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ORIGINAL INVESTIGATION

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Functional subsensitivity of 5-HT_{2A} and 5-HT_{2C} receptors mediating hyperthermia following acute and chronic treatment with 5-$HT_{2A/2C}$ receptor antagonists

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Abstract In the present study, we investigated the duration of attenuation of the temperature increases produced by (±) 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and *m*-chlorophenylpiperazine (*m*-CPP) which followed pretreatment with four serotonin (5-HT) antagonists; metergoline, mesulergine, mianserin and ritanserin. The duration of attenuation of *m*-CPP-induced hyperthermia lasted less than 1 day for ritanserin, more than 1 day for the 5 mg/kg doses of both mianserin and metergoline and more than 2 days for the 5 mg/kg dose of mesulergine. The duration of attenuation of DOI-induced hyperthermia lasted less than 1 day for ritanserin, more than 1 day for mianserin, more than 2 days for the 5 mg/kg dose of metergoline and more than 4 days for mesulergine. Daily administration of a low (1.0 mg/kg per day) dose of ritanserin for 14 days led to an attenuation of the temperature increases produced by *m*-CPP given 24 h after the last dose of ritanserin, but did not cause a similar desensitization of DOI-induced hyperthermia. On the other hand, daily administration of both low (1.0 mg/kg per day) and high (5.0 mg/kg per day) doses of mianserin for 14 days caused desensitization of DOI-induced hyperthermia but did not cause desensitization of *m*-CPP-induced hyperthermia when these agonists were administered 48 h after the last dose of mianserin. These findings demonstrate functional subsensitivity of both 5-HT_{2A} and 5-HT_{2C} receptors mediating hyperthermia following both acute and chronic administration of 5-HT_{2A}/5-HT_{2C} receptor antagonists; some differences in time course and in responses to individual antagonists at 5-HT_{2A} versus 5-HT_{2C} sites were also observed.

Key words Metergoline · Mesulergine · Mianserin · Ritanserin · *m*-Chlorophenylpiperazine · (±)1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane

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Introduction

In radioligand binding studies, metergoline has been reported to have similar high affinity for various 5-HT receptor subtypes, including 5-HT_{2A} and 5-HT_{2C}, whereas mesulergine, mianserin and ritanserin have been reported to have much higher affinity at 5-HT_{2C} and 5-HT_{2A} sites than other 5-HT receptor subtypes (Hoyer 1988). Both 5-HT_{2A} and 5-HT_{2C} receptors have been shown to have similar molecular structures (Julius et al. 1988; Pritchett et al. 1988), both are coupled to guanine nucleotide regulatory proteins (Hartig 1989) and, furthermore, stimulation of each of these receptors increases phosphatidylinositol hydrolysis (Hoyer et al. 1989). These similarities led to the recent nomenclature change in which the former 5-HT_{1C} receptor was renamed 5-HT_{2C}, and the former 5-HT_2 receptor designated 5-HT_{2A} (Humphrey et al. 1993).

There is a variety of evidence from studies in rats that the serotonergic neurotransmitter system can influence feeding, locomotor activity, temperature and plasma hormones such as prolactin, adrenocorticotropic hormone (ACTH), corticosterone and growth hormone. In recent years, the use of 5-HT receptor subtype-selective agonists and antagonists has permitted partial characterization of receptor subtypes that mediate these effects. Stimulation of 5-HT_{2A} receptors produces hypophagia (Schechter and Simanski 1988; Aulakh et al. 1995a) hyperthermia (Pranzatelli 1990; Aulakh et al. 1994; Mazzola-Pomietto et al. 1995) and increases in plasma concentrations of prolactin (Gartside and Cowen 1990), ACTH and corticosterone (Alper 1990). Similarly, stimulation of 5-HT_{2C} receptors also produces hypophagia (Kennett and Curzon 1991), hypolocomotion (Kennett and Curzon 1988), hyperthermia (Mazzola-Pomietto et al. 1996) and increases in plasma concentrations of prolactin (Aulakh et al. 1992), ACTH (Bagdy et al. 1989) and corticosterone (Fuller 1990).

By using various 5-HT receptor subtype-selective antagonists, as well as tolerance and cross-tolerance studies, we have recently demonstrated that hyperthermia induced by (±)1-(2,5-dimethoxy-4-iodophenyl)-2-amino-

propane (DOI) and *m*-chlorophenylpiperazine (*m*-CPP) is mediated by selective stimulation of 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Mazzola-Pomietto et al. 1995; 1996). In these studies, pretreatment with metergoline, mesulergine, mianserin and ritanserin (1 mg/kg) injected 30 min prior to *m*-CPP or DOI was shown to attenuate both DOI-induced (Mazzola-Pomietto et al. 1995) and *m*-CPP-induced (Mazzola-Pomietto et al. 1996) hyperthermia. In the present study, we investigated the duration of this attenuation of both DOI-induced and *m*-CPP-induced hyperthermia produced by acute administration of 1.0 mg/kg and 5.0 mg/kg doses of metergoline, mesulergine, mianserin and ritanserin. In addition, we also studied the effects of chronic (13–14 days) administration of both mianserin and ritanserin on DOI-induced as well as *m*-CPP-induced hyperthermia in rats.

Materials and methods

Male Wistar rats (Charles River, Kingston, New York, USA) weighing approximately 225 g at the beginning of the study were used. Animals were housed three per cage in a temperature-controlled (21±1°C) room with a 12-h light/dark cycle (light on at 6:00 a.m.). Animals had free access to food and water at all times.

For studying the duration of attenuation of *m*-CPP and DOI-induced hyperthermia by metergoline, mesulergine, mianserin and ritanserin, separate groups of animals were injected IP with either vehicle or a low dose (1.0 mg/kg) or a high dose (5.0 mg/kg) of each 5-HT antagonist. The animals were challenged 1, 2, 3, 4 or 6 days later with a 2.5 mg/kg dose of either *m*-CPP or DOI. The selection of the 2.5 mg/kg dose of *m*-CPP and DOI was based on our previous work (Aulakh et al. 1995b; Mazzola-Pomietto et al. 1995).

For the study of chronic serotonin antagonist treatment, separate groups of animals were injected IP with either vehicle or a low dose (1.0 mg/kg) or a high dose (5.0 mg/kg) of either ritanserin or mianserin every day for 6 or 7 days and 13 or 14 days for DOI or *m*-CPP challenge, respectively. In the case of ritanserin, the animals were challenged 24 h after the last injection with a 2.5 mg/kg dose of either *m*-CPP or DOI. In the case of mianserin, the animals were challenged 48 h after the last injection with a 2.5 mg/kg dose of either *m*-CPP or DOI.

Rectal temperature was measured with a rectal probe and digital thermometer (Sensortek, Clifton, N.J., USA), all recordings being made between 10 a.m. and 1 p.m. Each animal received ten habituating exposures to the rectal probe, which was inserted 2.5 cm into the colon, while each rat was held lightly by the tail. The rectal temperature was recorded at baseline and 30 min or 60 min after *m*-CPP or DOI injection, since the peak effects for *m*-CPP occurred at 30 min and those for DOI occurred at 60 min (Mazzola-Pomietto et al. 1995; 1996).

For investigating the brain and plasma levels of metergoline or mesulergine, separate groups of animals were killed 60 min, 90 min or 24 h later following IP administration of 5 mg/kg dose of either metergoline or mesulergine, respectively. The rats were sacrificed by decapitation. The brains were dissected out and stored at –70°C until analyzed for drug levels. The trunk blood was collected in centrifuge tubes containing 0.5 ml ethylenediaminetetraacetic acid. Following centrifugation, plasma samples were collected and stored at –70°C. The brain and plasma levels of mesulergine and metergoline were measured by high pressure liquid chromatography (HPLC).

Extraction procedure

For plasma, 0.5 ml of standard or unknown sample, 100 µl (100 ng) of the internal standard, metergoline (for mesulergine) or mesulergine (for metergoline), 100 µl 2 M TRIS and 2 ml methyl-tert-butyl ether were added to a 5 ml polypropylene tube. Samples were vortex mixed and centrifuged (2000 *g*, 5 min, 4°C). The ether phase was transferred to another tube containing 200 µl 10 mM phosphoric acid. Following vortex mixing, the samples were centrifuged and the aqueous phase was injected onto the HPLC column.

For brain tissue, extractions were performed according to a modification of the method of Miller and DeVane (1986). Weighed brain samples of approximately 600–700 mg were added to 5 ml of 0.1 M perchloric acid and sonicated (model XL 2020, Heat Systems-Ultrasonics, Farmingdale, N.Y., USA) for 60 s at 4°C. To a 500 µl aliquot of brain homogenate, 100 µl 1 µM mesulergine (internal standard for metergoline) or 50 µl 1 µM metergoline (internal standard for mesulergine), 100 µl 2 M TRIS, and 2 ml 2% isoamyl alcohol in hexane was added. The sample then was vortex mixed and centrifuged (2000 *g*, 5 min, 4°C). The hexane phase was removed and added to a test tube containing 200 µl 10 mM phosphoric acid. Following vortex mixing and centrifuging, the hexane phase was removed, discarded and a 50 µl aliquot of the remaining aqueous phase was injected onto the HPLC column.

Mobile phase

The mobile phase was a mixture of 0.03 M monobasic potassium phosphate buffer, adjusted to pH 3.0 with phosphoric acid, 30% acetronitrile and 20% methanol containing 0.035% triethylamine and 2.69 µM EDTA. A constant flow rate of 0.8 ml/min was delivered by a LKB Model 2150 HPLC pump (Pharmacia, Piscataway, N.J., USA).

Instrumentation and conditions

Separation was achieved on a 3 µm, 15 cm length×4.6 mm i.d. Axxion c.8 reversed-phase column (Thomson Instrument Co., Springfield, Va., USA). Samples were injected onto the column by a Gilson Model 231/401 autosampler (Gilson, Middleton, Wisc., USA) fitted with a 50 µl sample loop. Samples were quantitated amperometrically using a Model LC-4B electrochemical detector (Bioanalytical Systems Inc., West Lafayette, Ind., USA) with a glassy carbon electrode, TL 8A, at a potential of 0.98 V versus a Ag/AgCl reference. Chromatograms were recorded on a LKB Model 2210 dual pen recorder.

Chemicals

HPLC grade phosphoric acid, monobasic potassium phosphate, methyl-tert-butyl ether and HPLC grade acetronitrile were obtained from Fisher Scientific (Pittsburgh, Pa., USA). Triethylamine was obtained from Aldrich Chemical (Milwaukee, Wisc., USA). Metergoline and mesulergine were obtained from Research Biochemicals (Natick, Mass., USA). Water was purified prior to use by a Milli-Q purification system (Millipore, Bedford, Mass., USA).

Drugs

The following drugs were used: metergoline (Farmitalia, Milan, Italy), *m*-CPP hydrochloride, DOI hydrochloride, mianserin hydrochloride, mesulergine hydrochloride and ritanserin (Research Biochemicals Natick, Mass., USA). DOI, *m*-CPP, mianserin and mesulergine were dissolved in 0.9% saline. Metergoline and ritanserin were dissolved in 100% dimethylsulfoxide (DMSO). The volume injected was 0.1 ml/100 g body weight. All drug doses given in the text refer to the salt.

Data analysis

The data were analyzed using one-way analysis of variance accompanied by contrasts (means comparisons) specified a priori

Table 1 Brain and plasma levels of metergoline or mesulergine at various time points following acute administration of a 5 mg/kg dose of metergoline or mesulergine, respectively. Values are expressed as means±SEM from eight animals

Time	Metergoline		Mesulergine	
	Brain (ng/mg)	Plasma (ng/ml)	Brain (ng/mg)	Plasma (ng/ml)
60 min	329±95	86±24	162±61	96±20
90 min	194±72	88±19	145±78	43±24
24 h	0±0	0±0	0±0	0±0

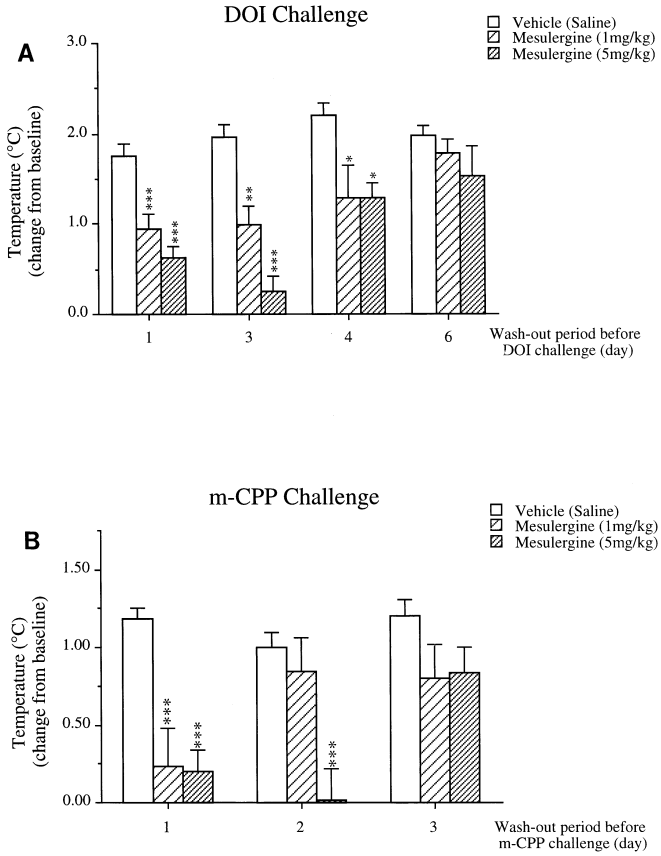

Fig. 1A, B Effects of pretreatment with low (1.0 mg/kg) or high (5.0 mg/kg) doses of mesulergine on DOI-induced (**A**) or *m*-CPP-induced (**B**) hyperthermia in rats. Values are expressed as means±SEM from six animals. **P*<0.05; ***P*<0.01; ****P*<0.001, significantly different from vehicle+DOI-treated or vehicle+*m*-CPP-treated animals

comparing independently each dose of 5-HT antagonist+*m*-CPP or DOI to the vehicle+*m*-CPP or DOI. The data used were change in rectal temperature from baseline at 30 min or 60 min, after *m*-CPP or DOI administration, respectively. All data are reported as means±SEM.

Results

The brain and plasma concentrations of both metergoline and mesulergine were generally higher at 60 than 90 min, and were nondetectable at 24 h (Table 1).

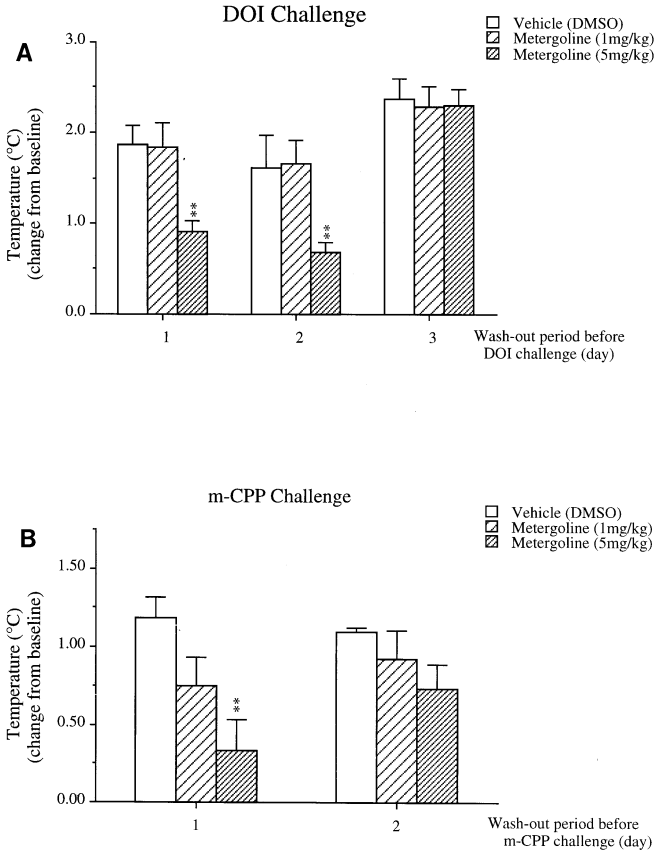

Fig. 2A, B Effects of acute pretreatment with low (1.0 mg/kg) or high (5.0 mg/kg) doses of metergoline or DOI-induced (**A**) or *m*-CPP-induced (**B**) hyperthermia in rats. Values are expressed as means±SEM from six animals. *P***<0.01, significantly different from vehicle+DOI-treated or vehicle+*m*-CPP-treated animals

For the effects of various doses of mesulergine on DOI-induced hyperthermia, analysis of variance showed an overall significant effect on day 1 [$F(2, 15)=16.5$, $P<0.001$], day 2 [$F(2, 15)=8.16$, $P<0.01$], day 3 [$F(2, 15)=4.34$, $P<0.05$] and day 4 [$F(2, 15)=4.54$, $P<0.05$] but not on day 6 [$F(2, 15)=1.13$, $P>0.05$]. Further analysis revealed that on days 1, 3 and 4, both doses of mesulergine significantly attenuated DOI-induced hyperthermia (Fig. 1A). For the effects of various doses of mesulergine on *m*-CPP-induced hyperthermia, analysis of variance showed an overall significant effect on day 1 [$F(2, 15)=11.21$, $P<0.01$] and day 2 [$F(2, 16)=7.75$, $P<0.01$] but not on day 3 [$F(2, 15)=1.69$, $P>0.05$]. Further analysis revealed that on day 1, both doses of mesulergine significantly attenuated *m*-CPP-induced hyperthermia; on day 2, only the high dose (5.0 mg/kg) of mesulergine significantly attenuated *m*-CPP-induced hyperthermia (Fig. 1B). The baseline values in saline-treated, low dose and high dose mesulergine-treated animals varied between 37.1°C and 37.6°C on various days and were not significantly different from saline-treated animals.

For the effects of various doses of metergoline on DOI-induced hyperthermia, analysis of variance showed an overall significant effect on day 1 [$F(2, 15)=6.68$,

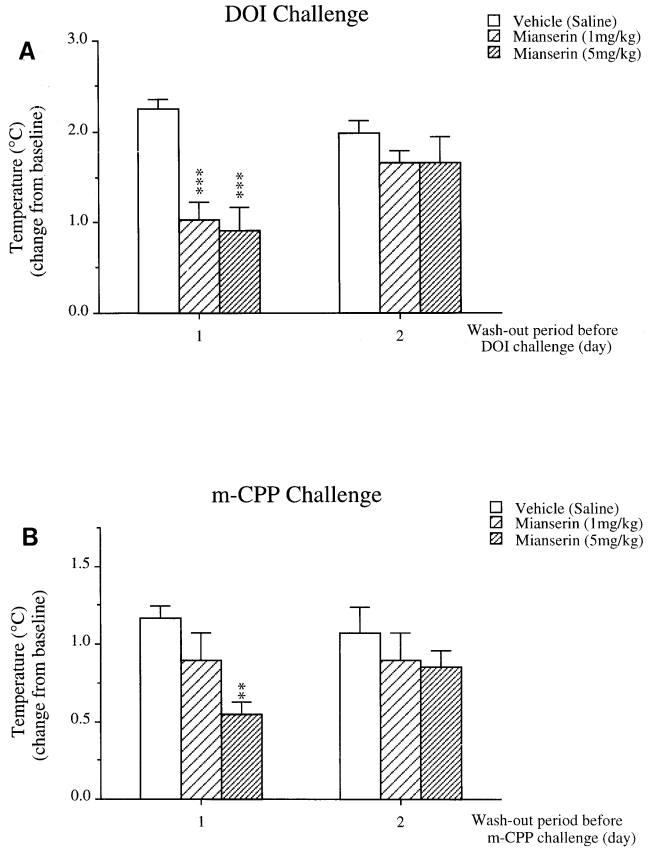

Fig. 3A, B Effects of acute pretreatment with low (1.0 mg/kg) or high (5.0 mg/kg) doses of mianserin on DOI-induced (**A**) or *m*-CPP-induced (**B**) hyperthermia in rats. Values are expressed as means±SEM from six animals. ***P*<0.01; ****P*<0.001, significantly different from vehicle+DOI-treated or vehicle+*m*-CPP-treated animals

P<0.01] and day 2 [*F*(2, 15)=4.81, *P*<0.05] but not on day 3 [*F*(2, 15)=0.04, *P*>0.05]. Further analysis revealed that on both day 1 and day 2, only the high dose (5.0 mg/kg) of metergoline significantly attenuated DOI-induced hyperthermia (Fig. 2A). For the effects of various doses of metergoline on *m*-CPP-induced hyperthermia, analysis of variance showed an overall significant effect on day 1 [*F*(2, 15)=3.82, *P*<0.05] but not on day 2 [*F*(2, 15)=2.51, *P*>0.05]. Further analysis revealed that on day 1, only the high dose (5.0 mg/kg) of metergoline significantly attenuated *m*-CPP-induced hyperthermia (Fig. 2B). The baseline values in saline-treated, low dose and high dose metergoline-treated animals varied between 37.1°C and 37.5°C on various days and were not significantly different from saline-treated animals.

For the effects of various doses of mianserin on DOI-induced hyperthermia, analysis of variance showed an overall significant [*F*(2, 15)=15.45, *P*<0.001] effect on day 1 but not on day 2 [*F*(2, 15)=0.89, *P*>0.05]. Further analysis revealed that on day 1, both doses (1.0 and 5.0 mg/kg) of mianserin significantly attenuated DOI-induced hyperthermia (Fig. 3A). For the effects of various doses of mianserin on *m*-CPP-induced hyperthermia, analysis of variance showed an overall significant [*F*(2, 15)=5.98, *P*<0.05] effect on day 1 but not on day 2 [*F*(2, 15)=0.56, *P*>0.05]. Further analysis revealed that on day 1, only the high dose (5.0 mg/kg) of mianserin significantly attenuated *m*-CPP-induced hyperthermia (Fig. 3B). The baseline values in saline-treated, low dose and high dose mianserin-treated animals varied between 37.0°C and 37.8°C. There was a small but significant decrease in the baseline temperature in mianserin-treated animals on day 2 but not on day 1.

For the effects of various doses of ritanserin on DOI-induced or *m*-CPP-induced hyperthermia, analysis of variance showed an overall non-significant effect for both DOI [*F*(2, 15)=0.17, *P*>0.05] and *m*-CPP [*F*(2, 15)=2.14, *P*>0.05]. The baseline values in saline-treated, low dose and high dose ritanserin-treated animals varied between 37.0°C and 37.3°C and were not significantly different from saline-treated animals.

For the effects of chronic administration of various doses of ritanserin on DOI-induced hyperthermia, analysis of variance showed an overall significant effect [*F*(2, 15)=5.43, *P*<0.05] on day 13. Further analysis revealed that only the high dose (5.0 mg/kg) of ritanserin significantly attenuated DOI-induced hyperthermia (Fig. 4A). For the effects of chronic administration of various doses of ritanserin on *m*-CPP-induced hyperthermia, analysis of variance showed an overall significant effect [*F*(2, 14)=38.01, *P*<0.001] on day 14. Further analysis revealed that both doses of ritanserin significantly attenuated *m*-CPP-induced hyperthermia (Fig. 4B). The baseline values in chronic saline-treated, low dose and high dose ritanserin-treated animals varied between 37.6°C and 37.9°C and were not significantly different from chronic saline-treated animals.

For the effects of chronic administration of various doses of mianserin on DOI-induced hyperthermia, analysis of variance showed an overall significant effect on day 6 [*F*(2, 15)=6.93, *P*<0.01] and day 13 [*F*(1, 10)=19.59, *P*<0.01]. Further analysis revealed that DOI-induced hyperthermia was significantly attenuated by high dose on day 6 and by low dose on day 13 (Fig. 5A). For the effects of chronic administration of various doses of mianserin on *m*-CPP-induced hyperthermia, analysis of variance did not show an overall significant effect either on day 7 [*F*(2, 15)=0.56, *P*>0.05] or on day 14 [*F*(2, 17)=0.04, *P*>0.05]. The baseline values in chronic saline-treated, low dose and high dose mianserin-treated animals varied between 37.0°C and 37.8°C. There was a small but significant decrease in the baseline temperature in chronic mianserin-treated animals relative to chronic saline-treated animals.

Discussion

The present study demonstrated that single doses of 5-HT_{2A}/5-HT_{2C} receptor antagonists, including metergoline, mesulergine, and mianserin but not ritanserin, caused long-lasting attenuation of both DOI-induced and

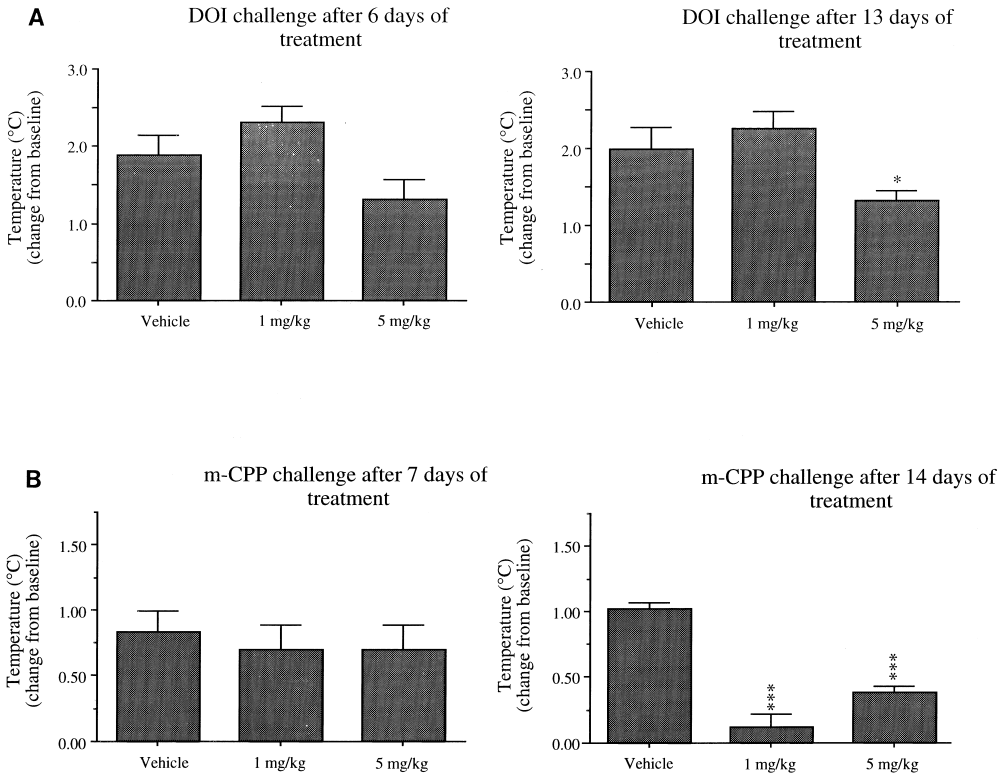

Fig. 4A, B Effects of chronic administration of low (1.0 mg/kg per day) or high (5.0 mg/kg per day) dose of ritanserin on DOI-induced (**A**) or *m*-CPP-induced (**B**) hyperthermia in rats. Values are expressed as means±SEM from six animals. **P*<0.05; ****P*<0.001, significantly different from chronic vehicle+DOI-treated or chronic vehicle+*m*-CPP-treated animals

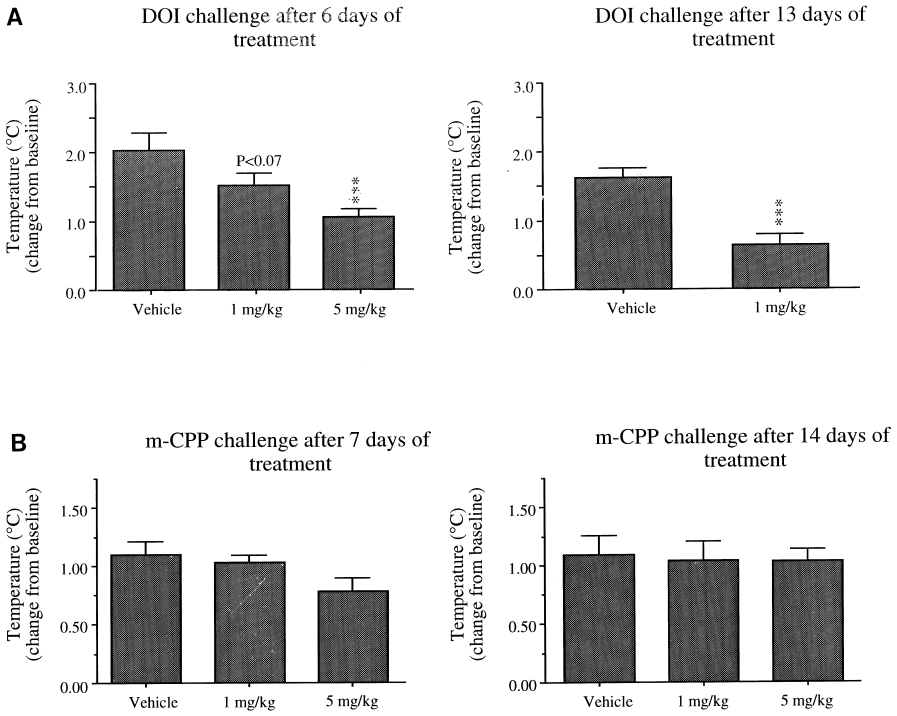

Fig. 5A, B Effects of chronic administration of low (1.0 mg/kg per day) or high (5.0 mg/kg per day) dose of mianserin on DOI-induced (**A**) or *m*-CPP-induced (**B**) hyperthermia in rats. Values are expressed as means±SEM from six animals. ****P*<0.001, significantly different from chronic vehicle+DOI-treated animals

m-CPP-induced hyperthermia in rats. Our results are consistent with a previous behavioral study in which acute administration of mianserin, metergoline and pizotifen was shown to block quipazine cue for 48 h (Smith et al. 1990). In two recent reports from this laboratory, we reported evidence from cross tolerance and antagonist studies indicating that hyperthermia induced by DOI and *m*-CPP is mediated by selective stimulation of 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Mazzola-Pomietto et al. 1995; 1996). Thus, the present findings suggest either direct receptor blockade, mediated by residual drug, or development of functional subsensitivity

of both 5-HT_{2A} and 5-HT_{2C} receptors following acute treatment with the 5-HT_{2A}/5-HT_{2C} receptor antagonists.

Pretreatment with a 1 mg/kg dose of mianserin, ritanserin, metergoline or mesulergine injected 30 min prior to *m*-CPP or DOI attenuated both *m*-CPP-induced (Mazzola-Pomietto et al. 1996) and DOI-induced hyperthermia (Mazzola-Pomietto et al. 1995). Therefore, it is possible that direct receptor blockade, mediated by residual drug, is responsible for the observed long-lasting attenuation of both 5-HT_{2A} and 5-HT_{2C} receptor-mediated hyperthermia following acute administration of 5-HT_{2A}/5-HT_{2C} receptor antagonists in the present study. However, this possibility seems unlikely due to the fact that the duration of attenuation of 5-HT_{2A} receptor-mediated hyperthermia persisted longer following the low dose of mianserin, the high dose of metergoline and both doses of mesulergine as compared to the attenuation of 5-HT_{2C} receptor-mediated hyperthermia in the present study. In radioligand binding studies, mianserin, metergoline and mesulergine have been reported to have similar high affinity at both 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer 1988). Also, both metergoline and mesulergine levels in the brain or plasma were undetectable at 24 h following the larger, 5 mg/kg dose, of both metergoline and mesulergine, respectively. Therefore, it is unlikely that the observed long-lasting attenuation of both 5-HT_{2A} and 5-HT_{2C} receptor mediated hyperthermia following acute administration of several 5-HT_{2A}/5-HT_{2C} receptor antagonists was due to direct residual effects of these antagonists.

Sanders-Bush and coworkers (1987) investigated the role of residual drug in mediating the loss of [^{3}H]ketanserin-labelled 5-HT_{2A} binding sites after acute treatment with a 5 mg/kg dose of mianserin. These investigators did not find any association between the levels of mianserin and the loss of binding sites, and therefore concluded that mianserin and other 5-HT antagonists decrease 5-HT_{2A} receptors by a unique mechanism, which is independent of a direct action of the drug. Similarly, Labrecque and coworkers (1995) demonstrated that decreases in 5-HT_{2C} receptor binding in intact Sf9 cells or membranes containing the 5-HT_{2C} receptor that followed treatment with several 5-HT antagonists including ritanserin, mianserin, metergoline and mesulergine were not due to the presence of residual antagonist. On the other hand, it is possible that the metabolites of the antagonists may be responsible for the observed long-lasting attenuation of both 5-HT_{2A} and 5-HT_{2C} receptor-mediated hyperthermia. However, this possibility seems unlikely due to the fact that several 5-HT antagonists with widely different chemical structures cause a loss of 5-HT_{2A} and 5-HT_{2C} binding sites (Blackshear et al. 1983; Leysen et al. 1986; Labrecque et al. 1995) as well as desensitize 5-HT_{2A} and 5-HT_{2C} receptor-mediated hyperthermia. Therefore, it seems unlikely that the effects of each of these compounds would be mediated by chemically different, active metabolites.

Acute administration of ritanserin (1–10 mg/kg) caused a decrease in B_{max} but not K_d values of [^{3}H]-ketanserin binding in washed frontal cortical membranes which lasted for 12 h (Leysen et al. 1986). Our results are consistent with this binding data since we did not observe attenuation of 5-HT_{2A}-receptor-mediated hyperthermia 24 h after acute administration of either the 1 mg/kg or 5 mg/kg dose of ritanserin in the present study. Unlike acute treatment, chronic treatment for 13–14 days with a high dose (5 mg/kg) of ritanserin caused apparent desensitization of both 5-HT_{2A} and 5-HT_{2C} receptors as indicated by a lack of hyperthermic response to either DOI or *m*-CPP administered 24 h after the last injection of ritanserin. Our pharmacologic response data are consistent with the study of Leysen et al. (1986), which demonstrated decreases in B_{max} values of [^{3}H]-ketanserin-labeled 5-HT_{2A} receptors in the frontal cortex following chronic oral administration of 10 mg/kg per day of ritanserin. Our results are also consistent with two reports demonstrating decreases in [^{3}H]-mesulergine-labeled 5-HT_{2C} binding sites in rat spinal cord (Pranzatelli et al. 1993) and brain stem (Pranzatelli and Tailor 1994) following chronic (30 days) administration of ritanserin (10 mg/kg per day). However, it is of note that chronic administration of a 1 mg/kg dose of ritanserin produced apparent desensitization of only 5-HT_{2C} but not 5-HT_{2A} receptors mediating hyperthermia in the present study, suggesting a differential effect of this dose of ritanserin, and, furthermore, ruling out the possibility that the observed desensitization was due to accumulation of ritanserin following its chronic treatment.

The present study also demonstrated that chronic administration of both 1 mg/kg and 5 mg/kg doses of mianserin produced desensitization of only 5-HT_{2A} but not 5-HT_{2C} receptors mediating hyperthermia; this, therefore, rules out the possibility that the observed desensitization was simply due to accumulation of mianserin following its chronic treatment. Our results are consistent with several binding studies demonstrating down-regulation of 5-HT_{2A} receptors labeled with either [^{3}H]-spiroperidol (Blackshear and Sanders-Bush 1982) or [^{3}H]-ketanserin (Roth and Ciaranello 1991) following chronic administration of mianserin. Furthermore, chronic treatment with mianserin has been shown to decrease the 5-HT_{2A} receptor-mediated head shake response to DOI (Brendsen and Broekkamp 1991) in rats as well as the head twitch response to 5-hydroxytryptophan (5-HTP) in mice (Goodwin et al. 1984). Our results are also consistent with those of Berendsen and Broekkamp (1991), who failed to observe modification of 5-HT_{2C} receptor-mediated penile erections induced by MK-212 following chronic treatment with mianserin in Wistar rats. Furthermore, chronic (30 days) administration of mianserin (10 mg/kg per day) did not down-regulate brain stem 5-HT_{2C} binding sites (Pranzatelli and Tailor 1994). On the other hand, our results are not concordant with those of Sanders-Bush and Breeding (1988), who observed decreases in [^{3}H]-mianserin-labeled 5-HT_{2C} receptor density in the choroid plexus following chronic (14 days) administration of mianserin. However, it is of note that the hypothalamus is involved in the regulation of temperature while the above mentioned study observed decreases in

5-HT_{2C} receptor binding in the choroid plexus. Also, there are other examples in which changes in serotonin receptor binding do not accompany changes in functional responses to serotonin agonists (Samanin et al. 1980; Leysen 1984; Smith et al. 1990).

The demonstration of desensitization of 5-HT_{2C} but not 5-HT_{2A} receptors following chronic administration of the 1 mg/kg dose of ritanserin on the one hand, and desensitization of 5-HT_{2A} but not 5-HT_{2C} receptors following chronic administration of mianserin on the other hand is very interesting. Although, there are very close structural similarities between 5-HT_{2A} and 5-HT_{2C} receptors as mentioned in the introduction, there are some differences also. For example, denervation of 5-HT neurons and/or decreases in brain serotonin produced either by the serotonin neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), or parachlorophenylalanine (PCPA) failed to elicit supersensitivity of 5-HT_{2A} receptors (Conn and Sanders-Bush 1986) but did produce supersensitivity of 5-HT_{2C} receptors (Conn and Sanders-Bush 1987). Daily administration of mianserin (15 mg/kg per day) for 1, 4, 10 or 21 days caused an increase in 5-HT_{2C} receptor mRNA levels but no change in 5-HT_{2A} receptor mRNA levels assessed by Northern blot solution hybridization, or in situ hybridization analysis (Roth et al. 1990). In two recent reports from our laboratory, we observed the development of tolerance to 5-HT_{2C} receptor-mediated hyperthermia, but not to 5-HT_{2A} receptor-mediated hyperthermia following daily administration of 5-HT agonists such as *m*-CPP, DOI and DOM (Aulakh et al. 1994; Mazzola-Pomietto et al. 1995; 1996).

In summary, this study demonstrates long lasting, apparent functional subsensitivity of both 5-HT_{2A} and 5-HT_{2C} receptors mediating hyperthermia following acute administration of 5-HT_{2A}/5-HT_{2C} receptor antagonists. Desensitization of 5-HT_{2A} receptors persists longer than desensitization of 5-HT_{2C} receptors following acute administration of mianserin, metergoline and mesulergine. Furthermore, chronic administration of ritanserin (1.0 mg/kg per day×14) causes desensitization of 5-HT_{2C} but not 5-HT_{2A} receptors, whereas similar chronic administration of mianserin caused desensitization of 5-HT_{2A} but not 5-HT_{2C} receptor-mediated hyperthermia.

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