

Direct Effects of 3,4-Methylenedioxymethamphetamine (MDMA) on Serotonin or Dopamine Release and Uptake in the Caudate Putamen, Nucleus Accumbens, Substantia Nigra Pars Reticulata, and the Dorsal Raphé Nucleus Slices

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ABSTRACT We examined the effects of pressure ejected 3,4-methylenedioxymethamphetamine (MDMA) from a micropipette on direct chemically stimulated release, and on electrically stimulated serotonin (5-HT) or dopamine (DA) release in the caudate putamen (CPu), nucleus accumbens (NAc), substantia nigra pars reticulata (SNr), and the dorsal raphé nucleus (DRN) brain slices of rat, using fast cyclic voltammetry (FCV). MDMA is electroactive, oxidising at +1100 mV. When the anodic input waveform was reduced from +1.4 to +1.0 volt, MDMA was not electroactive. Using this waveform, pressure ejection of MDMA did not release 5-HT or DA in brain slices prepared from any of the nuclei studied. MDMA significantly potentiated electrically stimulated 5-HT release in the SNr and DA release in CPu. In the DRN or in the NAc, MDMA was without effect on peak electrically stimulated 5-HT or DA release. The rates of neurotransmitter uptake, expressed as $t_{1/2}$, were in all cases significantly decreased after MDMA. The results indicate that MDMA, unlike (+)amphetamine, is not as a releaser of DA or 5-HT, it is a potent inhibitor of both DA and 5-HT uptake. **Synapse 36: 275–285, 2000.**

INTRODUCTION

Due to its euphoria-inducing and mild stimulant properties, 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”) has gained popularity as a recreational drug over the last decade. The major effects of MDMA can be categorised into acute and long-term effects. The acute effects of MDMA in common with (+)amphetamine and other drugs of abuse produce behavioural, neurochemical, and electrophysiological changes that can be correlated with elevation of the extracellular levels of serotonin (5-HT) and dopamine (DA) (Gold and Koob, 1988; Fitzgerald and Reid, 1990; Callaway et al., 1990; Gough et al., 1991; McKenna et al., 1991; Nash and Brodtkin, 1991; Johnson et al., 1991; Callaway and Geyer, 1992a,b; White et al., 1994; Green et al., 1995; Obradovic et al., 1998). However, in contrast to other drugs of abuse (e.g.,

(+)amphetamine or cocaine) its action is believed to be more prominent on 5-HT than on DA release (Berger et al., 1992; Gough et al., 1991; Schmidt et al., 1987; Sprouse et al., 1989; for reviews see McKenna and Peroutka, 1990; White et al., 1996).

Long-term exposure to MDMA causes a selective decrease in brain concentrations of 5-HT and DA. This diminution or loss of 5-HT and DA nerve terminals in

Abbreviations: CPu, caudate putamen; DA, dopamine; DRN, dorsal raphé nucleus; FCV, fast cyclic voltammetry; 5-HT, 5-hydroxytryptamine (serotonin); MDMA, 3,4-methylenedioxymethamphetamine; NAc, nucleus accumbens; SNr, substantia nigra pars reticulata. Current address for Z.L. Kruk: School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth PO1 2DT, UK.

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various brain regions of rodents and nonhuman primates has been associated with the neurotoxic effects of long-term use (Green et al., 1995). The neurotoxic effects of long-term exposure to MDMA, like its acute effects, are more prominent on 5-HT system. The MDMA-induced neurodegenerative changes include reduction of 5-HT reuptake sites (Battaglia et al., 1987) as well as deformation of the axon, varicosity, and nerve terminal morphology (see Molliver et al., 1990).

The acute effects of MDMA on brain monoamines and their metabolites result from the ability of the drug to modulate 5-HT release and uptake. With respect to release from synaptosomes (Nichols et al., 1982) or superfused brain slices (Johnson et al., 1986; Schmidt et al., 1987), MDMA is a more potent releaser of [3 H]5-HT than of [3 H]DA.

Rats treated with repeated MDMA injections exhibit behavioural and neurochemical sensitisation, expressed as increased locomotion and increased extracellular dopamine in the nucleus accumbens (Kalivas et al., 1998), similar to those following repeated treatments with (+)amphetamine or a D_2/D_3 agonist quinpirole (Muscat et al., 1993, 1996). The behavioural responses differ qualitatively, however. Rats treated with MDMA, though expressing a robust locomotor response, have a much reduced level of investigatory behaviour (Paulus and Geyer, 1992; Callaway et al., 1990; Krebs and Geyer, 1993; see also Geyer, 1996, for review). Blockade of serotonin uptake by fluoxetine has no effect on (+)amphetamine-induced locomotor activity but inhibits MDMA-induced hyperlocomotion, which is thought to be indirectly mediated through excitation of 5-HT $_{1b}$ receptors (Rempel et al., 1993). Furthermore, unlike (+)amphetamine-treated rats, animals treated with repeated injections of MDMA display low body posture, forepaw paddling, and head weaving (Spanos and Yamamoto, 1989), which are characteristics of the "serotonin syndrome."

Much is known about the medium- to long-term effects of MDMA on 5-HT and DA transmission, but in order to study the dynamics of MDMA's effect on 5-HT or DA, its effects must be studied in real time. We previously measured electrically evoked 5-HT and DA and reported the effects of direct application of (+)amphetamine by pressure ejection on DA and 5-HT release in the caudate putamen (CPu), nucleus accumbens (NAc), dorsal raphe nucleus (DRN), and substantia nigra pars reticulata (Iravani and Kruk, 1995, 1997; Iravani et al., 1999). At present, there is no data on real-time effects of MDMA on 5-HT or DA release. The aim of this study was to investigate the direct effects of MDMA on 5-HT and DA release in the DRN, SNr, NAc, and the CPu of the rat. MDMA was applied by pressure ejection into brain slices to study 1) its direct effects on 5-HT or DA release, 2) its effects on electrically stimulated 5-HT and DA release, and 3) its effects on 5-HT or DA uptake. Changes in DA or 5-HT

concentrations were measured in real time using fast cyclic voltammetry (FCV).

MATERIALS AND METHODS

Measurements of DA and 5-HT release in brain slices at a carbon fibre microelectrode following electrical stimulation has been described previously (O'Connor and Kruk, 1991, 1992; Iravani and Kruk, 1995, 1997; Iravani et al., 1996); only essential details are presented.

Male Wistar rats (Charles River, UK; 200–280 g) were killed by cervical dislocation and the brain rapidly and gently removed into artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 125, KCl 2.0, KH_2PO_4 1.25, $MgSO_4 \cdot 7H_2O$ 2.0, $NaHCO_3$ 25, D-glucose 11, $CaCl_2 \cdot 2H_2O$ 2.0, prepared using distilled water. ACSF was equilibrated at 32°C for 1 h with 95% O_2 : 5% CO_2 before use. Brain slices 350 μ m thick incorporating either the striatum (CPu and NAc), or the SNr, or the DRN equivalent to a brain slices 1.0–1.2 mm anterior to bregma (CPu, NAc), or 4.8–5.2 mm (SNr), and 5.4–6.0 mm (DRN) posterior to bregma (Paxinos and Watson, 1986) were prepared using an Oxford vibratome, and transferred to an incubation chamber as previously described (Trout and Kruk, 1992; O'Connor and Kruk, 1991). A carbon fibre electrode was placed 80 μ m below the surface of the slice in the desired region with the aid of a Nikon SMZ-U dissecting microscope with polarised light epi illumination. A bipolar tungsten stimulating electrode was located 200–400 μ m away from the carbon fibre electrode at the same depth below the surface of the slice.

Electrical stimulation

Electrical stimulation was controlled using a Neurolog signal conditioning apparatus, with constant voltage stimulation provided by a DS2 stimulator. The stimulus applied consisted of trains of 50 pulses at 50 Hz. The following protocol was followed. Trains of 50 p / 50 Hz were applied at 5-min intervals. MDMA (1 mM) was pressure-ejected at each recording site. Release of dopamine or serotonin following electrical stimulation and overflow following MDMA ejection was monitored. One minute after pressure application of MDMA, a 50 p / 50 Hz stimulation was applied. Four minutes later, this cycle was repeated.

Pressure ejection

Pressure ejection of 1 mM MDMA or (+)amphetamine was effected using a Neurophore apparatus (Medical Systems, Inc.) with drugs delivered from glass micropipettes (tip diameter 4–8 μ m) placed 20–40 μ m from the carbon fibre electrode. Drugs were ejected as bursts of 1 sec at pressure of 10 psi.

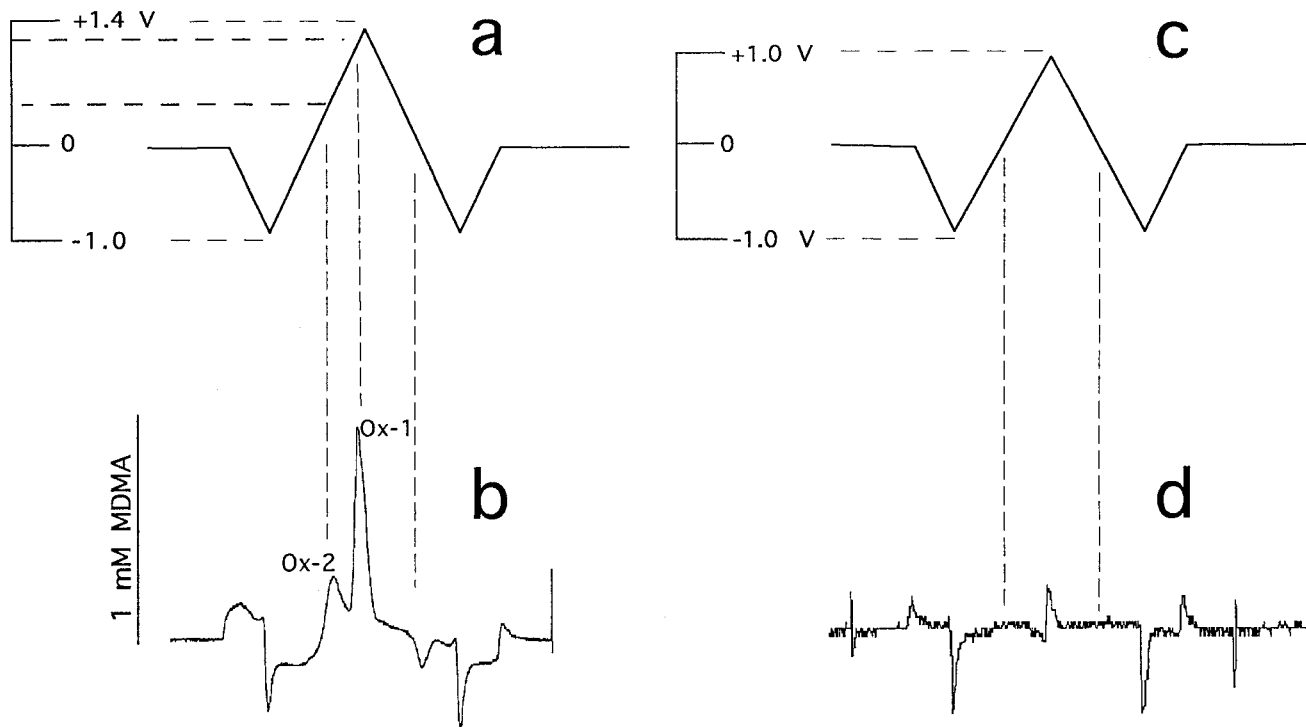


Fig. 1. The effect of input waveform on MDMA redox signal. Using the -1.0 V to +1.4 V waveform (a), MDMA was electroactive, with two oxidation peaks at +500 and +1100 mV (b). The first oxidation peak (+1100 mV, methamphetamine quinone, see Fig. 2) appeared on the first scan. With subsequent scans a second oxidation peak (at +500

mV, 3,4-dihydroxymethamphetamine, see Fig. 2) was seen. When the input waveform was set at -1.0 V to +1.0 V (c), MDMA was not electroactive (d). Further experiments were carried out using this waveform.

Fast cyclic voltammetry

FCV was effected with a Millar voltammeter (PD Systems, West Molesley, Surrey, UK) using a three-electrode system. The voltammetric output was monitored on a Nicolet 310 oscilloscope and fed into a CED 1401 interface running SIGAVG, with the voltammetric signals monitored on the computer screen and scans written to file for off-line inspection and analysis.

We used an "extended" or a "basic" waveform (see Stamford et al., 1992). The extended waveform as used in our previous studies (Iravani and Kruk, 1995, 1997; Iravani et al., 1996) consisted of $1\frac{1}{2}$ cycles of a 75 Hz triangular voltage ramp from -1.0 to +1.4 V scanning at a rate of 300 V.s^{-1} at 2 Hz. In preliminary in vitro experiments using the extended waveform it was shown that MDMA was electroactive, producing oxidation currents that interfered with the oxidation current of DA or 5-HT. In order to remove the artefacts produced as a result of the electrochemical property of MDMA molecule, we used the "basic" waveform. The "basic" waveform has cathodic and anodic limits of -1.0 to +1.0 volt, respectively (see Fig. 1). Using this waveform, MDMA was electrochemically inert. We found that alteration of the waveform had no significant effect on the sensitivity of FCV to DA or 5-HT.

When electrically stimulated release had been recorded, the sample and hold potential on the voltam-

eter was set to the peak oxidation potential obtained by examining the faradaic current signal on the oscilloscope; for detection of DA and 5-HT, this was invariably in the region of +590 to +640 mV. This monitored the time course of changes of the faradaic signal following electrical or chemical stimulation. The sample and hold outputs were monitored on a chart recorder; all potentials were related to an Ag/AgCl reference electrode. Estimates of concentration of amine released were made by reference to in vitro calibration. Briefly, signals were recorded with the same electrode at the end of each experiment when placed in 100 nM 5-HT or 1 μM DA in PBS (0.1 M phosphate buffer, pH 7.4, containing 0.9% w/v NaCl).

The rate of uptake was estimated by measuring the time(s) for the peak monoamine signal as recorded on the sample and hold trace to decline to half its maximum value ($t_{1/2}$).

Drugs and sources

Drugs were dissolved in water or solvent, diluted with ACSF to the final concentration, and perfused for the time indicated in the figures. (+)-Amphetamine sulphate ([+]- α -methylphenethylamine, dextro-amphetamine); dopamine hydrochloride; 5-hydroxytryptamine creatinine sulphate were obtained from Sigma (St. Louis, MO). S-(+)-3,4-Methylenedioxymeth-

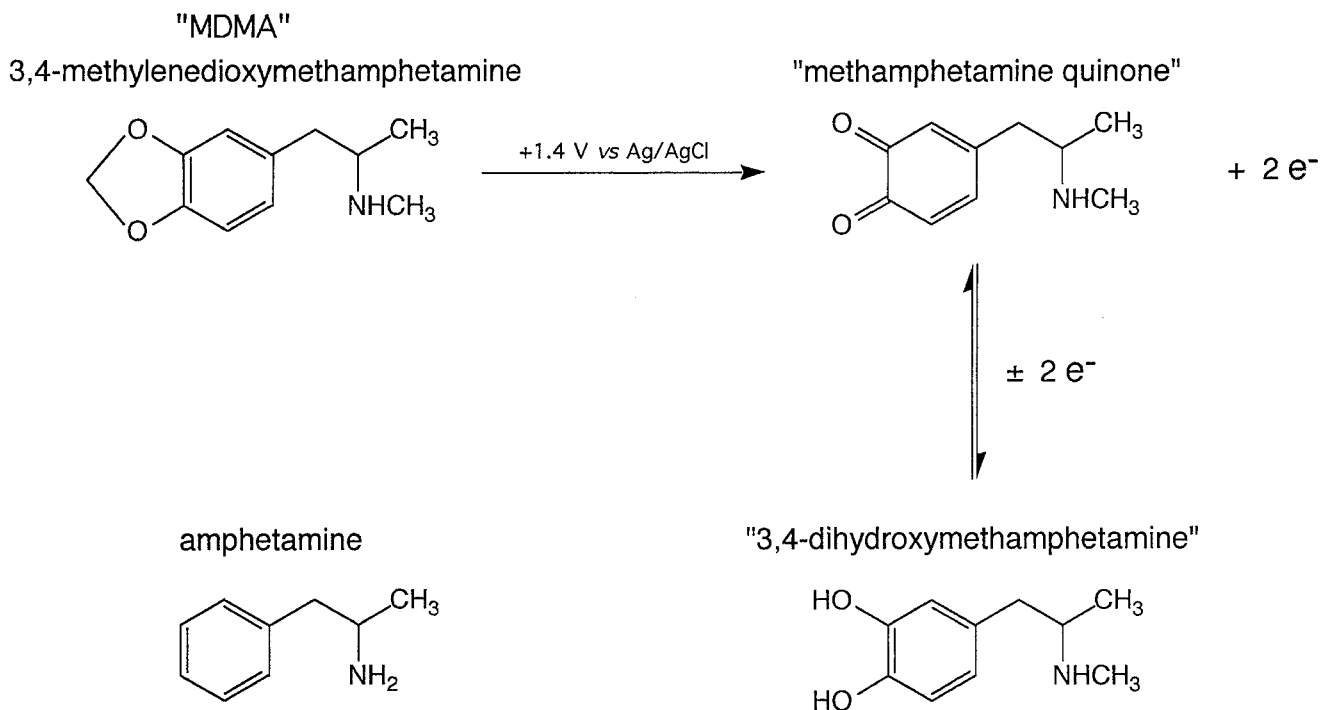


Fig. 2. The oxidation reactions of MDMA under an "extended" input waveform, scanning from -1.0 V to $+1.4$ V vs. Ag/AgCl. At the first scan, MDMA is oxidised to methamphetamine quinone (see oxi-

dation 1, Fig. 1). With subsequent scans this is further oxidised to 3,4-dihydroxymethamphetamine (see oxidation 2, Fig. 1). We suggest that the first reaction is irreversible, but the second is reversible.

amphetamine hydrochloride (MDMA) was obtained from RBI (Natick, MA). All salts for preparation of ACSF were obtained from BDH.

Statistical analysis

Estimates of absolute concentrations of released amine were made from calibration curves. Results are expressed as mean $\pm$ SEM; n = number of observations = number of slices. Groups were compared using paired or unpaired Student's t -test.

All calculations relating to time of the peak monoamine overflow and the $t_{1/2}$ values were made from the sample and hold outputs. These were then plotted using Kaleidograph software and all the subsequent x, y values were obtained from the software following computer digitisation.

RESULTS

Effect of MDMA on voltammetric signal

Using an extended waveform (anodic scan, $+1.4$ V), in vitro application of 1 mM MDMA in 0.1 M PBS resulted in two oxidation peaks appearing at $+1100$ mV (oxidation current 1) and $+500$ mV (oxidation current 2). The oxidation current 1 appeared within the first scan and subsequent scans resulted in generation of oxidation current 2. This is illustrated in Figure 1a,b. Switching the FCV waveform to "basic" mode ($+1.0$ V, anodic), resulted in elimination of the oxidation currents 1 and 2 following in vitro application of

MDMA. This change had no adverse effect on the sensitivity of the FCV for DA or 5-HT (results not shown). In vitro application of 1 mM (+)amphetamine (in the absence of brain slice) had no effect on the background voltammetric signal in either the "basic" or the "extended" waveform modes. It was concluded that MDMA, but not (+)amphetamine, under the condition of "extended" FCV waveform was electroactive. At $+1100$ mV, MDMA was converted into methamphetamine-quinone and this in turn was converted into 3,4-dihydroxymethamphetamine (Fig. 2).

In order to remove the interference of MDMA electro-oxidation on signals due to 5-HT or DA, all experiments reported hereafter were conducted using the "basic" waveform.

Pressure ejection of (+)amphetamine or MDMA

DA or 5-HT release could not be detected in slices from CPu, NAc or SNr, and DRN following 1 mM MDMA pressure ejection (1 sec / 10 psi). Small changes in the background voltammetric signals were infrequently detected in the SNr, but the chemical nature of these signals could not be identified. No voltammetrically identifiable signals were detected if the duration of MDMA pressure ejection was increased from 1 sec up to 10 sec. When 10 μ M MDMA was superfused together in the aCSF, no oxidation currents resembling 5-HT or DA were observed in any of the brain regions studied (results not shown).

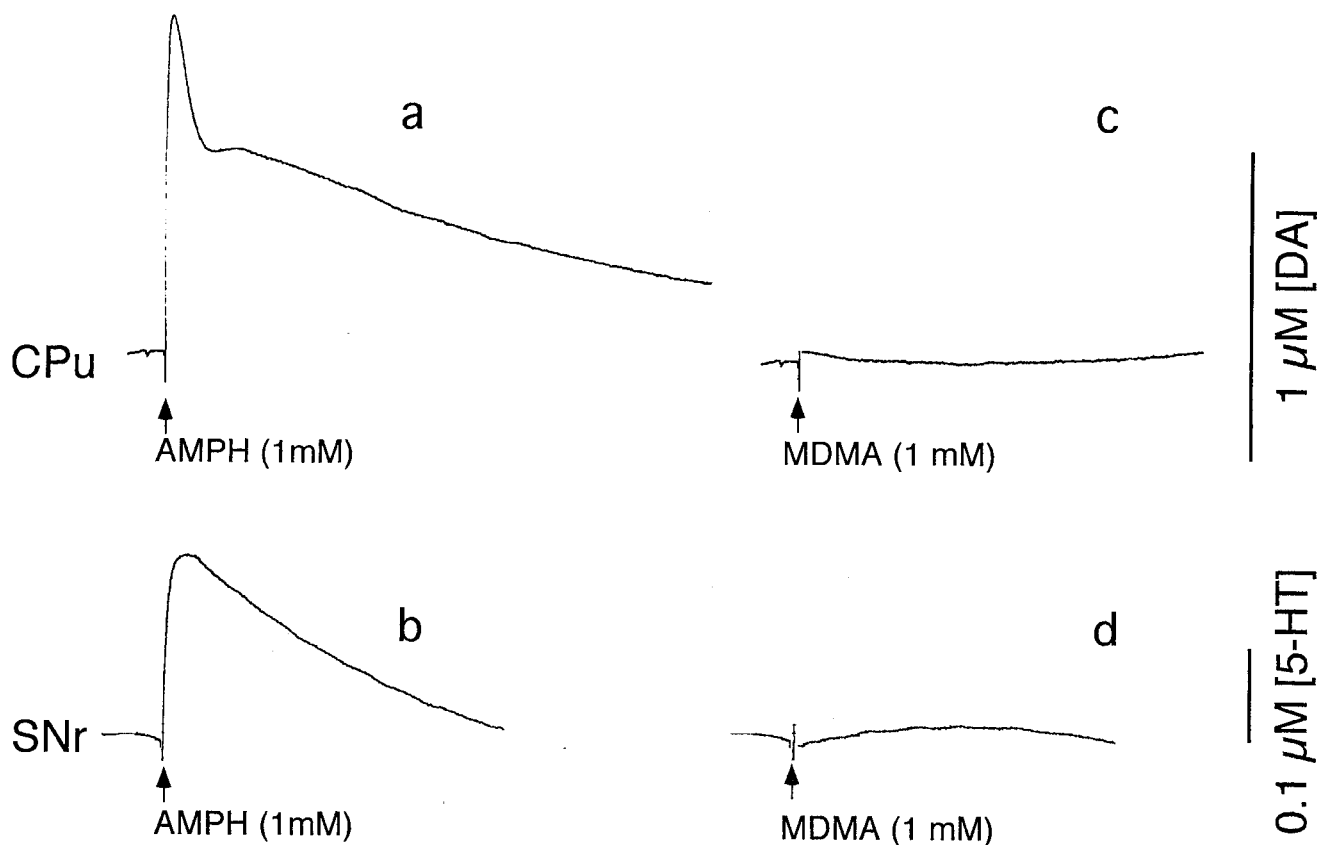


Fig. 3. Pressure ejection of 1 mM (+)amphetamine (AMPH) (a,b) and 1 mM MDMA (c,d) in CPu and SNr brain slices using +1.0 to -1.0 V waveform (see Fig. 2c). At this waveform, AMPH pressure

ejection (1 sec, 10 psi) resulted in a large release of DA and 5-HT in CPu and SNr, respectively. In contrast, pressure ejection of 1 mM MDMA did not release any detectable DA or 5-HT.

Pressure ejection of 1 mM (+)amphetamine (1 sec / 10 psi) resulted in large voltammetric signals. Analysis of these signals revealed that in the CPu or the NAc the voltammetric signal was due to DA, and in the SNr the signal was due to 5-HT (results not shown, but see Iravani and Kruk, 1995, 1997, respectively). We did not study the effect of (+)amphetamine in the DRN. Peak DA release following 1 mM (+)amphetamine pressure ejection was $0.6 \pm 0.2 \mu\text{M}$ ($n = 3$) and $0.76 \mu\text{M}$ ($n = 2$) in the CPu and NAc, respectively. In the SNr slices, peak 5-HT release following (+)amphetamine pressure ejection was $0.3 \pm 0.08 \mu\text{M}$ ($n = 3$; Fig. 3).

Effects of MDMA pressure ejection on electrically stimulated 5-HT or DA release

The effects of MDMA pressure ejection on electrically stimulated DA or 5-HT release are shown in Figure 4. Electrical stimulation with 50 p / 50 Hz resulted in robust release of DA in NAc or CPu brain slices. The peak release corresponded to $330 \pm 50 \text{ nM}$ DA ($n = 5$) and $360 \pm 40 \text{ nM}$ DA ($n = 4$) in the CPu and the NAc, respectively. In the DRN or in the SNr, the peak release by 50 p / 50 Hz stimulation was $54 \pm 5 \text{ nM}$ 5-HT ($n = 6$) and $50 \pm 6 \text{ nM}$ 5-HT ($n = 5$), respectively.

One minute after pressure ejection of 10 mM MDMA, electrically stimulated 5-HT release was potentiated by more than 200% to $113 \pm 21 \text{ nM}$ ($P < 0.05$; $n = 6$) in the SNr, whereas no statistically significant potentiation of 5-HT release was observed in the DRN. In the CPu, the DA signal was significantly potentiated to $620 \pm 140 \text{ nM}$ ($P < 0.05$; $n = 5$). MDMA had no significant effect on the peak DA levels in the NAc (control: $362 \pm 35 \text{ nM}$, MDMA: $380 \pm 39 \text{ nM}$; $n = 4$, $P = 0.479$). The results are summarised in Figure 5.

Effects of MDMA pressure ejection on the rate of reuptake of electrically stimulated DA or 5-HT

The effects of MDMA pressure ejection on electrically stimulated 5-HT and DA release are presented in Figure 6. Pressure ejection of MDMA 1 min before electrical stimulation resulted in a significant increase in the time taken for the 5-HT or DA signals to return to baseline in all areas studied. The $t_{1/2}$ values for 5-HT and DA are shown in Figure 6b. MDMA had a greater effect on 5-HT $t_{1/2}$ in the DRN and SNr than on the DA $t_{1/2}$ in the CPu and NAc. The effect of MDMA was

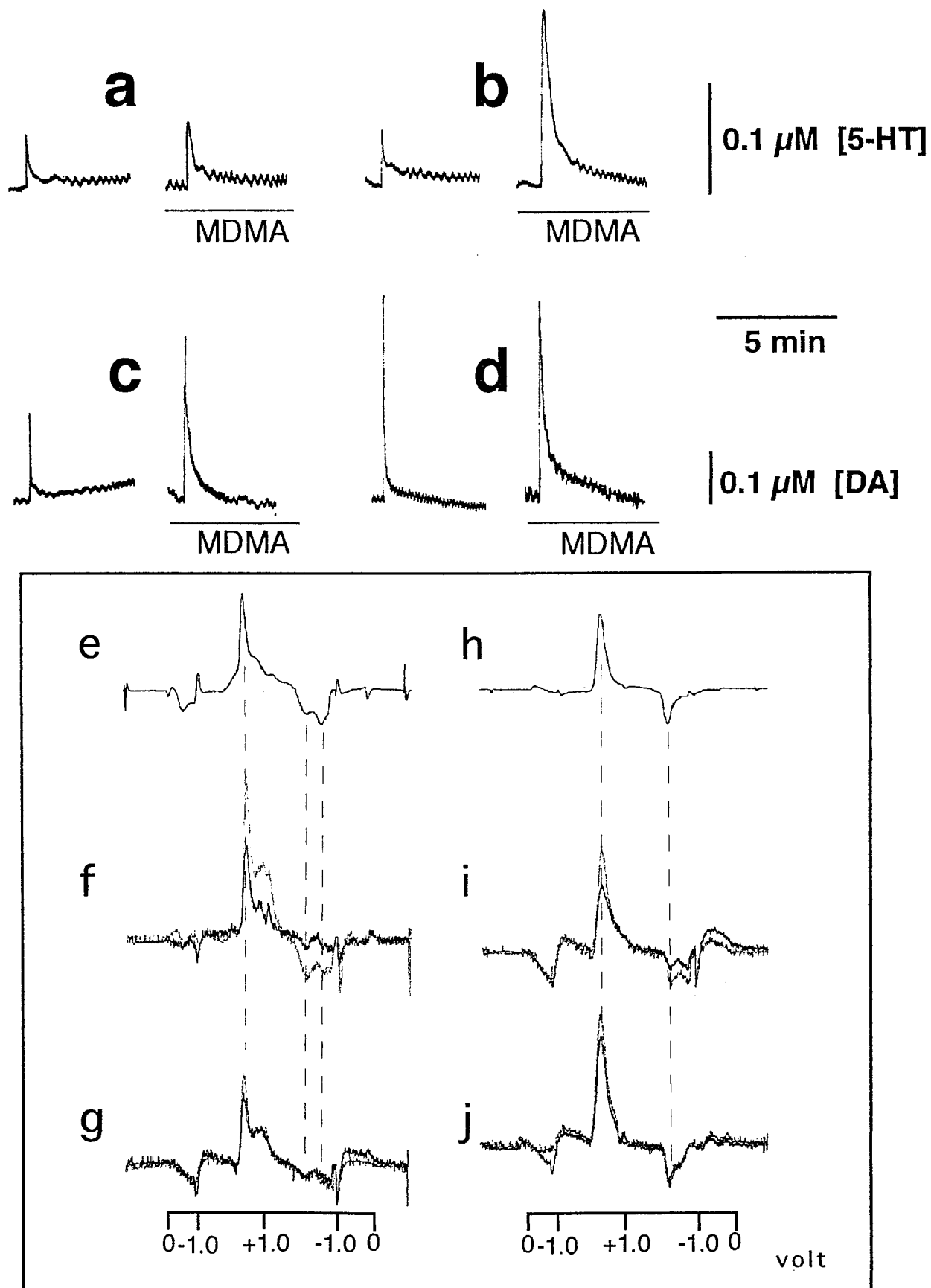


Fig. 4. Representative traces showing 5-HT (a,b) or DA (c,d) release before and after 1 mM MDMA pressure ejection in the DRN (a), SNr (b), CPu (c), and NAc (d). DA or 5-HT release was evoked by 50 pulses at 50 Hz. MDMA was pressure-ejected 4 min after electrical stimulation (50 p / 50 Hz), subsequent stimulus was delivered 1 min

after MDMA pressure ejection. Faradaic currents due to 5-HT and DA in vitro (e,h) and in brain slices. f-j: 5-HT and DA faradaic currents following 50 p / 50 Hz electrical stimulation, before (black traces) and after (gray traces) 1 mM MDMA pressure ejection in the SNr (f), DRN (g), CPu (i), and NAc (j).

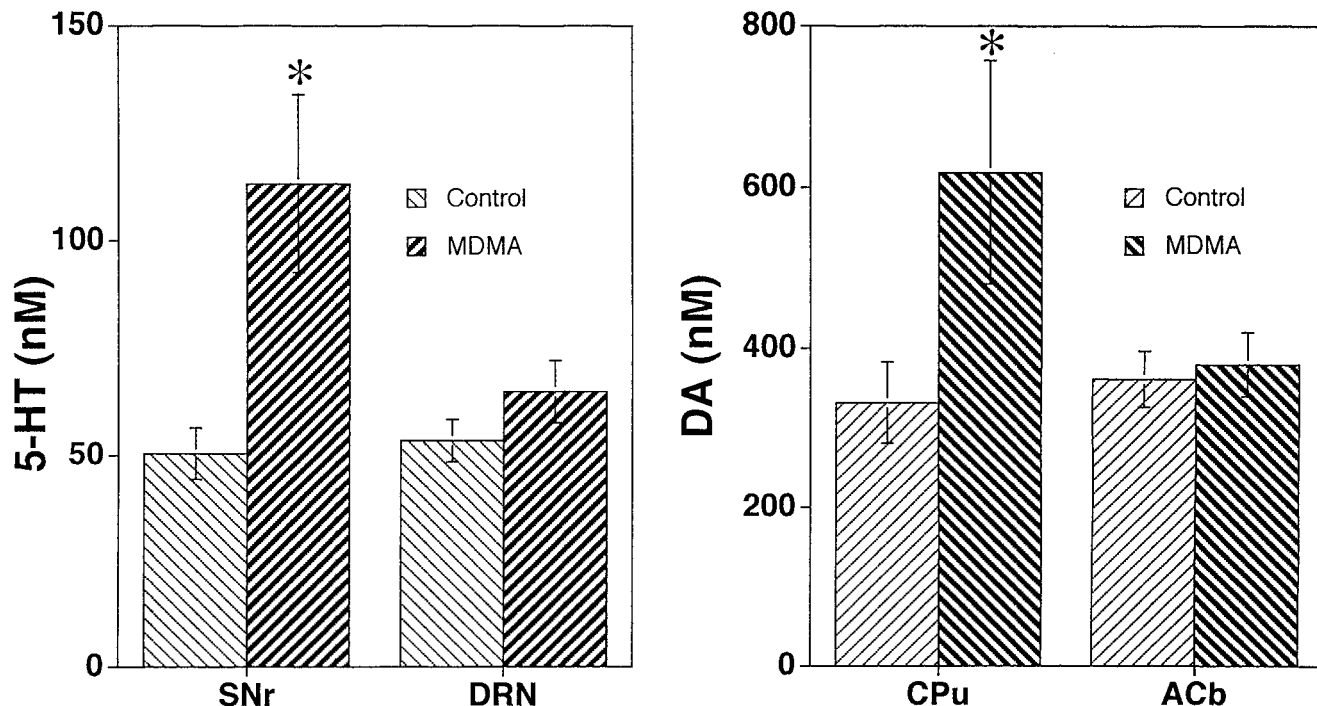


Fig. 5. The effect of MDMA pressure ejection on peak 5-HT release in the SNr and the DRN or peak DA release in the CPu and NAc in response to 50 p / 50 Hz stimulation. Each histogram shows mean $\pm$ SEM 5-HT or DA release in response to electrical stimulation, $n =$

4–6. Concentrations were calculated by postcalibration of electrodes in standard solutions of 5-HT or DA. * $P < 0.05$, Student's paired t -test.

greater on the $t_{1/2}$ in the SNr than in the DRN, but this difference did not reach statistical significance.

DISCUSSION

Voltammetric considerations

MDMA is a ring substituted phenylisopropylamine that is related in structure to (+)amphetamine and other hallucinogens such as mescaline (McKenna and Peroutka, 1990). The presence of the two single bond oxygen atoms on this molecule renders it extremely electroactive, and accordingly we obtained a strong oxidation signal at +1100 mV with the very first scan. We believe that the identity of this transient molecule resulting from electro-oxidation of MDMA to be a quinone, as is the case when DA or 5-HT are subjected to an anodic potential in FCV. This "methamphetamine quinone" is generated at a potential of +1100 mV. This transient molecule can in turn undergo a further electroreduction, which in turn can be oxidised to 3,4-dihydroxymethamphetamine at a potential of +500 mV (see Fig. 1, 2). Using the conventional extended waveform (−1.0 V to +1.4 V), the effects of MDMA on 5-HT or DA release cannot be studied because part of the electrochemical signature of MDMA overlaps that of the monoamines. However, when scans were adjusted to −1.0 V to +1.0 V, the problem of MDMA electroactivity was completely overcome. This alteration in measurement parameter had no effect on the sensitivity of FCV to 5-HT or DA whether in vitro or in

brain slice preparations. The calibration values, and estimates of extracellular concentration of 5-HT or DA using the "basic" waveform, did not differ significantly from those reported in other studies using the "extended" waveform (Iravani and Kruk, 1995, 1997; Iravani et al., 1996).

Direct effects of MDMA on DA or 5-HT release

In this study, using the "basic" waveform, and in previous studies from our laboratory using the "extended" waveform (Iravani and Kruk, 1995, 1997; Iravani et al., 1996), we have shown that when (+)amphetamine is pressure-ejected from a micropipette into CPu, NAc, or SNr slices, release of DA or 5-HT could be measured using FCV. This finding confirms observations made using a variety of neurochemical techniques (Fischer and Cho, 1978; Connor and Kuczenski, 1986; Butcher et al., 1988; Sulzer et al., 1993; Crespi et al., 1997) and is in line with the carrier-mediated mode of transmitter release (Levi and Raiteri, 1993). According to this model, (+)amphetamine acts as a substrate for the specific carrier and is taken up into nerve terminals. Once inside, (+)amphetamine redistributes DA from synaptic vesicles to the cytosol and promotes reverse transport (Sulzer et al., 1995). There is now evidence that the DA carrier is an obligatory target of (+)amphetamine, as this psychostimulant has no effect on locomotor activity or DA release and uptake in mice lacking the carrier protein (Giros et al., 1996; Jones et

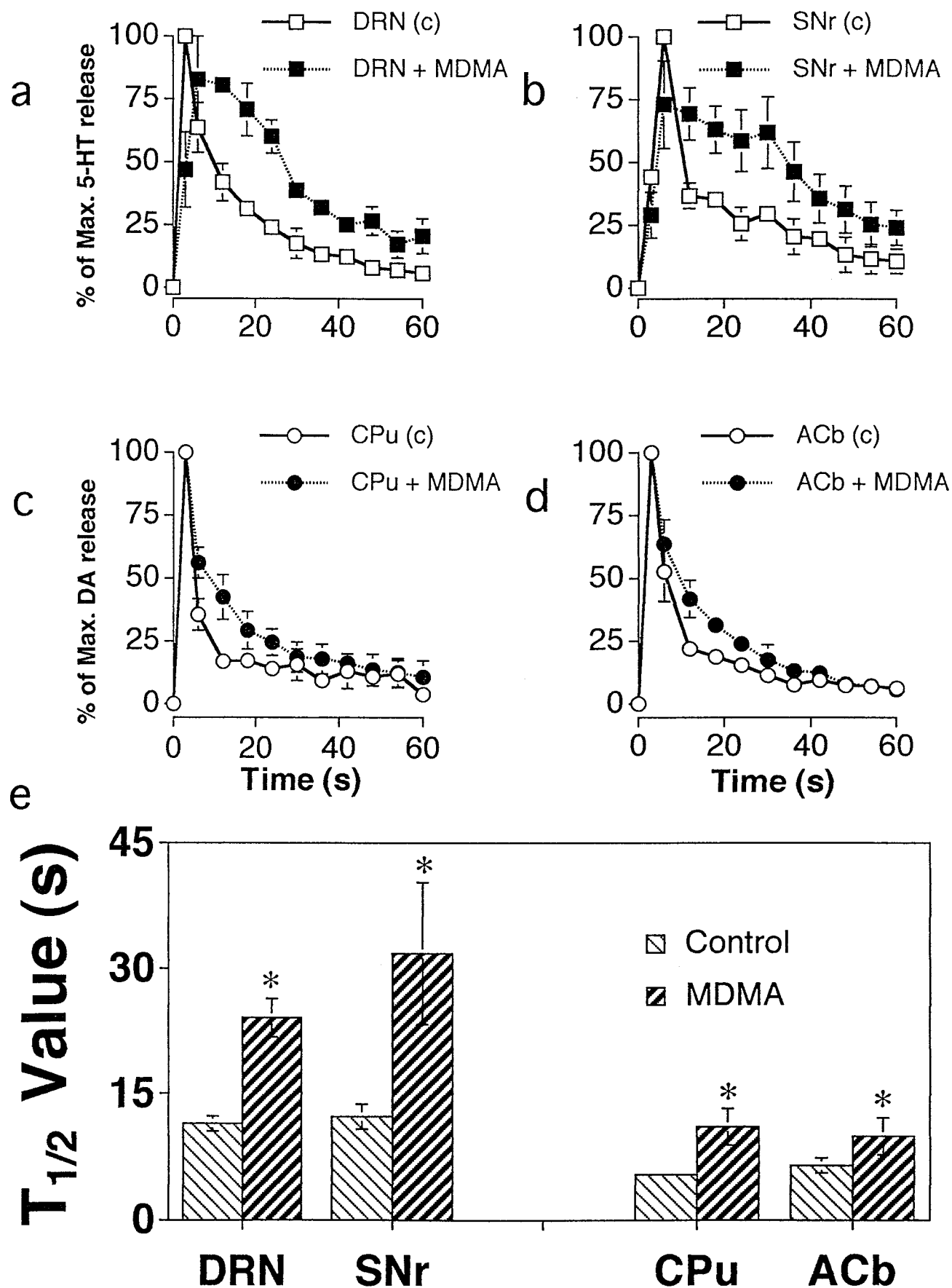


Fig. 6. Time course of electrically stimulated (50 p / 50 Hz) 5-HT and DA release monitored for 60 sec, before (blank data points) and after 1 mM MDMA pressure ejection (1 sec / 10 psi) (filled data points) in the DRN (a), SNr (b), CPu (c), and NAc (d). Peak 5-HT or DA

release was taken as 100%. Each data point is mean $\pm$ SEM of 4–6 experiments, * $P < 0.05$, paired Student's t -test. In e, each histogram is mean $\pm$ SEM of $t_{1/2}$ from 4–6 similar experiments. * $P < 0.05$, Student's paired t -test.

al., 1998). Indirect evidence also indicates that the serotonin carrier may also be a target for (+)amphetamine (Callaway et al., 1991; Barker et al., 1994; Iravani and Kruk, 1997; Kuczenski et al., 1987).

As an analog of (+)amphetamine, MDMA may be expected to act in a similar fashion to (+)amphetamine, releasing DA and 5-HT by a carrier-mediated process. Indeed, there are numerous *in vivo* (Callaway et al., 1990; Gough et al., 1991; Nash et al., 1991; White et al., 1994; Yamamoto et al., 1995; Gudelsky and Nash, 1996) and *in vitro* (Johnson et al., 1991; Wichems et al. 1995; Crespi et al., 1997) studies in which the neurochemical actions of MDMA on DA and 5-HT neurotransmission were investigated and there is a general consensus that MDMA is a potent releaser of 5-HT and, to some extent, DA, through a carrier-mediated process.

In two *in vivo* voltammetric studies, however, MDMA either increased DA levels in the CPu and NAc (Yamamoto and Spanos, 1988) or decreased it (Kelland et al., 1989). In the latter study the decrease was associated with the negative influence of 5-HT released by MDMA on DA transmission. In the present *in vitro* voltammetric study, we found that when 1 mM MDMA was applied locally by pressure ejection no 5-HT or DA signal was observed in the brain regions studied. This finding is rather surprising, because it contradicts the conclusions of previously published studies.

There may be several explanations for this apparently anomalous finding. First, lack of 5-HT or DA electrochemical detection following MDMA pressure ejection in the nuclei previously reported to release 5-HT or DA in response to chemical stimulation (i.e., (+)amphetamine, veratrine) may be due to the insensitivity of FCV to low concentrations of 5-HT or DA released. This explanation may be discarded because, first, we used relatively high concentrations of MDMA in our pressure ejection pipette (1 mM) and, although the actual extracellular concentration of MDMA is not known, this should still be in the micromolar range. In an earlier study from our laboratory, addition of 1 μ M (+)amphetamine by superfusion resulted in release of DA from CPu and NAc slices in concentrations in excess of 600 nM and 250 nM, respectively (Wieczorek and Kruk, 1994). In the present study, bath application of MDMA at 10 μ M had no effect on the background voltammetric signals in any of the nuclei studied (results not shown). Second, we were able to detect concentrations of 5-HT and DA as low as 10 nM and 50 nM, respectively, following electrical stimulation.

The other possible explanation for the lack of effect of MDMA on 5-HT or DA release may be the short duration of exposure of MDMA. It is possible that MDMA may require a longer exposure time in order for it to be taken up by the carrier molecules and initiate the process of reverse transport. However, we did not observe any changes in the voltammetric background

signal that could be positively identified as 5-HT or DA when 10 μ M MDMA was superfused for up to 60 min (results not shown).

The other possibility is that insufficient concentrations of MDMA were pressure-ejected. This possibility can also be discounted because when MDMA was pressure-ejected 1 min prior to electrical stimulation it had profound effects on electrically evoked 5-HT or DA release and reuptake in all regions tested (see below).

Effects of MDMA on electrically stimulated 5-HT or DA release and reuptake

MDMA potentiated the peak 5-HT release in both the DRN and the SNr and greatly extended the 5-HT $t_{1/2}$ values in these two regions. A general pattern observed in studying the effects of the monoamine reuptake blockers on DA or 5-HT release using FCV is prolongation of the detection time of DA or 5-HT, expressed as increases in the $t_{1/2}$ of the FCV signals. This finding is therefore consistent with MDMA having an inhibitory effect on 5-HT reuptake (Steele et al., 1987). The MDMA-mediated potentiation of peak 5-HT release was greater in the SNr than in the DRN. MDMA's apparent lack of effect (on peak 5-HT release) in the DRN may reflect the importance of 5-HT₁ autoreceptor-mediated inhibition of electrically stimulated 5-HT release in the DRN (Davidson and Stamford, 1995) but not in the SNr (Iravani and Kruk, 1997). The major effect of MDMA in prolonging the $t_{1/2}$ values in both the DRN and the SNr reflects the effect of MDMA on the 5-HT transporter (Bunin et al., 1997, 1998).

MDMA pressure ejection prior to electrical stimulation in the CPu or NAc led to significant potentiation of DA $t_{1/2}$ values in both regions, but only in the CPu was the peak DA release in response to electrical stimulation significantly potentiated. The degree of potentiation of the $t_{1/2}$ values in these regions was much less than those seen for 5-HT in the DRN and in the SNr. This finding is in agreement with previous data showing the preferential effect of MDMA on 5-HT neurotransmission. The differential effect of MDMA on peak DA release in CPu and NAc is very similar to the effects of (+)amphetamine on electrically stimulated DA release in these regions. We have already shown that (+)amphetamine enhances peak DA release in CPu in response to electrical stimulation, but inhibits electrically stimulated DA release in NAc (Wieczorek and Kruk, 1994; Iravani and Kruk, 1995).

The major difference between our study and other studies is methodology. FCV has a major advantage over other neurochemical detection techniques in that there is good spatial and temporal resolution. The probe size is small enough so that it can be accurately placed in distinct brain regions without causing mechanical damage, and sampling resolution is fast enough to detect subsecond changes in monoamine concentrations.

It is likely that in those studies in which release of 5-HT or DA by MDMA has been reported, increases in extracellular concentrations of 5-HT or DA are attributable to inhibition of monoamine uptake and not a direct release per se. Biotransformation of MDMA to a form capable of carrier-mediated 5-HT or DA release cannot be ruled out.

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