ELSEVIER

Effect of Chronic Serotonin-$_2$ Receptor Agonist or Antagonist Administration on Serotonin-$_{1A}$ Receptor Sensitivity

J.G. Hensler, Ph.D., and K.A. Truett, M.S.

We have investigated the effect of 5-HT$_2$ receptor agonist or antagonist administration on postsynaptic 5-HT$_{1A}$ receptor sensitivity assessed by two behavioral measures, reciprocal forepaw treading or hypothermia induced by acute injection of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. The effectiveness of these drug treatments to downregulate 5-HT$_{2A}$ receptors was confirmed by measuring the binding of [^{3}H]-ketanserin in cortical homogenates, because all of these drug treatments have been shown to result in the downregulation of 5-HT$_{2A}$ receptor sites. Acute or chronic treatment of rats with the 5-HT$_2$ receptor antagonist mianserin, or chronic administration of the 5-HT$_{2A}$ receptor antagonist ketanserin, did not alter 8-OH-DPAT-induced hypothermia or forepaw treading. These data indicate that downregulation of 5-HT$_{2A}$ receptors is not sufficient to alter these postsynaptic 5-HT$_{1A}$ receptor-mediated responses. Chronic treatment of rats with the 5-HT$_2$ receptor agonist DOI, however, resulted in the attenuation of both 5-HT$_{1A}$ receptor-mediated responses measured in separate experimental groups. The apparent desensitization of 5-HT$_{1A}$ receptors following chronic DOI treatment was not accompanied by a change in either the number or affinity of 5-HT$_{1A}$ receptor sites as measured by the binding of [^{3}H]-8-OH-DPAT in hippocampal homogenates. Chronic activation of 5-HT$_2$ receptors may be one mechanism by which the sensitivity postsynaptic 5-HT$_{1A}$ receptors can be regulated.

[Neuropsychopharmacology 19:354–364, 1998]

KEY WORDS: *Serotonin receptors; 5-HT$_{1A}$; 5-HT$_{2A}$; Downregulation; Forepaw treading; Hypothermia*

Of some 14 subtypes of receptor for the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) (see Hoyer et al. 1994), a tremendous amount of attention has been focused on the serotonin$_{1A}$ (5-HT$_{1A}$) receptor subtype. Drugs that act as agonists at this receptor have proved useful clinically as antidepressants (see Murphy et al. 1995) and anxiolytics (Coplan et al. 1995). The unique distribution of this serotonin receptor subtype in brain is consistent with its potential involvement in cognitive or integrative functions, as well as in emotional states. In terminal field areas of serotonergic innervation, the 5-HT$_{1A}$ receptor is located postsynaptically and is present in high density in cortical and limbic structures (i.e., hippocampus, entorhinal cortex, septum, amygdala, frontal cortex) (Vergé et al. 1986; Hensler et al. 1991; Khawaja 1995). The 5-HT$_{1A}$ receptor is also found in high density in serotonin cell body areas, such as the dorsal and median raphé, where it is believed to function as the somatodendritic autoreceptor modulating the activity of serotonergic neurons (see Aghajanian et al. 1990).

The sensitivity of behavioral (Goodwin et al. 1987; Hensler et al. 1991; Wieland et al. 1993; Maj et al. 1996) and electrophysiological (Blier and deMontigny 1983,

From the Department of Pharmacology, University of Texas Health Science Center, San Antonio, Texas, USA.

Address correspondence to: Julie G. Hensler, Ph.D., Department of Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7764, USA.

Received June 16, 1997; revised December 15, 1997; accepted February 16, 1998.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/98/$–see front matter
PII S0893-133X(98)00037-2

1985, 1987) responses mediated by the 5-HT$_{1A}$ receptor is decreased after chronic administration of 5-HT$_{1A}$ receptor agonists or antidepressants drugs. The desensitization of 5-HT$_{1A}$ receptor-mediated responses has also been reported to occur after acute or short-term agonist administration (Kennett et al. 1987; Larsson et al. 1990). Although some investigators have reported changes in 5-HT$_{1A}$ receptor number following agonist or antidepressant treatments (Welner et al. 1989; Fanelli and McMonagle-Strucko 1992), in many cases desensitization of 5-HT$_{1A}$ receptor-mediated responses has not been found to be accompanied by a decrease 5-HT$_{1A}$ receptor number (Larsson et al. 1990; Schecter et al. 1990; Hensler et al. 1991; Wieland et al. 1993). Changes in 5-HT$_{1A}$ receptor second messenger function have not been consistently reported to follow administration of antidepressants or 5-HT$_{1A}$ receptor agonists (Sleight et al. 1988; Varrault et al. 1991; Newman et al. 1992).

Interactions between serotonin receptor subtypes have been observed in behavioral studies. For example, 5-HT$_{2A}$ receptor-mediated head shake behavior in rats (Schreiber et al. 1995) elicited by the 5-HT$_2$ receptor agonist DOI is attenuated by co-administration of 5-HT$_{1A}$ receptor agonists (Arnt and Hyttel 1989; Yocca et al. 1990). Forepaw treading induced by acute injection of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT is potentiated by co-administration of DOI (Arnt and Hyttel; Berendsen and Broekkamp 1990) and attenuated by co-administration of the 5-HT$_{2C}$ receptor agonists MK 212 and mCPP (Berendsen and Broekkamp). 5-HT$_2$ receptor antagonists have also been reported to increase 8-OH-DPAT-induced forepaw treading (Backus et al. 1990). 8-OH-DPAT-induced spontaneous tail flick behavior is potentiated by agents with agonist properties at 5-HT$_{2C}$ receptors (Bervoets et al. 1990; Millan et al. 1997).

Historically, studies of receptor regulation have focused on effects of chronic under- or overexposure of receptor to its neurotransmitter. In view of evidence supporting acute interactions between serotonin receptor subtypes, the present study investigates the effect of 5-HT$_2$ receptor agonist or antagonist administration on postsynaptic 5-HT$_{1A}$ receptor sensitivity. The effectiveness of these drug treatment paradigms was confirmed by measuring the binding of [^{3}H]-ketanserin to 5-HT$_{2A}$ receptor sites in cortical homogenates, because all of these drug treatments have been shown to result in downregulation of 5-HT$_{2A}$ receptor sites. The sensitivity of postsynaptic 5-HT$_{1A}$ receptors was assessed *in vivo* by two behavioral measures, forepaw treading (Tricklebank et al. 1984; Scott et al. 1994; Thielen et al. 1996) or hypothermia (Bill et al. 1991; O'Connell et al. 1992; Millan et al. 1997) induced by acute injection of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. Because changes in 5-HT$_{1A}$ receptor second messenger function have not been consistently reported to follow administration of antidepressants or 5-HT$_{1A}$ receptor agonists (Sleight et al. 1988; Varrault et al. 1991; Newman et al. 1992), we chose not to measure second messenger responses associated with 5-HT$_{1A}$ receptor activation following 5-HT$_2$ receptor agonist or antagonist treatment.

Because drug treatments that result in the desensitization of 5-HT$_{1A}$ receptor-mediated responses (i.e., chronic administration of antidepressant or 5-HT$_{1A}$ receptor agonists) also result in desensitization or downregulation of 5-HT$_{2A}$ receptors (Eison and Yocca 1985; see Frazer et al. 1988; Sanders-Bush et al. 1989; Schecter et al. 1990; Wieland et al. 1993; Maj et al. 1996), we hypothesized that downregulation of 5-HT$_{2A}$ receptors may be a mechanism by which the sensitivity of postsynaptic 5-HT$_{1A}$ receptors is altered. In the present study, we report that acute or chronic treatment of rats with the 5-HT$_2$ receptor antagonist mianserin, or chronic administration of the 5-HT$_{2A}$ receptor antagonist ketanserin, did not alter 8-OH-DPAT-induced hypothermia or forepaw treading. Chronic treatment of rats with the 5-HT$_2$ receptor agonist DOI, however, resulted in the attenuation of both postsynaptic 5-HT$_{1A}$ receptor-mediated responses. The attenuation of 8-OH-DPAT-induced forepaw treading or hypothermia following chronic DOI administration was not accompanied by a change in 5-HT$_{1A}$ receptor sites as measured by [^{3}H]-8-OH-DPAT binding in hippocampal homogenates. Our results suggest that downregulation of 5-HT$_{2A}$ receptors is not sufficient to alter postsynaptic 5-HT$_{1A}$ receptor-mediated responses and that chronic activation of 5-HT$_2$ receptors may be one mechanism by which the sensitivity of postsynaptic 5-HT$_{1A}$ receptors is regulated.

METHODS

Animals

All experiments used male Sprague–Dawley rats weighing between 250 to 350 g. Animals were group housed, with lighting on a 14:10 hour day:night cycle and had *ad libitum* access to food and water. These studies were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health.

Drug Treatments

The following dose schedules were chosen from published reports showing 5-HT$_{2A}$ receptor downregulation following drug administration. Animals were injected with either saline or the following drugs: ketanserin (10 mg/kg, IP, b.i.d., for 21 days) (Gandolfi et al. 1985), mianserin (once at 2 mg/kg, SC) (Blackshear and Sanders-Bush 1982) or (once daily at 5 mg/kg, SC, for 14 days) (Gandolfi et al.), DOI (1 mg/kg, SC, once daily for 8 days) (McKenna et al. 1989). Separate control groups were used for each drug group.

5-HT_{1A} receptor function (i.e., 8-OH-DPAT-induced hypothermia) was assessed 24 hours after the last injection of ketanserin. Animals were sacrificed 48 hours after the last injection of ketanserin to allow for further clearance of any residual antagonist. For animals receiving acute mianserin treatment, 5-HT_{1A} receptor function (i.e., 8-OH-DPAT-induced forepaw treading) was determined 24 hours after a single injection of mianserin; animals were sacrificed following behavioral testing. For chronic mianserin-treated animals, 8-OH-DPAT-induced forepaw treading was measured 48 hours after the last injection of mianserin to allow clearance of residual antagonist. 8-OH-DPAT-induced forepaw treading has been shown to be potentiated by the co-administration 5-HT_2 receptor antagonists (Backus et al. 1990). Animals in this group were sacrificed after behavioral testing. For DOI-treated animals, 5-HT_{1A} receptor function was determined 24 hours after the last injection of DOI; animals were sacrificed after behavioral testing.

8-OH-DPAT-Induced Hypothermia

Measurement of core body temperature was performed, as previously described (Hensler et al. 1991). Core body temperature was measured with a digital thermometer (Fisher Scientific, Pittsburgh, PA) and rectal probe (YSI, Yellow Springs, OH). With the animal unrestrained, the probe was lubricated with petroleum jelly and inserted to a depth of 5 cm. Animals were acclimated to the procedure during the 2 days preceding the experiment. The procedure for acclimating the animal included handling the animal, allowing it to explore the test area and inserting the rectal probe for 1 min. Animals were habituated to the testing environment for at least 1 hour prior to temperature recording. Core body temperature was taken for 2 min at 10 or 15 min intervals. The temperature at the end of the 2-min period was recorded. Three temperature measurements were taken prior to SC injection of 8-OH-DPAT. Core body temperature immediately prior to 8-OH-DPAT injection was used as baseline. All experiments were performed between the hours of 2:30 P.M. and 5:00 P.M., at ambient temperature.

8-OH-DPAT-Induced Reciprocal Forepaw Treading

All animals were treated with reserpine (1 mg/kg, SC) 18 hours before behavioral testing, as previously described (Scott et al. 1994) to eliminate indirectly mediated catecholaminergic influences. Reciprocal forepaw treading in rats pretreated with reserpine has been shown to be closely associated with the activation of postsynaptic 5-HT_{1A} receptors (Tricklebank et al. 1984). Animals received SC injection of 8-OH-DPAT, and were placed into a Plexiglas cage on top of an Animex activity meter (Columbus Instruments, Columbus, OH; model WLI 1182). Activity counts were recorded every 2 min during the 30-min session with the aid of an 8-channel MiniCounter and PC-based MiniCounter software (Columbus Instruments, Columbus, OH). In rats pretreated with reserpine, the activity measured following saline injection was low, 50 counts/2 min or less. Such activity represents activity counts not attributable to forepaw treading, or base-line activity of the animal in the apparatus. In the presence of 8-OH-DPAT, the activity recorded was primarily attributable to forepaw treading, the repetitive dorsal–ventral treading of the forepaws as described by Lucki et al. 1984. Behavioral observations were made by a trained observer every 2 min during the experimental session. Animals were habituated to the testing environment for at least 1 hour prior to the experiment. All experiments were performed between the hours of 10:00 A.M to 1:30 P.M.

[^{3}H]-Ketanserin Binding

Whole cerebral cortices were removed on ice, flash-frozen on dry ice, and stored at −80°C until binding assays were performed. Cortical hemispheres were homogenized in lysis buffer (50 mM HEPES, 2 mM EDTA, 5 mM magnesium acetate, pH 7.4) and centrifuged for 15 min at 20,000 × *g*. The pellet was resuspended in 10 ml 50 mm Tris (pH 7.4 at 37°C). The suspension was incubated at 37°C for 10 min and centrifuged. The resultant pellet was washed twice and resuspended in 50 mM Tris (pH 7.4 at 3/°C). Saturation-binding experiments were performed using nine concentrations (0.1–10 nM) of [^{3}H]-ketanserin (80.1 Ci/mmol; NEN) in glass test tubes containing 100 nM prazosin and 100 nM pyrilamine to prevent the binding of [^{3}H]-ketanserin to alpha-adrenergic and histamine H_1 receptors, respectively. Nonspecific binding was defined by 10 μM methysergide. Binding was initiated by the addition of cortical homogenate (100–150 μg protein per tube). Assay tubes were covered and incubated for 1 hour at 37°C. Reactions were terminated by the addition of 5 ml of ice-cold buffer (50 mM Tris pH 7.4 at 4°C). Membranes were collected on glass fiber filters (Schleicher and Schuell, #25) that had been previously soaked in 0.3% polyethylenimine. Filters were washed three times with ice-cold buffer and incorporated radioactivity determined by liquid scintillation counting. Protein concentrations were determined by the method of Bradford (1976).

[^{3}H]-8-OH-DPAT Binding

Whole hippocampi were removed on ice, flash-frozen on dry ice, and stored at −80°C until binding assays were performed. For each animal, both hippocampi were pooled and homogenized in 50 mM Tris (pH 7.4 at 4°C) and centrifuged for 20 min at 20,000 × *g*. Hippo-

campal homogenate preparation was done as previously described (Chamberlain et al. 1993). Nine concentrations of [^{3}H]-8-OH-DPAT (0.1–8 nM) were used for saturation-binding experiments. Nonspecific binding was defined by 10 μM 5-HT. Saturation binding was performed in the presence of 2.5 mM $MgCl_2$ to promote ternary complex formation and high affinity agonist binding (Chamberlain et al.). Binding was initiated by the addition of hippocampal homogenate (40–60 μg protein per tube). Assay tubes were incubated for 15 min at 37°C. Reactions were terminated by addition of cold 20 mM Tris and filtration through glass fiber filters. Filters were washed three times with the same buffer, and radioactivity was determined by liquid scintillation counting. Protein concentrations were determined by the method of Bradford (1976).

Data Analysis and Statistical Comparisons

Saturation binding data were analyzed using KaleidaGraph. Data were fit by nonlinear regression to the model:

$$B = \text{Bound} = B_{max}/(1 + (K_d/[D]^{m1})) + m2[D],$$

where D = radioligand concentration, $m1$ = slope of total binding, and $m2$ = slope of nonspecific binding. B_{max} and K_d values obtained from radioligand binding experiments were analyzed by one-way analysis of variance (ANOVA). Time courses of hypothermia and forepaw treading were analyzed by repeated measured ANOVA. Individual points of the time courses were compared by Scheffe's post-hoc test. Data on the maximal effects of 8-OH-DPAT on hypothermia were analyzed by one-way ANOVA.

Materials

2,5-dimethoxy-4-iodophenylisopropylamine hydrochloride (DOI), 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), ketanserin tartrate, methysergide maleate, prazosin hydrochloride, and pyrilamine maleate were purchased from Research Biochemicals Inc. (Natick, MA). Mianserin hydrochloride, serotonin creatinine sulfate, and reserpine were purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

In confirmation of an earlier study (Frazer and Hensler 1990), acute injection of rats with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT caused a dose-related decrease in core body temperature. The time course of this response for three doses of 8-OH-DPAT is shown in Figure 1. For all three doses, the maximal response occurred 30 min postinjection. The decrease in body temperature produced by 0.05 mg/kg 8-OH-DPAT at 30 min was reproducible and statistically significant ($p < .001$, t-test) compared to that obtained for 0.01 mg/kg. We have used this dose of 8-OH-DPAT previously to assess the effect of chronic antidepressant treatment on 5-HT$_{1A}$ receptor function (Hensler et al. 1991). In the current study, this dose of 8-OH-DPAT (0.05 mg/kg) was used in subsequent experiments to determine the effect of 5-HT$_2$ receptor agonist or antagonist treatment on postsynaptic 5-HT$_{1A}$ receptor sensitivity.

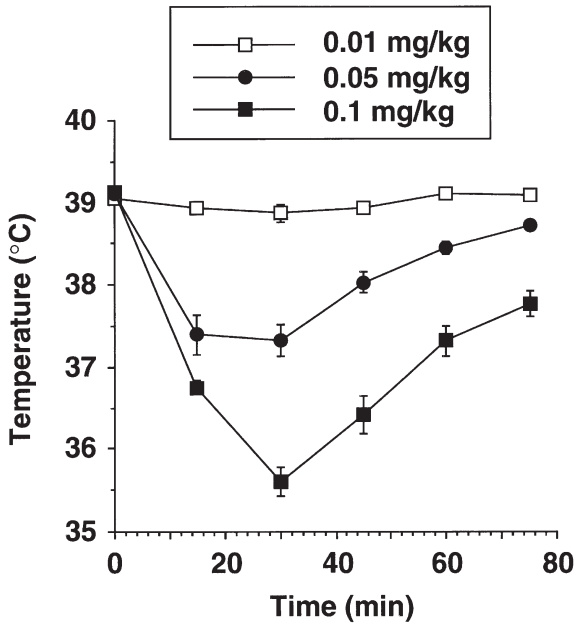

Figure 1. Time course of hypothermia elicited by acute injection of 8-OH-DPAT. Baseline core body temperature measurements were taken immediately prior to injection of 8-OH-DPAT (0.01, 0.05, or 0.1 mg/kg, SC) at $t = 0$. Body temperature, in degrees centigrade, was measured as described in METHODS at 10-min intervals for 60 min and then at $t = 75$ min after the SC injection of 8-OH-DPAT. Values shown are the means ± SEM ($n = 5$ in each experimental group).

Reciprocal forepaw treading, induced by acute injection of 8-OH-DPAT, was also assessed. 8-OH-DPAT elicited this behavioral response in a dose-dependent manner. The time course of this response for three doses of 8-OH-DPAT is shown in Figure 2. In rats pretreated with reserpine, the activity measured following saline injection was not attributable to forepaw treading, but represented base-line activity of the animal in the apparatus (Figure 2). In the presence of 8-OH-DPAT, the activity recorded was primarily attributable to forepaw treading. For all doses of 8-OH-DPAT tested, the maximal response occurred between 4 to 16 min postinjection. The lowest dose of 8-OH-DPAT that consistently produced observable forepaw treading behavior was 1.0 mg/kg. This dose of 8-OH-DPAT was used in subsequent experiments to assess postsynaptic

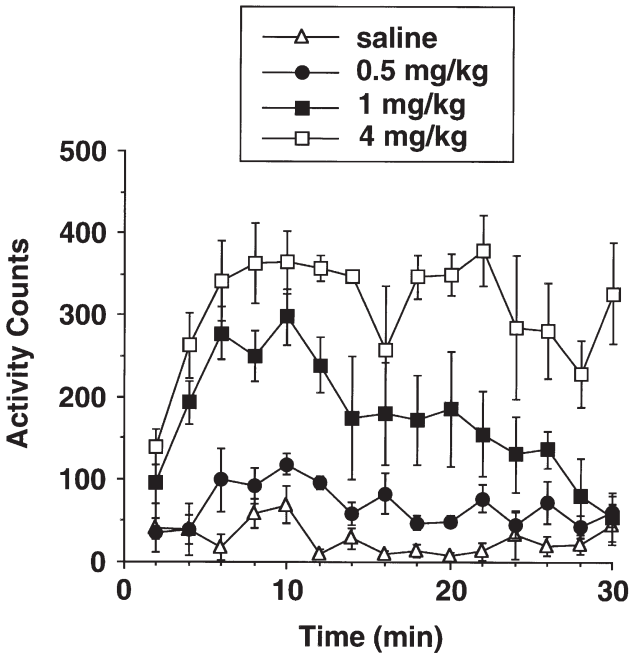

Figure 2. Time course of forepaw treading elicited by 8-OH-DPAT. Activity counts representing forepaw treading were measured as described in METHODS at 2-min intervals for 30 min immediately following injection of 8-OH-DPAT (0.05, 1.0, or 4.0 mg/kg, SC) or saline. Values shown are the means ± SEM (n = 4 in each experimental group).

5-HT_{1A} receptor sensitivity following 5-HT_2 receptor agonist or antagonist treatment.

To determine the effect of chronic 5-HT_2 receptor antagonist administration on postsynaptic 5-HT_{1A} receptor sensitivity, rats were treated for 21 days with the 5-HT_{2A} receptor antagonist ketanserin. The sensitivity of postsynaptic 5-HT_{1A} receptors was assessed by measuring 8-OH-DPAT-induced hypothermia 24 hours after the last injection of ketanserin. Chronic treatment with ketanserin did not alter 8-OH-DPAT-induced hypothermia ($F[1,8] = 0.2603$, $p = .6237$). Chronic ketanserin administration resulted in a significant decrease in the density (B_{max}) of 5-HT_{2A} receptors, as measured by the binding of [^{3}H]-ketanserin in cortical homogenates and a significant decrease in the affinity of the radioligand for this binding site as indicated by an increase in K_d value (Table 1). These data suggest that the sensitivity of this 5-HT_{1A} receptor-mediated response was not altered following chronic 5-HT_{2A} receptor antagonist administration.

To confirm that treatment with a 5-HT_2 receptor antagonist does not alter postsynaptic 5-HT_{1A} receptor sensitivity, animals were treated with the 5-HT_2 receptor antagonist mianserin and a second measure of postsynaptic 5-HT_{1A} receptor function, reciprocal forepaw treading, was assessed. 8-OH-DPAT-induced forepaw treading was assessed after a single injection of mianserin, which has been shown to downregulate 5-HT_{2A} receptors in brain (Blackshear and Sanders-Bush 1982). Acute mianserin administration did not alter 8-OH-DPAT-induced forepaw treading measured 24 hours after mianserin injection ($F[1,18] = 0.047$, $p = .8302$). To rule out the possibility that acute injection of mianserin was insufficient to alter the function of postsynaptic 5-HT_{1A} receptors, rats were treated for 14 days with mianserin. Because 8-OH-DPAT-induced forepaw treading has been shown to be potentiated by coadministration of 5-HT_2 receptor antagonists (Backus et al. 1990), this behavioral response was assessed 48 hours after the last mianserin injection to allow for clearance of antagonist from the animal. Chronic treatment of rats with mianserin did not result in a significant attenuation of 8-OH-DPAT-induced forepaw treading ($F[1,10] = 1.513$, $p = .2469$). Both acute

Table 1. [^{3}H]-Ketanserin Binding in Cortical Homogenates Following Treatment of Rats with 5-HT_2 Receptor Agonists or Antagonists

Treatment	n	K_d (nM)	B_{max} (fmol/mg protein)	% Downregulation
Chronic ketanserin				
Saline	6	0.95 ± 0.08	168 ± 7.9	27 ± 4.3%
Ketanserin	5	1.26 ± 0.10*	122 ± 7.1*	
Acute mianserin				
Saline	10	1.03 ± 0.09	191 ± 13	47 ± 2.5%
Mianserin	10	1.18 ± 0.06	100 ± 4.7*	
Chronic mianserin				
Saline	6	1.00 ± 0.10	219 ± 23	57 ± 5.4%
Mianserin	6	2.20 ± 0.25*	94.0 ± 12*	
Chronic DOI (hypothermia)				
Saline	5	1.34 ± 0.13	195 ± 13	24 ± 4.5%
DOI	5	1.59 ± 0.13	147 ± 9.8*	
Chronic DOI (forepaw treading)				
Saline	6	1.10 ± 0.07	156 ± 6.9	44 ± 3.6%
DOI	6	0.99 ± 0.18	87.3 ± 5.6*	

Radioligand binding experiments were performed in triplicate as described in METHODS. Shown are X ± SEM.
*$p < .05$, one-way ANOVA.

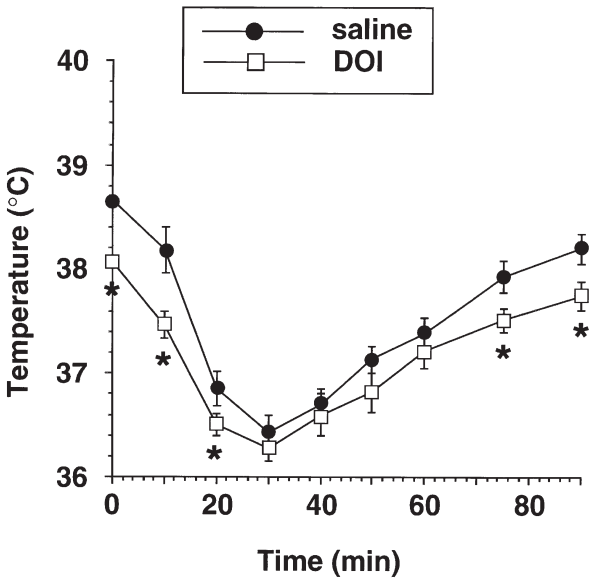

Figure 3. Hypothermic response to injection of 8-OH-DPAT in rats treated for 8 days with DOI. Hypothermia was measured 24 hours after the last injection of DOI or saline. Baseline core body temperature measurements were taken immediately prior to the subcutaneous injection of 8-OH-DPAT (0.05 mg/kg) at $t = 0$. Body temperature, in degrees centigrade, was measured at 10-min intervals for 60 min, and then at $t = 75$ and $t = 90$ min after 8-OH-DPAT injection. Values shown are the means ± SEM ($n = 5$ for saline-treated group; $n = 6$ for DOI-treated group). *$p < .05$.

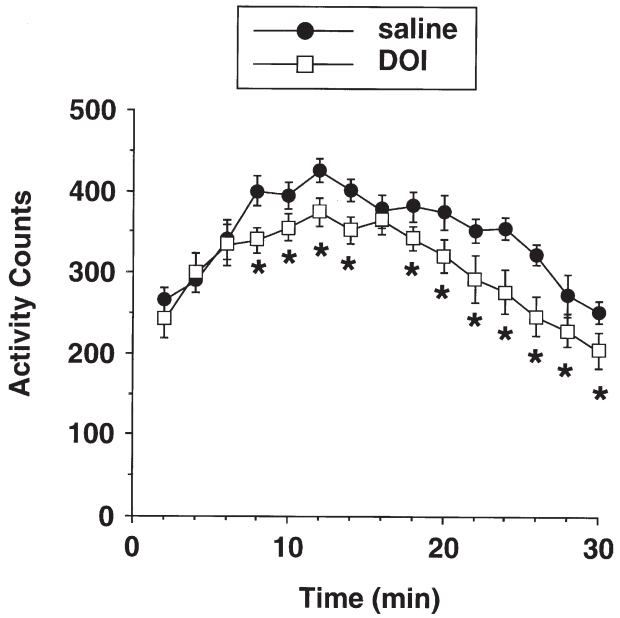

Figure 4. Forepaw treading induced by 8-OH-DPAT in rats treated for 8 days with DOI. Forepaw treading was measured 24 hours after the last injection of DOI or saline. Activity counts representing forepaw treading were measured at 2-min intervals for 30 min immediately following SC injection of 8-OH-DPAT (1 mg/kg). Values shown are the means ± SEM ($n = 6$ for saline-treated group; $n = 5$ for DOI-treated group). *$p < .05$.

and chronic mianserin treatments resulted in a decrease in the density of cortical 5-HT$_{2A}$ receptors (Table 1). Chronic mianserin treatment resulted in a significant decrease in the density of cortical 5-HT$_{2A}$ receptors (Table 1). Chronic mianserin treatment resulted in a significant decrease in the affinity of the radioligand for 5-HT$_{2A}$ receptor sites, as indicated by an increase in K_d value (Table 1). Thus, consistent with the lack of effect of chronic ketanserin treatment on 5-HT$_{1A}$ receptor-mediated hypothermia, mianserin administration did not alter the sensitivity of 5-HT$_{1A}$ receptor-mediated forepaw treading. These data suggest that the sensitivity of postsynaptic 5-HT$_{1A}$ receptors was not altered following 5-HT$_2$ receptor antagonist administration.

To determine if chronic 5-HT$_2$ receptor activation alters the sensitivity of postsynaptic 5-HT$_{1A}$ receptor-mediated responses, animals were treated for 8 days with the 5-HT$_2$ receptor agonist DOI. The sensitivity of postsynaptic 5-HT$_{1A}$ receptors was assessed by measuring in separate experimental groups either 8-OH-DPAT-induced hypothermia or 8-OH-DPAT-induced forepaw treading. A repeated measures ANOVA performed on the time course of 8-OH-DPAT-induced hypothermia showed a significant effect of drug treatment following chronic DOI administration ($F[1,8] = 10.978$, $p = .0106$). Post-hoc analysis revealed that the treatment groups were significantly different at t_0, t_{10}, and t_{20}, but not at t_{30}, when the maximal drop in temperature occurred for both groups (Scheffe's, $p < .05$) (Figure 3). Because of the significant difference in baseline temperatures (t_0) between the two groups, the percentage change from baseline at t_{30} was calculated. The average percentage drop in temperature at 30 min for saline-treated versus DOI-treated animals was significantly different (saline-treated: 5.1 ± 0.2%; DOI-treated: 3.6 ± 0.7%, $p = .003$, one-way ANOVA). Chronic treatment of rats with DOI also resulted in the attenuation of 8-OH-DPAT-induced forepaw treading (Figure 4). Repeated measures ANOVA showed a significant effect of drug treatment ($F[1,10] = 9.105$, $p = .0145$). The mean maximal number of activity counts calculated using the average for each animal for the time points, t_8, t_{10}, t_{12}, and t_{14} was 405 ± 14 for control animals and 355 ± 15 for DOI-treated rats, respectively ($p = .03$, one-way ANOVA). These time points were chosen, because this is when the greatest amount of activity attributable to forepaw treading occurred in the control group (Figure 4). Chronic treatment of rats with DOI resulted in a significant decrease in the density of cortical 5-HT$_{2A}$ receptors (B_{max}), without a change in affinity of the receptor site for radioligand (K_d) (Table 1). These data suggest that chronic administration of the 5-HT$_2$ receptor agonist

DOI alters the sensitivity of postsynaptic 5-HT_{1A} receptor-mediated responses.

To determine if the attenuation of 8-OH-DPAT-induced forepaw treading or 8-OH-DPAT-induced hypothermia following chronic DOI administration was accompanied by a change in 5-HT_{1A} receptor sites, [^{3}H]-8-OH-DPAT binding was performed in hippocampal homogenates. Neither B_{max} (fmol/mg protein) nor K_d (nM) values were significantly different between saline- and DOI-treated groups (saline: $B_{max} = 547.3 \pm 76.2$, $K_d = 1.15 \pm 0.11$; DOI: $B_{max} = 594.4 \pm 70.2$, $K_d = 1.01 \pm 0.16$, $n = 6$ per group). Thus, the apparent desensitization of 5-HT_{1A} receptor-mediated responses following chronic DOI administration was not accompanied by a change in 5-HT_{1A} receptor sites.

DISCUSSION

In the present study, we investigated the effect of 5-HT_2 receptor agonist or antagonist administration of postsynaptic 5-HT_{1A} receptor function. The effectiveness of these drug treatment paradigms was confirmed by measuring the binding of [^{3}H]-ketanserin to 5-HT_{2A} receptor sites in cortical homogenates, because all of these drug treatments have been shown to result in the downregulation of 5-HT_{2A} receptor sites. The sensitivity of postsynaptic 5-HT_{1A} receptors was assessed by two behavioral measures, forepaw treading or hypothermia induced by acute injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT. Neither of these 5-HT_{1A} receptor-mediated behavioral responses were altered following acute or chronic treatment of rats with the 5-HT_2 receptor antagonist mianserin or following chronic administration of the 5-HT_{2A} receptor antagonist ketanserin. Chronic treatment of rats with the 5-HT_2 receptor agonist DOI, however, resulted in the attenuation of both 5-HT_{1A} receptor-mediated responses measured in separate experimental groups. The apparent desensitization of 5-HT_{1A} receptor-mediated behavioral responses following chronic DOI treatment was not accompanied by a change in either the number or affinity of 5-HT_{1A} receptor sites as measured by the binding of [^{3}H]-8-OH-DPAT in hippocampal homogenates. These data indicate that the sensitivity of postsynaptic 5-HT_{1A} receptor-mediated responses can be regulated by chronic administration of 5-HT_2 receptor agonists.

Acute injection of rats with the 5-HT_{1A} receptor agonist 8-OH-DPAT causes a dose-related decrease in body temperature (Frazer and Hensler 1990; current study). Acute administration of the 5-HT_{1A} receptor agonists gepirone, ipsapirone, or buspirone also produces this response, although less potently (Koenig et al. 1988; Scott et al. 1994). The pharmacology of this response has been well characterized, demonstrating that 8-OH-DPAT-induced hypothermia is mediated by activation of 5-HT_{1A} receptors (Millan et al. 1997; Scott et al. 1994; Thielen et al. 1996). Although it has been suggested that 8-OH-DPAT-induced hypothermia in rats is mediated by presynaptic receptors (Goodwin et al. 1987b), the majority of evidence supports the view that in rats, the hypothermic response elicited by 5-HT_{1A} receptor agonists is mediated by postsynaptic receptors (Bill et al. 1991; O'Connell et al. 1992; Millan et al. 1997 and discussion therein). 5-HT_{1A} receptor-mediated hypothermia has been used to assess the sensitivity of 5-HT_{1A} receptors following a variety of antidepressant treatments (Goodwin et al. 1987a; Hensler et al. 1991), and has been proposed as a model to screen and identify novel 5-HT_{1A} receptor agonists and antagonists (Millan et al. 1997).

Reciprocal forepaw treading is one component of the serotonin syndrome, a constellation of behaviors that can be induced by drugs that increase brain 5-HT release or directly activate postsynaptic 5-HT receptors (Tricklebank et al. 1984). These behaviors include forepaw treading, head weaving, hindlimb abduction, rigidity, resting tremor, and Straub tail (see Tricklebank 1985). However, several of the behavioral components can be blocked by dopamine or α_1-adrenergic antagonists (Tricklebank et al. 1984), indicating that the syndrome as a whole is not specific enough to be used as unambiguous evidence of serotonergic activation, as had been originally suggested. Of the six symptoms of the serotonin syndrome, only forepaw treading and flat body posture are present following the depletion of monoamines by administration of reserpine, consistent with the involvement of catecholaminergic neurons in the other components of the serotonin syndrome (see Tricklebank 1985). 8-OH-DPAT-induced forepaw treading in reserpine-treated animals is inhibited by antagonists at the 5-HT_{1A} receptor (Tricklebank et al. 1984; Scott et al. 1994; Thielen et al. 1996), demonstrating that this behavioral response is mediated by 5-HT_{1A} receptor activation.

In the current study, none of the 5-HT_2 receptor antagonist treatment paradigms (i.e., acute or chronic administration of the 5-HT_2 receptor antagonist mianserin, or chronic administration of the 5-HT_{2A} receptor antagonist ketanserin) altered 8-OH-DPAT-induced hypothermia or 8-OH-DPAT-induced forepaw treading. All of these 5-HT_2 receptor antagonist treatments, however, resulted in the downregulation of 5-HT_{2A} receptor sites. Because of the lack of receptor reserve for 5-HT_{2A} receptors in cerebral cortex (Sanders-Bush 1990), 5-HT_{2A} receptor downregulation is expected to result in attenuation of 5-HT_{2A} receptor function. Chronic administration of antagonists or antidepressants that cause a decrease in the density of the 5-HT_{2A} receptor binding sites in cerebral cortex have been shown to cause a decrease in 5-HT_{2A} receptor-mediated phosphoinositide hydrolysis (Conn and Sanders-Bush 1986; Sanders-Bush et al. 1989). In the current study, chronic administration of either antagonist, ketanserin or mianserin, also resulted in a decrease in the affinity of the radioligand for 5-HT_{2A} receptor

binding sites, as indicated by an increase in K_d value. Although this may be caused by the presence of residual drug in the binding assay, perhaps a more likely explanation in view of the ample wash-out period (i.e., cortical homogenates were prepared from animals sacrificed 48 hours after the last injection of antagonist), is that chronic antagonist administration alters the affinity of the receptor. Our data indicate that the sensitivity of 5-HT$_{1A}$ receptor-mediated forepaw treading or hypothermia is not altered by 5-HT$_{2A}$ receptor antagonist treatments, suggesting that the downregulation of 5-HT$_{2A}$ receptors is insufficient to alter postsynaptic 5-HT$_{1A}$ receptor-mediated responses.

Chronic treatment of rats with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (Larsson et al. 1990) or with a variety of antidepressants, including monoamine oxidase inhibitors, tricyclic antidepressants, and selective serotonin reuptake inhibitors (Goodwin et al. 1987a; Hensler et al. 1991) results in the attenuation of 8-OH-DPAT-induced hypothermia. The serotonin syndrome, of which forepaw treading is a component, is also attenuated by chronic antidepressant treatment (Lucki and Frazer 1982; Goodwin et al. 1987a; Maj et al. 1996) or chronic 5-HT$_{1A}$ receptor agonist administration (Kennett et al. 1987; Larsson et al.; Wieland et al. 1993). These drug treatments, which result in the desensitization of 5-HT$_{1A}$ receptor-mediated responses (i.e., chronic administration of antidepressant or 5-HT$_{1A}$ receptor agonists), also result in the desensitization or downregulation of 5-HT$_{2A}$ receptors (Eison and Yocca 1985; see Frazer et al. 1988; Sanders-Bush et al. 1989; Schecter et al. 1990; Wieland et al. 1993; Maj et al. 1996). However, the lack of effect of 5-HT$_2$ receptor antagonist administration on responses mediated by postsynaptic 5-HT$_{1A}$ receptors suggests that downregulation of 5-HT$_{2A}$ receptors does not seem to be a mechanism by which the sensitivity of postsynaptic 5-HT$_{1A}$ receptors is altered.

Chronic mianserin treatment has also been shown to downregulate 5-HT$_{2C}$ receptors in spinal cord (Pranzatelli et al. 1993) and several areas of brain (Rocha et al. 1994), as measured by the binding of [^{3}H]-mesulergine. Mianserin, which has equal affinity for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor, and ketanserin, which is 100-fold selective for the 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptor, also have high nanomolar affinity for alpha-adrenergic receptors (Leysen et al. 1981). Although drugs interacting acutely with alpha-adrenergic receptors or dopaminergic receptors modulate 8-OH-DPAT-induced hypothermia in mice (Bill et al. 1991; Durcan et al. 1991), this has been shown not to be the case for 8-OH-DPAT-induced hypothermia in rats (Millan et al. 1997). However, if mianserin or ketanserin treatment had modified 8-OH-DPAT-induced hypothermia or forepaw treading in the current study, it would have been necessary to address the potential involvement of alpha-adrenergic receptors or 5-HT$_{2C}$ receptors by investigating the effect of chronic administration of the alpha-1 antagonist prazosin or the selective 5-HT$_{2C}$ receptor antagonist SB 206553 (Kennett et al. 1996) on these 5-HT$_{1A}$ receptor mediated behaviors. In the absence of an effect of either ketanserin or mianserin on 8-OH-DPAT-induced hypothermia or forepaw treading, we did not investigate this further.

Chronic administration of the 5-HT$_2$ receptor agonist DOI resulted in attenuation of both 8-OH-DPAT-induced hypothermia and 8-OH-DPAT-induced forepaw treading. Our data are in contrast with those of Nash et al. (1989), who found that chronic DOI treatment (1 mg/kg IP once daily for 7 days) did not alter 8-OH-DPAT-induced hypothermia. The difference in observations between our study and that of Nash et al. may be explained by differences in the procedures used to measure core body temperature (i.e., animals were restrained for body temperature measurement in the study of Nash et al.), or may be due to differences in the length of DOI treatment or mode of injection (SC vs. IP). In the current study, the apparent desensitization of these 5-HT$_{1A}$ receptor-mediated responses as a result of chronic administration of DOI was not accompanied by a change in the density of 5-HT$_{1A}$ receptor binding sites or in the affinity of 5-HT$_{1A}$ receptor sites for the agonist radioligand [^{3}H]-8-OH-DPAT. These findings are in agreement with those of Buckholtz et al. (1988), who report no change in the binding of [^{3}H]-8-OH-DPAT following chronic administration of DOI.

The effect of chronic DOI administration on postsynaptic 5-HT$_{1A}$ receptor-mediated responses in the current study may be mediated through either 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors, because the agonist DOI has equal affinity for both the 5-HT$_{2A}$ and the 5-HT$_{2C}$ receptor (see Hoyer et al. 1994). Chronic DOI treatment results in the downregulation of cortical 5-HT$_{2A}$ receptors (Buckholtz et al. 1988; McKenna et al. 1989; current report), as well as 5-HT$_{2C}$ receptors in spinal cord (Pranzatelli et al. 1993), and in brain (Aulakh et al. 1995). However, although chronic DOI treatment results in downregulation of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, as is observed following 5-HT$_2$ receptor antagonist administration, 5-HT$_{1A}$ receptor-mediated responses were attenuated following chronic administration of 5-HT$_2$ receptor agonist. Thus, chronic 5-HT$_2$ receptor activation may be one mechanism by which the sensitivity of 5-HT$_{1A}$ receptors is regulated.

The attenuation of 8-OH-DPAT-induced hypothermia and reciprocal forepaw treading observed in the current study as a result of chronic DOI treatment was modest. One possible explanation may be the nonselective nature of DOI for 5-HT$_2$ receptor subtypes. DOI, with equal affinity for members of the 5-HT$_2$ receptor family, may activate 5-HT$_2$ receptors that have opposing effects on these 5-HT$_{1A}$ receptor-mediated behaviors. For example, forepaw treading in rats induced by 8-OH-DPAT is potentiated by co-administration of DOI

(Arnt and Hyttel 1989; Berendsen and Broekkamp 1990) and attenuated by co-administration of the 5-HT_{2C} receptor agonists MK 212 and mCPP (Berendsen and Broekkamp). An alternative explanation for the modest effect of chronic DOI treatment on 8-OH-DPAT-induced hypothermia and forepaw treading may be the partial agonist activity of DOI. DOI, and a closely related cogener (1-(2,5-dimethoxy-4-phenylmethyl)-2-aminopropane (DOM), are partial agonists at the 5-HT_{2A} receptor in cortical tissue (Pierce and Peroutka 1988; Sanders-Bush et al. 1988; Edwards et al. 1992) and at the 5-HT_{2C} receptor in choroid plexus (Hoyer et al. 1989; Sanders-Bush and Breeding 1991).

It is possible that DOI may exert its effects on 8-OH-DPAT-induced forepaw treading and hypothermia by means of a non-5-HT_2 receptor mechanism. Buckholtz et al. (1988) observed a significant increase in alpha-1 adrenergic binding sites and a significant decrease in beta-adrenergic binding sites, following treatment of rats with DOI (1 mg/kg) for 8 days. To the best of our knowledge, the effects of these adrenergic receptor changes on 8-OH-DPAT-induced forepaw treading or hypothermia have not been described. Acute administration of DOI, systemically or directly into the dorsal raphé, inhibits the firing of raphé serotonergic neurons and decreases extracellular 5-HT in the frontal cortex measured by microdialysis (Wright et al. 1990; Garratt et al. 1991). The action of DOI, however, was not blocked by the 5-HT_2 receptor antagonists ketanserin or ritanserin, or by the β-adrenergic/5-HT_{1A} receptor antagonist pindolol (Wright et al. 1990; Garratt et al. 1991). Chronic administration of DOI has been reported to reduce the inhibition of serotonergic cell firing and cortical serotonin release by 5-HT_{1A} somatodendritic autoreceptor activation (Kidd et al. 1991). Attenuation of 5-HT_{1A} somatodendritic autoreceptor function may serve to increase serotonergic neurotransmission, which, over time, would be expected to result in desensitization of postsynaptic 5-HT_{1A} receptors. By contrast, chronic treatment with mianserin does not significantly alter the function of 5-HT_{1A} autoreceptors, as assessed by examining the ability of 8-OH-DPAT to reduce 5-HT release in the hippocampus and striatum (Kreiss and Lucki 1995).

In conclusion, downregulation of 5-HT_{2A} receptors does not seem to be a mechanisms by which the sensitivity of postsynaptic 5-HT_{1A} receptors is altered. The lack of effect of 5-HT_2 receptor antagonist administration on 5-HT_{1A} receptor-mediated responses suggests that the downregulation of 5-HT_2 receptors is not sufficient to regulate postsynaptic 5-HT_{1A} receptor sensitivity. Chronic activation of 5-HT_2 receptors may be one mechanism by which the sensitivity of postsynaptic 5-HT_{1A} receptors is regulated. Further characterization of the effect of chronic DOI administration on 5-HT_{1A} receptor-mediated forepaw treading or hypothermia awaits the availability of selective agonists for either the 5-HT_{2A} or 5-HT_{2C} receptor.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Rick Thielen for assistance with behavioral experiments measuring reciprocal forepaw treading, and thank Drs. Alan Frazer and Rick Thielen for many helpful discussion. This work was supported by United States Public Health Service Grant MH 52369 (JGH), and research funds from the National Alliance for Research in Schizophrenia and Depression (JGH).

REFERENCES

Aghajanian GK, Sprouse JS, Sheldon P, Rasmussen K (1990): Electrophysiology of the central serotonin system: Receptor subtypes and transducer mechanisms. Ann NY Acad Sci 600:93–103

Arnt J, Hyttel J (1989): Facilitation of 8-OH-DPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI. Eur J Pharmacol 161:45–51

Aulakh CS, Mazzolla-Pomietto P, Hulihan-Giblin BA, Murphy DL (1995): Lack of cross-tolerance for hypophagia induced by DOI vs. m-CPP suggests separate mediation by 5-HT_{2A} and 5-HT_{2C} receptors, respectively. Neuropsychopharmacology 13:1–8

Backus LI, Sharp T, Grahame-Smith DG (1990): Behavioral evidence for a functional interaction between central 5-HT_2 and 5-HT_{1A} receptors. Br J Pharmacol 100:793–799

Berendsen HHG, Broekkamp CLE (1990): Behavioral evidence for functional interactions between 5-HT-receptor subtypes in rats and mice. Br J Pharmacol 101:667–673

Bervoets K, Millan MJ, Colpaert FC (1990): Agonist action at 5-HT_{1C} receptors facilitates 5-HT_{1A} receptor-mediated spontaneous tail-flicks in the rat. Eur J Pharmacol 191: 185–195

Bill DJ, Knight M, Forster EA, Fletcher A (1991): Direct evidence for an important species difference in the mechanism of 8-OH-DPAT-induced hypothermia. Br J Pharmacol 103:1857–1864

Blackshear MA, Sanders-Bush E (1982): Serotonin receptor sensitivity after acute and chronic treatment with mianserin. J Pharmacol Exp Ther 221:303–308

Blier P, deMontigny C (1983): Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. J Neurosci 3:1270–1278

Blier P, deMontigny C (1985): Serotonergic but not noradrenergic neurons in rat CNS adapt to long-term treatment with monoamine oxidase inhibitors. Neuroscience 16:949–955

Blier P, deMontigny C (1987): Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: Electrophysiological studies in the rat brain. Synapse 1:470–480

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

the principal of protein-dye binding. Analyt Biochem 72:248–254

Buckholtz NS, Zhou D, Freedman DX (1988): Serotonin$_2$ agonist administration downregulates rat brain serotonin$_2$ receptors. Life Sci 42:2439–2445

Chamberlain J, Offord SJ, Wolfe BB, Tyau LS, Wang H, Frazer A (1993): Potency of 5-hydroxytryptamine$_{1A}$ agonists to inhibit adenylyl cyclase activity is a function of affinity for the "low affinity" state of [^{3}H]-8-hydroxy-N,N-dipropylaminotetralin ([^{3}H[-8-OH-DPAT) binding. J Pharmacol Exp Ther 266:618–625

Conn PJ, Sanders-Bush E (1986): Regulation of serotonin-stimulated phosphoinositide hydrolysis: Relation to the serotonin 5-HT-2 binding site. J Neurosci 6:3669–3675

Coplan JD, Wolk SI, Klein DF (1995): Anxiety and serotonin 1A receptor. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation of Progress, Raven Press, NY, pp 1301–1310

Durcan MJ, Wozniak KM, Linnoila M (1991): Modulation of the hypothermic and hyperglycaemic effects of 8-OH-DPAT by alpha 2-adrenoceptor antagonists. Br J Pharmacol 102:222–226

Edwards E, Ashby CR, Wang RY (1992): ±-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and α-methyl-5-HT: 5-HT_2 receptor agonistic action on phosphatidylinositol metabolism in the rat fronto-cingulate and entorhinal cortex. Neuropharmacology 31:615–621

Eison AS, Yocca FD (1985): Reduction in cortical 5-HT_2 receptor sensitivity after continuous gepirone treatment. Eur J Pharmacol 111:389–392

Fanelli RJ, McMonagle-Strucko K (1992): Alteration of 5-HT_{1A} receptor binding sites following chronic treatment with ipsapirone measured by quantitative autoradiography. Synapse 12:75-81

Frazer A, Offord SJ, Lucki I (1988): Regulation of serotonin receptors and responsiveness in the brain. In Sanders-Bush E (ed), The Serotonin Receptors, Humana Press, Totowa, NJ, pp 319–362

Frazer A, Hensler JG (1990): 5-HT_{1A} receptors and 5-HT_{1A}-mediated responses: Effects of treatments that modify serotonergic neurotransmission. Ann NY Acad Sci 600: 460–475

Gandolfi O, Barbaccia ML, Costa E (1985): Different effects of serotonin antagonists on ^{3}H-mianserin and ^{3}H-ketanserin recognition sites. Life Sci 36:713–721

Garratt JC, Kidd EJ, Wright IK, Marsden CA (1991): Inhibition of 5-hydroxytryptamine neuronal activity by the 5-HT agonist, DOI. Eur J Pharmacol 199:349–355

Goodwin GM, De Souza RJ, Green AR (1987a): Attenuation by electroconvulsive shock and antidepressant drugs of the 5-HT_{1A} receptor-mediated hypothermia and serotonin syndrome produced by 8-OH-DPAT in the rat. Psychopharmacology 91:500–505

Goodwin GM, De Souza RJ, Green AR, Heal DJ (1987b): The pharmacology of the behavioral and hypothermic responses of rats to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). Psychopharmacology 91:506–511

Hensler JG, Kovachich GB, Frazer A (1991): A quantitative autoradiographic study of Serotonin$_{1A}$ receptor regulation: Effect of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology 4:131–144

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin DR, Mylecharane EJ, Saxena PR, Humphrey PPA (1994): Proceedings VII international union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46:157–203

Hoyer D, Waeber C, Schoeffter P, Palacios JM, Dravid A (1989): 5-HT_{1C} receptor-mediated stimulation of inositol phosphate production in pig chroroid plexus. A pharmacological characterization. Naunyn-Schmiedeberg's Arch Pharmacol 339:252–258

Kennett GA, Marcou M, Dourish CT, Curzon G (1987): Single administration of 5-HT_{1A} agonists decreases presynaptic, but not postsynaptic receptor-mediated responses: Relationship to antidepressant-like action. Eur J Pharmacol 138:53–60

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn, TP (1996): *In vitro* and *in vivo* profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434

Khawaja X (1995): Quantitative autoradiographic characterization of the binding of [^{3}H]WAY-100635, a selective 5-HT_{1A} receptor antagonist. Brain Res 673:217–225

Kidd EJ, Garratt JC, Marsden CA (1991): Effects of repeated treatment with 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on the autoregulatory control of dorsal raphe 5-HT neuronal firing and cortical 5-HT release. Eur J Pharmacol 200:131–139

Koenig JI, Meltzer HY, Gudelsky GA (1988): 5-hydroxytryptamine$_{1A}$ receptor-mediated effects of buspirone, gepirone, and ipsapirone. Pharmacol Biochem Behav 29:711–715

Kreiss DS, Lucki I (1995): Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. J Pharmacol Exp Ther 274:866-876

Larsson LG, Renyi L, Ross SB, Svensson B, Angeby-Moller K (1990): Different effects of the responses of functional pre- and postsynaptic 5-HT_{1A} receptors by repeated treatment of rats with the 5-HT_{1A} receptor agonist 8-OH-DPAT. Neuropharmacology 29:85–91

Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J, Janssen PAJ (1981): Receptor binding profile of R 41 468, a novel antagonist at 5-HT_2 receptors. Life Sci 28:1015–1022

Lucki I, Frazer A (1982): Prevention of the serotonin syndrome in rats by repeated administration of monoamine oxidase inhibitors but not tricyclic antidepressants. Psychopharmacology 77:205–211

Lucki I, Nobler MS, Frazer A (1984): Differential actions of serotonin antagonists on two behavioral models of serotonin receptor activation in the rat. J Pharmacol Exp Ther 228:133–139

Maj J, Bijak M, Dziedzicka-Waslyewskam M, Rogóz R, Rogóz Z, Skuza G, Tokarski T (1996): The effects of paroxetine given repeatedly on the 5-HT receptor subpopulations in the rat brain. Psychopharmacology 127:73–82

McKenna DJ, Nazarali AJ, Himeno A, Saavedra JM (1989): Chronic treatment with DOI, a psychotomimetic 5-HT_2 agonist, downregulates 5-HT_2 receptors in rat brain. Neuropsychopharmacology 2:81–87

Millan MJ, Girardon S, Bervoets K (1997): 8-OH-DPAT-induced tail-flicks in the rat are facilitated by the selective serotonin (5-HT)$_{2C}$ agonist, RO 60-0175: blockade of its actions by the novel 5-HT$_{2C}$ receptor antagonist SB 206,553. Neuropharmacology 36:743–745

Murphy DL, Mitchell PB, Potter WZ (1995): Novel pharmacological approaches to the treatment of depression. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation of Progress, Raven Press, NY, pp 1143–1153

Nash JF, Meltzer HY, Gudelsky GA (1989): Selective cross-tolerance to 5-HT$_{1A}$ and 5-HT$_2$ receptor-mediated temperature and corticosterone responses. Pharmacol Biochem Behav 33:781–785

Newman ME, Shapira B, Lerer B (1992): Regulation of 5-hydroxytryptamine1A receptor function in rat hippocampus by short- and long-term administration of 5-hydroxytryptamine1A agonists and antidepressants. J Pharmacol Exp Ther 260:16–20

O'Connell JT, Sarna GS, Curzon G (1992): Evidence for postsynaptic mediation of the hypothermic effect of 5-HT1A receptor activation. Br J Pharmacol 106:603–609

Pierce PA, Peroutka SJ (1988): Antagonism of 5-hydroxytryptamine2 receptor-mediated phosphatidylinositol turnover by d-lysergic acid diethylamide. J Pharmacol Exp Ther 247:918–925

Pranzatelli MR, Murthy JN, Tailor PT (1993): Novel regulation of 5-HT$_{1C}$ receptors: Downregulation induced by 5-HT$_{1C/2}$ receptor agonists and antagonists. Eur J Pharmacol 244:1–5

Rocha B, Rigo M, DiScala G, Sandner B, Hoyer D (1994): Chronic mianserin or eltoprazine treatment in rats: Effect on the elevated plus-maze and on limbic 5-HT2C receptor levels. Eur J Pharmacol 262:125-131

Sanders-Bush E (1990): Adaptive regulation of central serotonin receptors linked to phosphoinositide hydrolysis. Neuropsychopharmacology 3:411–416

Sanders-Bush E, Burris KD, Knoth K (1988): Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. J Pharmacol Exp Ther 246:924–928

Sanders-Bush E, Breeding M (1991): Choroid plexus epithelial cells in primary culture: A model of 5-HT$_{1C}$ receptor activation by hallucinogenic drugs. Psychopharmacology 105:340–346

Sanders-Bush E, Breeding M, Knoth K, Tsutsumi M (1989): Sertraline-induced desensitization of the serotonin 5-HT$_2$ transmembrane signaling system. Psychopharmacology 99:64–69

Schecter LE, Bolanos FJ, Gozlan H, Lanfumey L, Haj-Dahmane S, Laporte AM, Fattaccini CM, Hamon MJ (1990): Alterations of central serotoninergic and dopaminergic neurotransmission in rats treated chronically with ipsapirone: Biochemical and electrophysiological studies. J Pharmacol Exp Ther 255:1335–1347

Schreiber R, Brocco M, Audinot B, Gobert A, Veiga S, Millan MJ (1995): (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine 5-HT$_{2A}$ receptors: Modulation by novel 5-HT$_{2A/2C}$ antagonist, D$_1$ antagonist and 5-HT$_{1A}$ agonists. J Pharmacol Exp Ther 273:101–112

Scott PA, Chou JM, Tang H, Frazer A (1994): Differential induction of 5-HT$_{1A}$-mediated responses *in vivo* by three chemically dissimilar 5-HT$_{1A}$ agonists. J Pharmacol Exp Ther 260:198–208

Sleight AJ, Marsden CA, Palfreyman MG, Mir AK, Lovenberg W (1988): Chronic MAO$_A$ and MAO$_B$ inhibition decreases the 5-HT$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase. Eur J Pharmacol 154:255–261

Thielen RJ, Fangon NB, Frazer A (1996): 4(2′-methoxyphenyl)-1-[2′-[N-(2″-pyridinyl)-p-iodobenzamido]ethyl]piperazine and 4-(2′-methoxyphenyl)-1-[2′-[N-(2′-pyridinyl)-p-fluorobenzamido]ethyl] piperazine. Two new antagonists at pre- and postsynaptic serotonin-1A receptors. J Pharmacol Exp Ther 277(2):661–670

Tricklebank MD, Forler C, Fozard JR (1984): The involvement of subtypes of the 5-HT$_1$ receptor and of catecholaminergic systems in the behavioral response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. Eur J Pharmacol 106:271–282

Tricklebank, MD (1985): The behavioral response to 5-HT receptor agonists and subtypes of the central 5-HT receptor. Trends Pharmacol Sci 6:403–407

Varrault A, Leviel V, Bockaert J (1991): 5-HT$_{1A}$-sensitive adenylyl cyclase of rodent hippocampal neurons: Effects of antidepressant treatments and chronic stimulation with agonists. J Pharmacol Exp Ther 257:433–438

Vergé D, Daval G, Marcinkiewicz M, Patey A, El Mestikawy S, Gozlan H, Hamon M (1986): quantitative autoradiography of multiple 5-HT$_1$ receptor subtypes in the brain of control of 5,7-dihydroxytryptamine-treated rats. J Neurosci 6:3473–3482

Welner SA, DeMonitigny C, Desroches J, Desjardins P, Suranyi-Cadotte BE (1989): autoradiographic visualization of serotonin (5-HT)$_{1A}$ binding sites in rat brain following antidepressant drug treatment. Synapse 4:347–352

Wieland S, Fischette CT, Lucki I (1993): Effect of chronic treatments with tandospirone and imipramine on serotonin-mediated behavioral responses and monoamine receptors. Neuropharmacology 32:561–573

Wright IK, Garratt JC, Marsden CA (1990): Effects of a selective 5-HT$_2$ agonist, DOI on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex. Br J Pharmacol 99:221–222

Yocca FD, Wright RN, Margraf RR, Eison AS (1990): 8-OH-DPAT and buspirone analogs inhibit the ketanserin-sensitive quipazine-induced head shake response in rats. Pharmacol Biochem Behav 35:251–254