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Short communication

Effects of methylenedioxymethamphetamine on the release of monoamines from rat brain slices

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The effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamine release were investigated in superfused slices of rat striatum and hippocampus. MDMA (10 μM) increased the resting release of radioactivity from slices incubated in [^{3}H]dopamine, [^{3}H]5-hydroxytryptamine or [^{3}H]noradrenaline. These effects of MDMA (10 μM) were blocked by the neuronal uptake inhibitors, cocaine (10 μM), fluoxetine (1 μM) and desmethylimipramine (1 μM), respectively. MDMA (10 μM) enhanced the stimulation-induced efflux of radioactivity from slices incubated in [^{3}H]noradrenaline but not from slices incubated in [^{3}H]5-hydroxytryptamine or [^{3}H]dopamine. These results demonstrate for the first time a direct noradrenaline-releasing action of MDMA and differential effects of MDMA on the stimulation-induced release of noradrenaline, dopamine and 5-hydroxytryptamine from rat superfused brain slices.

MDMA (3,4-methylenedioxymethamphetamine); Monoamine release; Brain slices (rat, superfused); Noradrenaline; 5-HT (5-hydroxytryptamine, serotonin); Dopamine

1. Introduction

Methylenedioxymethamphetamine (MDMA) has been shown to release dopamine (Johnson et al., 1986) and 5-hydroxytryptamine (Nichols et al., 1982; Johnson et al., 1986; Schmidt et al., 1987; Hekmatpanah and Peroutka, 1990), and to block the neuronal uptake of monoamines in the brain (Steele et al., 1987). The monoamine-releasing effects of MDMA can be blocked with neuronal uptake blockers (Schmidt et al., 1987; Hekmatpanah and Peroutka, 1990).

While MDMA-induced dopamine and 5-hydroxytryptamine release has been investigated, there are no reports either on the effects of MDMA on noradrenaline release or on the effects of MDMA on the electrical stimulation-induced (S-I) release of noradrenaline, 5-hydroxytryptamine and dopamine. This study compares the effects of MDMA on the resting and S-I release of the three monoamines from rat brain slices.

2. Materials and methods

Female Sprague-Dawley rats (250-300 g) were decapitated and 500 μm coronal slices of hippocampus and striatum were obtained. Slices were equilibrated for 30 min in physiological salt solution of the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, KH_2PO_3 1.03, $MgSo_4$ 0.45, D-glucose 11.1, ascorbic acid 0.14, Na_2EDTA 0.067. Slices were then incubated in physiological salt solution containing either 0.1 μM [^{3}H]dopamine (5.4 μCi/ml), 0.1 μM [^{3}H]5-hydroxytryptamine (4.4 μCi/ml), or 0.2 μM

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[^{3}H]noradrenaline (2.5 μCi/ml) for 30 min. Following incubation, slices were rinsed three times with 2 ml physiological salt solution, then transferred singly to glass superfusion chambers (1 ml internal volume) where they were held by nylon mesh between two platinum plate electrodes, and superfused at 37° C at a rate of 1 ml/min.

A priming stimulation (5 Hz, 1 min) was delivered 45 min after the commencement of superfusion. Slices were subjected to two further periods of electrical stimulation (S_1 and S_2) 90 and 132 min after the commencement of superfusion. Stimulation at 8 V/cm consisted of square wave pulses of 2 ms duration at a frequency of 5 Hz for 1 min.

The superfusate was collected in 3-min fractions starting 6 min prior to S_1 and continuing for 15 min after S_2. MDMA was introduced 21 min prior to S_2 and where indicated fluoxetine, cocaine or desmethylimipramine (DMI) was present throughout superfusion. At the end of superfusion, slices were deproteinated in 0.2 M $HClO_4$ for 12 h, sonicated and tissue radioactivity determined.

The efflux of radioactivity was expressed as a percentage of the tissue radioactive content at the time of fraction collection. The resting efflux of radioactivity preceding each period of electrical stimulation was taken as the mean of the fractional release of radioactivity in the two 3-min periods prior to stimulation. The S-I outflow of radioactivity (FR) was calculated as the difference between five times the resting outflow of radioactivity and the sum of the fractional release of radioactivity in the five 3-min periods from the start of stimulation. The S-I efflux of radioactivity, taken as an index of transmitter release, was calculated as the ratio of S-I outflow evoked by S_2 to that evoked by S_1 (FR_2/FR_1).

Results are expressed as means and S.E., and n indicates the number of animals tested.

The drugs used in this study were: cocaine hydrochloride (MacFarlane-Smith, Australia), desmethylimipramine hydrochloride (Ciba-Geigy, U.K.), fluoxetine hydrochloride (Lilly, U.S.A.) and (±)-3,4-methylenedioxymethamphetamine hydrochloride (Makor, Israel). Radiolabelled monoamines were obtained from Amersham (U.K.).

3. Results

3.1. MDMA-induced release of radioactivity

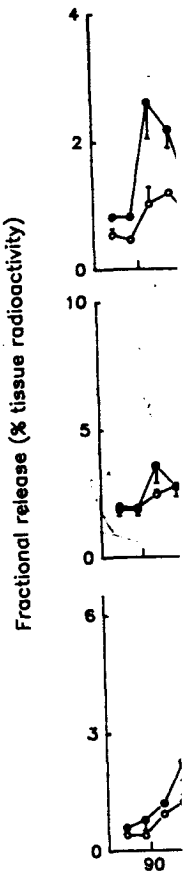
MDMA (10 μM) caused an increase in the fractional release of radioactivity from striatal slices incubated in [^{3}H]dopamine and from hippocampal slices incubated in [^{3}H]5-hydroxytryptamine or [^{3}H]noradrenaline (fig. 1). To determine the MDMA-induced change in resting efflux of radioactivity, the resting efflux of radioactivity just prior to S_2 was expressed as a percentage of the resting efflux of radioactivity prior to S_1 (R_2/R_1). MDMA (10 μM) increased the resting efflux of radioactivity to the same extent from slices incubated in [^{3}H]dopamine, [^{3}H]5-hydroxytryptamine or [^{3}H]noradrenaline, enhancing R_2/R_1 from 88.8 ± 4.3% (n = 4) to 340.5 ± 71.8% (n = 3), from 80.3 ± 2.4% (n = 4) to 348.1 ± 45.9% (n = 4) and from 79.6 ± 3.1% (n = 4) to 312.6 = 57% (n = 5), respectively.

Blockers of the neuronal uptake of dopamine (cocaine, 10 μM), 5-hydroxytryptamine (fluoxetine, 1 μM) and noradrenaline (DMI, 1 μM), abolished the MDMA-induced increases in resting fractional release of radioactivity (fig. 1).

3.2. Effect of MDMA on the S-I efflux of radioactivity

Electrical stimulation of slices incubated in each of the three monoamines caused an outflow of radioactivity (fig. 1). MDMA (10 μM) had no effect on the S-I efflux of radioactivity (FR_2/FR_1) from slices incubated in [^{3}H]5-hydroxytryptamine. Similarly, MDMA did not appear to affect the S-I efflux of radioactivity from slices incubated in [^{3}H]dopamine, although in this case a stable resting outflow of radioactivity prior to S_2 was not clearly established. In contrast, the S-I efflux of radioactivity (FR_2/FR_1) from slices incubated in [^{3}H]noradrenaline was markedly enhanced in the presence of 10 μM MDMA from a control value of 0.785 ± 0.021 (n = 4) to 5.898 ± 0.856 (n = 5). This enhancement was blocked by 1 μM DMI.

When present throughout superfusion, fluoxetine (1 μM), cocaine (10 μM) or DMI (1 μM) had no effect on S-I efflux (FR_2/FR_1), either in the



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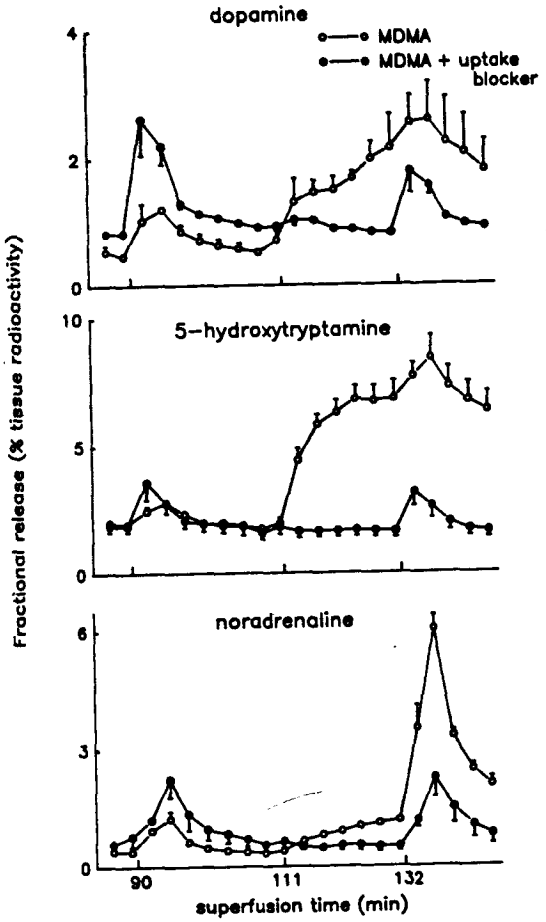

Fig. 1. The fractional release of radioactivity, expressed as a percentage of tissue radioactivity at the time of fraction collection, from rat striatal slices incubated in [^{3}H]dopamine, and hippocampal slices incubated in [^{3}H]5-hydroxytryptamine or [^{3}H]noradrenaline. Two periods of electrical stimulation (5 Hz, 1 min) were delivered 90 and 132 min after the beginning of superfusion. Open circles designate experiments in which MDMA (10 μM) was added to the superfusion medium 111 min after the beginning of superfusion. Closed circles represent experiments in which the respective neuronal uptake blockers of dopamine, 5-hydroxytryptamine and noradrenaline, i.e. cocaine (10 μM), fluoxetine (1 μM) and DMI (1 μM), were present throughout superfusion. Each point is the mean and vertical bar the S.E. of values from three to five animals.

presence or absence of MDMA (10 μM), from slices incubated in [^{3}H]5-hydroxytryptamine, [^{3}H]dopamine or [^{3}H]noradrenaline, respectively.

4. Discussion

In this study, it has been demonstrated for the first time that MDMA directly causes the release of radioactivity from rat hippocampal slices incubated in [^{3}H]noradrenaline. It was also shown that MDMA causes the release of radioactivity from brain slices incubated in either [^{3}H]dopamine or [^{3}H]5-hydroxytryptamine, in agreement with previous reports (Nichols et al., 1982; Johnson et al., 1986; Schmidt et al., 1987; Hekmatpanah and Peroutka, 1990). In brain slice superfusion systems, resting radioactive efflux is comprised mainly of radioactive metabolites (Herdon et al., 1985). However, the MDMA-induced increase in resting radioactive efflux in the present study is likely to consist of predominantly monoamine transmitter, since Herdon et al. (1985) have demonstrated that amphetamine-induced release from rat striatal slices consists of mainly transmitter with very little deaminated metabolite.

MDMA markedly enhanced the S-I release of radioactivity from slices incubated in [^{3}H]noradrenaline. In contrast, MDMA had no effect on the S-I efflux of radioactivity (FR_2/FR_1) from slices incubated in [^{3}H]dopamine or [^{3}H]5-hydroxytryptamine, although resting release (R_2/R_1) was enhanced to a simular extent from slices incubated in [^{3}H]dopamine, [^{3}H]5-hydroxytryptamine and [^{3}H]noradrenaline. This suggests that the differential effects of MDMA on the S-I release of transmitter are not due to differential effects on resting efflux.

The actions of MDMA were blocked by neuronal uptake blockers, as previously reported (Hekmatpanah and Peroutka, 1990; Schmidt et al., 1987), suggesting that the neuronal uptake carrier is critical in the acute monoamine-releasing actions of MDMA.

Behavioural effects caused by MDMA, such as enhanced locomoter activity (Gold and Koob, 1988) and lowering of brain stimulation reward thresholds (Hubner et al., 1988), are considered to be mediated primarily by alterations in dopaminergic and serotonergic neurotransmission. Noradrenaline is also thought to be involved in these behaviours (Hornykiewicz, 1982). Since the results of the present study have shown that MDMA has

effects on noradrenergic neurotransmission, a role of noradrenaline should perhaps be considered in the behavioural effects of MDMA.

Acknowledgements

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