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Short communication

Methylenedioxymethamphetamine induces opposite changes in central pre- and postsynaptic 5-HT_{1A} receptors in rats

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Abstract

The present study examined the short- and long-term effects of single and repeated administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on somatodendritic and postsynaptic 5-HT_{1A} receptors of the rat brain. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) was used to label 5-HT_{1A} receptors in the brain stem region containing the dorsal raphe nucleus and in the frontal cortex. As expected, both schedules of treatment reduced the serotonin (5-hydroxytryptamine, 5-HT) content and [^{3}H]paroxetine binding in the frontal cortex but not in the brain stem. Multiple but not single MDMA administration significantly reduced 5-HT_{1A} receptor density in the selected brain stem region. In the frontal cortex, both MDMA treatments increased or tended to increase 5-HT_{1A} receptor number, the effect being more marked after repeated drug administration.

Keywords: MDMA (3,4-methylenedioxymethamphetamine); 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{1A} receptor

1. Introduction

The administration of a single high dose or of multiple doses of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats produces long-term alterations in different serotonergic parameters. A marked reduction in the content of serotonin (5-hydroxytryptamine, 5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) along with a decrease in tryptophan hydroxylase activity in various brain regions have been described (Stone et al., 1986; Schmidt, 1987). Immunocytochemical evidence indicates that MDMA selectively damages fine axon terminals which arise from cell bodies located in the dorsal raphe nucleus of the midbrain which remain spared (O'Hearn et al., 1988). The loss of axon terminals is supported by the finding that MDMA administration significantly decreases the number of [^{3}H]paroxetine-labeled 5-HT uptake sites (Battaglia et al., 1987).

The 5-HT_{1A} receptor is both an inhibitory somatodendritic autoreceptor on raphe cells and a postsynaptic receptor in 5-HT terminal fields such as the hippocampus and the frontal cortex (Vergé et al., 1986). Since adaptative changes in 5-HT_{1A} receptor number or function may be induced by different psychotropic drugs affecting 5-HT dynamics (Vergé et al., 1986; De Montigny et al., 1993; Jolas et al., 1994), the present study was aimed at assessing the short- and long-term effects of MDMA on 5-HT_{1A} receptors located in the brain stem region containing the dorsal raphe nucleus and in the frontal cortex of the rat. The results indicate that MDMA regulates in an opposite manner 5-HT_{1A} receptors in these two brain regions.

2. Materials and methods

2.1. Animals and treatments

Male Wistar rats (200–250 g) were housed in plastic cages in a temperature-controlled room (22° C) and maintained on a 12 h light-dark cycle with free access to food and water. Two different schedules of treatment with MDMA were used in this study. Acute treatment consisted of a single high dose of MDMA (30 mg/kg i.p.), the rats being killed 3 h or 7 days later.

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These two time points were selected because a maximal effect of MDMA on 5-HT release has been described between 3 and 6 h after a single drug injection (Schmidt, 1987). It is known that a 7-day survival time allows the detection of neurodegenerative changes (Battaglia et al., 1987; Schmidt, 1987). The same survival times were selected when the same dose of MDMA was given b.i.d. for 4 consecutive days. MDMA doses refer to the hydrochloride.

2.2. Brain dissection

In all cases animals were killed by decapitation and their brains were removed rapidly and placed on ice. The frontal cortex and the brain stem region including the entire dorsal raphe nucleus were dissected free. To dissect out the latter region, the brain was bisected through the intercollicular commissure down the surface of the fourth ventricle. The tissue surrounding the midline was then excised throughout the aqueduct up to the third ventricle. The brain regions were frozen on dry ice and stored at −80° C until chromatographic studies were performed. Binding studies were carried out with fresh tissue.

2.3. 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 (Pérez-Otaño et al., 1991).

2.4. [^{3}H]Paroxetine binding

[^{3}H]Paroxetine binding studies were performed according to the procedure described by Marcusson et al. (1988), with minor modifications. The brain regions studied were homogenized in 15 ml of ice-cold buffer (Tris-HCl 50 mM, 120 mM NaCl, 5 mM KCl, pH 7.4) and centrifuged at 48 000 × g for 10 min at 4° C. The pellet was resuspended in buffer and incubated at 37° C for 10 min. After a second centrifugation in the same conditions the resultant pellet was resuspended in buffer (1.5 mg tissue/400 μl buffer). The incubation mixture contained 400 μl of tissue suspension, 200 μl of increasing concentrations of [^{3}H]paroxetine (0.02–0.4 nM) and 1.4 ml of incubation buffer in the absence and presence of fluoxetine 10 μM. Tubes were incubated for 60 min at 22° C. After rapid filtering 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).

2.5. [^{3}H]8-OH-DPAT binding

[^{3}H]8-Hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]8-OH-DPAT) binding studies were performed according to the procedure described by Gozlan et al. (1983) with minor modifications. Briefly, freshly dissected frontal cortices and brain stem regions were homogenized in ice-cold buffer Tris-HCl 50 mM (pH 7.7) and centrifuged at 49 000 × 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 in 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 12.5 mg wet weight/ml for the frontal cortex and brain stem region respectively. The incubation mixture contained 100 μl of tissue suspension, 50 μl of increasing concentrations of the labelled 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. The following steps were the same as above.

The sources of the drugs used were as follows: MDMA-HCl was either from Sigma (UK) or was a gift from the 'Servicio de Restricción de Estupefacientes' (Dr. L. Domínguez, Madrid, Spain); [^{3}H]8-OH-DPAT (148.5 Ci/mmol) and [^{3}H]paroxetine (22.5 Ci/mmol) were obtained from New England Nuclear (Boston, MA, USA); buspirone-HCl, 5-HT creatinine sulfate and 5-HIAA were from Sigma (UK); fluoxetine-HCl was donated by Eli-Lilly and Co. (Indianapolis, IN, USA); all other chemicals were from Merck (Darmstadt, Germany).

3. Results

3.1. Short- and long-term effects of MDMA on 5-HT and 5-HIAA brain levels

The content of 5-HT in the frontal cortex and in the brain stem region after single or multiple administrations of MDMA is depicted in Table 1. A single injection of a high dose of MDMA significantly decreased the 5-HT content in the frontal cortex both 3 h and 7 days after drug administration. No significant change was found in the brain stem region containing the dorsal raphe at either time point. The effect of repeated MDMA treatment was much more marked. A reduction of approximately 90% in 5-HT content in the frontal cortex was found 3 h after the last MDMA injection. Seven days later, 5-HT levels remained significantly decreased. Repeated administration of MDMA produced again no significant change in 5-HT

levels in the analyzed brain stem region. Both schedules of treatment with MDMA reduced 5-HT and 5-HIAA levels not only in the frontal cortex but also in other terminal fields of the serotonergic system such as hippocampus, striatum and hypothalamus (not shown).

3.2. 5-HT uptake site density

MDMA caused a substantial reduction in the density of 5-HT uptake sites in the frontal cortex of the rat 7 days after a single administration, the effect being more marked after repeated drug administration. In the brain stem region, no significant change in the density of 5-HT uptake sites was observed after repeated MDMA treatment at either time point (Table 1).

3.3. Effects of single and repeated MDMA administration on somatodendritic and postsynaptic 5-HT_{1A} receptor density

In vitro, MDMA did not inhibit at concentrations up to 10 μM the binding of a fixed concentration (2 nM) of [^{3}H]8-OH-DPAT to rat brain cortical homogenates.

A single high dose of MDMA (30 mg/kg i.p.) did not elicit any significant change in the density of somatodendritic 5-HT_{1A} autoreceptors at either time point (3 h or 7 days). In the frontal cortex, a slight increase in the number of 5-HT_{1A} postsynaptic receptors was observed 3 h after a single MDMA injection, the effect being significant 7 days after drug administration (Fig. 1).

Repeated MDMA treatment (30 mg/kg i.p. b.i.d. for 4 days) caused opposite effects on the 5-HT_{1A} receptor density depending on the area studied. In the dorsal raphe region, B_{max} was significantly reduced 3 h and 7 days after the last MDMA injection. In the frontal cortex, multiple high doses of MDMA produced a significant increase in B_{max} 3 h and 7 days after the MDMA treatment (Fig. 1). No treatment produced a significant change in the K_d values, which were in the range of 0.9–1.5 nM.

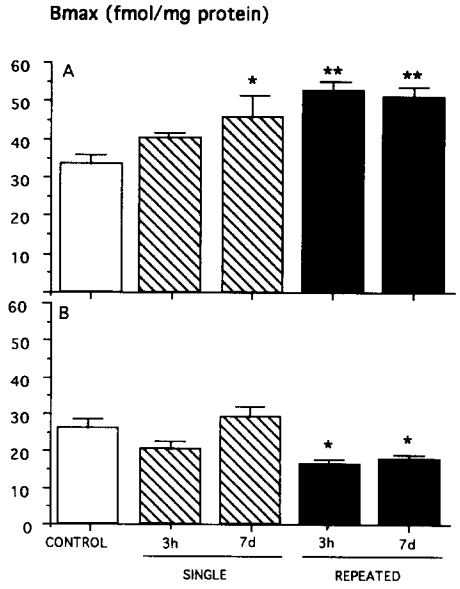

Fig. 1. Effect of single (30 mg/kg i.p.) and repeated (30 mg/kg i.p. b.i.d., 4 consecutive days) administration of MDMA on 5-HT_{1A} receptor density (B_{max}) in the frontal cortex (A) and brain stem region including the dorsal raphe (B) of the rat. Animals were killed 3 h or 7 days after the last MDMA injection. Data are the means ± S.E.M. for 5–6 rats. * $P < 0.05$, ** $P < 0.01$ vs. control group (one-way analysis of variance followed by Dunnett's t-test).

4. Discussion

The results of the present study indicate that repeated administration to rats of MDMA, a drug with negligible in vitro affinity for brain 5-HT_{1A} receptors (Battaglia et al., 1988; our own results), increases the density of this 5-HT receptor subtype in the frontal cortex and reduces 5-HT_{1A} receptor density in the brain stem region containing the dorsal raphe nucleus.

Table 1
Effect of single and repeated administration of MDMA on 5-HT levels and [^{3}H]paroxetine binding sites in the frontal cortex and brain stem of the rat

Treatment		Survival time	Frontal cortex		Brain stem	
			5-HT levels (pg/mg wet tissue)	[^{3}H]Paroxetine binding B_{max} (fmol/mg protein)	5-HT levels (pg/mg wet tissue)	[^{3}H]Paroxetine binding B_{max} (fmol/mg protein)
Single	Control		722 ± 50	306.2 ± 28.5	1002 ± 37	–
	MDMA	3 h	105 ± 14 [a]	284.0 ± 13.6	951 ± 44	–
	MDMA	7 days	450 ± 45 [a]	197.7 ± 15.2 [a]	1017 ± 89	–
Repeated	Control		739 ± 32	318.4 ± 29.6	1043 ± 74	397.7 ± 16.6
	MDMA	3 h	87 ± 18 [a]	140.5 ± 16.5 [a]	1121 ± 90	349.0 ± 12.9
	MDMA	7 days	201 ± 16 [a]	168.7 ± 16.1 [a]	963 ± 76	368.6 ± 14.3

Animals received saline (control group), MDMA (30 mg/kg i.p.) or MDMA (30 mg/kg i.p. b.i.d. for 4 days) for single or repeated treatment respectively. Rats were killed 3 h or 7 days after the last MDMA injection. Values are means ± S.E.M. from 5–10 rats (MDMA treatments) or 12–17 rats (controls). [a] $P < 0.01$ vs. control group using one-way analysis of variance followed by Dunnett's t-test.

As repeatedly described (e.g. Stone et al., 1986; Schmidt, 1987), a single high dose of MDMA decreased 5-HT levels in different 5-HT terminal fields but not in the dorsal raphe region. This decrease was more pronounced after repeated MDMA treatment and in both cases there was a partial recovery after a 7-day survival time. Likewise, [^{3}H]paroxetine binding sites were significantly decreased in the frontal cortex 7 days after a single MDMA injection and at any of the two selected time points after repeated MDMA treatment, suggesting a degeneration of 5-HT nerve terminals (Battaglia et al., 1987).

The decrease in [^{3}H]paroxetine binding sites was correlated with an increase of [^{3}H]8-OH-DPAT-labeled 5-HT$_{1A}$ receptors in the frontal cortex. Preliminary experiments appear to indicate that there is also such a correlation in the hippocampus (not shown). These findings can be probably interpreted as adaptative changes to compensate for the loss of serotonergic nerve terminals. In the dorsal raphe region, MDMA may produce a carrier-mediated 5-HT release and a subsequent activation of inhibitory somatodendritic autoreceptors of the 5-HT$_{1A}$ subtype (Sprouse et al., 1989). Since the activation of 5-HT$_{1A}$ autoreceptors causes a profound reduction in the firing activity of 5-HT neurons (De Montigny et al., 1993), the observed long-term decrease in the density of somatodendritic 5-HT$_{1A}$ receptors might represent an attempt to compensate for the loss in 5-HT nerve terminals. The reduction in somatodendritic 5-HT$_{1A}$ receptor number was only apparent after repeated MDMA administration, which produced a more marked reduction in 5-HT levels and 5-HT nerve terminals in the frontal cortex. In spite of the lack of in vitro affinity of MDMA at brain 5-HT$_{1A}$ receptors, it has been previously reported that some behavioural effects of MDMA in rats such as the induction of forepaw treading and spontaneous tail-flicks are 5-HT$_{1A}$ receptor-mediated (Millan and Colpaert, 1991). In line with these behavioural studies, the present report shows that MDMA is also able to produce adaptative changes in 5-HT$_{1A}$ receptor number.

Even though MDMA has been assigned to the Schedule I status by the Drug Enforcement Administration, there is no consensus on the public health implications of MDMA abuse, particularly on the risk for sustained serotonergic neuronal damage in humans. Moreover, MDMA has been used as an adjunct to psychotherapy because of its proposed anxiolytic effect (Greer and Tolbert, 1990), which may promote self-disclosure and trust. It is of interest that the effects of typical pyrimidinylpiperazine anxiolytics such as buspirone appear to be mediated through somatodendritic 5-HT$_{1A}$ receptors. Electrophysiological investigations suggest that chronic administration of antidepressants may desensitize somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus or make postsynaptic 5-HT$_{1A}$ receptors hypersensitive to 5-HT (De Montigny et al., 1993), though receptor density may not be affected (Jolas et al., 1994).

The predominance of 5-HT$_{1A}$ receptors in the limbic system suggests that many effects of 5-HT and 5-HT receptor ligands in emotional mechanisms could be mediated by 5-HT$_{1A}$ receptors (Iversen, 1984). Interestingly, the results obtained with MDMA in the present study show adaptative changes of 5-HT$_{1A}$ receptors and raise the intriguing possibility of an involvement of 5-HT$_{1A}$ receptors in the behavioural effects of MDMA.

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