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Interactions of methylenedioxymethamphetamine with monoamine transmitter release mechanisms in rat brain slices

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Summary. This study investigates the effects of methylenedioxymethamphetamine (MDMA) and amphetamine on monoamine release from rat superfused brain slices in both the presence and absence of vesicular stores of transmitter. MDMA caused the release of radioactivity from slices incubated with [^{3}H]5-hydroxytryptamine, [^{3}H]noradrenaline or [^{3}H]dopamine with EC_{50} values of 1.9 μmol/l (95% confidence limits 1.5–2.3 μmol/l), 4.5 μmol/l (2.3–8.7 μmol/l), and greater than 30 μmol/l, respectively. In contrast, amphetamine (0.1–300 μmol/l) was more effective in releasing radioactivity from slices incubated with [^{3}H]dopamine than [^{3}H]noradrenaline or [^{3}H]5-hydroxytryptamine. When Ca^{2+} was excluded from the superfusion fluid, the MDMA-induced release of radioactivity from slices incubated with [^{3}H]dopamine was unaltered, but that from slices incubated with [^{3}H]noradrenaline or [^{3}H]5-hydroxytryptamine was enhanced. MDMA (10 μmol/l) facilitated the stimulation-induced (5 Hz, 1 min) outflow of radioactivity from slices incubated with [^{3}H]noradrenaline or [^{3}H]5-hydroxytryptamine to 7.5-fold and 2.1-fold of control values, respectively, but had no effect on that from slices incubated with [^{3}H]dopamine. Amphetamine (1 μmol/l) increased the stimulation-induced outflow from slices incubated with [^{3}H]noradrenaline, but not that from slices incubated with [^{3}H]5-hydroxytryptamine or [^{3}H]dopamine.

Inhibition of monoamine oxidase by a 30-min incubation with pargyline (100 μmol/l) enhanced the releasing action of MDMA on all three monoamines. Pargyline (100 μmol/l) also enhanced the facilitation caused by MDMA, of the stimulation-induced outflow of radioactivity from slices incubated with [^{3}H]noradrenaline, [^{3}H]5-hydroxytryptamine or [^{3}H]dopamine.

In some experiments, slices were obtained from reserpinised rats (2.5 mg/kg s.c. 24 h prior) and pre-exposed for 30 min to the monoamine oxidase inhibitor pargyline (100 μmol/l). Under these conditions, electrical stimulation evoked a small residual stimulation-induced outflow of radioactivity from slices incubated with [^{3}H]noradrenaline, and failed to evoke an outflow of radioactivity from slices incubated with [^{3}H]5-hydroxytryptamine or [^{3}H]dopamine. However, a Ca^{2+}-dependent stimulation-induced outflow of radioactivity was evoked in the presence of either MDMA (10 μmol/l) or amphetamine (1 μmol/l) from slices incubated with either [^{3}H]dopamine or [^{3}H]noradrenaline, but not from slices incubated with [^{3}H]5-hydroxytryptamine. The stimulation-induced outflow of radioactivity from slices incubated with [^{3}H]noradrenaline was enhanced in the presence of desipramine (1 μmol/l), however this enhancement was less than that caused by 10 μmol/l MDMA or 1 μmol/l amphetamine. The Ca^{2+}-dependent response to electrical stimulation in the presence of MDMA from slices incubated with [^{3}H]noradrenaline was greatly reduced when rats were pretreated with a higher dose of reserpine (10 mg/kg s.c.).

This study demonstrates that MDMA and amphetamine release radioactivity from brain slices incubated with [^{3}H]noradrenaline, [^{3}H]dopamine and [^{3}H]5-hydroxytryptamine, but their order or potency as releasers of brain monoamines differs. The results also suggest that MDMA and amphetamine have significant effects on the exocytotic release of monoamines and may interact with both vesicular and cytoplasmic monoamines.

Key words: Amphetamine – Rat brain slice – Methylenedioxymethamphetamine – Monoamine release – Reserpine

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Introduction

Single or repeated doses of 3,4-methylenedioxymethamphetamine (MDMA) in rats reduce brain concentrations of 5-hydroxytryptamine (5-HT) (Schmidt et al. 1987; Battaglia et al. 1987; Commins et al. 1987b), but do not re-

duce brain concentrations of dopamine or noradrenaline (Commins et al. 1987b). It has recently been postulated that the neurotoxic effects of MDMA on serotonergic nerves may be related to its action as a releaser of non-vesicular dopamine or 5-HT (Commins et al. 1987a; Stone et al. 1989; Johnson et al. 1991; McKenna et al. 1991).

The mechanism of monoamine release induced by amphetamine and other indirectly acting sympathomimetic amines is well characterized, and involves the displacement of monoamines from intraneuronal stores and their subsequent release by a carrier-mediated process (Brodie et al. 1969; Arnold et al. 1977; Raiteri et al. 1977; Fischer and Cho 1979; Raiteri et al. 1979; Maura et al. 1982). The MDMA-induced release of dopamine, 5-HT and noradrenaline can be blocked by neuronal uptake inhibitors (Schmidt et al. 1987; Hekmatpanah and Peroutka 1990; Fitzgerald and Reid 1990) and is unaffected by reserpine pretreatment (Johnson et al. 1991). Furthermore, although there is no evidence that MDMA can enter monoaminergic nerve terminals, it has been demonstrated that the N-demethylated metabolite of MDMA, methylenedioxyamphetamine (MDA) can be taken up into synaptosomes (Zaczek et al. 1990). These findings suggest that MDMA has a mechanism of action similar to that of indirectly acting amines, such as amphetamine.

The aim of the present study was to describe and compare the mechanisms by which MDMA and amphetamine cause monoamine release and interact with electrical stimulation-induced (S−I) release of radiolabelled monoamines from rat superfused brain slices in the presence and absence of vesicular transmitter stores.

Methods

Slice preparation and superfusion. The methods used in this study were modified slightly from those reported previously (Fitzgerald and Reid 1990). Male and female Sprague-Dawley rats (250−300 g) were decapitated, the brain was carefully removed, and 400 µm coronal slices of hippocampus and striatum were obtained. In some experiments male Sprague-Dawley rats (250−300 g) were given a subcutaneous injection of reserpine (2.5, 5 or 10 mg/kg) 24 h prior to the experiment, to deplete transmitter stores and to prevent vesicular storage of monoamines. Slices were equilibrated for 30 min in medium of the following composition (in mmol/l): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.3, $NaHCO_3$ 25.0, KH_2PO_3 1.3, $MgSO_4$ 1.2, D-glucose 11.1, ascorbic acid 0.14, Na_2EDTA 0.0067. In some experiments the medium contained 100 µmol/l pargyline during the 30-min equilibration period. Striatal slices were then incubated in a medium containing 0.1 µmol/l [^{3}H]dopamine (5.4 µCi/ml), and hippocampal slices were incubated with either 0.1 µmol/l [^{3}H]5-HT (4.4 µCi/ml) or 0.2 µmol/l [^{3}H]noradrenaline (2.5 or 8.7 µCi/ml) for 30 min. Following incubation, slices were rinsed three times with 2 ml medium, then transferred singly to glass superfusion chambers, where they were held by glass mesh between 2 platinum plate electrodes, and superfused with medium at 37 °C at a rate of 1 ml/min. In some experiments, Ca^{2+}-free medium was used.

In experiments using amphetamine, ring-labelled [^{3}H]noradrenaline (8.7 µCi/ml) was used whereas 7,8-labelled [^{3}H]noradrenaline (2.5 µCi/ml) was used in experiments with MDMA. The specific activity of ring-labelled [^{3}H]noradrenaline was higher than that of 7,8-labelled [^{3}H]noradrenaline, resulting in an elevation in the absolute amount of radioactivity accumulated by the slices. Control experiments were performed to ensure that the proportional effects of drugs on both resting and S-I release of radioactivity were the same, irrespective of whether slices had been incubated with ring-labelled or 7,8-labelled [^{3}H]noradrenaline (data not shown).

MDMA- and amphetamine-induced release of monoamines. Slices were superfused for 45 min to remove loosely bound radioactivity. Following the washout period, 3-min samples of superfusate were collected. After 15 min, MDMA (0.1−400 µmol/l) or amphetamine (0.1−300 µmol/l) was added to the superfusion medium for 39 min. At the end of the experiment, slices were deproteinated in 0.1 mol/l $HClO_4$ for 12 h and sonicated.

The radioactive content in superfusate fractions and deproteinated tissues was determined by liquid scintillation counting. Counting efficiency, determined by automatic external standardization, was approximately 25%. The radioactive outflow in each sample was expressed as a fraction of the total radioactivity present in the slice at the time of collection. The initial resting outflow of radioactivity was calculated by averaging the fractional outflow measured in the 3 samples collected immediately before the addition of drug. MDMA- and amphetamine-induced release of radioactivity was determined by expressing the sum of the radioactive outflow in the 13 samples collected after the addition of drug, as a percentage of 13 times the initial resting outflow of radioactivity.

Electrical S-I release of radioactivity. Forty-five minutes after the commencement of superfusion, a priming stimulation (5 Hz, 1 min, 2 ms pulse duration, 8 V/cm, 25 mA) was delivered. Slices were then given two further periods of electrical stimulation ($Stim_1$ and $Stim_2$) 90 and 132 min after the commencement of superfusion, at a frequency of 5 Hz for 1 min. MDMA or amphetamine was added 21 min before $Stim_2$. The superfusate was collected in 3-min samples starting 6 min before $Stim_1$ and counting for 15 min after $Stim_2$. Slices were deproteinated and sonicated as described in the previous section.

The radioactive outflow in each 3-min sample was expressed as a fraction of the total radioactivity present in the slice at the time of collection. The resting outflow of radioactivity was taken as the mean of the fractional outflow of radioactivity in the two 3-min samples immediately before each stimulation period (R_1 and R_2). The ratio R_2/R_1 was used as an index of drug-induced release of radioactivity. The S-I outflow of radioactivity was calculated as the difference between five times the resting outflow of radioactivity and the sum of the fractional outflow in the five 3-min periods from the start of stimulation. To determine the effects of drugs on the S-I outflow of radioactivity, the S-I outflow evoked by $Stim_2$ was expressed as a fraction of that evoked by $Stim_1$ (S_2/S_1).

In experiments using slices from reserpinised animals, the radioactive outflow in each 3-min sample was expressed as disintegrations per min (dpm). The S-I outflow of radioactivity is referred to as the outflow of radioactivity evoked by electrical stimulation above the resting outflow of radioactivity. The resting and S-I outflows of radioactivity were not quantified in experiments using slices from reserpinized rats as stable levels of resting outflow of radioactivity were not observed in the presence of either amphetamine or MDMA.

Drugs and chemicals. The drugs used in this study were: (±)amphetamine sulphate (Sigma, St.-Louis, Mo., USA), cocaine hydrochloride (Glaxo, Australia), desipramine hydrochloride (Sigma), (±)-3,4-methylenedioxymethamphetamine HCl (Makor, Israel), pargyline HCl (Sigma) and reserpine (Sigma). Reserpine was prepared for injection by dissolving 125 mg of reserpine and 125 mg of anhydrous citric acid in a solution consisting of 1 ml benzyl alcohol and 5 ml polysorbat tween 80. The solution was then made up to 50 ml with distilled H_2O.

5-[1,2-^{3}H(N)]-Hydroxytryptamine (24.7 Ci/mmol) and (−)-[ring 2,5,6-^{3}H]-noradrenaline (43.7 Ci/mmol) were obtained from New England Nuclear (USA), and [7,8-^{3}H]-dopamine and (−)-[7,8-^{3}H]-noradrenaline (15 Ci/mmol) were obtained from Amersham (UK).

Statistics. Results are expressed as means±SEM and *n* indicates the number of animals tested. Significant differences between means were determined by an unpaired two-tailed Student's *t*-test after a one-way

analysis of variance (ANOVA), where $P<0.05$ denotes statistical significance.

Regression lines were fitted to the linear portion of the log concentration-response curves for MDMA and amphetamine by the method of least squares. EC_{50} values were interpolated from the regression lines, and correspond to a concentration producing 50% of the maximal response.

Results

Drug-induced release of radioactivity

MDMA enhanced the radioactive outflow from striatal slices incubated with [^{3}H]dopamine and from hippocampal slices incubated with [^{3}H]5-HT or [^{3}H]noradrenaline in a concentration-dependent manner (Fig. 1). The releasing action of MDMA was maximal at approximately 10 µmol/l for slices incubated with either [^{3}H]5-HT or [^{3}H]noradrenaline. Maximal MDMA-induced release of radioactivity from slices incubated with [^{3}H]dopamine had not been reached at a MDMA concentration of 400 µmol/l (Fig. 1). MDMA was equipotent in releasing radioactivity from slices incubated with [^{3}H]5-HT and [^{3}H]noradrenaline (EC_{50} values: 1.9 µmol/l, 95% confidence limits 1.5–2.3 µmol/l, and 4.5 µmol/l, 2.3–8.7 µmol/l, respectively), however MDMA was less potent in releasing radioactivity from slices incubated with [^{3}H]dopamine, with an EC_{50} value greater than 30 µmol/l.

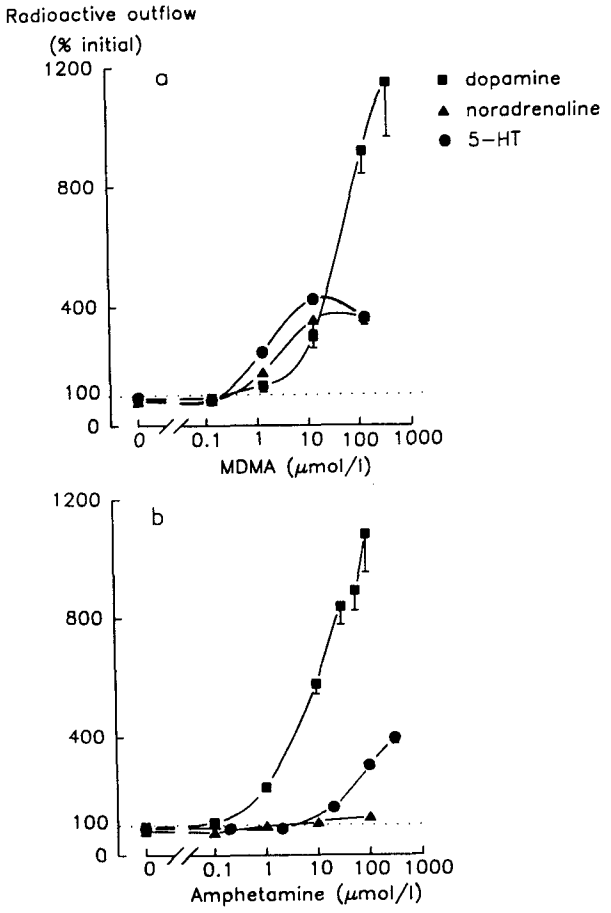

Fig. 1. Concentration-response curves for the effect of MDMA (**a**) and amphetamine (**b**) to release radioactivity from slices incubated with [^{3}H]dopamine, (*filled squares*), [^{3}H]noradrenaline (*filled triangles*) or [^{3}H]5-hydroxytryptamine (5-HT) (*filled circles*). The drug-induced release of radioactivity is expressed as a percentage of the initial resting outflow of radioactivity before the addition of the drug. *Each point* denotes the mean and *vertical bar* the SEM of 3–6 experiments

Effects of MDMA and amphetamine on S-I release of radioactivity

The S-I outflow of radioactivity (S_2/S_1) from slices incubated with [^{3}H]noradrenaline was greatly enhanced by 10 µmol/l MDMA (Table 1, Fig. 2) and 1 µmol/l amphetamine (Fig. 3). The S-I outflow from slices incubated with [^{3}H]5-HT was enhanced to a lesser degree by 10 µmol/l MDMA (Table 1, Fig. 2), and was not affected by 10 µmol/l amphetamine (Fig. 3). MDMA (10 µmol/l) (Table 1, Fig. 2) or amphetamine (1 µmol/l) (Fig. 3) had no effect on the S-I outflow from slices incubated with [^{3}H]dopamine.

Table 1. The effects of MDMA on the stimulation-induced (S-I) release of radioactivity from rat brain slices incubated with [^{3}H]dopamine, [^{3}H]noradrenaline or [^{3}H]5-hydroxytryptamine

Treatment	*n*	S_1 (% tissue radioactivity)	S_2/S_1
Dopamine			
Control	4	2.576 ± 0.279	0.789 ± 0.043
MDMA	4	2.437 ± 0.706	0.804 ± 0.039
Control, pargyline	5	0.797 ± 0.129[a]	0.747 ± 0.050
MDMA, pargyline	4	0.518 ± 0.112[b]	2.012 ± 0.192[c,d]
Noradrenaline			
Control	5	2.542 ± 0.197	0.785 ± 0.021
MDMA	5	2.052 ± 0.280	5.898 ± 0.856[c]
Control, pargyline	4	0.891 ± 0.077[a]	0.909 ± 0.056
MDMA, pargyline	4	0.915 ± 0.113[b]	9.532 ± 1.074[c,d]
5-Hydroxytryptamine			
Control	4	1.461 ± 0.233	0.929 ± 0.163
MDMA	4	1.676 ± 0.292	1.907 ± 0.347[c]
Control, pargyline	4	0.188 ± 0.076[a]	0.527 ± 0.072
MDMA, pargyline	5	0.207 ± 0.040[b]	4.975 ± 0.596[c,d]

Slices were subjected to two periods ($Stim_1$ and $Stim_2$) of electrical stimulation (5 Hz, 1 min) 42 min apart. MDMA (10 µmol/l) was added to the superfusion fluid 21 min before $Stim_2$. The S-I outflow of radioactivity was calculated as the ratio of the S-I outflow evoked by $Stim_2$ to that evoked by $Stim_1$ (S_2/S_1). In some experiments slices were preincubated with pargyline (100 µmol/l) for 30 min. The number of experiments is denoted by *n* and results are expressed as means ± SEM

[a] Denotes a significant difference of control-pargyline from control for S_1 ($P<0.05$, unpaired Student's *t*-test after ANOVA)

[b] Denotes a significant difference of MDMA-pargyline from MDMA for S_1 ($P<0.05$, unpaired Student's *t*-test after ANOVA)

[c] Denotes a significant difference of MDMA from control for S_2/S_1 ($P<0.05$, unpaired Student's *t*-test after ANOVA)

[d] Denotes a significant difference of MDMA-pargyline from MDMA for S_2/S_1 ($P<0.05$, unpaired Student's *t*-test after ANOVA)

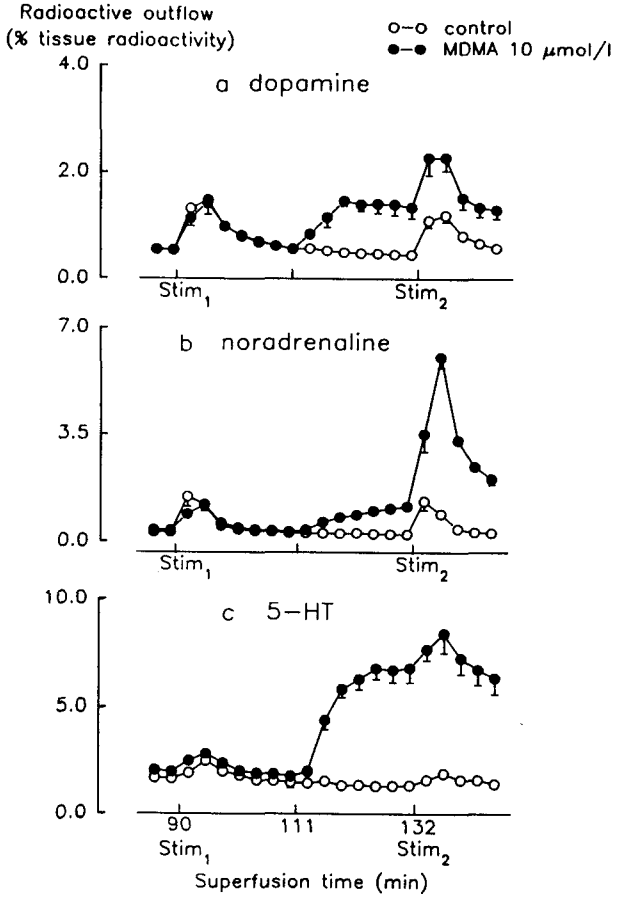

Fig. 2. Effect of MDMA on the radioactive outflow from rat brain slices incubated with [^{3}H]dopamine (**a**), [^{3}H]noradrenaline (**b**) or [^{3}H]5-hydroxytryptamine (5-HT) (**c**). Slices were given two periods of electrical stimulation 90 and 132 min after the beginning of superfusion ($Stim_1$ and $Stim_2$), at a frequency of 5 Hz for 1 min. MDMA (10 µmol/l) was added 111 min after the start of superfusion, in the experiments denoted by the filled circles. Control experiments (no MDMA) are denoted by the *open circles*. The radioactive content in each sample of the superfusion fluid is expressed as a percentage of the total tissue radioactivity at the time of sample collection. *Each point* represents the mean and *vertical bar* the SEM from 3–6 experiments

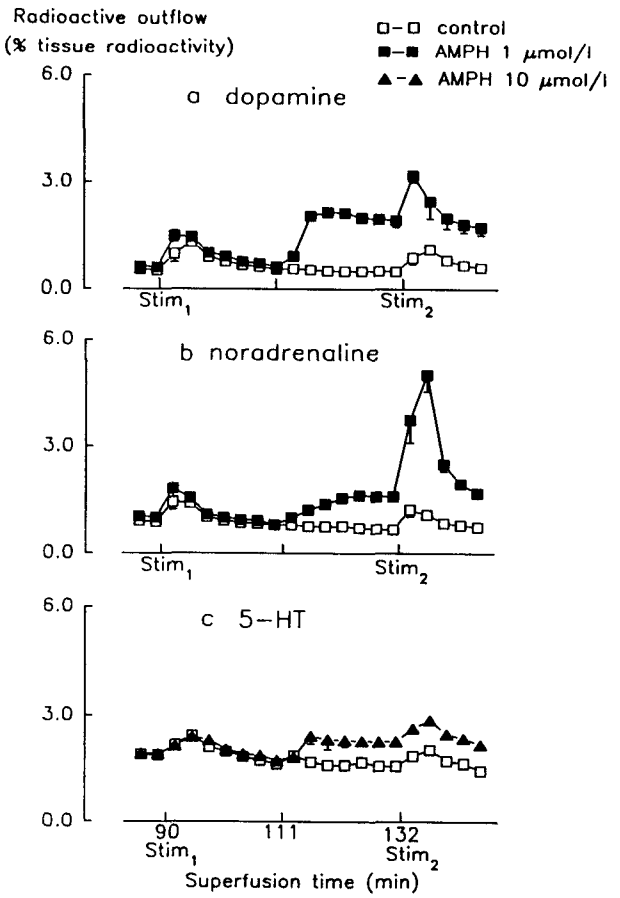

Fig. 3. Effect of amphetamine on the radioactive outflow from rat brain slices incubated with [^{3}H]dopamine (**a**), [^{3}H]noradrenaline (**b**) or [^{3}H]5-hydroxytryptamine (5-HT) (**c**). Slices were given two periods of electrical stimulation 90 and 132 min after the beginning of superfusion ($Stim_1$ and $Stim_2$), at a frequency of 5 Hz for 1 min. Amphetamine (AMPH, 1 or 10 µmol/l) was added 111 min after the start of superfusion in the experiments denoted by the *filled squares* and *filled diamonds* respectively. Control experiments (no amphetamine) are denoted by the *open squares*. The radioactive content in each 3-min sample of the superfusion fluid is expressed as a percentage of the total tissue radioactivity at the time of sample collection. *Each point* represents the mean and *vertical bar* the SEM from 3–6 experiments

Effect of MDMA in Ca^{2+}-free superfusion fluid

Exclusion of Ca^{2+} from the superfusate reduced the resting outflow of radioactivity (R_1) from slices incubated with [^{3}H]noradrenaline and increased the resting outflow of radioactivity from slices incubated with [^{3}H]dopamine (Table 2). The S-I outflow of radioactivity was abolished in Ca^{2+}-free superfusion fluid. When Ca^{2+} was excluded from the superfusion fluid, the MDMA-induced release of radioactivity from slices incubated with [^{3}H]5-HT or [^{3}H]noradrenaline was significantly enhanced, but was unchanged from slices incubated with [^{3}H]dopamine (Table 2).

Effect of MDMA after pre-incubation with pargyline

Pargyline pre-incubation did not significantly affect the total tissue radioactivity when compared to control conditions (Table 3).

The resting outflow of radioactivity prior to the first period of stimulation (R_1) from slices incubated with either of the three [^{3}H]monoamines was significantly reduced by 100 µmol/l pargyline (Table 2). The MDMA-induced release of radioactivity (R_2/R_1) from slices incubated with [^{3}H]dopamine, [^{3}H]5-HT or [^{3}H]noradrenaline was significantly enhanced by pargyline (Table 2), the enhancement being greater from slices incubated with [^{3}H]noradrenaline than for slices incubated with either [^{3}H]dopamine or [^{3}H]5-HT (Table 2).

Pargyline (100 µmol/l) pre-incubation reduced the absolute amount of S-I outflow of radioactivity evoked by the first period of stimulation (S_1) for all three [^{3}H]monoamines (Table 1). The facilitatory action of MDMA on the S-I outflow of radioactivity (S_2/S_1) from slices incubated with [^{3}H]noradrenaline or [^{3}H]5-HT was enhanced after pargyline pre-incubation (Table 1). In contrast with results obtained in the absence of pargyline, the S-I out-

ble 2. The effects of MDMA on the resting outflow of radioactivity
m rat brain slices incubated with [^{3}H]dopamine, [^{3}H]noradrenaline
[^{3}H]5-hydroxytryptamine

eatment	n	R_1 (% tissue radioactivity)	R_2/R_1
)pamine			
Control	4	0.550 ± 0.027	0.846 ± 0.027
MDMA	4	0.557 ± 0.036	2.451 ± 0.309[c]
Control, Ca^{2+}-free	4	1.019 ± 0.084[a]	0.993 ± 0.060
MDMA, Ca^{2+}-free	4	1.220 ± 0.120	2.939 ± 0.254
Control, pargyline	5	0.185 ± 0.020[b]	0.741 ± 0.033
MDMA, pargyline	4	0.188 ± 0.012	5.955 ± 0.936[d]
oradrenaline			
Control	4	0.321 ± 0.012	0.796 ± 0.031
MDMA	5	0.396 ± 0.040	3.126 ± 0.570[c]
Control, Ca^{2+}-free	5	0.252 ± 0.008[a]	0.856 ± 0.015
MDMA, Ca^{2+}-free	5	0.275 ± 0.008	8.246 ± 0.378[e]
Control, pargyline	4	0.193 ± 0.007[b]	0.731 ± 0.059
MDMA, pargyline	4	0.192 ± 0.016	12.540 ± 2.096[d]
-Hydroxytryptamine			
Control	4	1.660 ± 0.072	0.803 ± 0.024
MDMA	4	1.977 ± 0.120	3.481 ± 0.459[c]
Control, Ca^{2+}-free	4	1.733 ± 0.053	0.847 ± 0.011
MDMA, Ca^{2+}-free	4	1.761 ± 0.050	5.773 ± 0.205[e]
Control, pargyline	5	0.816 ± 0.057[b]	0.984 ± 0.008
MDMA, pargyline	5	0.853 ± 0.095	5.713 ± 0.563[d]

lices were subjected to two periods ($Stim_1$ and $Stim_2$) of electrical timulation 42 min apart, and MDMA (10 µmol/l) was added to the uperfusion fluid 21 min before $Stim_2$. The resting outflow of radioactivity before $Stim_2$ was expressed as a fraction of that before $Stim_1$ (R_2/R_1). In some experiments Ca^{2+} was excluded from the superfusion fluid from the beginning of superfusion (Ca^{2+}-free), or slices were preincubated with pargyline (100 µmol/l) for 30 min. The number of experiments is denoted by n and results are expressed as means ± SEM

[a] Denotes a significant difference of control-Ca^{2+}-free from control for R_1 ($P<0.05$, unpaired Student's t-test after ANOVA)

[b] Denotes a significant difference of control-pargyline from control for R_1 ($P<0.05$, unpaired Student's t-test after ANOVA)

[c] Denotes a significant difference of MDMA from control for R_2/R_1 ($P<0.05$, unpaired Student's t-test after ANOVA)

[d] Denotes a significant difference of MDMA-pargyline from MDMA for R_2/R_1 ($P<0.05$, unpaired Student's t-test after ANOVA)

[e] Denotes a significant difference of MDMA-Ca^{2+}-free from MDMA for R_2/R_1 ($P<0.05$, unpaired Student's t-test after ANOVA)

Table 3. Radioactive content of brain slices incubated with either [^{3}H]dopamine, [^{3}H]noradrenaline or [^{3}H]5-hydroxytryptamine (5-HT)

Treatment	Tissue radioactivity (dpm)[a]		
	Dopamine	Noradrenaline	5-HT
Control	895560 ± 186360	558930 ± 37332	422804 ± 128887
Pargyline[b]	597398 ± 118373	427572 ± 101087	380740 ± 43695
Reserpine[c]	376231 ± 71016[d]	205820 ± 26189[d]	183116 ± 13399

[a] Superfused slices were subjected to two periods ($Stim_1$ and $Stim_2$) of electrical stimulation 42 min apart and superfusion ceased 15 min after $Stim_2$. At the end of superfusion, the total tissue radioactivity was determined after tissues were deproteinated in 0.1 µmol/l $HClO_4$ for 12 h and sonicated. Results are expressed as means ± SEM of 5 – 9 experiments

[b] Slices were pre-incubated with 100 µmol/l pargyline for 30 min prior to incubation with either [^{3}H]dopamine, [^{3}H]noradrenaline or [^{3}H]5-HT

[c] Slices obtained from rats pretreated with reserpine (2.5 mg/kg s.c. 24 h before the experiment)

[d] Denotes a significant difference from control ($P<0.05$, two-tailed Student's t-test)

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flow of radioactivity (S_2/S_1) from slices incubated with [^{3}H]dopamine was also enhanced by MDMA after exposure to pargyline.

Drug-induced release of radioactivity from slices obtained from reserpinised rats

The total tissue radioactivity was reduced in striatal slices incubated with [^{3}H]dopamine and hippocampal slices incubated with [^{3}H]noradrenaline obtained from reserpinised (2.5 mg/kg) rats (Table 3).

In slices obtained from rats pretreated with 2.5 mg/kg reserpine, MDMA (10 µmol/l) and amphetamine (1 or 10 µmol/l) enhanced the resting outflow of radioactivity from slices incubated with either [^{3}H]dopamine, [^{3}H]5-HT or [^{3}H]noradrenaline (Figs. 4, 5). The MDMA- and amphetamine-induced release of radioactivity were not reduced when Ca^{2+} was omitted from the superfusion fluid. However the MDMA-induced release of radioactivity from slices incubated with [^{3}H]dopamine and the amphetamine-induced release of radioactivity from slices incubated with [^{3}H]5-HT, was enhanced above that in superfusion fluid containing Ca^{2+} (Figs. 4, 5).

Effects of MDMA, amphetamine and neuronal uptake inhibitors on the S-I release of radioactivity from slices obtained from reserpinised rats

In tissues obtained from rats pretreated with 2.5 mg/kg reserpine, the first period of electrical stimulation failed to evoke a S-I outflow of radioactivity from slices incubated with [^{3}H]dopamine or [^{3}H]5-HT in either the presence or absence of Ca^{2+} (Figs. 4, 5). There was however, a small residual response to electrical stimulation, which was abolished in Ca^{2+}-free solution, from slices incubated with [^{3}H]noradrenaline (Figs. 4, 5). The second period of stimulation, in the presence of MDMA or amphetamine, evoked a S-I outflow of radioactivity from slices incubated with [^{3}H]dopamine or [^{3}H]noradrenaline, but not from slices incubated with [^{3}H]5-HT (Figs. 4, 5). The S-I outflow in the presence of MDMA or amphetamine was abolished when Ca^{2+} was excluded from the superfusion fluid (Figs. 4, 5). The S-I outflow of radioactivity from slices incubated with [^{3}H]noradrenaline or [^{3}H]dopamine was also increased by the neuronal uptake inhibitors, 1 µmol/l desipramine and 1 µmol/l cocaine, respectively, but the increases were less than those caused by 10 µmol/l MDMA (Fig. 6).

In slices obtained from rats pretreated with 10 mg/kg reserpine, the first period of electrical stimulation failed to evoke a S-I outflow of radioactivity from slices incubated in either of the three monoamines (Fig. 7). In slices incubated with [^{3}H]noradrenaline, electrical stimulation evoked a much smaller S-I outflow of radioactivity in the presence of MDMA compared to that from slices ob-

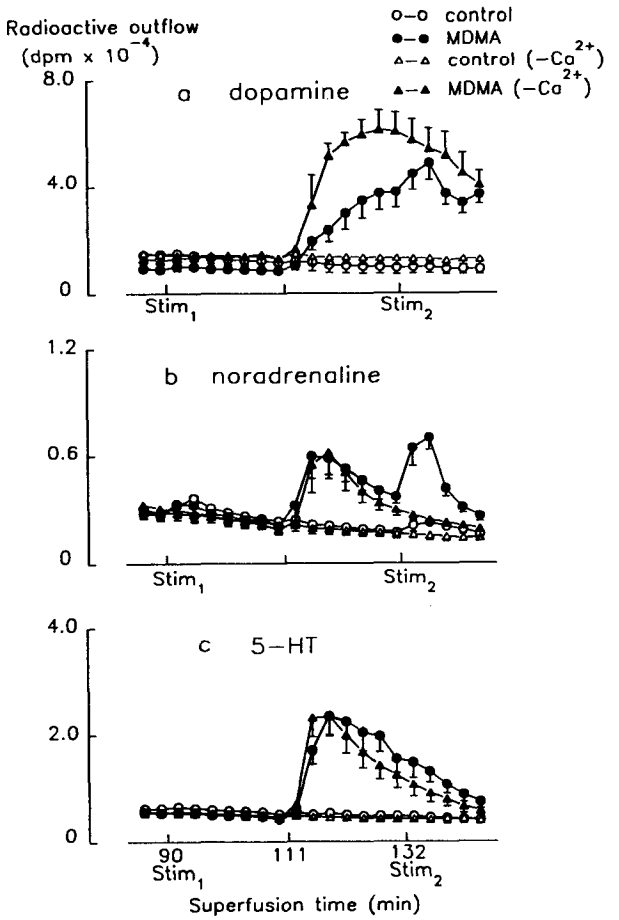

Fig. 4. Effect of MDMA on the radioactive outflow from brain slices obtained from rats pretreated with 2.5 mg/kg reserpine. Slices were incubated with [^{3}H]dopamine (**a**), [^{3}H]noradrenaline (**b**) or [^{3}H]5-hydroxytryptamine (5-HT) (**c**), and then given two periods of electrical stimulation ($Stim_1$, $Stim_2$) 90 and 132 min after the beginning of superfusion, at a frequency of 5 Hz for 1 min. MDMA (10 μmol/l) was added 111 min after the start of superfusion in experiments denoted by the *filled circles*. Control experiments (no MDMA) are denoted by the *open circles*. Calcium was excluded from the superfusion fluid ($-Ca^{2+}$) in the experiments denoted by *open triangles* (control) and *filled triangles* (10 μmol/l MDMA). The radioactive content in each 3-min sample of the superfusion fluid is expressed as disintegrations per minute (dpm). *Each point* represents the mean and *vertical bar* the SEM from 3–6 experiments

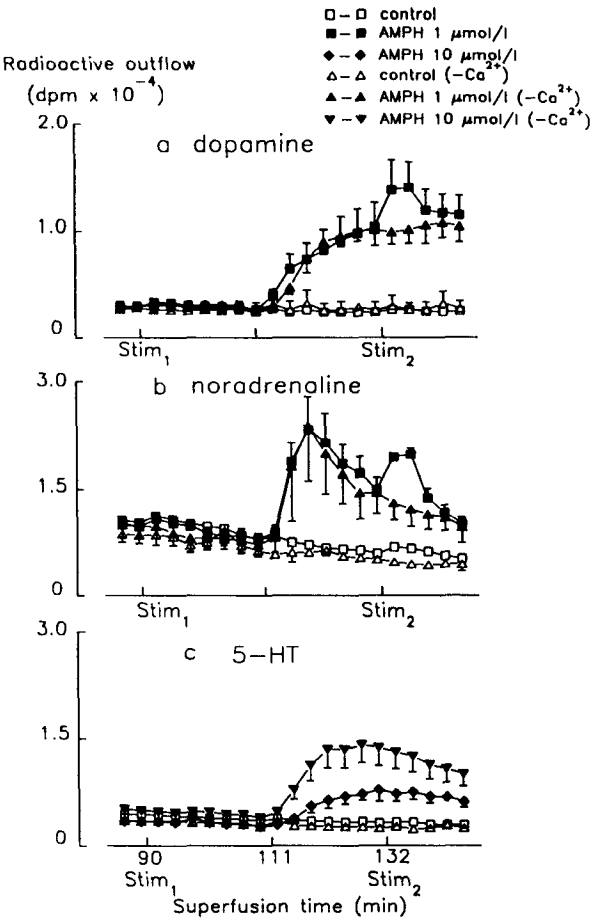

Fig. 5. Effect of amphetamine on the radioactive outflow from brain slices obtained from rats pretreated with 2.5 mg/kg reserpine. Slices were incubated with [^{3}H]dopamine (**a**), [^{3}H]noradrenaline (**b**) or [^{3}H]5-hydroxytryptamine (5-HT) (**c**), and then given two periods of electrical stimulation ($Stim_1$, $Stim_2$) 90 and 132 min after the beginning of superfusion, at a frequency of 5 Hz for 1 min. Amphetamine (AMPH, 1 or 10 μmol) was added 111 min after the start of superfusion in the experiments denoted by the *filled squares* and *filled diamonds* respectively. Control experiments (no AMPH) are denoted by the *open squares*. Calcium was excluded from the superfusion fluid ($-Ca^{2+}$) in the experiments denoted by *open triangles* (control), *filled triangles* (1 μmol/l AMPH) and *filled inverted triangles* (10 μmol/l AMPH). The radioactive content in each 3-min sample of the superfusion fluid is expressed as disintegrations per minute (dpm). *Each point* represents the mean and *vertical bar* the SEM from 3–6 experiments

tained from rats pretreated with 2.5 mg/kg reserpine (compare Fig. 4 and Fig. 7). Electrical stimulation still evoked a small S-I outflow of radioactivity from slices incubated in [^{3}H]dopamine in the presence of 10 μmol/l MDMA (Fig. 7). In slices obtained from rats pretreated with 10 mg/kg reserpine, there was no response to electrical stimulation in either the presence or absence of MDMA (10 μmol/l) from slices incubated with [^{3}H]5-HT (Fig. 7).

In all cases, results obtained from rats pretreated with 10 mg/kg reserpine were similar to those obtained from rats pretreated with 5 mg/kg reserpine, suggesting that 10 mg/kg reserpine was a supramaximal dosage (data not shown).

Discussion

Effects of MDMA and amphetamine in the presence of vesicular stores of transmitter

MDMA and amphetamine were shown to release radioactivity in a concentration-dependent manner from striatal slices incubated with [^{3}H]dopamine and from hippocampal slices incubated with [^{3}H]noradrenaline or [^{3}H]5-HT. MDMA was more potent (albeit less effective) in releasing [^{3}H]5-HT than [^{3}H]dopamine, in accord with McKenna et al. (1991), who showed that at a concentration of 1 μmol/l, MDMA released [^{3}H]5-HT and [^{3}H]dopamine to approximately 180% and 130% of resting release, respectively; these values are in close

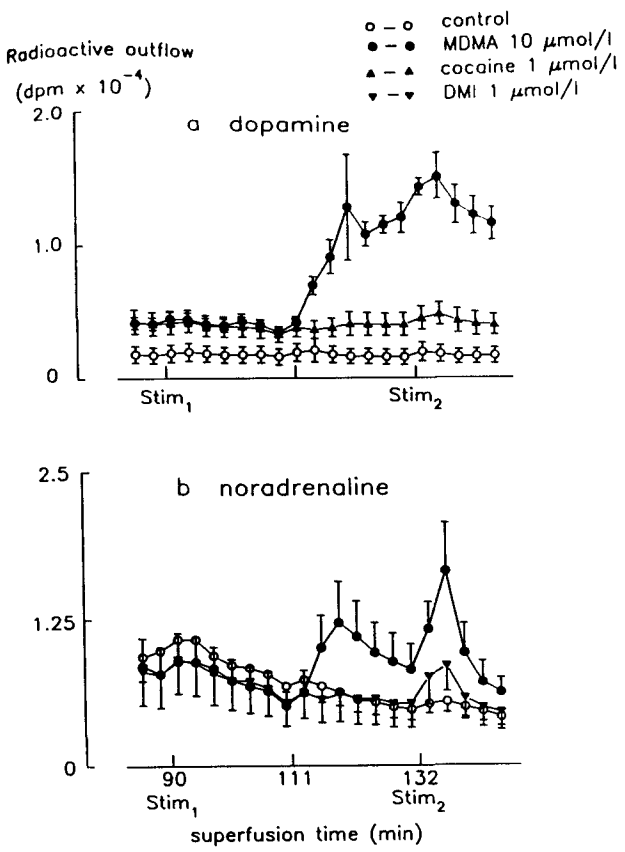

Fig. 6. Effect of MDMA, cocaine and desipramine (DMI) on the radioactive outflow from brain slices obtained from rats pretreated with 2.5 mg/kg reserpine. Slices were incubated with [^{3}H]dopamine (**a**), or ([^{3}H]noradrenaline (**b**), and then given two periods of electrical stimulation ($Stim_1$, $Stim_2$) 90 and 132 min after the beginning of superfusion, at a frequency of 5 Hz for 1 min. MDMA (10 μmol/l, *filled circles*), cocaine (1 μmol/l, *filled triangles*), or DMI (1 μmol/l, *inverted filled triangles*), were added 111 min after the start of superfusion. Control experiments (no drug) are denoted by the *open circles*. The radioactive content in each 3-min sample of the superfusion fluid is expressed as disintegrations per min (dpm). *Each point* represents the mean and *vertical bar* the SEM from 3–4 experiments

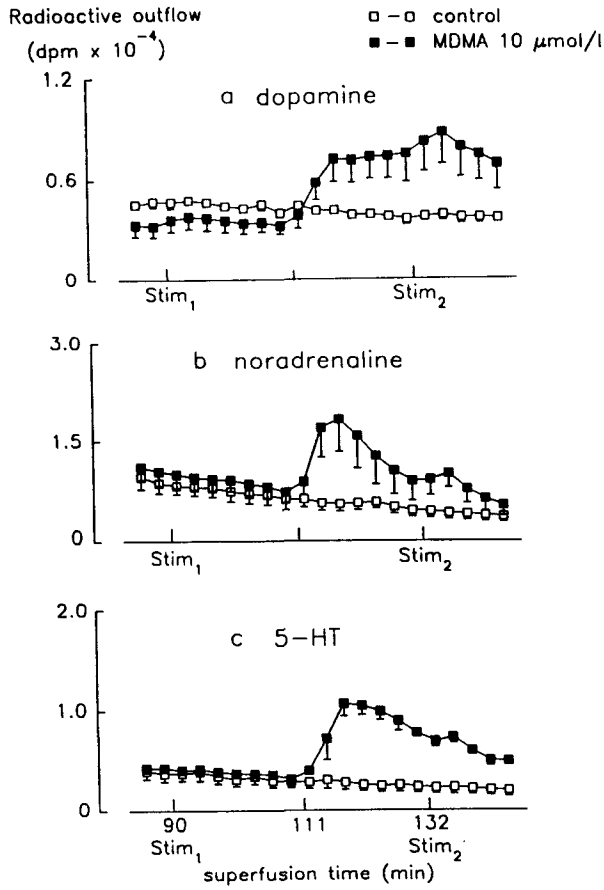

Fig. 7. Effect of MDMA on the radioactive outflow from brain slices obtained from rats pretreated with 10 mg/kg reserpine. Slices were incubated with [^{3}H]dopamine (**a**), [^{3}H]noradrenaline (**b**) or [^{3}H]5-hydroxytryptamine (5-HT) (**c**) and then given two periods of electrical stimulation ($Stim_1$, $Stim_2$) 90 and 132 min after the beginning of superfusion, at a frequency of 5 Hz for 1 min. MDMA (10 μmol/l) was added 111 min after the start of superfusion in experiments denoted by the *filled squares*. Control experiments (no MDMA) are denoted by the *open squares*. The radioactive content in each 3-min samples of the superfusion fluid is expressed as disintegration per minute (dpm). *Each point* represents the mean and *vertical bar* the SEM from 3–6 experiments

agreement with those obtained in the present study. MDMA was more potent than amphetamine in releasing [^{3}H]5-HT (see Fig. 1).

Amphetamine caused a greater release of [^{3}H]dopamine than of [^{3}H]5-HT or [^{3}H]noradrenaline. This is in accordance with the report of Azzaro and Rutledge (1973), who compared the release of the three monoamines induced by amphetamine at concentrations up to 1 mmol/l; when corrected for spontaneous release, the amphetamine-induced release was greatest for [^{3}H]dopamine in both the rat striatum and cerebral cortex.

The release of [^{3}H]noradrenaline by amphetamine was dependent on the experimental protocol. In the absence of electrical stimulation, amphetamine caused very little release of [^{3}H]noradrenaline, even at a concentration of 100 μmol/l, in agreement with Raiteri et al. (1975) who reported amphetamine to have only a small noradrenaline-releasing action in superfused synaptosomes. However, when slices had been previously subjected to electrical stimulation, 1 μmol/l amphetamine released [^{3}H]noradrenaline to an appreciable extent (compare Fig. 1 b and Fig. 3 b). It is unclear why this difference was observed, and why such a difference occurred with noradrenaline, and not with dopamine or 5-HT. Carrier-mediated release is only possible when the amine is present in the cytoplasm, and the relative distribution in the cytoplasm will be higher for the more lipophilic amines which are completely synthesized within the cytoplasm (i.e. dopamine and 5-HT). Thus it is possible that any change in the intraneuronal distribution of noradrenaline may be more readily observed than for dopamine or 5-HT. However, if the difference in noradrenaline release between experimental protocols was related to the distribution of cytoplasmic noradrenaline, then a similar difference between protocols should have been observed in experiments using MDMA; such a difference with MDMA was not evident.

It was not possible to determine the EC_{50} values for amphetamine- and MDMA-induced release of [^{3}H]dopamine as maximal effects were not reached. This is in agreement with previous studies (Azzaro and Rutledge 1973; Parker and Cubeddu 1988) where the amphetamine-induced release of [^{3}H]dopamine from rat striatal slices did not reach a maximal effect up to a concentration of 1 or 10 mmol/l. Nevertheless, from the data obtained in the present study, over a concentration range of 0.1 – 100 µmol/l, it appears that amphetamine is approximately an order of magnitude more effective than MDMA in releasing [^{3}H]dopamine. Both MDMA and amphetamine caused release of a much greater magnitude of [^{3}H]dopamine than of [^{3}H]5-HT or [^{3}H]noradrenaline (see Fig. 1). This result is not due to the higher density of dopaminergic nerve terminals in the striatum compared to noradrenergic or serotonergic terminals in the hippocampus, as the data are expressed as a percentage of the total tissue radioactive content of the slice. The results also cannot be explained by differences in the affinity of MDMA or amphetamine for different monoamine uptake sites, since MDMA has higher affinity for 5-HT and noradrenaline uptake sites than for dopamine uptake sites (Battaglia et al. 1988). Therefore it is suggested that there are differences in the availability of each of the monoamines to be released from intraneuronal stores by MDMA and amphetamine, perhaps based on the different lipophilicities of the monoamines.

The release of monoamines by amphetamine is generally considered to be Ca^{2+}-independent (Arnold et al. 1977; Marquardt et al. 1978; Kamal et al. 1981). In the present study, the MDMA-induced release of [^{3}H]dopamine was unaltered in Ca^{2+}-free superfusion fluid, in accordance with the Ca^{2+}-independent release of [^{3}H]dopamine induced by amphetamine (Arnold et al. 1977; Kamal et al. 1981). The resting outflow of radioactivity from slices incubated in [^{3}H]dopamine was enhanced in Ca^{2+}-free solution, as reported by Starke et al. (1978) for rabbit striatal slices. In contrast to [^{3}H]dopamine, the MDMA-induced release of [^{3}H]noradrenaline and [^{3}H]5-HT was enhanced when Ca^{2+} was excluded from the superfusate. The small 30% reduction in resting outflow of radioactivity from slices incubated in [^{3}H]noradrenaline cannot account for the 160% enhancement of MDMA-induced release of [^{3}H]noradrenaline caused by Ca^{2+} omission. In contrast to the present findings, Russ et al. (1991) reported an increase in the spontaneous outflow of [^{3}H]noradrenaline and its metabolites from isolated vas deferentia in the absence of extracellular Ca^{2+}; the increase was reduced by 1.5 µmol/l desipramine and therefore appeared to be due to increased outward transport. The contribution of outward transport of transmitter to the resting outflow of radioactivity has not been evaluated in the present study, however, the conflicting results suggest that different mechanisms are operating to establish spontaneous release in the present study and in the study by Russ et al. (1991).

The enhancement of [^{3}H]5-HT release in Ca^{2+}-free solution is unlikely to be due to an interaction between MDMA and endogenous 5-HT synthesis, as the activity of tryptophan hydroxylase is not affected by MDMA in vitro (Schmidt and Taylor 1987). There results are not consistent with the findings by a number of investigators that the release of noradrenaline (Arnold et al. 1977; Marquardt et al. 1978) and 5-HT (Johnson et al. 1986) caused by amphetamine or its derivatives is Ca^{2+}-independent. The disparity may be related to differences in experimental protocol, such as the use of different brain regions (Arnold et al. 1977), different tissue preparations (Marquardt et al. 1978), monoamine oxidase inhibition (Johnson et al. 1986) or different levels of tissue hypoxia (Russ et al. 1991).

In agreement with previous reports (Kamal et al. 1983; Fitzgerald and Reid 1990), amphetamine and MDMA facilitated the S-I outflow of radioactivity from slices incubated with [^{3}H]noradrenaline. In contrast, the S-I outflow from slices incubated with [^{3}H]5-HT was only slightly enhanced by MDMA, and was unaffected by amphetamine. Neither amphetamine nor MDMA affected the S-I outflow of radioactivity from slices incubated with [^{3}H]dopamine. Kamal et al. (1983) however, have previously reported an inhibitory action of 1 µmol/l amphetamine on S-I [^{3}H]dopamine release from rabbit caudate. The reason for this discrepancy is not clear, but it is interesting to note that the magnitude of the direct releasing action of amphetamine was considerably greater in the present study than in the study reported by Kamal et al. (1983).

Amphetamine is known to be a reversible inhibitor of monoamine oxidase (Green 1971; Mantle et al. 1976). However, it is unlikely that monoamine oxidase inhibition plays a significant role in the pharmacological effects of this compound because the inhibition is relatively weak (Mantle et al. 1976).

Although the ability of MDMA to inhibit monoamine oxidase has not been reported, it is possible that such an action may partially mediate the effects of MDMA on [^{3}H]monoamine release. Therefore, we investigated the effects of MDMA after pre-incubation of slices with the irreversible monoamine oxidase inhibitor, pargyline. As previously observed for rabbit and rat striatal slices incubated with [^{3}H]dopamine (Zumstein et al. 1981; Kamal et al. 1983; Dwoskin and Zahniser 1986), inhibition of monoamine oxidase with pargyline reduced the resting outflow of radioactivity from slices incubated with either of the three [^{3}H]monoamines.

Pargyline pre-incubation also resulted in a significant reduction in the S-I outflow of radioactivity from slices incubated with [^{3}H]dopamine, [^{3}H]noradrenaline and [^{3}H]5-HT in the absence of MDMA. Under normal conditions, acidic deaminated metabolites such as 3,4-diphenylacetic acid (DOPAC) account for a major proportion of the total radioactive outflow, both at rest and during stimulation (Zumstein et al. 1981). The inhibition of the production of these metabolites in the presence of pargyline allows the re-incorporation of previously released [^{3}H]transmitter into vesicles and thus a reduced S-I outflow of radioactivity (Zumstein et al. 1981).

The MDMA-induced release of [^{3}H]dopamine, [^{3}H]noradrenaline and [^{3}H]5-HT were all enhanced by pre-incubation with pargyline, which is consistent with reports that the amphetamine-induced release of dopa-

mine and noradrenaline are enhanced by monoamine oxidase inhibition (Liang and Rutledge 1982; Niddam et al. 1985; Parker and Cubeddu 1986a). Monoamine oxidase inhibition enhanced MDMA-induced release of radioactivity from slices incubated with [^{3}H]noradrenaline to a greater extent than that of [^{3}H]5-HT or [^{3}H]dopamine. It is not clear why this is the case, but it may relate to a difference in the distribution of cytoplasmic/vesicular [^{3}H]noradrenaline compared to that of the other monoamines.

Following pargyline pre-incubation, MDMA caused a greater enhancement of the S-I outflow of [^{3}H]noradrenaline and [^{3}H]5-HT than in the absence of pargyline. The S-I outflow of [^{3}H]dopamine was enhanced after monoamine oxidase inhibition, whereas no enhancement had been observed with dopamine when monoamine oxidase was intact. Parker and Cubeddu (1986b) also observed that amphetamine enhanced the S-I release of dopamine, although they measured endogenous release and did not need to inhibit monoamine oxidase for the enhancement to become evident. The heightening of the facilitatory actions of amphetamine on resting and S-I release of monoamines following monoamine oxidase inhibition has previously been attributed to the enhancement of axoplasmic concentrations of monoamine (Parker and Cubeddu 1986b). The enhancement of the S-I outflow may be related to a greater reincorporation of transmitter released by the first period of stimulation, as evidenced by a reduced S_1 (Table 1) in slices pre-incubated with pargyline. Since the actions of MDMA on S_2 remain relatively unchanged by pargyline, a reduction in S_1 following pargyline pre-incubation would subsequently result in an elevation of the ratio of S_2/S_1 in the presence of MDMA, and an apparent enhancement of the actions of MDMA.

Effects of MDMA and amphetamine in slices obtained from reserpinised rats

Reserpine depletes endogenous stores of dopamine, noradrenaline and 5-HT by blocking vesicular storage of monoamines (Carlsson 1965) and subsequently abolished the S-I release of transmitter (Parker and Cubeddu 1986a). Inhibition of monoamine oxidase prior to incubation of tissue from reserpinised animals with [^{3}H]monoamine allows the examination of cytosolic stores of [^{3}H]monoamine transmitter (Paton 1976; Masuoka et al. 1982). Using this model, the present study demonstrated that both MDMA and amphetamine released [^{3}H]dopamine, [^{3}H]noradrenaline and [^{3}H]5-HT, as previously reported for amphetamine (Niddam et al. 1985; Parker and Cubeddu 1986a). It was noted that the MDMA-induced release of radioactivity from slices incubated with [^{3}H]dopamine and the amphetamine-induced release of radioactivity from slices incubated with [^{3}H]5-HT were higher in Ca^{2+}-free medium (compare Figs. 4a and 5c), although amphetamine-induced release of radioactivity from slices incubated with [^{3}H]dopamine and MDMA-induced release of radioactivity from slices incubated with [^{3}H]5-HT were unchanged in Ca^{2+}-free medium. As there is no clear pattern to the results, the reason for the increased release in Ca^{2+}-free medium is not clear.

In slices obtained from rats pretreated with 2.5 mg/kg reserpine, electrical stimulation failed to evoke a S-I outflow of [^{3}H]dopamine or [^{3}H]5-HT, and caused only a slight residual S-I outflow of [^{3}H]noradrenaline. This confirmed that the vesicular storage of [^{3}H]monoamines was virtually abolished. In the presence of MDMA and amphetamine however, electrical stimulation evoked as S-I outflow of [^{3}H]dopamine and [^{3}H]noradrenaline, but not of [^{3}H]5-HT. This S-I outflow of [^{3}H]dopamine and [^{3}H]noradrenaline in the presence of MDMA or amphetamine was Ca^{2+}-dependent, suggesting that electrical stimulation induced the exocytotic release of monoamines from vesicular stores. This was not due to "washout" of reserpine, as responses to the second period of electrical stimulation in the absence of MDMA or amphetamine were identical to the responses to the first period of electrical stimulation in control experiments.

A S-I outflow from slices incubated with [^{3}H]noradrenaline or [^{3}H]dopamine was also evoked in the presence of neuronal uptake blockade using desipramine and cocaine, respectively. However, the S-I outflow in the presence of MDMA was not solely due to neuronal uptake blockade by MDMA, as the response to electrical stimulation in the presence of neuronal uptake inhibition was less pronounced than that in the presence of MDMA. The concentration of desipramine used to block the neuronal uptake of noradrenaline has previously been shown to completely block the MDMA-induced release of [^{3}H]noradrenaline (Fitzgerald and Reid 1990).

The S-I release of vesicular transmitter in the presence of MDMA was reduced when the dose of reserpine was increased from 2.5 to 5 or 10 mg/kg. This indicates that, in accordance with previous reports, there is a dose-related effect of reserpine on vesicular storage of monoamines (see Maxwell et al. 1976).

It is speculated that the large S-I outflow of radioactivity from slices incubated with [^{3}H]noradrenaline or [^{3}H]dopamine in the presence of MDMA or amphetamine, is due to both the blockade of neuronal uptake and an interaction of the amines at the level of the vesicular membrane. Vesicles are still intact and even undergo exocytosis after reserpine pretreatment, because although monoamine release is inhibited, the vesicular enzyme dopamine β-hydroxylase can still be released (Cubeddu and Weiner 1975; Thoa et al. 1975). Amphetamine uptake into isolated synaptic vesicles is not blocked by reserpine (Lentzen and Philippu 1981). Once inside the vesicle, indirectly acting amines can be "trapped" by being protonated in the acid environment (Johnson et al. 1982; Knoth et al. 1984). It is therefore proposed that amphetamine and MDMA may enter vesicles, and reduce the pH gradient across the vesicular membrane (Phillips 1982; Johnson et al. 1982). Reserpine binding is slowed by a reduction in the pH gradient across the vesicular membrane (Near and Mahler 1983; Scherman and Henry 1984), and it is apparent that reserpine binding may be partially reversible after 24 h following the low dose (2.5 mg/kg) used in the present study (Norn and Shore 1971; Darchen et al. 1989). Thus the apparent "reversal" of reserpine

blockade of vesicular uptake may possibly come about through indirect changes in the binding of reserpine caused by MDMA and amphetamine.

The failure of electrical stimulation to evoke a S-I outflow of [^{3}H]5-HT in the presence of MDMA or amphetamine illustrates a possible difference in the time taken for vesicles in serotonergic neurones to recover function following reserpine pretreatment compard with vesicles in noradrenergic or dopaminergic neurones. Reserpine is equipotent in blocking the vesicular storage of all three monoamines (Shore 1962; Carlsson 1965) and thus the failure of electrical stimulation to evoke a S-I outflow of radioactivity from slices incubated with [^{3}H]5-HT, in the presence of MDMA or amphetamine, is unlikely to be due to stronger binding of reserpine in serotonergic nerve terminals. Rather it may indicate that serotonergic vesicles take longer to recover from the effects of reserpine. The life span of spinal 5-HT-containing granules is similar to spinal noradrenaline-containing granules (Dahlström et al. 1973), however there are fundamental differences between serotonergic and catecholaminergic vesicles such as vesicular adenosine triphosphate content (Barasch et al. 1987), regulation of intravesicular pH, and the types of intravesicular proteins (Tamir and Gershon 1987).

In summary, the results suggest that MDMA interacts with neuronal and vesicular uptake and storage of monoamines in a manner similar to that of amphetamine. In both the presence and absence of vesicular stores of transmitter, MDMA and amphetamine were found to have differential effects on the three monoamine systems studied. It is possible that the inability of electrical stimulation to evoke a S-I outflow of 5-HT from slices obtained from reserpinised animals reflects a difference between monoaminergic neurones, and may give insight into the selective neurotoxicity of MDMA at serotonergic nerve terminals.

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