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Short-term effects of 3,4-methylenedioximethamphetamine on noradrenergic activity in locus coeruleus and hippocampus of the rat

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Abstract

This study was undertaken to investigate the effects of the administration of 3,4-methylenedioxymethamphetamine (MDMA) on the locus coeruleus firing rate, on the sensitivity of the α_2-adrenoceptors which regulate neuronal activity and on the in vivo tyrosine hydroxylase activity in hippocampus. The basal firing rate was not modified by either a single dose or repeated doses of MDMA, although the latter produced a shift to the right in the dose-response curve for clonidine-induced inhibition of the firing rate (ED_{50} increased by 59%) and a reduction in tyrosine hydroxylase activity (20%) in the hippocampus. However, 8 days after the final dose α_2-adrenoceptor sensitivity and tyrosine hydroxylase activity had returned to control values. Our results show a desensitization of α_2-adrenoceptors in locus coeruleus and the existence of short-term changes in the noradrenergic system.

Keywords: 3,4-methylenedioxymethamphetamine; α_2-adrenoceptors; Locus coeruleus; In vivo; Tyrosine hydroxylase activity; Single-unit extracellular recordings; Hippocampus

(±)3,4-methylenedioxymethamphetamine (MDMA), commonly known by the name of 'ecstasy', is structurally related to amphetamine and mescaline. The normal dose of this drug required to obtain the effects desired by recreational users is 1–4 mg/kg, but nowadays the repeated use of MDMA for a short period of time (e.g. a weekend) is also frequent and, therefore, the total quantity taken borders the toxic doses described for different animal species. The effects of MDMA have been mainly attributed to its interaction with the central serotonergic system, as relevant reductions in the content of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), accompanied by decreases in the tryptophan hydroxylase activity, have been described in diverse brain regions after the repeated administration of MDMA [20]. The intravenous administration of MDMA has been seen to depress the firing rate of noradrenergic neurons in the locus coeruleus (LC), an effect which was only partially reversed by administering an α_2-adrenoceptor antagonist [16]. However, the few studies carried out to date which have evaluated the modification of noradrenergic neurotransmission after repeated treatments with this drug, have yielded controversial results [12,14]. Noradrenergic innervation to the brain principally originates in the LC, whose neurons are regulated by α_2-adrenoceptors [2], and the hippocampus receives noradrenergic fibres exclusively arising from this nucleus [17]. LC is densely innervated by serotonergic fibres and there is evidence that 5-HT plays a role in the modulation of LC activity [9,11,19]. Therefore, we considered it of interest to study whether MDMA-induced alteration of serotonergic neurotransmission influences the activity of the noradrenergic system. To do this, we evaluated the effects of a single and repeated administration of this drug on the LC firing rate, on somatodendritic α_2-adrenoceptor sensitivity and on in vivo tyrosine hydroxylase activity in hippocampus.

Male Sprague–Dawley rats with an initial weight of 180–200 g were divided into different groups: I: this group received a single dose of MDMA (20 mg/kg, s.c.) and the animals were subjected to the experimental procedure 18 h later (ACUTE). II: the rats received MDMA (20 mg/kg, s.c., at 10:00 and 16:00 h) for two consecutive days, then had a 3 day drug free period, after which another 2 day MDMA treatment was administered and the experiments were carried out 18 h after receiving the last drug dose ($MDMA^1$). III: this group was subjected to the same drug

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regime as MDMA[1], but the experiments were carried out 8 days after administration of the last dose (MDMA[2]). IV: the animals received two daily injections of saline solution (1 ml/kg, s.c.) following the same protocol as MDMA[1] (CONTROL). V: this group received *p*-chlorophenylalanine (PCPA) (150 mg/kg/24 h, i.p.) for 3 days and the rats were killed 24 h after the last dose (PCPA). VI: the rats received PCPA (150 mg/kg/24 h, i.p.) for 3 days followed by a 2 day MDMA treatment; they were then subjected to a further 3 days of PCPA administration before being given a final 2 day treatment of MDMA, and were killed 18 h after the last dose (PCPA + MDMA). The MDMA regime used was chosen in an attempt to emulate the way in which recreational users take this drug (i.e. several doses followed by a drug-free period of some days and then further doses).

For the in vivo electrophysiological studies, the rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and then, the jugular vein was cannulated for the administration of both additional injections of anaesthetic and the α_2-adrenoceptor agonist, clonidine. The animals were placed in a stereotaxic frame with the head oriented at 15° to the horizontal plane (nose down). A bur hole was drilled in the occipital bone over the LC (coordinates 3.7 mm caudal and 1.1 mm lateral lambda). Body temperature was maintained at 37 °C by means of an electric heating pad. Procedures for a single-extracellular recording from LC cells were carried out as described previously [19]. The extracellular signal from the electrode was amplified through a high-input impedance amplifier and monitored on an oscilloscope and an audiomonitor. Individual neuronal spikes were discriminated and the firing rate was recorded using 'Spike-2' software (CED, Cambridge, England). LC cells were identified by standard anatomical and electrophysiological criteria [19]. The firing rate in the different groups was measured before (basal: mean firing rate during 3–5 min) and after administration of clonidine (0.625--40 μg/kg, injected at 1 min intervals in doubling doses, until maximal inhibition was reached). Dose-effect curves for clonidine were analyzed using GraphPad (Prisma 3.0).

The in vivo activity of tyrosine hydroxylase (TH) (EC 1.14.16.2) was determined by measuring the accumulation of 3,4-dihydroxyphenylalanine (DOPA) 30 min after inhibition of the aromatic L-amino acid decarboxylase by 3-hydroxybenzylhydrazine (NSD-1015) (100 mg/kg, i.p.). After the dissection of the hippocampus on an ice-cooled plate, the tissues were treated as described in a previous study [17] and the compounds of interest were quantified by high-pressure liquid chromatography (HPLC) (Waters Assoc., USA) with electrochemical detection (LC-4B, Bioanalytical Systems Inc., USA). Prior to subjecting the samples to HPLC to determine 5-HT and 5-HIAA content, one aliquot of the purified supernatants was filtered, whilst the rest was processed in order to evaluate DOPA and noradrenaline (NA) levels following the method used in the above-cited study.

After both the acute treatment (single dose) and 18 h after the last dose of the repeated treatment with MDMA, the average basal firing rate of LC noradrenergic neurons was similar to that of the control group, although 8 days after completing the treatment it was slightly diminished (Table 1). The administration of a single dose of MDMA, did not modify the dose-response curve for clonidine-induced inhibition of cellular activity (Table 1). However, the repeated treatment with MDMA led to a marked reduction in the ability of the agonist to inhibit the LC firing rate (ED_{50} increased by 59%) (Table 1), which was reflected by a shift to the right of the dose-effect curve (Fig. 1). Nevertheless, α_2-adrenoceptor sensitivity had returned to control values by the 8th day after the repeated treatment (Table 1 and Fig. 1).

The administration of a single dose of MDMA did not affect basal DOPA accumulation nor NA content in hippocampus in relation to the control group, while 18 h after the last dose of the repeated treatment, DOPA synthesis was found to be decreased (20%), returning to values similar to that of control after 8 drug-free days (Fig. 2). In neither of the groups which received a repeated treatment with MDMA did the NA concentrations change in comparison with those of the control group (Fig. 2). In the animals that received PCPA and MDMA sequentially (PCPA + MDMA), there was a reduction in DOPA accumulation (64 ± 4, $n = 8$; $P < 0.008$ vs. control value; Kruskal–Wallis followed by Mann–Whitney *U*-test according to the Bonferroni correction) which was similar (25%) to that obtained in the group treated with only MDMA, without there being changes in NA content (330 ± 17, $n = 8$). The administration of PCPA alone did not lead to modifications in DOPA accumulation (96 ± 4, $n = 8$) and, as expected, reduced 5-HT and 5-HIAA (89% and 90%, respectively;

Table 1
Effect of acute and repeated treatment with MDMA on the basal firing rate and on the parameters of dose-effect curves for the clonidine-induced inhibition of firing rate of LC neurons in anaesthetized rats[a,b]

			Parameters of dose-effect curve	
	FR Basal (Hz)	(*n*)	DE_{50} (μg/kg)	(*n*)
Control	2.3 ± 0.1	71	2.7 ± 0.3	5
Acute	2.0 ± 0.2	32	2.3 ± 0.3	6
MDMA[1]	2.0 ± 0.1	29	4.3 ± 0.6*	7
MDMA[2]	1.7 ± 0.1*	37	2.7 ± 0.3■	7

[a] The values are mean ± S.E.M of *n* cells for group. Dose-effect curves for the different groups were constructed by analyzing, with a logistic equation, the reductions in firing rates of LC neurons following i.v. administration of increasing doses of clonidine (0.625–40 μg/kg) until the maximal effect was achieved ($E_{max} = 100\%$).

[b] FR Basal *$P < 0.05$ when compared to the control group (one-way ANOVA followed by DMS test). ED_{50} *$P < 0.05$ when compared to the control group and ■$P < 0.05$ when compared to the MDMA[1] group (one-way ANOVA followed by Newman Keuls test).

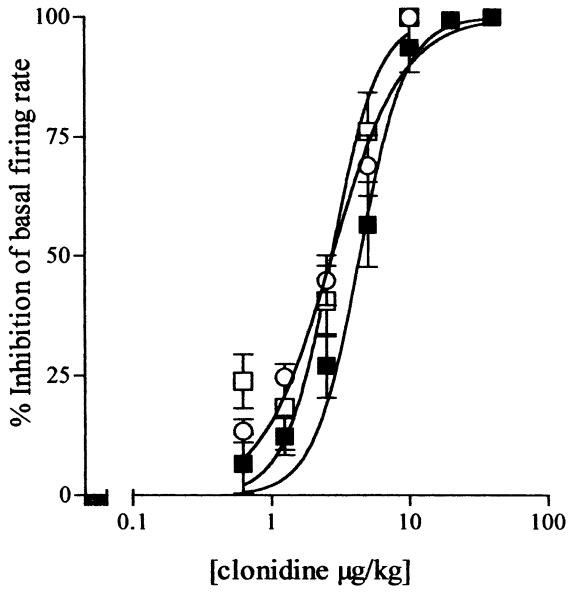

Fig. 1. Effects of MDMA on α_2-adrenoceptors in the locus coeruleus. Dose-effect curves for clonidine (0.625–40 μg/kg, i.v.)-induced inhibition of the LC firing rate in control (○, $n = 5$) and in MDMA[1] (■, $n = 7$) or MDMA[2] (□, $n = 7$) groups. Each point is the mean ± SEM of the changes in firing rate expressed as the percentage of the baseline.

$P < 0.008$, Kruskal–Wallis followed by Mann–Whitney *U*-test) contents in relation to control values (208 ± 23 and 296 ± 40 ng/g; $n = 11$). There were also relevant decreases in the levels of 5-HT (77% and 57%, $n = 6$) and its metabolite (87% and 89%, $n = 6$) in the groups of rats which received either PCPA and MDMA or only MDMA when compared to the control group ($n = 11$).

The results of the present study show that the repeated administration of MDMA leads to a transitory desensitisation of the α_2-adrenoceptors which regulate LC noradrenergic neurons. The relatively low affinity of MDMA for α_2-adrenoceptor binding sites [1] together with the fact that its estimated half-life in the rat is only between 74 and 101 min [3] makes it unlikely that the reduced sensitivity of the α_2-adrenoceptors is caused by a direct action of the drug. Moreover, as the electrophysiological experiments in this study were carried out 18 h after administration of the last dose, the existence of a residual effect of MDMA may be ruled out. Functional interaction between serotonergic and noradrenergic systems in the LC has been described and an enhancement in the spontaneous firing of NA-LC neurons has been found after the inhibition of 5-HT synthesis [5]. On the other hand, it has been seen that the local application of a selective 5-HT uptake inhibitor in the LC increases extracellular NA concentration in this area [13]. The administration of each dose of MDMA during the repeated treatment may provoke an acute release of 5-HT [20], in turn, facilitating the NA release which leads to α_2-adrenoceptor desensitisation. In fact, by the 8th day after finishing the repeated treatment, a recovery of α_2-adrenoceptor functionality in the LC had taken place, accompanied by a reduction in the basal LC firing rate, which could reflect the existence of a compensatory mechanism secondary to the reduced serotonergic control of LC. Moreover, the capacity of MDMA to induce NA release in the central nervous system has been described [7]. Therefore, both the 5-HT induced NA release, which occurs after the administration of each dose of MDMA, and the drug's direct NA-releasing capacity lead to the desensitisation of the receptors. In line with this, a subsensitivity of the somatodendritic α_2-adrenoceptors has been seen following the subchronic administration of amphetamine [18].

The lack of change observed in DOPA accumulation in the hippocampus after the administration of a single dose of MDMA is in accordance with the short-lasting increase in TH activity [15]. However, we found a reduction in DOPA accumulation after the repeated administration of MDMA probably due to an enhancement of synaptic NA which, through the activation of α_2-adrenoceptors, inhibits the synthesis of DOPA. In fact, the interaction between serotonergic and noradrenergic systems in hippocampus has been thoroughly studied and the presence of serotonergic receptors has been described as facilitating NA release [4,6,10]. In this context, despite the relevant decreases of 5-HT found in MDMA-treated rats after using a similar regime to that used in the present study, the extracellular basal 5-HT and that obtained in response to stimulation remained unchanged [8]. We found that by the 8th day after finishing the repeated treatment with MDMA, TH activity had returned to control values, which suggests the involvement of NA in the effect induced by the drug. Furthermore, the

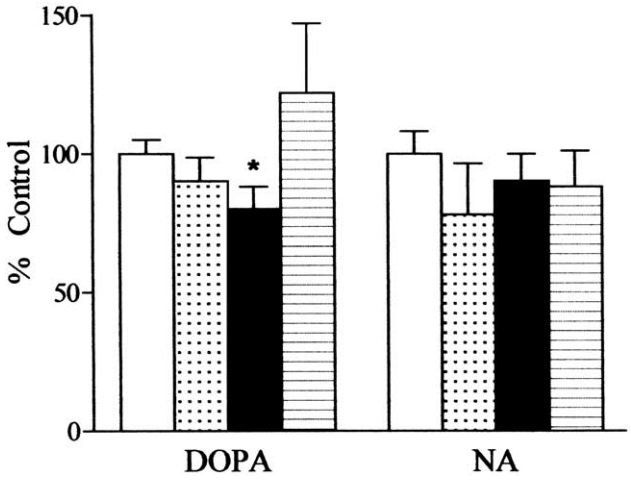

Fig. 2. Effects of acute and repeated treatments with MDMA on DOPA accumulation and on NA content in hippocampus. All experimental groups were injected with NSD-1015 (100 mg/kg, i.p) 30 min before decapitation. Bars represent the percentages ± SEM of the values obtained in the control group (□) (86 ± 4 ng/g and 280 ± 23 ng/g, for DOPA and NA respectively; $n = 20$). Acute (▤, $n = 7$), MDMA[1] (■, $n = 15$) and MDMA[2] (⬓, $n = 15$). *$P < 0.017$ vs. control group (Kruskal–Wallis followed by Mann–Whitney *U*-test according to the Bonferroni correction).

implication of the noradrenergic neurotransmitter is further supported by the fact that TH activity was not modified by the inhibition of 5-HT synthesis and the reduction of this activity was similar both when PCPA and MDMA were sequentially administered and when only the latter drug was given. The results provide evidence that repeated treatment with MDMA modifies central noradrenergic activity and these modifications seem to be dependent on the drug's capacity to induce an enhancement of NA availability.

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