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The 5-HT_{1A} Receptor Antagonist p-MPPI Blocks Responses Mediated by Postsynaptic and Presynaptic 5-HT_{1A} Receptors

ANGELA R. ALLEN,* ASHISH SINGH,* ZHI-PING ZHUANG,‡
MEI-PING KUNG,‡ HANK F. KUNG†‡ AND IRWIN LUCKI*†[1]

*Departments of Psychiatry, †Pharmacology and ‡Radiology, University of Pennsylvania,
3600 Market Street, Room 808, Philadelphia, PA 19104-2649

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ALLEN, A. R., A. SINGH, Z.-P. ZHUANG, M.-P. KUNG, H. F. KUNG AND I. LUCKI. *The 5-HT_{1A} receptor antagonist p-MPPI blocks responses mediated by postsynaptic and presynaptic 5-HT_{1A} receptors.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 301–307, 1997.—The present experiments examined the ability of the novel 5-HT_{1A} receptor antagonist to block responses mediated by postsynaptic and presynaptic 5-HT_{1A} receptors in vivo. Pretreatment with p-MPPI reduced or blocked the effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on two responses mediated by postsynaptic 5-HT_{1A} receptors, reduction of body temperature and the 5-HT behavioral syndrome. Administration of p-MPPI alone did not alter body temperature or produce symptoms of the 5-HT syndrome. Pretreatment with p-MPPI also blocked the ability of 8-OH-DPAT to reduce extracellular 5-HT in the striatum, a response mediated by presynaptic 5-HT_{1A} receptors in the dorsal raphe nucleus, but did not alter striatal 5-HT when administered alone. These results indicate that p-MPPI is an effective 5-HT_{1A} receptor antagonist in vivo with no intrinsic activity. p-MPPI may prove to be a useful pharmacological tool for studying 5-HT_{1A} receptors and their involvement in anxiety and affective disorders. © 1997 Elsevier Science Inc.

Serotonin p-MPPI 8-OH-DPAT Microdialysis Hypothermia 5-HT syndrome

RECEPTORS for 5-hydroxytryptamine (5-HT) have been classified into a number of subtypes based on their pharmacological, cellular and structural properties (9,15). There is special interest in the pharmacology of 5-HT_{1A} receptors because buspirone, a partial agonist at 5-HT_{1A} receptors (1,22,23), has been found useful in the clinical treatment of generalized anxiety disorder (33,37) and depression (32,34).

Understanding the functional effects of 5-HT_{1A} receptors is complicated because they are located both on serotonergic cell bodies as presynaptic autoreceptors and postsynaptically in the terminal fields that receive serotonergic innervation from the raphe nuclei (29,44). Selective 5-HT_{1A} receptor agonists have been described, such as 8-hydroxy-2-(di-n-propyl)aminotetralin (8-OH-DPAT; 26), and pharmacological and behavioral responses associated with the activation of presynaptic and postsynaptic 5-HT_{1A} receptors have been characterized (see 20, for review). In comparison, the development of selective 5-HT_{1A} receptor antagonists has been rather slow and unsuccessful (see 8). Several drugs have been proposed as 5-HT_{1A} receptor antagonists, such as spiperone (30), (S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin (UH-301; 3), pindolol and propranolol (42), but these drugs also have equivalent or higher affinity for other receptors. A number of compounds, such as 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-aza-spirol-[4]-dec ane-7,9-dione (BMY 7378; 47) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190; 10), and methyl 4{4-[4-(1,1,3-trioxo-2H-1,2-benzo-isothiazol-2-yl)butyl]-1-piperaz inyl}1H-indole-2-carboxylate (SDZ 216-525; 36) were developed as selective 5-HT_{1A} receptor antagonists. Initial evidence for their antagonist effects in vivo was based on their ability to block activity of functional effects due to the activation of postsynaptic 5-HT_{1A} receptors. However,

[1]Requests for reprints should be addressed to: Dr. Irwin Lucki, Departments of Psychiatry and Pharmacology, University of Pennsylvania, 3600 Market Street, Room 808, Philadelphia, PA 19104-2649 USA. Phone: (215)573-3305, Fax: (215)573-2149.

these compounds were demonstrated subsequently to be agonists at presynaptic 5-HT_{1A} receptors and produce responses mediated by presynaptic 5-HT_{1A} receptors, such as the inhibition of raphe neuronal discharge rates (24,27,43), attenuation of 5-HT synthesis and reduction of extracellular levels of 5-HT (14,38,39). More recently, N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropa namide (WAY 100135; 6) and several piperazine derivatives (WAY 100635; 7) have been proposed as selective and "silent" 5-HT_{1A} receptor antagonists and have been demonstrated to block behavioral and pharmacological effects produced by 8-OH-DPAT at both postsynaptic and presynaptic 5-HT_{1A} receptors (6,27,35).

The development of a new 5-HT_{1A} receptor antagonist, 4-(2′-methoxy-phenyl-1-[2′-n-2′′-pyridinyl)-p-iodobenzamido]- ethyl-piperazine (p-MPPI), was recently described. p-MPPI is a structural analog of WAY 100635 with a high affinity (K_d = 0.36 nM) in vitro for the 5-HT_{1A} receptor (18,19). The initial characterization studies suggested that p-MPPI was a 5-HT_{1A} receptor antagonist based on its binding characteristics in the presence of guanine nucleotides (19). p-MPPI also blocked the ability of 8-OH-DPAT to inhibit forskolin-stimulated adenylyl cyclase activity from hippocampal homogenates without demonstrating significant effects of its own at up to 100-fold higher concentrations (18).

The present series of experiments were conducted in order to examine p-MPPI's potential as an antagonist of responses mediated by postsynaptic and presynaptic 5-HT_{1A} receptors in vivo. Two responses produced in rats by systemic administration of 8-OH-DPAT were used to examine p-MPPI's effects at postsynaptic 5-HT_{1A} receptors: hypothermia (2,12) and the 5-HT behavioral syndrome (42,46). A preliminary study reported that single doses of p-MPPI blocked hypothermia and forepaw treading, one of the symptoms of the 5-HT syndrome produced by 8-OH-DPAT (41). Subsequently, p-MPPI was also examined for its effects at presynaptic 5-HT_{1A} receptors by examining its ability to modulate extracellular levels of 5-HT using in vivo microdialysis. The ability of pretreatment with p-MPPI to block the 8-OH-DPAT-induced reduction of extracellular 5-HT levels in the striatum was examined as indicative of antagonist activity, and the effects of p-MPPI when given alone measured its potential agonist activity at presynaptic 5-HT_{1A} receptors. Extracellular levels of 5-HT in the striatum are decreased by 8-OH-DPAT by the activation of 5-HT_{1A} autoreceptors located on the dorsal raphe nucleus (4,17). The results show that p-MPPI is an effective antagonist of responses mediated by both presynaptic and postsynaptic 5-HT_{1A} receptors.

METHODS

Animals

Male Sprague–Dawley rats (Charles River Co., Wilmington, MA) 250–300g were housed in groups of two or three in polycarbonate cages, and maintained on a 12 h light schedule (lights on 0700–1900), in a temperature-controlled (22°C) colony room. Free access was provided to standard rat chow and water. Rats were handled and allowed several days to adjust to the laboratory housing conditions prior to the initiation of experiments.

Temperature Regulation

The rectal temperature of rats was measured at room temperature (21–23°C) after removal from their home cage using a temperature probe linked to a digital thermometer (Yellow Springs Instrument Co.). The lubricated probe was inserted 6 cm into the rectum, remained in place for approximately 60 s using gentle restraint, and the animal was returned to his home cage. Four baseline temperature measurements, at 15 min intervals, were obtained for each rat prior to drug administration. The first reading was discarded because of the significant variability associated with this time point. The other three temperature measurements were averaged to determine the mean baseline temperature for each rat. After the determination of baseline temperature, each rat was injected with either p-MPPI (3, 10 or 30 mg/kg, SC) or saline. Fifteen min later, the rats received a second injection of either 1.0 mg/kg 8-OH-DPAT or saline. Following the second injection, four additional temperature measurements were made at 15 min intervals. Rats (n = 6 rats/group) were tested at weekly intervals until the completion of the dose-response curves with each dose combination. This interval was sufficient to prevent the development of tolerance. The magnitude of the temperature reduction associated with each time point was obtained by subtracting the actual temperature from the baseline preinjection temperature for each rat. After completion of the above studies, the same groups of rats were used to examine whether the hypothermic effects of a lower dose of 8-OH-DPAT (0.5 mg/kg) were antagonized by pretreatment with 30 mg/kg p-MPPI.

5-HT Behavioral Syndrome

The animals were observed individually, in clear polycarbonate test cages (45 × 24 × 20 cm) with the cage floor covered with fresh sawdust. All rats were placed in the test cages 10 min prior to drug administration. Separate groups (n = 6 rats/group) then received an injection of either p-MPPI (1, 3, 10 or 30 mg/kg) or saline, followed 15 min later by an injection of either 2.0 mg/kg 8-OH-DPAT or saline. Rats were observed for 30 min following the second injection. Testing was conducted at weekly intervals until the dose-response functions were completed. This retest interval was sufficient to prevent the development of tolerance. Each rat was evaluated for the presentation of the following symptoms: (1) repetitive dorsoventral treading of the forepaws; (2) abduction of the hindlimbs; (3) lateral head-weaving; (4) flat body posture, an outstretched posture with the abdomen resting close to the cage floor; and (5) Straub tail. Each symptom was rated on an intensity scale (0 = absent, 1 = weak, 2 = moderate, 3 = intense) with an intensity score of 2 or greater necessary for the symptom to be considered as prominently presented. In addition, rats were scored as showing the composite 5-HT behavioral syndrome if at least three of the five symptoms were prominently present during the observation period. Similar procedures have been used previously to quantify the 5-HT behavioral syndrome (16,21,45).

Separate groups of rats (n = 6 rats/group) were used to assess the capacity of 8-OH-DPAT to produce, and of p-MPPI to antagonize, the 5-HT syndrome after reserpine pretreatment because of previous reports that the effects of antagonists on the 5-HT syndrome may depend on the depletion of endogenous 5-HT (42). The rats were treated with reserpine (1 mg/kg) 18 h prior to testing. Each rat received an injection of either 30 mg/kg p-MPPI or saline, followed 15 min later by a second injection of either 2.0 mg/kg 8-OH-DPAT or saline. The 5-HT syndrome was rated using the criteria described above.

Microdialysis

Concentric microdialysis probes used in this study were constructed using a 3.0 mm length of cupramonium rayon fiber of 12 m wall thickness and 224 m outer diameter with a 35,00 molecular weight cut-off (Terumo Medical Corporation, Somerset, NJ). Artificial cerebrospinal fluid (ACSF; composition (mM): NaCl 147; KCl 4.0; $MgCl_2$ 0.9; $CaCl_2$ 1.7, unadjusted pH = 6.5) entered the dialysis probe via a central fused-silica tube of ID 76 m and OD 144 m (Polymicro Technologies, Phoenix, AZ). The ACSF then washed back over this fused silica tubing where it came in contact with the dialysis membrane and exited the probe through a second length of fused-silica tubing.

Dialysis probes were implanted into the striatum on the day prior to the experiment. Rats were anesthetized with chloral hydrate (350 mg/kg, IP), placed in a Kopf stereotaxic frame with the nose bar set at −3.5 mm, and the probe was lowered slowly into the striatum through a burr hole drilled in the cranium to the following coordinates relative to bregma: AP + 0.2; ML + 3.0; DV −8.0 (28). The probe was fixed to the skull using bone screws and dental cement. After surgery, the rats were placed in a cylindrical microdialysis chamber (12 inches in diameter) and the tubing was attached to a counterweight and a dual channel swivel that allowed the animals to move freely (Instech Laboratories, Plymouth Meeting, PA). The probe was continuously perfused with ACSF at a flow rate of 0.6 μl/min (Instech infusion pump, model 2000).

Experiments were conducted approximately 16–21 h after surgery in awake, unrestrained animals. Dialysate samples were collected in microcentrifuge vials placed on ice at 20 min intervals and frozen immediately at −80°C for storage until assayed. After the collection of six baseline samples, the rats received the first of two injections. The first consisted of either p-MPPI (30 mg/kg) or saline, followed 5 min later by the second injection consisting of either 8-OH-DPAT (1.0 mg/kg) or saline. Dialysis samples were then collected for a further four hours.

The samples were analyzed using high performance liquid chromatography (HPLC) linked to an electrochemical detector (BAS; Bioanalytical Systems, West Lafayette, IN). The HPLC separation system consisted of a 5 μl sample loop linked in series to a sepstik microbore column (ODS 3 m; 100 × 1 mm; BAS). The mobile phase consisted of 32 mM NaH_2PO_4, 0.67 M ethylenediaminetetracetic acid, 0.43 mM octyl sodium sulfate and 8% v/v acetonitrile adjusted to a pH of 4.0. The flow rate through the column was 80–100 μl/min, and the electrochemical detector was set at a potential of + 0.70 V relative to a Ag/AgCl reference electrode. Samples were injected by a refrigerated (5°C) microsampler (Thermo Separation; Fremont, CA) in a 6.5 μl volume.

Standard concentrations of 5-HT were prepared each day from stock solutions stored at −80°C and injected regularly between dialysate samples. Based on a signal-to-noise ratio of 2:1, the sensitivity of the system for detecting 5-HT was 0.5 fmol. Peaks for 5-HT were identified by comparison of their retention time with standards. Dialysate concentrations of 5-HT were determined by interpolation of the plot of peak heights of standard concentrations.

Drugs

All experimental drugs were dissolved in deionized water and administered subcutaneously (SC), with dosage calculated as mg/kg base. 8-OH-DPAT and reserpine were administered in a volume of 1 ml/kg, and p-MPPI was administered in a volume of 3.2 ml/kg. Dissolution of p-MPPI required gentle heating and stirring. The pH of the p-MPPI solution was increased to ≈4, using dilute NaOH, prior to injection. For the temperature regulation studies control rats received an acidic saline injection (pH ≈4) to compensate for the acidity of the p-MPPI injection. Because preliminary studies indicated that acidic saline did not alter values obtained in the 5-HT behavioral syndrome or the microdialysis studies, normal saline was used in these studies.

8-Hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT) and reserpine were purchased from Research Biochemicals Inc. (Natick, MA). 4-(2′-methoxy-phenyl-1-[2′-n-2′′pyridinyl)-p-iodobenzamido]-ethyl-piperazine (p-MPPI) was synthesized according to a method reported previously (48).

Statistics

For the hypothermia experiment, temperatures were converted into change scores (temperature following drug treatment minus baseline temperature) in order to normalize temperature measurements across rats. A two-factor ANOVA was conducted, including dose and repeated measures on time as factors. Temperature changes produced by drug treatments were compared with corresponding baseline values using Dunnett's test, two-tailed. Temperature changes at corresponding time points were compared between treatment conditions using Newman–Keul's test. Behavioral scores of the 5-HT behavioral syndrome from controls were compared with drug-treated groups using Mann–Whitney U tests. The microdialysis data were analyzed using a two-factor repeated-measures ANOVA, with repeated measures for time. Corresponding values between the saline control and drug-treated groups were compared using Dunnett's test.

RESULTS

Hypothermia

The effects of p-MPPI and 8-OH-DPAT on rectal temperature are shown in Figs. 1A and 1B. Figure 1A shows that the 5-HT_{1A} receptor agonist 8-OH-DPAT (1.0 mg/kg) produced a time-dependent decrease in body temperature, with the maximal effect occurring 45–60 min post-injection. Furthermore, pretreatment with p-MPPI dose-dependently antagonized this effect of 8-OH-DPAT. ANOVA indicated a significant effect of p-MPPI dose ($F(3, 60) = 12.18$; $p < 0.001$), time ($F(4, 60) = 124.32$; $p < 0.001$) and of their interaction ($F(3, 60) = 8.64$; $p < 0.001$). 8-OH-DPAT produced smaller temperature decreases when it was preceded by p-MPPI than by saline, according to Newman–Keul's test. At 30 min post-injection, 3, 10 and 30 mg/kg p-MPPI significantly impaired the hypothermic response to 1.0 mg/kg 8-OH-DPAT. At 45 and 60 min post-injection, 10 and 30 mg/kg p-MPPI continued to impair the hypothermic response to 1.0 mg/kg 8-OH-DPAT.

An additional experiment examined the ability of 30 mg/kg p-MPPI to block a lower dose of 8-OH-DPAT (0.5 mg/kg) because the two highest doses of p-MPPI tested diminished, but did not prevent, the hypothermic effects of 1.0 mg/kg 8-OH-DPAT. These data are shown in Fig. 1B. ANOVA indicated significant effects of dose ($F(1, 20) = 33.92$; $p < 0.01$), time ($F(4, 20) = 27.02$; $p < 0.001$) and a significant dose by time interaction ($F(1, 20) = 13.38$; $p < 0.001$). Significant hypothermic effects were produced by 0.5 mg/kg 8-OH-DPAT at 30, 45 and 60 min post-injection, although its overall effect was less than 1.0 mg/kg 8-OH-DPAT. When 0.5 mg/kg 8-OH-

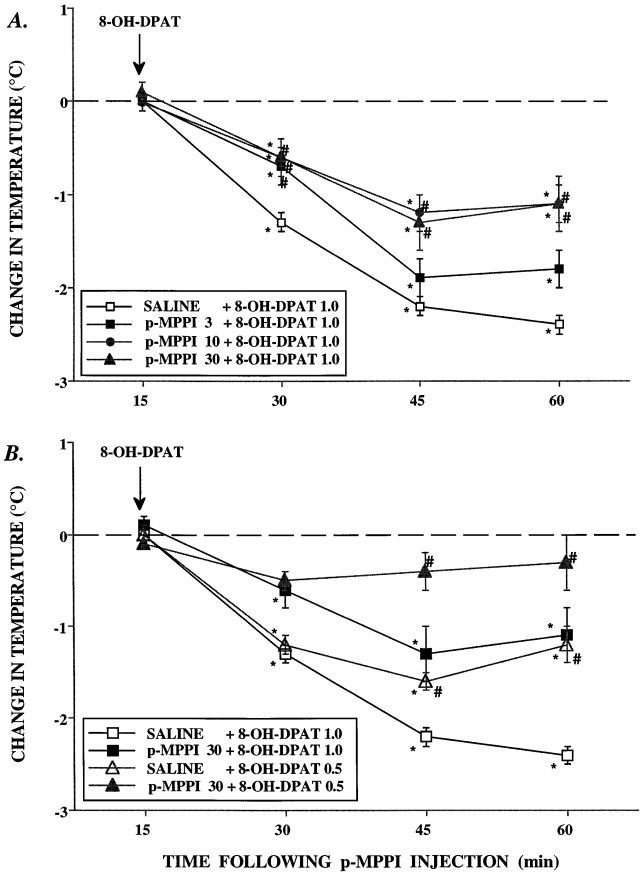

FIG. 1. Change in body temperature from baseline (mean ± 1 SEM) as a function of time. Panel A shows the hypothermic effects of 1.0 mg/kg 8-OH-DPAT following pretreatment with either saline or varying doses of p-MPPI. Panel B shows the hypothermic effects of 0.5 and 1.0 mg/kg 8-OH-DPAT following pretreatment with either saline or 30 mg/kg p-MPPI. *Indicates values for 8-OH-DPAT-induced hypothermia that differed significantly from the corresponding baseline values for each group, according to Dunnett's test, $p < 0.05$. #Indicates values for 8-OH-DPAT-induced hypothermia following pretreatment with p-MPPI that differed from corresponding values following pretreatment with saline, $p < 0.05$. $n = 6$ rats/group.

DPAT was preceded by 30 mg/kg p-MPPI, the hypothermic effect of 8-OH-DPAT was blocked when compared either with its own baseline or with corresponding effects following pretreatment with saline.

As shown in Fig. 2, p-MPPI (3–30 mg/kg) was also administered alone to different groups of rats to determine whether it produced hypothermia. ANOVA indicated no significant effect for dose ($F(3, 60) = 3.16$; NS), but significant effects for time ($F(4, 60) = 3.16$; $p < 0.05$), and for the interaction between dose and time ($F(3, 60) = 4.69$; $p < 0.001$). No dose of p-MPPI produced significant changes in body temperature when compared with baseline values.

5-HT Behavioral Syndrome

Table 1 shows the effects of 8-OH-DPAT on the composite 5-HT behavioral syndrome and the ratings of individual symptoms of the 5-HT behavioral syndrome after pretreatment with either saline or p-MPPI. 8-OH-DPAT (2.0 mg/kg) promi-

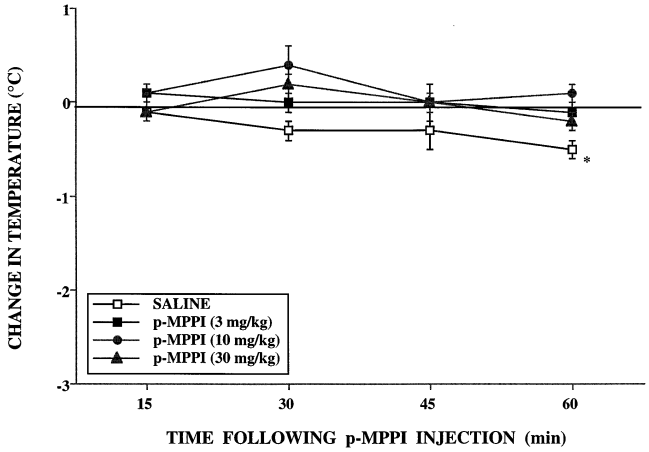

FIG. 2. The effects of p-MPPI (3–30 mg/kg) or saline on body temperature as a function of time. Asterisk indicates values that differed significantly from corresponding baseline values, according to Dunnett's test, $p < 0.05$. No significant effects of p-MPPI on body temperature were measured. $n = 6$ rats/group.

nently produced all of the symptoms of the 5-HT behavioral syndrome in 100% of the rats tested. Pretreatment of rats with 30 mg/kg p-MPPI blocked the 5-HT behavioral syndrome. Inspection of individual symptoms showed that the intensity rating of forepaw treading, head weaving, and Straub tail elicited by 2.0 mg/kg 8-OH-DPAT was significantly reduced by 30 mg/kg p-MPPI. Although there was a trend for intensity ratings for the symptoms of hindlimb abduction and flat body posture to be reduced by p-MPPI, these values were not statistically significant ($p = 0.052$, Mann–Whitney U-test). Pretreatment with a lower dose of p-MPPI (10 mg/kg) did not antagonize the 5-HT behavioral syndrome (data not shown).

p-MPPI was also administered alone to determine whether it produced the 5-HT behavioral syndrome (Table 1). Doses up to 30 mg/kg p-MPPI did not produce the composite 5-HT behavioral syndrome or any of the individual symptoms of the 5-HT behavioral syndrome.

A separate group of rats was tested for the 5-HT syndrome after the administration of 1.0 mg/kg reserpine, 18 h prior to testing. Table 1 shows that 2.0 mg/kg 8-OH-DPAT elicited the 5-HT behavioral syndrome in 100% of the rats tested. However, pretreatment with 30 mg/kg p-MPPI decreased the incidence of the 5-HT behavioral syndrome to 0%. When tested alone in reserpinized rats, 30 mg/kg p-MPPI did not produce any of the symptoms of the 5-HT behavioral syndrome. When the intensity ratings of individual symptoms produced by 8-OH-DPAT were considered separately in reserpinized rats (Table 1), all of the symptoms, including hindlimb abduction and flat body posture, were significantly attenuated by 30 mg/kg p-MPPI.

Extracellular Levels of 5-HT

The effects of p-MPPI and 8-OH-DPAT on extracellular 5-HT levels in the striatum are shown in Fig. 3. There was an overall significant effect of treatment ($F(3, 26) = 4.99$; $p < 0.01$), a significant effect of time ($F(12, 312) = 4.17$, $p < 0.001$), but no significant interaction ($F(36, 312) = 1.09$, NS). As expected, 8-OH-DPAT (1.0 mg/kg) reduced extracellular 5-HT levels to about 55% below baseline. Values were significantly lower than corresponding saline control values from 40

TABLE 1

EFFECTS OF p-MPPI (30.0 mg/kg) ON SYMPTOMS OF THE 5-HT BEHAVIORAL SYNDROME PRODUCED BY 8-OH-DPAT (2.0 mg/kg)

Symptom	Saline	8-OH-DPAT	p-MPPI	p-MPPI + 8-OH-DPAT
No reserpine pretreatment				
Forepaw treading	0.0	2.9 ± 0.1	0.0	0.0**
Flat body posture	0.0	3.0	0.0	2.1 ± 0.3
Hindlimb abduction	0.0	3.0	0.0	2.1 ± 0.3
Head weaving	0.0	3.0	0.0	0.9 ± 0.3**
Straub tail	0.0	2.2 ± 0.5	0.0	0.0**
Composite score (%)	0.0	100.0	0.0	16.7
Reserpine pretreatment				
Forepaw treading	0.0	3.0	0.0	1.5 ± 0.3**
Flat body posture	0.0	3.0	0.0	0.5 ± 0.2**
Hindlimb abduction	0.0	3.0	0.0	0.7 ± 2.0**
Head weaving	0.0	1.0 ± 0.4	0.0	0.0**
Straub tail	0.0	2.0 ± 0.5	0.0	0.0**
Composite score (%)	0.0	100.0	0.0	0.0

Values represent mean scores (± 1 SEM) rated for the intensity of individual symptoms of the 5-HT behavioral syndrome on a 0–3 scale. $n = 6$ rats per group. Values are shown with no SEM if all animals were rated with the same score. Asterisks indicate values which differ significantly from those rated when 8-OH-DPAT was given alone, according to the Mann–Whitney test ($p < 0.01$). Composite scores are based on the percentage of animals that prominently displayed 3 of the 5 symptoms.

to 180 min post injection, according to Dunnett's test. Extracellular 5-HT levels after the combination of p-MPPI and 8-OH-DPAT did not differ significantly from those of saline, indicating that p-MPPI antagonized the reduction of striatal 5-HT levels produced by 8-OH-DPAT. In addition, p-MPPI did not significantly change striatal 5-HT levels when given alone.

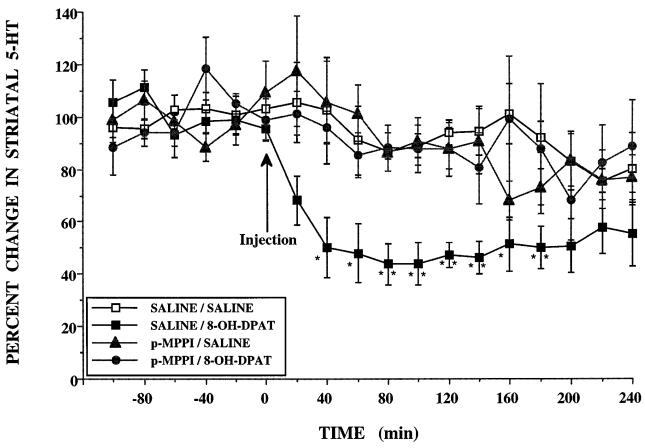

FIG. 3. Extracellular levels of 5-HT in the striatum after injection of either 1.0 mg/kg 8-OH-DPAT or saline following pretreatment with either 30 mg/kg p-MPPI or saline. Values are expressed as a percentage change from baseline levels, measured for 2 h prior to injection (time − 100 to 0 min.). Baseline values for individual groups were: saline/saline, 14.3 ± 2.0 fmoles/6.5 μl, $n = 6$; saline/8-OH-DPAT, 20.7 ± 3.9 fmoles/6.5 μl, $n = 7$; p-MPPI/saline, 13.2 ± 2.6 fmoles/6.5 μl, $n = 10$; p-MPPI/8-OH-DPAT, 13.2 ± 5.3 fmoles/6.5 μl, $n = 7$. Asterisks indicate values differed significantly from corresponding values of the saline control group, according to Dunnett's test: *$p < 0.05$; **$p < 0.01$.

DISCUSSION

p-MPPI was proposed as a selective 5-HT$_{1A}$ receptor antagonist on the basis of its high affinity for 5-HT$_{1A}$ receptors (18), its ability to block 8-OH-DPAT-induced inhibition of forskolin-stimulated adenylyl cyclase (18) and alterations of its binding in the presence of guanine nucleotides (19). The results of the present studies extend observations of the antagonist effects of p-MPPI to responses mediated by postsynaptic and presynaptic 5-HT$_{1A}$ receptors in vivo. Hypothermia (2,12) and the 5-HT behavioral syndrome (42,46) are two responses known to be mediated by postsynaptic 5-HT$_{1A}$ receptors. p-MPPI reduced the degree of hypothermia produced by 1.0 mg/kg 8-OH-DPAT and prevented the hypothermic effects by 0.5 mg/kg 8-OH-DPAT. Increasing doses of p-MPPI reduced the intensity of or prevented the 5-HT behavioral syndrome elicited by 2.0 mg/kg 8-OH-DPAT. It also reduced or prevented the incidence of forepaw treading in reserpine pretreated rats produced by 8-OH-DPAT, an important component behavior of the 5-HT behavioral syndrome associated with postsynaptic 5-HT$_{1A}$ receptors (42). These observations are similar to and extend the preliminary studies of Thielen and Frazer (41).

There were no signs of partial agonist activity for p-MPPI on the responses mediated by postsynaptic 5-HT$_{1A}$ receptors, as has been reported for other partial 5-HT$_{1A}$ receptor agonists, such as ipsapirone or buspirone. Although ipsapirone reduced body temperature, the magnitude of its effect was less than the full agonist 8-OH-DPAT, and pretreatment with ipsapirone reduced hypothermia produced by 8-OH-DPAT (11). In the present study, p-MPPI did not alter body temperature when given alone. Buspirone blocked the elicitation of the full 5-HT behavioral syndrome, but it produced two of its component symptoms, hindlimb abduction and flat body posture (40), and buspirone produced the full 5-HT syndrome when rats were pretreated with reserpine (13). In contrast, p-MPPI did not produce any behavioral symptoms when given alone with or

without reserpine pretreatment. These findings indicate that p-MPPI is a 5-HT_{1A} receptor antagonist without partial agonist effects.

The effects of p-MPPI were also examined on the regulation of extracellular 5-HT in the striatum, a response mediated by presynaptic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (17). p-MPPI (30 mg/kg) did not alter striatal 5-HT levels when given alone, and blocked the ability of 8-OH-DPAT to produce a 55% reduction of striatal 5-HT. Previous studies had demonstrated that a number of drugs that appeared to be 5-HT_{1A} receptor antagonists on the basis of postsynaptic 5-HT_{1A} receptors demonstrated partial agonist activity at presynaptic 5-HT_{1A} receptors, such as BMY 7378 (38), NAN-190 (14), or SDZ 216-525 (39). This is possibly due to the large receptor reserve associated with presynaptic 5-HT_{1A} autoreceptors (5,25). Therefore, like WAY 100135 (35), p-MPPI failed to produce agonist-like effects on one of the responses that is most sensitive for discriminating partial agonists from antagonists with no intrinsic activity.

The ability of p-MPPI to antagonize 8-OH-DPAT's effects varied somewhat with different testing conditions and between responses. Although 30 mg/kg p-MPPI completely abolished the composite 5-HT behavioral syndrome produced by 2.0 mg/kg 8-OH-DPAT, it blocked the symptoms of hindlimb abduction and flat body posture only in reserpinized rats. This observation for p-MPPI is similar to those of Tricklebank et al. (42), who reported that pindolol blocked hindlimb abduction and flat body posture only in rats pretreated with reserpine and suggested that the presence of endogenous 5-HT or catecholamines may interfere with the effects of 5-HT antagonists. Although 30 mg/kg p-MPPI completely blocked the hypothermia produced by 0.5 mg/kg 8-OH-DPAT, it only reduced the hypothermia produced by 1.0 mg/kg 8-OH-DPAT. In contrast, 30 mg/kg p-MPPI was sufficient to completely prevent the decrease in 5-HT release caused by 1.0 mg/kg 8-OH-DPAT. At present, it appears that some responses produced by 8-OH-DPAT may be more easily antagonized by p-MPPI than others.

In summary, p-MPPI is an effective pharmacological tool as a 5-HT_{1A} receptor antagonist in vivo because it was shown to be effective at blocking responses mediated by 5-HT_{1A} receptors without agonist activity. The development of 5-HT_{1A} receptor antagonists provides an important tool for studying the role of this receptor in potential therapeutic targets, such as anxiety, affective disorders and cognition (8).

Note added in proof: Recently, Thielen et al. (J. Pharmacol. Exp. Ther., 277:661–670; 1996) also demonstrated that p-MPPI can block changes in 5-HT metabolism, forepaw treading and hypothermia produced by 8-OH-DPAT.

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