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Short communication

Intravenous administration of ecstasy (3,4-methylendioxymethamphetamine) enhances cortical and striatal acetylcholine release in vivo

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Abstract

The effect of intravenous administration of 3,4-methylendioxymethamphetamine (MDMA), in a range of doses (0.32–3.2 mg/kg) that have been shown to maintain self-administration behaviour in rats, on in vivo acetylcholine release from rat prefrontal cortex and dorsal striatum was studied by means of microdialysis with vertical concentric probes. Intravenous administration of MDMA dose-dependently increased basal acetylcholine release from the prefrontal cortex to 57 ± 21%, 98 ± 20%, 102 ± 7% and 141 ± 14% above baseline, at doses of 0.32, 0.64, 1.0 and 3.2 mg/kg, respectively. MDMA also stimulated striatal acetylcholine release at the dose of 3.2 mg/kg i.v. (the maximal increase being 32 ± 3% above baseline) while at the dose of 1 mg/kg i.v., MDMA failed to affect basal acetylcholine output. Administration of MDMA also dose-dependently stimulated behaviour. The results of the present study show that MDMA affects measures of central cholinergic neurotransmission in vivo and suggest that at least some of the psychomotor stimulant actions of MDMA might be positively coupled with an increase in prefrontal cortical and striatal acetylcholine release.

Keywords: Acetylcholine; (±)-3,4-Methylendioxymethamphetamine (MDMA, ecstasy); Microdialysis; Prefrontal cortex; Striatum

1. Introduction

"Ecstasy" or "Adam" (3,4-methylendioxymethamphetamine or MDMA) misuse has seen a constant growth in the last few years among youngsters of western countries, despite the fact that it has been included in the list of illicit compounds and despite user awareness that Ecstasy pills very often have a variable and unknown composition (Green et al., 1995). As in the case of other psychotropic compounds (Freud's prescriptions of cocaine can be taken as an example), MDMA was also originally used in therapy (Downing, 1986; Green et al., 1995) but was soon after withdrawn because of its adverse acute effects.

Many studies report about the MDMA mechanism of acute and long-term neurotoxicity (Green et al., 1995). MDMA has been reported to act, acutely, as a potent serotonin releasing agent, both in vitro (Schmidt et al., 1987) and in vivo (Rudnick and Wall, 1992), and to release dopamine as well (Hiramatsu and Cho, 1990). However, to our knowledge there have not been studies that investigated the actions of MDMA on the central cholinergic system, apart from a recent study, which reports that MDMA increases acetylcholine release from rat striatal slices (Fischer et al., 2000).

Cholinergic neurotransmission in the rat frontal cortex and hippocampus positively correlates with the behavioural activation induced by sensory stimuli or by drugs (Acquas et al., 1996; Day et al., 1991; Mizuno et al., 1991). Thus, the release of acetylcholine from the rat frontal cortex has been reported to be stimulated by D-amphetamine (Day and Fibiger, 1992), methylphenidate (Acquas and Fibiger, 1996) or fenfluramine (Hirano et al., 1995). Similarly, cocaine and D-amphetamine affect in vivo striatal acetylcholine release (Acquas and Fibiger, 1998; Consolo et al., 1992; Damsma et al., 1991) and

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striatal acetylcholine release also correlates, in some instances, with behavioural activation (Day et al., 1991).

The aim of the present study was to characterize for the first time the effects of MDMA on cholinergic neurotransmission in vivo in the rat prefrontal cortex and dorsal striatum. To this end, we studied the effect of intravenous administration of MDMA at doses known to maintain self-administration in rats (Ratzenboeck et al., 2001).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (275–300 g) were housed in groups of two to three per cage for at least 3 days before use and were maintained on a 12:12-h light/dark cycle (lights on at 7:30 AM) with food and water available ad libitum. After surgery, the rats were individually housed in hemispherical bowls, which also served as the experimental environment. Experiments were carried out between 9:00 AM and 4:00 PM at least 24–30 h after surgery. The experimental protocol was approved by the Ethics Committee of the University of Cagliari and experiments were performed in strict accordance with the EC regulations for the care and use of experimental animals (CEE/NE 86/609).

2.2. Surgery and microdialysis

Rats were anaesthetised with chloral hydrate (Carlo Erba, Italy) (400 mg/kg i.p.) before stereotaxical implantation of concentric microdialysis probes aimed at the prefrontal cortex and at the dorsal striatum, the coordinates being AP = +3.6 mm, DV = −4.8 mm, ML = −0.7, and AP = +0.7 mm, DV = −6.5 mm, ML = +3.0, respectively, according to Paxinos and Watson (1998). In addition, a polyethylene catheter was inserted in the left femoral vein and tunneled subcutaneously to exit at the nape of the neck (Crane and Porrino, 1989). In the experiments in which the doses of 0.32 and 0.64 mg/kg i.v. of MDMA were studied, rats were implanted with a single vertical concentric microdialysis probe in the left prefrontal cortex, while the effects of vehicle, 1.0 and 3.2 mg/kg i.v. of MDMA were studied in rats implanted with two microdialysis probes aimed at the right prefrontal cortex and the left dorsal striatum. The membrane for microdialysis, a polyacrylonitrile/sodium methallyl sulphonate copolymer (AN 69, Hospal, Italy), was covered with epoxy glue along its whole length except for 3 mm corresponding to the area of dialysis. On the day of the experiment, rats were connected to a perfusion pump by polyethylene tubing connected to a 2.5-ml glass syringe containing normal Ringer; the perfusion flow rate was set at 1.25 μl/min. Samples were collected every 10 min into a 20-μl sample loop and subsequently injected in a manually operated injector valve. To achieve consistently detectable amounts of acetylcholine in the dialysates, the reversible acetylcholine esterase inhibitor, neostigmine bromide (0.1 μM) (Sigma, MO, USA), was added to normal Ringer containing 147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$, in twice-distilled water. Acetylcholine was assayed by high-pressure liquid chromatography (HPLC) coupled with electrochemical detection in conjunction with an enzyme reactor (Damsma et al., 1987). Acetylcholine was separated on a reverse-phase Chromspher C_{18} 5 μm (Merck, FRG) column (75 × 2.1 mm). The mobile phase passed directly through the enzyme reactor containing acetylcholine esterase (ED 3.1.1.7; type VI-S, Sigma) and choline oxidase (EC 1.1.3.17; Sigma) covalently bound to glutaraldehyde-activated LiChrosorb 10-NH_2; acetylcholine was quantitatively converted into hydrogen peroxide, which was detected electrochemically at a platinum working electrode set at 500 mV versus an Ag/AgCl reference electrode (LC-4B, BAS, IN, USA). The mobile phase was an aqueous potassium phosphate buffer (1.9 mM K_2HPO_4, 0.2 mM tetramethyl ammonium hydroxide, pH = 8) delivered at a constant flow of 0.4 ml/min by an HPLC pump (Bishoff 2200, Germany). The detection limit of the assay was about 50 fmol/sample. Injections of an acetylcholine standard (20 μl, 0.1 μM) were made every 60 to 90 min in order to monitor changes in electrode sensitivity, and sample concentrations were corrected accordingly.

2.3. Behavioural measures

The behavioural analysis was started immediately after vehicle or drug administration and lasted 60 min (corresponding to six dialysis samples). Spontaneous behaviour was classified as: *Still, Sedated*: resting, lying down with eyes closed or half open; *Still, Aroused*: eyes wide open, movements of the head and of the whiskers, chewing; *Active*: upward sniffing and rearing, locomotor activity accompanied by sniffing (exploratory behaviour), wet-dog shakes, digging in the bedding, grooming and at, the highest intensity, circling.

2.4. Drugs

(±)-3,4-Methylendioxymethamphetamine HCl (National Institute of Drug Abuse, NIDA, USA) was dissolved in saline and injected intravenously in a volume of 1 ml/kg; all doses are expressed as mg/kg of the free base.

2.5. Statistics

Values are expressed as percent changes with respect to baseline (100%). Baseline was set as the average of the last six pre-treatment samples, not differing by more than 15%. One-way and two-way analyses of variance (ANOVA), with time as the repeated measure, were used to analyse the treatment effects. Tukey's post-hoc analyses

were applied for multiple comparisons, with statistical significance set at $P < 0.05$.

3. Results

Basal acetylcholine output in cortical and striatal dialysates was 62 ± 3 ($n = 30$) and 104 ± 5 ($n = 14$) fmol/min ± S.E.M., respectively. The intravenous administration of saline (1 ml/kg) failed to significantly affect basal acetylcholine release from the prefrontal cortex [$F(8,16) = 0.95$, N.S.] and the striatum [$F(8,16) = 1.74$, N.S.] (one-way ANOVA, Fig. 1, top and bottom panels).

Administration of MDMA stimulated acetylcholine release from the prefrontal cortex at the doses of 0.32 mg/kg [$F(8,48) = 4.94$, $P < 0.0001$], 0.64 mg/kg [$F(8,56) = 8.18$, $P < 0.00001$], 1.0 mg/kg [$F(8,72) = 12.06$, $P < 0.00001$] and 3.2 mg/kg [$F(8,32) = 5.91$, $P < 0.0001$]. Two-way ANOVA showed a significant effect of dose [$F(4,28) = 14.57$, $P < 0.00001$] and time [$F(8,224) = 8.54$, $P < 0.00001$], and a significant dose × time interaction [$F(32,224) = 1.98$, $P < 0.002$], the effect of 1.0 and 3.2 mg/kg being significantly greater than that of 0.32 and 0.64 mg/kg (Tukey's post hoc test, $P < 0.05$). No differences were observed between 0.32 and 0.64 mg/kg or between 1.0 and 3.2 mg/kg of MDMA (Tukey's post hoc test, $P > 0.05$).

MDMA, at the dose of 1.0 mg/kg, did not affect acetylcholine release from the striatum [$F(8,32) = 1.9$, N.S.] (one-way ANOVA), while at the dose of 3.2 mg/kg, MDMA significantly increased it [$F(6,30) = 5.11$, $P < 0.001$] (one-way ANOVA). Two-way ANOVA revealed a significant effect of dose of MDMA [$F(2,11) = 14.04$, $P < 0.0009$] and the increase after 3.2 mg/kg was significantly greater than that after vehicle or MDMA 1.0 mg/kg ($P < 0.05$ at Tukey's post hoc analysis).

As shown in Fig. 2, the administration of vehicle did not elicit any change in the spontaneous behaviour of rats, which remained still, resting (*Still, Sedated*). The effects of MDMA on behaviour were characterized by episodes of grooming (*Active*) lasting 10 min after doses of 0.32 mg/kg, and by chewing, wet-dog shakes and episodes of sniffing with rearing and/or locomotion (exploration) (*Active*) lasting 15–20 min after doses of 0.64 mg/kg. Doses of 1.0 mg/kg elicited a pattern of behavioural activation characterized by chewing, wet-dog shakes, sniffing at the air and circling (*Still, Aroused* and *Active*), which lasted for 30–40 min after drug administration,

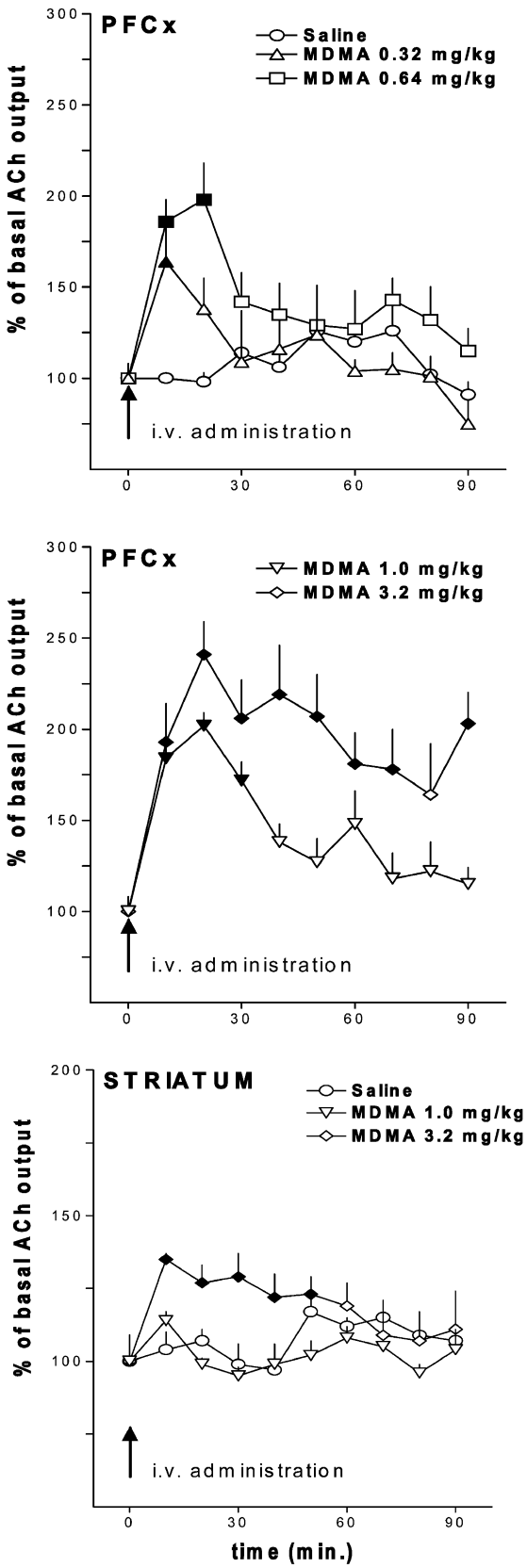

Fig. 1. Effects of intravenous administration of saline ($n = 3$), MDMA 0.32 mg/kg ($n = 7$), MDMA 0.64 mg/kg ($n = 8$) (top panel), MDMA 1.0 mg/kg ($n = 7$) and MDMA 3.2 mg/kg ($n = 5$) (middle panel) on basal prefrontal cortical acetylcholine release, and effects of saline ($n = 3$), MDMA 1.0 mg/kg ($n = 5$) and MDMA 3.2 mg/kg ($n = 6$) on basal striatal acetylcholine release (bottom panel). Values are expressed as percentages of baseline. Vertical bars represent S.E.M. Arrows indicate the last pretreatment sample. Filled symbols indicate values significantly different from baseline ($p < 0.05$ at Tukey's post hoc test).

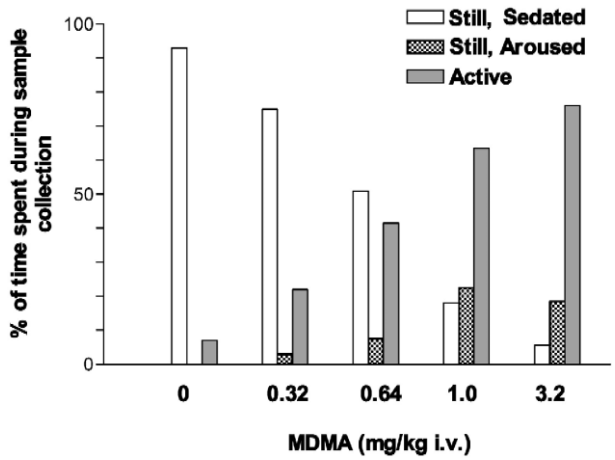

Fig. 2. Behavioural effects of vehicle or MDMA (0.32, 0.64, 1.0, and 3.2 mg/kg i.v.) administration. Behaviors were analysed according to three main categories as follows: *Still, Sedated* in which rats were resting, lying down with eyes closed or half open; *Still, Aroused* in which rats had their eyes wide open and showed movements of the head and of the whiskers, and chewing; *Active* in which rats showed upward sniffing and rearing, locomotor activity accompanied by sniffing (exploratory behaviour), wet-dog shakes, digging in the bedding, grooming and, at the highest intensity, circling.

while after doses of 3.2 mg/kg of MDMA rats showed an immediate activation with intense circling and sniffing at the air within the first 20 min after MDMA, followed by episodes of chewing, sniffing with locomotion and circling behaviour (*Still, Aroused* and *Active*), which lasted longer than 60 min.

4. Discussion

The results of the present study show that intravenous administration of MDMA, at doses of 0.32, 0.64, 1.0 and 3.2 mg/kg, stimulates dose dependently the release of acetylcholine from the rat prefrontal cortex and striatum (at doses of 1.0 and 3.2 mg/kg,). MDMA also produced a dose-related behavioural activation.

Although MDMA is normally taken by humans per os, we used the intravenous route of administration in order to avoid the artifact of handling on cortical acetylcholine release (Acquas et al., 1996; Moore et al., 1993) and to mimic to a certain extent self-administration in rats (Ratzenboeck et al., 2001).

Acetylcholine release from the prefrontal cortex and striatum was increased by MDMA and these increases were paralleled by a dose-dependent activation of spontaneous behaviour. The mechanism of the stimulation of acetylcholine release by MDMA is unclear. Similar effects on cortical (and hippocampal) and striatal acetylcholine release have been reported for other psychomotor stimulants such as D-amphetamine (Day and Fibiger, 1992; Imperato et al., 1993), cocaine (Imperato et al., 1993) and methylphenidate (Acquas and Fibiger, 1996), effects antagonized by the dopamine D_1 receptor antagonist SCH 23390 (Acquas and Fibiger, 1996; Day and Fibiger, 1992; Imperato et al., 1993). Therefore, dopamine seems to play an important role in the MDMA-induced increase in acetylcholine release. However, it is unclear if this role of dopamine is a direct or an indirect one. In the striatum, the increase in acetylcholine elicited by MDMA might be directly related to the release of dopamine in this area (Acquas and Di Chiara, 1999), while in the prefrontal cortex it might be the indirect result of the psychostimulant property of the drug. A further possibility is that MDMA stimulates acetylcholine neurotransmission by releasing serotonin and stimulating 5-HT_2 receptors which have been demonstrated to be involved in the fenfluramine-induced increase in acetylcholine release from the rat frontal cortex (Hirano et al., 1995).

A role of H_1 histamine receptors acetylcholine release by MDMA in the striatum has been recently suggested (Fischer et al., 2000); thus, it is reasonable to speculate that these effects in vivo can be controlled by histamine H_1 receptors.

In conclusion, our study indicates that the acute effects of MDMA are associated with the stimulation of acetylcholine release in the prefrontal cortex and striatum and that these increases might be related to the psychomotor stimulant properties of ecstasy.

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