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MDMA ('Ecstasy') enhances 5-HT_{1A} receptor density and 8-OH-DPAT-induced hypothermia: blockade by drugs preventing 5-hydroxytryptamine depletion

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Abstract

One week after a single administration of 3,4-methylenedioxymethamphetamine (MDMA · HCl, 30 mg/kg i.p.), 5-HT_{1A} receptor density was significantly increased by approximately 25–30% in the frontal cortex and hypothalamus of rats. The increased density correlated with the potentiation of the hypothermic response to the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 1 mg/kg s.c.). Hypothalamic 5-HT_7 receptors, which also bind 8-OH-DPAT, were not changed, however, by MDMA. Fluoxetine (5 mg/kg s.c.), ketanserin (5 mg/kg s.c.) or haloperidol (2 mg/kg i.p.), given 15 min prior to MDMA, prevented the depletion of 5-hydroxytryptamine (5-HT) induced by MDMA and also blocked the effects of this neurotoxin on 5-HT_{1A} receptor density and on 8-OH-DPAT-induced hypothermia. The protection afforded by drugs against 5-HT loss did not correlate, however, with the antagonism of the acute hyperthermic effect of MDMA. The present results indicate that drugs able to prevent or to attenuate MDMA-induced 5-HT loss also prevent the changes in 5-HT_{1A} receptor density as well as the enhanced hypothermic response to the 5-HT_{1A} receptor agonist 8-OH-DPAT in MDMA-treated rats. © 1998 Elsevier Science B.V.

Keywords: MDMA (3,4-methylenedioxymethamphetamine); 5-HT (serotonin, 5-hydroxytryptamine); 5-HT_{1A} receptor; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); Temperature

1. Introduction

It has been widely reported that single or repeated administration of high doses of the ring-substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') produces long-lasting changes in various 5-HT parameters in the brain of rodents and nonhuman primates (McKenna and Peroutka, 1990). Different findings support this contention: a reduction of 5-hydroxytryptamine (5-HT) and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) in several brain regions (Stone et al., 1986; Schmidt, 1987), a decline in the activity of tryptophan hydroxylase (Stone et al., 1986), a decrease in the number of [^{3}H]paroxetine-labeled 5-HT uptake sites (Battaglia et al., 1987) and degeneration of serotonergic terminals (O'Hearn et al., 1988; Slikker et al., 1988).

Although the mechanism by which MDMA damages 5-HT terminals remains elusive, different drugs interfering with central serotonergic or dopaminergic systems, such as blockers of 5-HT or dopamine uptake, 5-HT_2 and dopamine receptor antagonists, the dopamine synthesis inhibitor α-methyl-*p*-tyrosine or previous lesioning of brain dopamine pathways with the neurotoxin 6-hydroxydopamine, prevent the depletion of brain 5-HT following MDMA administration to rats (Schmidt, 1987; Stone et al., 1988; Schmidt et al., 1990b,c; Hewitt and Green, 1994). Other drugs, like dextromethorphan, chlormethiazole or dizocilpine, with no direct action on the serotonergic or dopaminergic system also prevent the neurotoxic effects of MDMA (Finnegan et al., 1990; Colado et al., 1993; Colado and Green, 1994). In a previous study, we reported a significant increase in 5-HT_{1A} receptor number in the rat frontal cortex 7 days after single or repeated MDMA administration (Aguirre et al., 1995). It appeared of interest to determine whether different drugs that presumably prevent 5-HT depletion, a landmark in MDMA-induced neurotoxicity, would be also able to prevent the change in 5-HT_{1A} receptor density

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induced by this psychedelic amphetamine. The neuroprotective drugs used were ketanserin, a 5-HT_2 receptor antagonist, haloperidol, an antagonist at D_2 and other dopamine receptor subtypes, and fluoxetine, a selective 5-HT uptake inhibitor.

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) is a selective 5-HT_{1A} receptor agonist (Hjorth et al., 1982; Middlemiss and Fozard, 1983) that induces hypothermia in mice and rats (Goodwin et al., 1985; Hjorth, 1985; Gudelsky et al., 1986). In rats, the hypothermia appears to be postsynaptically mediated (e.g., Millan et al., 1993). Since a single high dose of MDMA increases 5-HT_{1A} receptor density in the frontal cortex of the rat (Aguirre et al., 1995), we examined the possible functional correlation of this change with the hypothermic response to 8-OH-DPAT as well as the effect of drugs that prevent MDMA-induced neurotoxicity. Changes in body temperature elicited by 8-OH-DPAT should be exerted through 5-HT_{1A} receptors located in the hypothalamus (Cox et al., 1980). Consequently, the effect of MDMA on hypothalamic 5-HT_{1A} receptor density was also evaluated. The affinity of 8-OH-DPAT, the typical 5-HT_{1A} receptor ligand, at 5-HT_7 receptors (To et al., 1995) suggested the additional interest of measuring the eventual effect of MDMA on 5-HT_7 receptor density in the hypothalamus, where there is an abundant expression of this 5-HT receptor subtype (Shen et al., 1993; Branchek et al., 1994).

It has been recently suggested that some drugs may prevent the long-term neurochemical deficits induced by MDMA by decreasing the core temperature of the rat (Farfel and Seiden, 1995; Miller and O'Callaghan, 1995; Malberg et al., 1996). In the present study, we also determined the ability of the above-mentioned neuroprotective drugs to prevent the marked hyperthermia that follows the administration of MDMA (Schmidt et al., 1990a; Gordon et al., 1991; Dafters, 1994).

The obtained results indicate that MDMA increases 5-HT_{1A} receptor density in the frontal cortex and in the hypothalamus and also potentiates the hypothermia induced by 8-OH-DPAT in rats. These effects are not observed when MDMA is given in combination with drugs preventing the neurotoxicity of this substituted amphetamine.

2. Materials and methods

2.1. Animals and treatments

Male Wistar rats (220–240 g) were housed in plastic cages in a temperature-controlled room (22 ± 2°C) with free access to food and water and maintained on a 12/12-h light/dark cycle (lights on at 0700). Rats were distributed into the following groups: saline/saline (control group); fluoxetine (5 mg/kg s.c.); haloperidol (2 mg/kg i.p.) or ketanserin (5 mg/kg s.c.) followed by saline or MDMA (30 mg/kg i.p. of MDMA · HCl, corresponding to approximately 25 mg/kg of the base) 15 min later. In a different set of experiments, rats received MDMA (30 mg/kg i.p. twice daily for 4 days) or multiple saline injections followed by a single dose of MDMA (30 mg/kg i.p.) or saline (control group). In all cases, the doses of MDMA refer to the hydrochloride. Seven days after MDMA, rats received 8-OH-DPAT (1 mg/kg s.c.) to assess the hypothermic response or, alternatively, were killed by decapitation and their brains were removed rapidly and placed on ice. The appropriate brain regions were then frozen on dry ice and stored at −80°C until chromatographic and binding studies were performed.

2.2. Determination of 5-HT and 5-HIAA

The concentrations of 5-HT and 5-HIAA in the brain regions of the rats were determined by high-performance liquid chromatography with electrochemical detection as previously described (Perez-Otaño et al., 1991).

2.3. [^{3}H]8-OH-DPAT binding

[^{3}H]8-OH-DPAT binding studies were carried out according to the procedure previously described by Gozlan et al. (1983) with minor modifications. Briefly, the brain region was homogenized in ice-cold buffer Tris–HCl 50 mM (pH 7.7) and centrifuged at $49\,000 \times g$ for 15 min at 4°C. The pellet was resuspended in the same buffer and incubated at 37°C for 15 min. After a second centrifugation under the same conditions, the resultant pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.7) containing $CaCl_2$ 4 mM at a final tissue concentration of approximately 17 and 20 mg/ml (wet tissue weight) for the frontal cortex and hypothalamus respectively. The incubation mixture contained 100 μl of tissue suspension, 50 μl of six increasing concentrations of the labeled ligand (0.4–2 nM) and 50 μl of incubation buffer with or without buspirone 10 μM. Tubes were incubated for 15 min at 37°C. After rapid filtration of the incubation mixture through GF/C Whatman filters, the filters were rinsed with 4×5 ml of ice-cold buffer and placed in vials containing 4 ml of liquid scintillation cocktail (Biogreen3, Scharlau). All the determinations were carried out in duplicate. Data were subjected to Scatchard analysis to determine the number of binding sites (B_{max}: fmol/mg of protein) and the dissociation constant (K_d: nM).

2.4. [^{3}H]5-CT binding to 5-HT_7 receptors

[^{3}H]5-Carboxamidotryptamine ([^{3}H]5-CT) was used to label 5-HT_7 receptors in rat hypothalamus homogenates according to the method described by To et al. (1995) with minor modifications. Briefly, rat hypothalamus was homogenized in 50 mM Tris–HCl buffer, pH 7.4 (100 ml/g wet tissue), and centrifuged once at 4°C for 10 min at

48 000 × *g*. The tissue pellet was rehomogenized and incubated at 37°C for 20 min and was then centrifuged twice under the same conditions as above. After the last centrifugation, tissues were homogenized (10 mg/ml) in 50 mM Tris–HCl, pH 7.4, containing 4 mM $CaCl_2$, 1 mg/ml ascorbate, 0.01 mM pargyline and 1 μM (−)-pindolol. The incubation mixture contained 400 μl of tissue suspension, 50 μl of increasing concentrations of [^{3}H]5-CT (0.1–2 nM) and 50 μl of incubation buffer with or without 5-HT 10 μM. Tubes were incubated for 120 min at 23°C. The membrane fraction was separated by rapid filtration through GF/C Whatman filters and then the filters were rinsed with 4 × 5 ml of ice-cold 50 mM Tris–HCl buffer, pH 7.4. All the determinations were carried out in duplicate.

2.5. Temperature measurements

The rectal temperature of rats was measured with a lubricated digital thermometer probe (pb 0331, Panlab, Barcelona) inserted 4 cm into the rectum and maintained until the temperature stabilized. Readings were taken 15 min before the administration of any drug and at different times after MDMA. In other experiments, 8-OH-DPAT (1 mg/kg s.c.) was given 7 days after MDMA; temperature readings were taken 15 min before and 30 and 60 min after 8-OH-DPAT.

2.6. Drugs

The source of the drugs used were as follows: MDMA · HCl was either purchased from Sigma (UK) or was a generous gift from the 'Servicio de Restricción de Estupefacientes' (Dr. Luis Domínguez, Madrid); [^{3}H]8-OH-DPAT (148.5 Ci/mmol) was from New England Nuclear (Boston, MA); 5-carboxamido [^{3}H]tryptamine trifluoroacetate (88.0 Ci/mmol) was from Amersham (UK); haloperidol, (−)-pindolol and 8-hydroxy-2-(di-*n*-propylamino)tetralin–HBr (8-OH-DPAT) were from Research Biochemicals (Natick, MA); buspirone–HCl, pargyline, 5-HT creatinine sulfate and 5-HIAA were from Sigma (UK); fluoxetine–HCl and ketanserin were kindly donated by Eli-Lilly (Indianapolis, IN) and Janssen Life Science Products (Beerse, Belgium) respectively; all other chemicals were from Merck (Darmstadt, Germany).

2.7. Statistics

The data were analysed by one-way analysis of variance (ANOVA) with Dunnett's *t*-test for comparing control to MDMA (single or repeated treatments). In experiments where preventive pretreatments were used, data were analysed by two-way ANOVA followed by Tukey post-hoc test. Student's *t*-test was used to compare cerebral concentrations of 5-HT and 5-HIAA between saline/saline with saline/MDMA group.

3. Results

3.1. 5-HT content

As expected, MDMA produced a significant decrease ($P < 0.05$ or better) of 5-HT levels in all of the brain areas studied (Table 1). Fluoxetine (5 mg/kg s.c.) and haloperidol (2 mg/kg i.p.) completely prevented the loss of 5-HT. Ketanserin (5 mg/kg s.c.) pretreatment also prevented the MDMA-induced 5-HT depletion, although the 5-HT content was still lower than in controls in the hippocampus. Pretreatment with the different antagonists/reuptake inhibitors did not produce any change in the concentration of 5-HT in any of the brain regions examined. Similar changes in 5-HIAA levels were found after the different drug treatments (not shown).

Table 1
Effect of drugs on 5-HT depletion induced by MDMA in different rat brain regions. Animals were killed 7 days after drug treatments. Drugs (fluoxetine 5 mg/kg s.c.; ketanserin 5 mg/kg s.c.; or haloperidol 2 mg/kg i.p.) were given 15 min before saline or MDMA (30 mg/kg of MDMA · HCl). Values are means ± S.E.M. from 6 to 10 rats

Treatment	5-HT (pg/mg wet tissue)			
	Frontal cortex	Striatum	Hippocampus	Hypothalamus
Saline/Saline	382.0 ± 13.6	581.7 ± 44.3	461.7 ± 27.7	1198.0 ± 91.7
Fluoxetine/Saline	376.8 ± 18.1	593.5 ± 37.6	420.3 ± 30.2	1093.4 ± 77.8
Ketanserin/Saline	399.9 ± 23.0	560.2 ± 41.3	482.1 ± 26.1	1080.6 ± 53.4
Haloperidol/Saline	405.7 ± 13.0	566.3 ± 35.5	463.3 ± 40.1	1101.1 ± 60.6
Saline/MDMA	192.0 ± 8.1[b]	371.7 ± 55.2 [b]	146.8 ± 26.4[b]	802.8 ± 61.4[a]
Fluoxetine/MDMA	355.2 ± 23.1[c]	553.0 ± 31.2[c]	409.3 ± 35.1[c]	1093.8 ± 57.6[c]
Ketanserin/MDMA	325.0 ± 19.0[c]	510.0 ± 54.3[c]	382.0 ± 30.1[c]	1006.3 ± 13.8[c]
Haloperidol/MDMA	412.0 ± 19.6[c]	526.3 ± 39.5[c]	473.3 ± 34.1[c]	1129.3 ± 50.0[c]

[a] $P < 0.01$ and [b] $P < 0.001$ vs. saline/saline group.
[c] $P < 0.05$ or better vs. saline/MDMA using two-way ANOVA followed by Student's *t*-test or Tukey's test, respectively.

Table 2
Effect of MDMA and of combined treatments of MDMA with other drugs on 5-HT_{1A} receptor density in rat frontal cortex. Animals were killed 7 days after drug treatments. Drugs (fluoxetine 5 mg/kg s.c.; ketanserin 5 mg/kg s.c.; or haloperidol 2 mg/kg i.p.) were given 15 min before saline or MDMA (30 mg/kg of MDMA·HCl). Data are means ± S.E.M. for five rats

Treatment	B_{max} (fmol/mg of protein)
Saline/Saline	34.7 ± 2.1
Fluoxetine/Saline	33.3 ± 2.4
Ketanserin/Saline	35.4 ± 3.1
Haloperidol/Saline	33.9 ± 2.7
Saline/MDMA	45.9 ± 2.6[a]
Fluoxetine/MDMA	35.9 ± 0.9[b]
Ketanserin/MDMA	36.8 ± 1.4[b]
Haloperidol/MDMA	39.0 ± 1.3

[a] $P < 0.05$ vs. control group (saline/saline).
[b] $P < 0.05$ vs. MDMA-treated group (saline/MDMA) using two-way ANOVA followed by Tukey's test.

3.2. 5-HT_{1A} receptor density in the frontal cortex

As shown in Table 2, [^{3}H]8-OH-DPAT binding to rat frontal cortex was significantly increased in rats receiving a single injection of MDMA (30 mg/kg i.p.) one week before. However, no significant difference was found when MDMA was administered together with fluoxetine (5 mg/kg s.c.), ketanserin (5 mg/kg s.c.) or haloperidol (2 mg/kg i.p.) 7 days before the binding studies.

3.3. 5-HT_{1A} and 5-HT_7 receptor density in the hypothalamus

[^{3}H]8-OH-DPAT binding was significantly enhanced in the hypothalamus 7 days after single or repeated MDMA administration. However, no significant difference was found in [^{3}H]5-CT binding to rat hypothalamic 5-HT_7 receptors 7 days after MDMA treatment (Fig. 1).

3.4. Hypothermia induced by 8-OH-DPAT

Seven days prior to the administration of 8-OH-DPAT (1 mg/kg s.c.) rats received either single or repeated injections of MDMA (30 mg/kg i.p. twice daily for 4 days). As shown in Fig. 2, both acute and subacute MDMA treatment significantly potentiated the hypothermia induced by 8-OH-DPAT, 30 and 60 min after drug administration. In a parallel set of experiments, rats were injected with fluoxetine (5 mg/kg s.c.), ketanserin (5 mg/kg s.c.) or haloperidol (2 mg/kg i.p.) 15 min before saline or a single administration of MDMA. Seven days later, rats were injected with 8-OH-DPAT (1 mg/kg s.c.) and rectal temperatures were recorded. The potentiating effect of MDMA on the hypothermia induced by 8-OH-DPAT was prevented by all of the combined treatments (antagonist/uptake inhibitor + MDMA). The 8-OH-DPAT-induced hypothermia in rats receiving any pretreatment and saline was not significantly different from that seen in the saline/saline group (data not shown). The results obtained 60 min after 8-OH-DPAT administration are shown in Fig. 3.

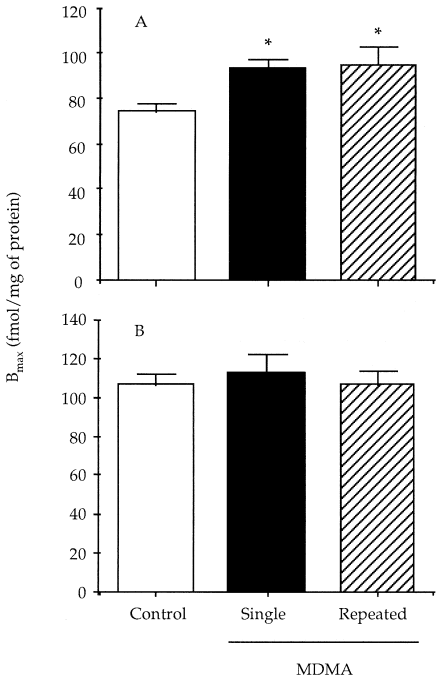

Fig. 1. Long-term effects of single or repeated MDMA administration on hypothalamic 5-HT_{1A} (A), and 5-HT_7 (B) receptor density. Animals were killed 7 days after MDMA (30 mg/kg i.p. twice daily for 4 days), multiple saline injections followed by a single dose of MDMA (30 mg/kg i.p.) or saline (control group). B_{max} values are means ± S.E.M. in fmol/mg of protein, from four to eight rats. * $P < 0.05$ vs. control group using ANOVA followed by Dunnett's *t*-test.

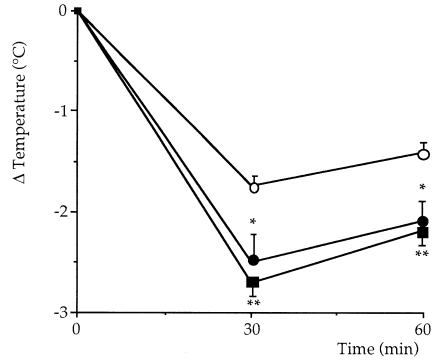

Fig. 2. Effects of MDMA (30 mg/kg i.p. b.i.d. for 4 consecutive days, ■), multiple saline injections followed by a single dose of MDMA (30 mg/kg, ●) or saline (○, control group $n = 10$) on the hypothermia induced by 8-OH-DPAT (1 mg/kg s.c.) in rats. Animals received saline or MDMA ($n = 6$ in both groups) 7 days before 8-OH-DPAT. Data were analysed by ANOVA followed by Dunnett's *t*-test. * $P < 0.05$, ** $P < 0.01$ compared to control group.

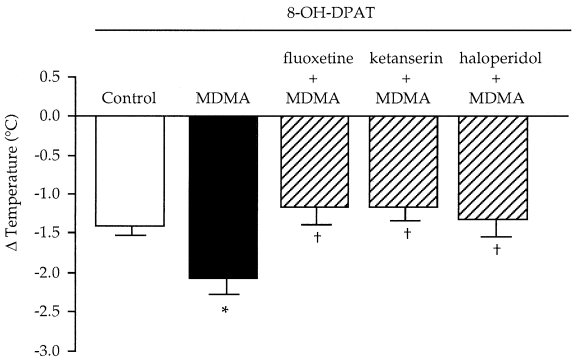

Fig. 3. Hypothermia in rats 60 min after the administration of 8-OH-DPAT (1 mg/kg). Seven days before, the animals received saline/saline (control group), saline/MDMA (30 mg/kg) or combined treatments of different drugs and MDMA. In combined treatments, drugs (doses as in legend to Fig. 1) were given 15 min before MDMA. Values are means ± S.E.M. ($n = 6–10$). Data were analysed by two-way ANOVA followed by Tukey's test. $* P < 0.05$ vs. control group; $^{\dagger} P < 0.05$ vs. saline/MDMA.

3.5. MDMA-induced hyperthermia

MDMA (30 mg/kg i.p.) produced marked hyperthermia in rats, averaging approximately 2°C, 30 and 60 min after injection. Fifteen minutes before saline or MDMA administration, rats were pretreated with drugs that prevent 5-HT loss. Ketanserin pretreatment fully prevented the MDMA-induced hyperthermia, while the combination of haloperidol and MDMA not only prevented the hyperthermia but induced a mild hypothermia. Fluoxetine had no effect on the MDMA-induced hyperthermia. No pretreatment had any significant effect on temperature compared to the effect of saline/saline injection (data not shown). Changes in core temperature 60 min after MDMA are depicted in Fig. 4.

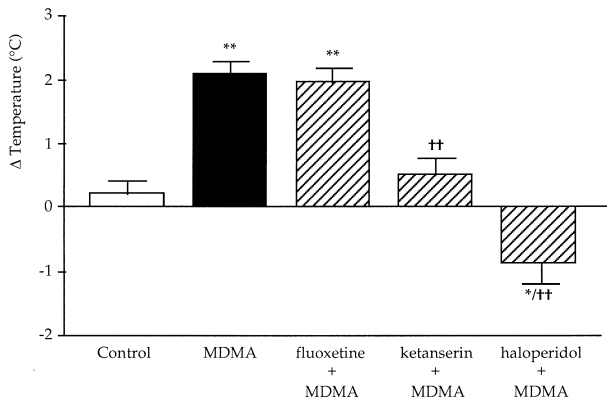

Fig. 4. Changes in rectal temperature of rats 60 min after the administration of saline/saline (control group), saline/MDMA (30 mg/kg) or combined treatments of different drugs and MDMA. In combined treatments, drugs (doses as in the legend to Fig. 1) were given 15 min before MDMA. Values are means ± S.E.M. ($n = 6–10$). Data were analysed by two-way ANOVA followed by Tukey's test. $* P < 0.05$, $** P < 0.01$ vs. control group, $^{\dagger\dagger} P < 0.01$ vs. saline/MDMA-treated animals.

4. Discussion

Both the single and repeated MDMA treatments used in the present study have been repeatedly reported to produce a long-term reduction in brain 5-HT content, a decrease in the activity of tryptophan hydroxylase and a degeneration of fine 5-HT terminals projecting from the dorsal raphe nucleus to different brain areas of the rat (Stone et al., 1986; Battaglia et al., 1987; Schmidt, 1987; O'Hearn et al., 1988). In the present experiments, one week after a single high dose of MDMA, a marked reduction in 5-HT and 5-HIAA levels was observed in different terminal fields of the serotonergic system of the rat. In keeping with previous reports (Schmidt, 1987; Stone et al., 1988; Schmidt et al., 1990b,c; Hewitt and Green, 1994), the selective 5-HT reuptake inhibitor, fluoxetine, as well as ketanserin, a 5-HT_2 receptor antagonist, and haloperidol, an antagonist at dopamine receptors, were able to protect against the lasting 5-HT depletion induced by MDMA. Full protection by all three drugs was found in the frontal cortex and in the striatum. However, only partial protection was found with ketanserin in the hippocampus. The results obtained with ketanserin are in line with previous studies in which 5-HT_2 receptor antagonists only provided incomplete protection against MDMA-induced neurotoxicity (Schmidt et al., 1992).

As previously reported (Aguirre et al., 1995, 1997), MDMA (20–30 mg/kg) upregulates postsynaptic 5-HT_{1A} receptors in the frontal cortex and enhances the expression of the corresponding mRNA. In the present study, single or repeated MDMA administration also increased significantly [^{3}H]8-OH-DPAT binding in the hypothalamus. Since this typical 5-HT_{1A} receptor ligand also shows affinity at 5-HT_7 receptors (To et al., 1995), which are abundant in the rat hypothalamus (Shen et al., 1993; Branchek et al., 1994), it appeared of interest to study the possibility of a change by MDMA in 5-HT_7 receptor number in this brain region. No change in the density of the latter receptor was found after acute or subacute MDMA, so it seems that the enhanced [^{3}H]8-OH-DPAT binding induced by this serotonergic neurotoxin in the hypothalamus also corresponds to an upregulation of 5-HT_{1A} receptors. The increase in postsynaptic 5-HT_{1A} receptors could be initially interpreted as an attempt to compensate for the loss of 5-HT terminals. Yet, another 5-HT neurotoxin such as 5,7-dihydroxytryptamine does not produce such an adaptative change (Hensler et al., 1991). The neurotoxicity induced by *p*-chloroamphetamine appears similar to that seen after MDMA, but drugs such as dizocilpine or deprenyl or previous lesioning of brain dopamine pathways with the neurotoxin 6-hydroxydopamine is able to prevent the long-term neurotoxicity of MDMA but not that of *p*-choloroamphetamine (Green et al., 1995; Perry et al., 1995; Sprague et al., 1996). In the present study, we found that different drugs repeatedly used to prevent MDMA-induced neurotoxicity, such as 5-HT_2/dopamine receptor

antagonists or monoamine uptake inhibitors (see above), which are without any intrinsic effect on 5-HT_{1A} receptor density, were able to prevent the upregulation of this 5-HT receptor subtype by MDMA. Even though an inverse correlation was found between 5-HT levels and 5-HT_{1A} receptor number, other mechanisms may also account for this unique regulatory effect of MDMA on 5-HT_{1A} receptor density. It should be mentioned in this regard that MDMA is able to alter after 7 days, as in the present study, 5-HT_{1A} receptor mRNA expression in the frontal cortex and in the hippocampus (Aguirre et al., 1997). However, MDMA produced no change in hippocampal 5-HT_{1A} receptor mRNA after 14 days (Yau et al., 1994), and it is known that at this time significant 5-HT depletion should still be found in different rat brain regions (e.g., Malberg et al., 1996). As recently suggested in a behavioural study with *p*-chloroamphetamine (Santucci et al., 1996), it is always possible to speculate that acute 5-HT release, and not long-term depletion, may determine, by a hitherto unknown mechanism, the regulation of 5-HT_{1A} receptors induced by MDMA.

8-OH-DPAT is a selective 5-HT_{1A} receptor agonist (Hjorth et al., 1982; Middlemiss and Fozard, 1983) that induces hypothermia in mice and rats (Goodwin et al., 1985; Hjorth, 1985; Gudelsky et al., 1986). In the mouse, there is good evidence for the involvement of somatodendritic 5-HT_{1A} receptors in 8-OH-DPAT-induced hypothermia (Goodwin et al., 1985; Heal et al., 1989; Bill et al., 1991). However, there has been some controversy over the last few years as to whether pre- or postsynaptic 5-HT_{1A} receptors are involved in the hypothermic response to 8-OH-DPAT in the rat. Goodwin et al. (1987) reported that the hypothermia induced by 8-OH-DPAT was mediated by presynaptic 5-HT_{1A} receptor stimulation because it was attenuated by previous 5-HT depletion with *p*-chlorophenylalanine or by direct injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine into the third ventricle. Other reports by Higgins et al. (1988) and Hillegaart (1991) agree with the data of Goodwin et al. (1987) because hypothermia was induced by injecting 8-OH-DPAT into the dorsal raphe. However, data from other authors seem to contradict these observations because *p*-chlorophenylalanine pretreatment tended to increase the hypothermic effect of 8-OH-DPAT in rats (Hjorth, 1985; Hutson et al., 1987). In another study (Bill et al., 1991), both *p*-chlorophenylalanine and 5,7-dihydroxytryptamine counteracted the hypothermic response to 8-OH-DPAT in mice but not in rats, suggesting presynaptic 5-HT_{1A} receptor activation by this drug only in the former species. More recent studies have also presented direct evidence for a postsynaptic mediation of the hypothermic effect of 5-HT_{1A} receptor activation in the rat. Thus, the buspirone analogue BMY 7378 (8-[2[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]8-azaspirol[4,5]-decane-7,9-dione dihydrochloride), which is a high-efficacy agonist at 5-HT_{1A} receptors in the dorsal raphe, but a low-efficacy agonist at postsynaptic sites (Vandermaelen et al., 1987; Sharp et al., 1990; Middlemiss and Tricklebank, 1992), showed no hypothermic effect when directly infused into the dorsal raphe (O'Connell et al., 1992) and only mild hypothermia at high doses when administered systemically (Millan et al., 1993).

In order to examine a possible functional correlate of changes in 5-HT_{1A} receptor density, 8-OH-DPAT was given to rats one week after single and repeated administration of MDMA, and their rectal temperature was measured. It was found that MDMA pretreatment significantly enhanced 8-OH-DPAT-induced hypothermia. According to the opposite changes induced by MDMA on pre- and postsynaptic 5-HT_{1A} receptors, which are down- or upregulated respectively by MDMA (Aguirre et al., 1995), if somatodendritic 5-HT_{1A} autoreceptors were involved, an attenuation of 8-OH-DPAT-induced hypothermia 7 days after MDMA treatment would have been expected. Conversely, the potentiation of the hypothermia argues in favour of the involvement of postsynaptic 5-HT_{1A} receptors. These results are also in accordance with other reports in which prior depletion of rat neuronal 5-HT with *p*-chlorophenylalanine or damage to 5-HT neurons with 5,7-dihydroxytryptamine did not affect or rather tended to increase the hypothermic response to 8-OH-DPAT (Hjorth, 1985; Bill et al., 1991; O'Connell et al., 1992; Millan et al., 1993). Interestingly, drug treatments that prevent the loss of 5-HT and 5-HIAA in all of the brain areas assayed also attenuated the enhanced hypothermic response to 8-OH-DPAT in rats pretreated 7 days before with MDMA. In keeping with these findings, functional alterations of 5-HT_{1A} receptor-coupled neuroendocrine responses 2 weeks after a single dose of MDMA have been reported (Poland, 1990).

It should be noted however that in a recent study (McNamara et al., 1995), no change was found in the hypothermic response to 8-OH-DPAT in rats that had previously received a subacute MDMA treatment. Different doses of MDMA and of 8-OH-DPAT may explain the disparate findings. Moreover, in this previous study reductions in 5-HT and 5-HIAA content were only found in the frontal cortex and amygdala of MDMA-treated rats and not in the hippocampus or striatum as in the present study and in many other reports (e.g., Green et al., 1995). The lack of 5-HT depletion by MDMA in some of the terminal fields of the serotonergic system may simply explain the failure to detect a change in the hypothermic response to 8-OH-DPAT.

It has been recently suggested that the mechanism of protection of some drugs against MDMA-induced neurotoxicity may be their ability to decrease the core temperature of the rat (Farfel and Seiden, 1995; Miller and O'Callaghan, 1995; Malberg et al., 1996). Accordingly, we also measured the ability of the same neuroprotective agents to prevent the hyperthermia that follows the administration of MDMA. These agents were used at doses that, in keeping

with previous studies (Hewitt and Green, 1994; Malberg et al., 1996), did not change core temperature on their own. Although the treatments used prevented or markedly attenuated the loss of 5-HT and 5-HIAA in all of the brain regions examined, our results showed that fluoxetine did not prevent MDMA-induced hyperthermia, as already reported (Malberg et al., 1996). The failure of fluoxetine to prevent the hyperthermic effect of MDMA is in line with other studies (Schmidt et al., 1990a; Colado et al., 1993), indicating that this functional effect is not always associated with the long-term neurochemical deficits produced by the drug.

5. Conclusion

The present results indicate that MDMA induces adaptative changes in postsynaptic 5-HT_{1A} receptors. Different drug treatments that prevented the 5-HT depletion induced by MDMA also prevented the increased 5-HT_{1A} receptor density and the augmented hypothermic response to postsynaptic 5-HT_{1A} receptor activation. The results suggest a role for 5-HT_{1A} receptors in the psychopathological changes elicited by MDMA in humans.

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