streptomycin, gentamycin sulfate, phenylmethylsulfonyl fluoride (PMSF), serotonin and polyethylenimine were obtained from Sigma Chemical Co. (St. Louis, MO). D-MEM/F-12 (Dulbecco's Modified Eagle Medium: nutrient mixture F-12 (Ham) (1:1)) and geneticin (G 418) were from Life Technologies (Grand Island, NY). Guanosine-5′-*O*-(3-thiotriphosphate) (GTP-$\gamma$-S) was from Roche Applied Science (Mannheim, Germany). Bicinchoninic acid (BCA) reagent kit for protein estimation was from Pierce (Rockford, IL). [$^3$H]8-OH-DPAT (sp. activity = 135.0 Ci/mmol) and [$^3$H]*p*-MPPF (sp. activity = 70.5 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA). GF/B glass microfiber filters were obtained 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.

### Cell Culture and Prolonged Ligand Treatment of Serotonin$_{1A}$ Receptors

CHO cells stably expressing the human serotonin$_{1A}$ receptor have been described earlier (Kalipatnapu *et al.*, 2004). These cells were maintained in D-MEM/F-12 (1:1) supplemented with 2.4 g/L of sodium bicarbonate, 10% fetal calf serum, 60 $\mu$g/mL penicillin, 50 $\mu$g/mL streptomycin, 50 $\mu$g/mL gentamycin sulfate, and 200 $\mu$g/mL geneticin in a humidified atmosphere with 5% $CO_2$ at 37°C. Prolonged treatment of the human serotonin$_{1A}$ receptor expressed in these cells with receptor-specific ligands was carried out by incubating cells for 18 h in medium containing either 8-OH-DPAT (10 $\mu$M), *p*-MPPF (1 $\mu$M), or *p*-MPPI (1 $\mu$M). Following this treatment, cells were rinsed with PBS and harvested for preparation of cell membranes.

### Preparation of Cell Membranes

Cell membranes were prepared as described previously (Kalipatnapu *et al.*, 2004). Confluent cells were harvested by treatment with ice-cold buffer containing 10 mM Tris, 5 mM EDTA, 0.1 mM PMSF, pH 7.4. Cells were then homogenized for 10 s at 4°C at maximum speed with a Polytron homogenizer. The cell lysate was centrifuged at 500 × *g* for 10 min at 4°C and the resulting post-nuclear supernatant was centrifuged at 40,000 × *g* for 30 min at 4°C. The pellet thus obtained (cell membranes) was suspended in 50 mM Tris buffer, pH 7.4. Total protein concentration in cell membranes was determined using BCA reagent (Smith *et al.*, 1985).

### Radioligand Binding Assays

Receptor binding assays were carried out as described earlier (Kalipatnapu *et al.*, 2004). Briefly, tubes in duplicate containing cell membranes were incubated

in a total volume of 1 mL of buffer A (50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM $MnCl_2$, pH 7.4) for agonist binding assays, or in 1 mL of buffer B (50 mM Tris, 1 mM EDTA, pH 7.4) for antagonist binding assays. Tubes were incubated with the radiolabeled agonist [$^3$H]8-OH-DPAT (final concentration = 0.29 nM) or antagonist [$^3$H]*p*-MPPF (final concentration = 0.5 nM) for 1 h at room temperature (25°C). Non-specific binding was determined by performing the assay either in the presence of 10 $\mu$M serotonin (for agonist binding assays) or in the presence of 10 $\mu$M *p*-MPPI (for antagonist binding assays). The binding reaction was terminated by rapid filtration under vacuum in a Brandel cell harvester (Gaithersburg, MD) through Whatman GF/B 2.5 cm diameter glass microfiber filters (1.0 $\mu$m pore size) which were presoaked in 0.15% (w/v) polyethylenimine for 1 h (Bruns *et al.*, 1983). The filters were then washed three times with 3 mL of cold water (4°C), dried and the retained radioactivity was measured in a Packard Tri-Carb 1500 liquid scintillation counter using 5 mL of scintillation fluid.

### GTP-$\gamma$-S Sensitivity Assay

Agonist binding assays were carried out in buffer A as described above in the presence of varying concentrations of GTP-$\gamma$-S as described previously (Harikumar and Chattopadhyay, 1999; Kalipatnapu *et al.*, 2004). The concentrations of GTP-$\gamma$-S leading to 50% inhibition of specific agonist binding ($IC_{50}$) were calculated by non-linear regression fitting of the data to a four parameter logistic function:

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

where $B$ is the specific binding of the agonist normalized to control binding (in the absence of GTP-$\gamma$-S), $x$ denotes concentration of GTP-$\gamma$-S, $a$ is the range ($y_{max}$–$y_{min}$) of the fitted curve on the ordinate ($y$-axis), $I$ is the $IC_{50}$ concentration, $b$ is the background of the fitted curve ($y_{min}$) and $S$ is the slope factor.

## RESULTS AND DISCUSSION

Chinese hamster ovary cells have previously been used to understand several pharmacological and signaling aspects of the serotonin$_{1A}$ receptor (Raymond *et al.*, 1999). We have earlier characterized CHO cells stably expressing the human serotonin$_{1A}$ receptor (Kalipatnapu *et al.*, 2004). The expression of the serotonin$_{1A}$ receptor in these cells is under the control of the Rous Sarcoma virus (RSV) long terminal repeat (Banerjee *et al.*, 1993), and the pharmacology and cellular signaling characteristics of the receptor have been shown to be essentially similar to the native hippocampal serotonin$_{1A}$ receptor (Banerjee *et al.*, 1993; Kalipatnapu *et al.*, 2004). Importantly, the radiolabeled agonist [$^3$H]8-OH-DPAT displays a maximum number of binding sites ($B_{max}$) of 1.24 pmol/mg protein while the radiolabeled antagonist [$^3$H]*p*-MPPF displays a $B_{max}$ of 3.93 pmol/mg protein in membranes isolated from these cells (Kalipatnapu *et al.*, 2004). This difference is attributed to the agonist 8-OH-DPAT preferentially binding to the G-protein coupled form of

the receptor while the antagonist *p*-MPPF binding equally well to the G-protein coupled and uncoupled forms of the receptor.

As a paradigm to understand desensitization processes involving the serotonin$_{1A}$ receptor, we analyzed the effect of prolonged exposure of ligands to CHO cells stably expressing the serotonin$_{1A}$ receptor on the ligand binding function of the receptor. The development of selective serotonin$_{1A}$ agonist (8-OH-DPAT) and antagonists (*p*-MPPI and *p*-MPPF), which bind serotonin$_{1A}$ receptors with high affinity and specificity, has contributed to our understanding of the pharmacology and signaling functions of the receptor (Gozlan *et al*., 1983; Thielen *et al*., 1996; Barr and Manning, 1997; Harikumar and Chattopadhyay, 1999). We analyzed the effect of prolonged (18 h) treatment of cells with 8-OH-DPAT and *p*-MPPF on specific agonist [$^3$H]8-OH-DPAT binding to membranes isolated from these cells. Prolonged treatment with 8-OH-DPAT leads to a significant reduction in specific binding of [$^3$H]8-OH-DPAT to isolated cell membranes (Fig. 1A). Thus, membranes isolated from 8-OH-DPAT-treated cells show ~46% reduction in binding of [$^3$H]8-OH-DPAT compared to those isolated from control cells. Interestingly, similar analysis carried out with membranes isolated from cells treated with *p*-MPPF does not show a significant effect on specific [$^3$H]8-OH-DPAT binding (Fig. 1A).

Because agonists such as 8-OH-DPAT bind to receptors coupled to G-proteins, whereas antagonists like *p*-MPPF bind to both G-protein coupled and uncoupled forms of the receptor (Harikumar and Chattopadhyay, 1999; Javadekar-Subhedar and Chattopadhyay, 2004; Pucadyil *et al*., 2005), their relative binding abilities can be used to differentially discriminate the extent of interaction between the receptor and G-proteins. To analyze the effect of prolonged ligand treatment on the total (G-protein coupled and uncoupled) serotonin$_{1A}$ receptor population, we monitored binding of the specific antagonist [$^3$H]*p*-MPPF under such conditions. As shown in Fig. 1B, specific [$^3$H]*p*-MPPF binding does not appear to be significantly different in membranes isolated from cells treated with either 8-OH-DPAT or *p*-MPPF. In fact, prolonged treatment with 8-OH-DPAT appears to induce a moderate (~23%) increase in specific [$^3$H]*p*-MPPF binding (Fig. 1B).

The analysis of receptor ligand binding upon prolonged treatment of cells with specific ligands can be complicated due to the possible effects on cellular biosynthesis of receptors. Thus, any effect on ligand binding of the receptor can be interpreted to arise from changes either in the binding affinity of the ligand or in the total number of receptors expressed in cells. On the basis of our results where prolonged treatment of cells does not appear to significantly affect specific [$^3$H]*p*-MPPF binding (which reflects the total population of serotonin$_{1A}$ receptors), the effect of agonist treatment on the binding of [$^3$H]8-OH-DPAT (Fig. 1A) could reflect desensitization of serotonin$_{1A}$ receptors by a mechanism that does not involve downregulation of receptor biosynthesis. Moreover, the trivial possibility of reduction in specific [$^3$H]8-OH-DPAT binding observed when cells are treated with 8-OH-DPAT (Fig. 1A) due to competition between the labeled and unlabeled forms of 8-OH-DPAT can be ruled out because such competition would have led to a reduction in specific binding of [$^3$H]*p*-MPPF as well, which is not the case (Fig. 1B).

Interestingly, there appears to be a lack of consensus in previous literature on the effect of prolonged agonist treatment on the binding of [$^3$H]8-OH-DPAT

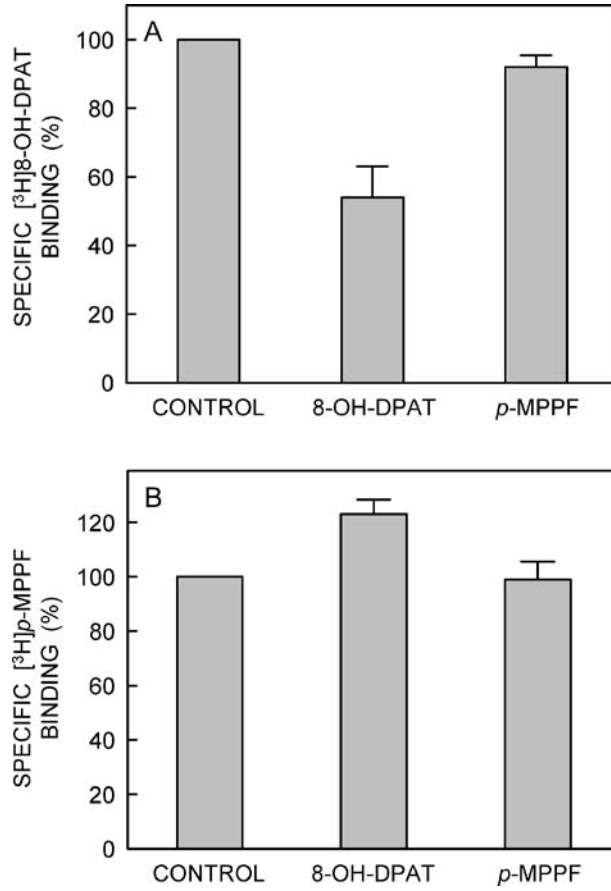


**Fig. 1.** Specific binding of the (A) agonist [$^3$H]8-OH-DPAT and (B) antagonist [$^3$H]*p*-MPPF to membranes isolated from CHO cells stably expressing the human serotonin$_{1A}$ receptor that were treated with either the agonist 8-OH-DPAT (10 $\mu$M), or the antagonist *p*-MPPF (1 $\mu$M) for 18 h. Values are expressed as percentages of specific radioligand binding obtained in membranes isolated from untreated cells (control). The data shown are the means $\pm$ SE of duplicate points from at least six independent experiments. See Materials and Methods section for other details.

to serotonin$_{1A}$ receptors. While long-term treatment of serotonin$_{1A}$ receptors expressed in HeLa cells with 8-OH-DPAT has been found to result in a loss of high-affinity agonist binding sites (Harrington *et al.*, 1994), another study with the agonist 5-carboxamidotryptamine (5-CT) showed no significant effect on binding of [$^3$H]8-OH-DPAT to serotonin$_{1A}$ receptors expressed in P11 cells (Hensler *et al.*, 1996). In this context, our analysis of the effect of prolonged treatment with 8-OH-DPAT and *p*-MPPF on binding of both the agonist [$^3$H]8-OH-DPAT and the antagonist [$^3$H]*p*-MPPF to serotonin$_{1A}$ receptors assumes relevance.

Desensitization of serotonin$_{1A}$ receptors upon prolonged treatment with agonists has earlier been speculated to occur due to alterations in receptor-G-protein coupling (Hensler and Durgam, 2001) that possibly leads to a reduction in the ability of the receptor to affect downstream signaling events (Harrington *et al.*, 1994). The serotonin$_{1A}$ receptor agonists are known to specifically activate the $G_i/G_o$ class of G-proteins (Raymond *et al.*, 1999). Therefore, agonist binding to such receptors displays sensitivity to agents that uncouple the normal cycle of guanine nucleotide exchange at the G-protein $\alpha$ subunit (such as GTP-$\gamma$-S, a non-hydrolyzable analogue of GTP) caused by activation of the receptor. We find that treatment of cells with 8-OH-DPAT leads to a reduction in specific agonist [$^3$H]8-OH-DPAT binding to cell membranes, whereas specific antagonist [$^3$H]*p*-MPPF binding displays a moderate increase (Fig. 1). We analyzed if the reduction in specific binding of [$^3$H]8-OH-DPAT upon prolonged treatment of cells with 8-OH-DPAT is due to a reduction in G-protein coupling of the serotonin$_{1A}$ receptor by monitoring the sensitivity of agonist binding to GTP-$\gamma$-S (Harikumar and Chattopadhyay, 1999). Figure 2 shows the inhibition of specific [$^3$H]8-OH-DPAT binding to serotonin$_{1A}$ receptors in the presence of GTP-$\gamma$-S in a characteristic concentration-dependent manner in membranes isolated from control and 8-OH-DPAT-treated cells. The half maximal inhibition concentration ($IC_{50}$) value for inhibition of specific [$^3$H]8-OH-DPAT binding by GTP-$\gamma$-S is ~3.3 nM for membranes isolated from control cells, similar to what we reported earlier (Kalipatnapu *et al.*, 2004). Interestingly,

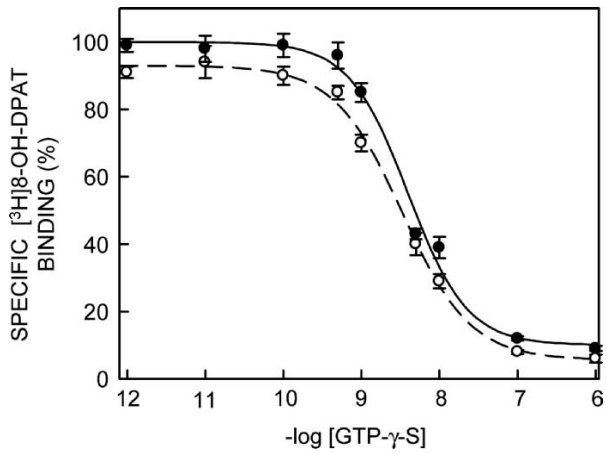


**Fig. 2.** Effect of increasing concentrations of GTP-$\gamma$-S on specific [$^3$H]8-OH-DPAT binding to human serotonin$_{1A}$ receptors in membranes isolated from control (—○—) and 8-OH-DPAT-treated (—●—) cells. The conditions for 8-OH-DPAT treatment are as described in Fig. 1. Values are expressed as percentages of the specific [$^3$H]8-OH-DPAT binding obtained in the absence of GTP-$\gamma$-S. The curves are non-linear regression fits to the experimental data using Eq. (1). Data represent the means ± SE of duplicate points from five independent experiments. See Materials and Methods section for other details.

the extent of inhibition in [$^3$H]8-OH-DPAT binding to membranes isolated from 8-OH-DPAT-treated cells by GTP-$\gamma$-S does not appear to be significantly different ($IC_{50}$ value of $\sim$3.8 nM). On the basis of the sensitivity of [$^3$H]8-OH-DPAT binding to serotonin$_{1A}$ receptors to GTP-$\gamma$-S, we conclude that G-protein coupling of the receptor does not display any significant change upon prolonged treatment with the agonist 8-OH-DPAT. Our results therefore suggest the possibility of an alternate mechanism for the observed loss in [$^3$H]8-OH-DPAT binding to membranes isolated from 8-OH-DPAT-treated cells.

Earlier reports have described that the binding parameters of *p*-MPPI to serotonin$_{1A}$ receptors appear to be similar to that of *p*-MPPF (Thielen *et al.*, 1996; Barr and Manning, 1997; Kalipatnapu *et al.*, 2004). Moreover, these two ligands have earlier been found to antagonize the response generated upon in vivo administration of the serotonin$_{1A}$ receptor specific agonist 8-OH-DPAT in animal models (Thielen and Frazer, 1995). Interestingly, based on the ability of ligands to reduce constitutive activity of serotonin$_{1A}$ receptors expressed in *Spodoptera frugiperda* cells, *p*-MPPF has been categorized as a neutral antagonist, whereas *p*-MPPI has been shown to display partial inverse agonist activity on serotonin$_{1A}$ receptors (Barr and Manning, 1997). To analyze if *p*-MPPI indeed differs from *p*-MPPF from the perspective of desensitization of serotonin$_{1A}$ receptors, we monitored the effect of prolonged treatment with *p*-MPPI on ligand binding ability of serotonin$_{1A}$ receptors. As shown in Fig. 3, ligand binding to serotonin$_{1A}$ receptors in membranes isolated from cells treated with *p*-MPPI for 18 h show a dramatically different result compared to what

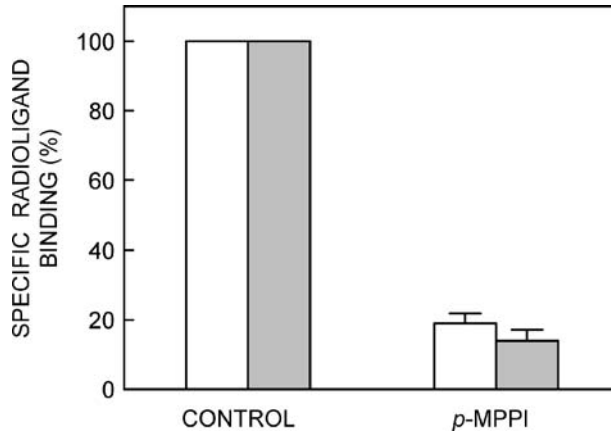


**Fig. 3.** Specific binding of the agonist [$^3$H]8-OH-DPAT (white bars) and antagonist [$^3$H]*p*-MPPF (gray bars) to membranes isolated from CHO cells stably expressing the human serotonin$_{1A}$ receptor that were treated with *p*-MPPI (1 $\mu$M) for 18 h. Values are expressed as percentages of specific radioligand binding obtained in membranes isolated from untreated cells (control). Data represent the means $\pm$ SE of duplicate points from at least six independent experiments. See Materials and Methods section for other details.

was observed when cells were treated with the antagonist *p*-MPPF (Fig. 1). Thus, membranes isolated from *p*-MPPI-treated cells display a marked (>80%) reduction in specific binding of the agonist [$^3$H]8-OH-DPAT and antagonist [$^3$H]*p*-MPPF (Fig. 3). To the best of our knowledge, this is the first report describing the effect of prolonged treatment of serotonin$_{1A}$ receptors to *p*-MPPI in terms of binding of the specific agonist [$^3$H]8-OH-DPAT and antagonist [$^3$H]*p*-MPPF. An earlier report, which monitored serotonin$_{1A}$ receptor function by analyzing binding of [$^{125}$I]*p*-MPPI, described an upregulation in serotonin$_{1A}$ receptor number in CHO cells after long-term exposure of cells to *p*-MPPI (Cowen *et al.*, 1997). This increase in receptor number was attributed to an increase in transcriptional activity of the cytomegalovirus (CMV) promoter that regulated expression of the serotonin$_{1A}$ receptor in these cells. On the basis of the criteria of specific binding of the classical agonist [$^3$H]8-OH-DPAT and antagonist [$^3$H]*p*-MPPF, our results show a contrary trend. Importantly, our results on the reduction in ligand binding to serotonin$_{1A}$ receptors upon *p*-MPPI treatment (Fig. 3) bring out a previously unappreciated difference in the long-term action of the antagonists *p*-MPPF, and its iodinated analogue *p*-MPPI. In light of the widespread use of *p*-MPPI and *p*-MPPF as an antagonist to serotonin$_{1A}$ receptor function in animal models (Thielen and Frazer, 1995; Theilen *et al.*, 1996; Teng *et al.*, 2003), our results suggest a more detailed analysis of the pharmacology of *p*-MPPF, and its iodinated analogue *p*-MPPI before their antagonist properties on the serotonin$_{1A}$ receptor can be assumed in animal studies.

In summary, we show that prolonged treatment of serotonin$_{1A}$ receptors in a cellular environment to specific ligands induces significant effects on ligand binding of the receptor that are specific to the type of ligand used. We observe a reduction in specific binding of the agonist [$^3$H]8-OH-DPAT but not the antagonist [$^3$H]*p*-MPPF upon prolonged treatment with the agonist 8-OH-DPAT, in a manner that is independent of receptor-G-protein coupling. Importantly, we find novel differences in ligand binding to serotonin$_{1A}$ receptors in response to prolonged treatment with the classical antagonist *p*-MPPF, and its iodinated analogue *p*-MPPI. As mentioned earlier, long-term treatment with serotonin$_{1A}$ receptor agonists and antagonists constitutes a powerful therapeutic approach in anxiety- or stress-related disorders, and in developmental disorders (Blier *et al.*, 1990; Griebel, 1999; Azmitia, 2001). While the identification of the serotonin$_{1A}$ receptor as an important therapeutic target in a variety of complex behavioral traits represents a significant step (Gingrich and Hen, 2001), the success of therapeutic approaches that involve the administration of ligands specific to the serotonin$_{1A}$ receptor depends on the long-term effect of such treatments on the serotonin$_{1A}$ receptor function. Our analysis on ligand binding of serotonin$_{1A}$ receptors under conditions of prolonged treatment with ligands represents an attempt in this direction.

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