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Effects of MDMA ('ecstasy') on firing rates of serotonergic, dopaminergic, and noradrenergic neurons in the rat

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3,4-Methylenedioxymethamphetamine (MDMA), a non-hallucinogenic drug of abuse, potently depressed firing rates of a subpopulation of serotonin neurons in the dorsal and median raphe. High neurotoxic doses depressed those serotonin neurons unresponsive to low doses. Noradrenaline neurons in the locus coeruleus were also depressed by moderate doses. Dopamine neurons were unaffected. It is concluded that MDMA's unique psychological effects are mediated through a subpopulation of serotonergic and noradrenergic neurons, presumably through effects on release mechanisms.

INTRODUCTION

MDMA (3,4-methylenedioxymethamphetamine), or 'ecstasy', is a chemical cross between mescaline (a hallucinogen) and amphetamine (a stimulant). Although originally synthesized as an ethical anti-appetite drug, it was never marketed as such, and has now attained the status of a 'designer' drug for elicit users[6,9,15,17]. When 1–2 mg/kg of the drug is ingested, it produces a unique feeling of well being, an enhanced cognition, and an appreciation for the feelings of others, without eliciting hallucinations[6,9,15]. Some psychiatrists have used it as an aid to psychotherapy[11,25]. However, due to its increasing widespread recreational use and new knowledge about neurotoxicity, government agencies are rendering MDMA as an illegal drug of abuse[6,9,15].

Many investigators have shown that MDMA interacts with serotonergic (5-HT), dopaminergic (DA), and noradrenergic (NA) systems in the brain[6,9,15,19,23]. Although 1–2 mg/kg represents the clinically effective dose[6,9,15], most studies have used 10 mg/kg or more. They report that 5-HT neurons seem to be most susceptible to MDMA neurotoxicity, but relatively little is known about the neural systems responsible for eliciting MDMA's interesting psychological effects. In this study, we measured the effects of intravenously applied MDMA on nerve impulse rates of single 5-HT, NA, and DA neurons in the rat. We found that doses known to elicit the MDMA psychological syndrome in man depressed firing rates of some 5-HT and NA, but not DA, cells. The results demonstrate that MDMA is not amphetamine-like and may give clues into the psychobiological roles of 5-HT and NA systems.

MATERIALS AND METHODS

Preparation of animals

The techniques are similar to those previously utilized in this laboratory for recording from dopaminergic and serotonergic neurons[12,18]. Male Sprague–Dawley rats, 250–350 g, were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses (50–100 mg/kg, i.p.) were administered as needed to maintain anesthesia. The femoral artery and vein were cannulated for blood pressure measurement and drug administration, respectively. MDMA did not affect blood pressure in any consistent manner over the dose range used in these experiments. Body temperature was maintained at 37 °C using an isothermal pad.

Extracellular recording and electrode placement

Single-barrel Omega Dot tubing with an internal glass fiber were pulled by a Narashige microelectrode puller. The tips were broken back to 1 μm under microscopic control, and filled with a filtered 2 M NaCl solution saturated with Pontamine sky blue dye (Gurr). Standard electrophysiological recording techniques were used. Drug effects were measured as changes in firing rates as indicated by an integrated ratemeter output monitored throughout each experiment. Drug solutions were made in distilled water.

The atlas of Paxinos and Watson[16] was used for electrode placement. The coordinates were: dorsal raphe (DR) A 0.5–1.7 mm, L 0 mm, V 3.5–4.2 mm (at a posterior angle of 34° to the vertical axis); median raphe (MnR) A 1.0–2.0 mm, L 0 mm, V 1.1–2.0 mm (at an angle of 30° to the vertical axis), substantia nigra pars compacta (SNPC) A 3.0–4.0 mm, L 1.8–2.5 mm, V 7.0–7.5 mm; locus coeruleus (LC) P 0.3–1.3 mm, L 1.0–1.3 mm, V 2.5–3.0 mm (at an angle of 20° to the vertical axis). Serotonin neurons were

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identified as biphasic large positive-negative action potentials with slow and regular firing rates (approx. 0.8–2.5 spikes/s) according to the criteria of Aghajanian et al.[1]. DA neurons were identified by action potentials of >2.5 ms, shape, and firing patterns (<12 spikes/s) according to Bunney and Aghajanian[4]. Noradrenaline neurons were identified by their >2 ms positive–negative action potentials, regular firing rates of 0.5–5 spikes/s, according to the criteria of Cedarbaum and Aghajanian[5], and Graham and Aghajanian[10].

Histology

At the termination of each recording session, the location of the unit was identified by passing 10 μA cathodic current through the recording electrode for 10–20 min. The brain was then removed, fixed in 10% formalin, sectioned, stained, and the location of the dye spot within DR, MnR, LC, or SNPC was verified for each animal. Specifically for MnR, only recordings from cells precisely on the midline were accepted since the great majority of 5-HT cells in the MnR are so located[24].

RESULTS

Elicitation of jaw movements

Even though the animals were anesthetized, it was common for MDMA in doses of 1000 μg/kg or more to elicit repetitive jaw movements, a behavior also reported in human subjects[6,9,15]. This effect was usually transient in nature, although it varied in duration from animal to animal. The effect was not quantitatively evaluated beyond these casual observations.

Effect of MDMA in serotonin cells

Intravenous MDMA depressed all 23 5-HT cells studied. However, potencies for this effect varied greatly. Based on their sensitivities to MDMA, DR serotonin neurons could be placed into two subpopulations (Fig. 1). Intravenous MDMA depressed cells in the high-sensitivity DR group (ED_{50} <1000 μg/kg) with an ED_{50} of 128 ± 25 μg/kg, (mean ± S.E.M., n = 12). The low-sensitivity DR group (ED_{50} >1000 μg/kg) was depressed by MDMA with an ED_{50} of 1830 ± 425 μg/kg (n = 7). Cells in the MnR (n = 4) were all of the high sensitivity type with an ED_{50} of 86 ± 34 μg/kg.

The ED_{50}s from the high-sensitivity groups, of either DR or MnR, were significantly different from the low-sensitivity DR group ($P < 0.05$, t-test). Furthermore, the ED_{50} of the high-sensitivity DR group could not be statistically distinguished from the ED_{50} of the MnR cells. Neither spiperone, a 5-HT_{1A} antagonist[12], nor LY 53837, a 5-HT_2[7] antagonist reversed MDMA's effects in any of the 5-HT groups.

Effect of MDMA on catecholamine cells

Up to 3000 μg/kg intravenous MDMA did not depress SNPC DA cells (n = 5), although amphetamine did (Fig. 2). In contrast to its lack of effect on DA neurons, MDMA depressed all NA neurons tested in the LC. The

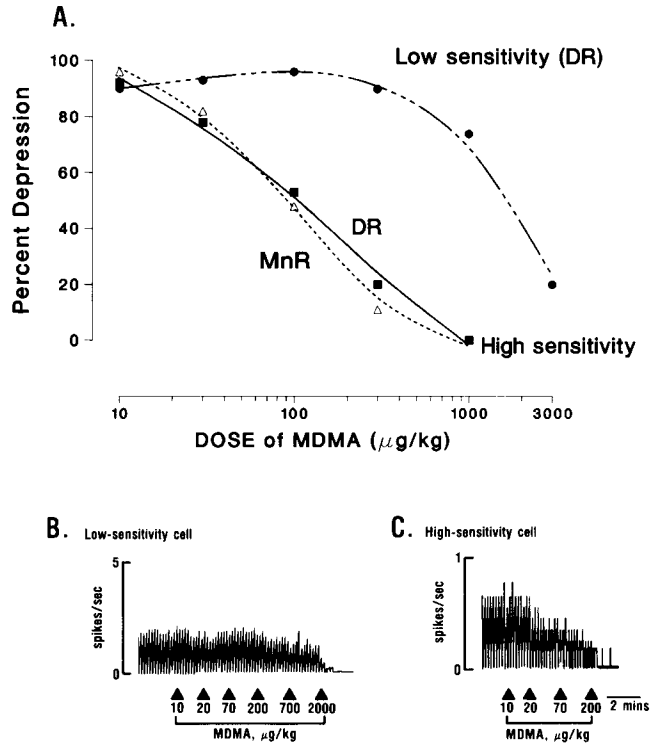

Fig. 1. Effects of MDMA on firing rates of serotonin neurons. A: population response. Low-sensitivity DR neurons (circles, n = 7), high-sensitivity DR neurons (squares, n = 12), MnR neurons (triangles, n = 4). Doses are cumulative. B: depression of firing rates of a low-sensitivity 5-HT cell (ED_{50} >1000 μg/kg) in DR. Non-cumulative doses and their times of injection are given on the abscissa. C: depression of firing rates of a high-sensitivity 5-HT cell (ED_{50} <1000 μg/kg).

potency for this effect (ED_{50} = 640 μg/kg, n = 5, Fig. 3) was sufficient to be actuated by clinically effective doses, and it was intermediate between the high- and low-

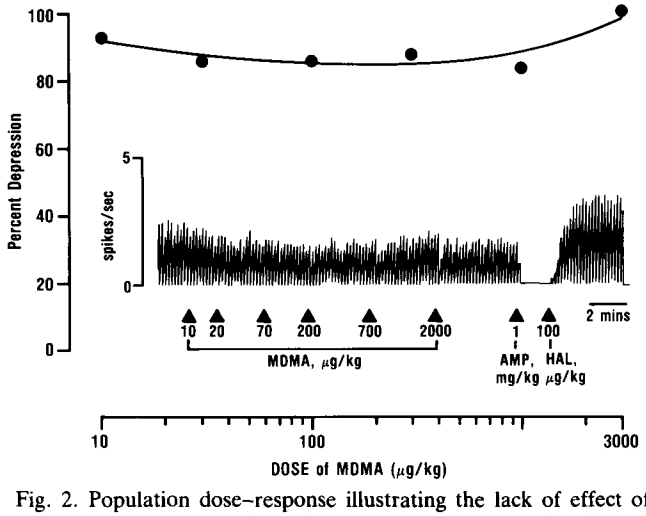

Fig. 2. Population dose–response illustrating the lack of effect of MDMA on SNPC cells (n = 4). Pen recording shows the effect of MDMA on a single DA neuron. Doses for the population curve are cumulative, while they are non-cumulative for the single cell recording.

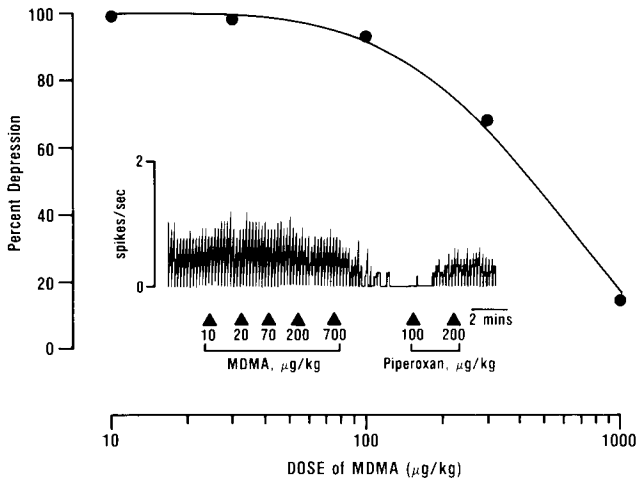

Fig. 3. Population dose–response illustrating the effects of cumulative MDMA doses in depressing LC cells (n = 5). Pen recording shows the effect of MDMA on a single NA neuron, the doses being non-cumulative.

sensitivity 5-HT cells. Piperoxan, an α2-antagonist, only partially reversed the effects of MDMA in the 5 cells tested (Fig. 3).

DISCUSSION

Because MDMA, originally synthesized as an anti-appetite drug, is structurally related to amphetamine, a DA releasing anti-appetite drug, we were interested in seeing if MDMA would reduce DA neuron firing rates similar to amphetamine[3,18]. Even with intravenous doses as high as 3000 μg/kg, MDMA was completely without effect. These results suggest that, although high MDMA doses weakly release DA[19,23], clinically effective doses do not. MDMA's unique psychological effects, which do not resemble those for amphetamine, apparently do not involve DA systems.

In contrast, MDMA potently interacted with 5-HT and NA neurons, depressing the firing rates of both cell types with submilligram/kg doses. The effects of MDMA on 5-HT and NA cell firing may result from negative feedback controls subsequent to neurotransmitter release in postsynaptic target areas. The lack of good reversal with autoreceptor antagonists (spiperone, piperoxan) suggests that these effects are not direct. Moreover, MDMA does not bind to the appropriate autoreceptors, the 5-HT_{1A} and α2-receptors (Lahti, R.A., Carrigan, K.J. and Sethy, V., personal communication). Using synaptosomes, Steele et al.[22] demonstrated that the active isomer, (+)-MDMA, blocked reuptake of NA and 5-HT into nerve terminals with approximately equal potencies; DA reuptake blockade required an order of magnitude higher doses. Direct postsynaptic effects of MDMA seem unlikely since the drug does not bind to 5-HT_2[13], α1-, nor α2- (Lahti, R.A., Carrigan K.J. and Sethy, V., personal communication) receptors, the major NA and 5-HT postsynaptic receptor subtypes.

The report that MDMA depresses 5-HT neurons in DR slice preparations[21] is thought to reflect actions on local 5-HT release within the slice. Since all cells in the slices were equally sensitive to MDMA, it seems likely that these more direct in vitro effects represent the low-sensitivity effects studied in vivo. Thus, while every DR cell may be able to respond to high MDMA doses, these effects could be masked in vivo by high-sensitivity feedback responses in cells having such properties. The contrary hypothesis, that the in vitro effects represent the high-sensitivity in vivo response, seems less likely since the low-sensitivity response would not be observed in vivo if every cell responded to low MDMA doses. Overall, the data suggest a more indirect (e.g. feedback) mechanism for the high-sensitivity group studied in vivo.

An interesting feature observed throughout these studies was that, although anesthetized, MDMA elicited transient jaw movements in some rats. Jaw clenching and teeth grinding are a frequent occurrence in human subjects taking MDMA[6,9,15], and aching jaw muscles are often reported on the day following usage. The occurrence of this effect illustrates that our dose in rats was producing some effects comparable to those observed with psychoeffective doses in man.

5-HT, DA, and NA are all neurotransmitters thought to play important roles in mind, emotions, and psychiatric illnesses. It was interesting that we found two subpopulations of 5-HT cells responding to MDMA in the DR. This selectivity is quite unusual. For example, both LSD-type hallucinogens and antidepressants depress all 5-HT cells non-selectively[1,20]. Knowledge of the axonal projections of the low- and high-sensitivity cells could shed light on the roles of specific 5-HT projections in emotional control. Fine fibrous axons with small 5-HT terminals innervating the cerebral cortex, thalamus, and striatum are selectively destroyed by neurotoxic doses of MDMA; more coarse 5-HT axons with large terminals in the hippocampal, basal forebrain, hypothalamic, and brainstem terminals are partially spared[2,14]. If the selective neurotoxicity of serotonergic systems were an extension of the selective pharmacology observed with lower doses, one might suspect cortical pathways to underlie the mood and cognitive effects of MDMA, and the striatum, already known to be associated with stereotyped movements[8], as a potential site for initiation of the repetitive jaw movements. However, the fine fibrous axons sensitive to MDMA neurotoxicity arise from the DR, while the more resistant, coarse axons arise from the MnR[2,14].

Although only a few MnR neurons were sampled, it is

clear that at least some of the cells in this region belong to the high-sensitivity group. Thus, the potent effects observed in this neurotoxicity-resistant area with clinically relevant low MDMA doses suggest that the psychotropic effects may not be due to neurotoxicity. It is not yet clear, however, if low-sensitivity neurons can also be found in the MnR. Given the rapid tolerance to MDMA's psychotropic effects, the results of multiple doses on MDMA sensitivities of 5-HT neurons are also of interest.

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