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Acute Effects of 3,4-methylenedioxymethamphetamine (MDMA) on 5-HT Cell Firing and Release: Comparison Between Dorsal and Median Raphe 5-HT Systems

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Summary—It is proposed that 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) is more toxic to 5-HT neurones projecting from the dorsal raphe nucleus (DRN) than to those from the median raphe nucleus (MRN). Since increased 5-HT release has been associated with MDMA-induced neurotoxicity, MDMA may have a DRN-selective 5-HT releasing effect. Here we have compared the effects of acute MDMA on DRN and MRN 5-HT pathways using *in vivo* electrophysiological and neurochemical techniques. MDMA inhibited the firing of 5-HT neurones in both the DRN and the MRN, and did so with similar potency (ED_{50} values, 0.589 ± 0.151 (8) and 0.588 ± 0.207 (6) mg/kg i.v., respectively). In both nuclei this inhibitory effect was reversed by the selective 5-HT_{1A} receptor antagonist, WAY 100635 (0.1 mg/kg i.v.). Microdialysis measurements were made in the frontal cortex and dorsal hippocampus, regions which receive a DRN- and an MRN-selective 5-HT innervation, respectively. A dose of 1 mg/kg i.v. MDMA increased extracellular 5-HT 3-fold in both the frontal cortex and dorsal hippocampus. A higher dose (3 mg/kg i.v.) increased 5-HT levels 8-fold in both regions. Overall, our data suggest that MDMA releases 5-HT from the cell body and terminal regions of both DRN and MRN 5-HT pathways, and does so in a qualitatively and quantitatively similar fashion. We conclude that any DRN-selectivity in the neurotoxic effects of MDMA is not due to a DRN-selective, acute 5-HT releasing action of the drug.

Keywords—3,4-methylenedioxymethamphetamine (MDMA), 5-HT, microdialysis, 5-HT neuronal activity.

The substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) is a recreational drug which has been shown in rats and non-human primates to be a selective 5-HT neurotoxin (Stone *et al.*, 1988; Battaglia *et al.*, 1988b; Commins *et al.*, 1987; Schmidt, 1987; O'Hearn *et al.*, 1988; Ricaurte *et al.*, 1988; Ricaurte and McCann, 1992). Anatomical evidence indicates that, in contrast to classical 5-HT neurotoxins, MDMA destroys only axon terminals whilst cell bodies are left apparently intact (O'Hearn *et al.*, 1988). Interestingly, there is evidence that MDMA (in common with other related substituted amphetamines) causes a selective loss of 5-HT axons of a particular morphological subtype ("fine" 5-HT fibres), whilst sparing others ("beaded" 5-HT fibres) (Mamounas and Molliver, 1988; Mamounas *et al.*, 1991). Furthermore, on the basis of anatomical evidence that fine 5-HT fibres project exclusively from the DRN whilst beaded 5-HT fibres project from the MRN (Kosofsky and Molliver, 1987; Molliver, 1987), it has been proposed that MDMA and related compounds are selectively neurotoxic to 5-HT neurones of the DRN (Molliver *et al.*, 1990; Mamounas *et al.*, 1991—but see McQuade and Sharp, 1995, and Discussion).

On acute administration, MDMA enters 5-HT cells via the 5-HT transporter in competition with 5-HT. Once inside the cell, MDMA causes the release of neuronal 5-HT (Schmidt and Kehne, 1990). Although the mechanism by which MDMA induces 5-HT neurotoxicity remains unclear, this acute release of 5-HT by MDMA may be necessary—although not sufficient—for the longer term neurotoxic action. Thus, pretreatment with a selective 5-HT uptake inhibitor blocks both the acute 5-HT releasing effect of MDMA (Battaglia *et al.*, 1988a; Hekmatpanah and Peroutka, 1990) and also its long-term neurotoxic effect (Schmidt, 1987). In addition, several studies have shown that 5-HT_{2A} receptor antagonists protect against the 5-HT neurotoxicity induced by MDMA and related compounds (Schmidt *et al.*, 1991, 1992). These latter data suggest that the MDMA-induced release of 5-HT onto 5-HT_{2A} receptors (which some

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anatomical studies have associated with DRN projections; Blue *et al.*, 1988) is an essential part of the mechanism responsible for MDMA-induced neurotoxicity. Whilst the release of dopamine appears to be necessary for the toxic effects of MDMA on 5-HT neurones, it is argued that the release of 5-HT is the first step in the sequence of events which leads to neurotoxicity (Sprague *et al.*, 1994; Sprague and Nichols, 1995).

Given this background, it is possible that any difference in the sensitivity of DRN and MRN 5-HT neurones to the neurotoxic effect of MDMA is the result of a difference in the sensitivity of these neurones to the acute 5-HT releasing effect of the drug. Indeed, it seems possible that MDMA releases 5-HT only, or preferentially, from those 5-HT neurones which arise from the DRN: an idea which has gained some popularity in the literature (see Series *et al.*, 1994; Graeff *et al.*, 1996; White *et al.*, 1996).

Here we compare the acute action of MDMA on 5-HT neurones of the DRN and MRN in two ways. Firstly, we examined the effect of MDMA on the firing rate of 5-HT neurones in the DRN and MRN. Previous *in vitro* electrophysiological studies have shown that MDMA inhibits the firing of 5-HT neurones in the DRN by increasing extracellular 5-HT in the region of somatodendritic 5-HT_{1A} autoreceptors (Sprouse *et al.*, 1989, 1990). Secondly, we used *in vivo* microdialysis to study the effect of MDMA on extracellular 5-HT levels in the frontal cortex and dorsal hippocampus. These forebrain areas are preferentially innervated by 5-HT axon terminals from the DRN and MRN, respectively (Azmitia and Segal, 1978; McQuade and Sharp, 1995).

METHODS

General surgical procedures

Animals were anaesthetized with chloral hydrate (500 mg/kg i.p., plus supplementary doses as necessary), and placed in a stereotaxic frame (Kopf) with the incisor bar set at −3.3 mm (flat skull position). A lateral tail vein was cannulated for administration of drugs, and body temperature was kept at 35 ± 0.5 °C by means of a homeothermic blanket connected to a rectal thermistor probe. The skull was exposed and burr holes were drilled to allow implantation of recording electrodes or microdialysis probes as appropriate.

Electrophysiological recording

Extracellular recordings were made from the DRN and MRN as described previously (Hajós *et al.*, 1995). Electrodes were made from glass micropipettes filled with 2 M NaCl saturated with Pontamine sky blue (4–8 MΩ impedance *in vitro*). The extracellular signal was amplified (× 1000), filtered (300–3000 Hz band-pass) and monitored by oscilloscope, chart recorder and audio speaker. The amplified signal was also recorded on digital audio tape for off-line analysis.

The recording electrode was positioned initially just above the DRN (stereotaxic co-ordinates in mm: anterior −7.7, lateral 0, ventral −4.5; relative to bregma and the dura surface according to Paxinos and Watson, 1986), and then lowered using a hydraulic microdriver. 5-HT neurones were identified on the basis of their stereotaxic position and electrophysiological properties [spontaneously active, firing single, broad (1–2 msec) action potentials in a slow (0.2–3 Hz) and regular pattern (Aghajanian and VanderMaelen, 1982)]. Neurones in the DRN were encountered 4.5–6.0 mm below the dura surface, whilst neurones in the MRN were detected deeper than 6.0 mm. At the end of each experiment, Pontamine sky blue was iontophoretically ejected from the recording electrode, and brains were removed for subsequent histological verification of the recording site.

After a baseline recording period, MDMA was given intravenously in doubling doses (starting dose 0.05 or 0.1 mg/kg) at approximately 2 min intervals, until inhibition of cell firing was achieved. In most cases the final dose of MDMA was followed by administration of a single dose of the 5-HT_{1A} receptor antagonist, WAY 100635 (0.1 mg/kg). Only one neurone per animal was studied.

Microdialysis procedure

Concentric microdialysis probes (Hospal AN69 membrane, 300 μm diameter, 2–2.5 mm window) were implanted into both the left dorsal hippocampus (anterior −4.0, lateral −2.5, ventral −4.5; relative to bregma and the dura surface according to Paxinos and Watson, 1986) and the right frontal cortex (anterior +3.2, lateral +3.0, ventral −4.5). Probes were cemented in place using dental acrylic. The microdialysis probes were perfused (2 μl/min) with artificial CSF (composition in mM: NaCl 140, KCl 3, $CaCl_2$ 2.4, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.27, glucose 7.2, pH 7.4). Samples of the dialysate were collected every 20 min and assayed for 5-HT. Once levels of 5-HT in the dialysates had stabilized (3–4 hr after probe implantation), MDMA (1 or 3 mg/kg) was injected intravenously. Samples of dialysate were collected for a further 2 hr. Following each experiment, the brain was removed, post-fixed in 4% paraformaldehyde, and the positions of the probes verified on slide-mounted sections.

HPLC-ED

Immediately following collection, dialysates were analysed for 5-HT by HPLC with electrochemical detection. 5-HT was resolved using a Microsorb™ column (C_{18} ODS 2, Rainin) with a mobile phase (methanol 12.5%, EDTA 0.03%, NaH_2PO_4 127 mM, octane sulphonic acid 15 or 20 μM; pH 4.0) pumped at 1.2 ml/min using an HPLC pump (LKB). Detection was by a BAS (LC-4) potentiometer with a working electrode (glassy carbon) set at +0.7 V.

Data analysis and statistics

Data in the figures and text are given as mean ± SEM

(n = number of determinations). Electrophysiological data are expressed either as absolute firing rate (spikes/10 sec) or as a percentage of baseline firing (100%). Baseline firing rates and ED_{50} values for MDMA were compared using Student's unpaired t-test. For the microdialysis experiments, data are expressed either as the absolute amount of 5-HT (fmol) in each 20 min sample, or as a percentage of baseline (100%). Basal 5-HT levels were compared between regions using paired Student's t-test, and drug effects were compared by calculating the area under the curves (0 = 120 min post-drug) followed by analysis of variance with repeated measures. Probability values of 0.05 or less were considered to be statistically significant.

Drugs

MDMA (3,4-methylenedioxymethamphetamine) was supplied by the National Institutes of Health, Bethesda, MD, U.S.A.: WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl)cyclohexanecarboxamide.3HCl) was a gift of Wyeth Pharmaceuticals, Maidenhead, UK.

RESULTS

Effect of MDMA on 5-HT cell firing in the DRN and MRN

5-HT neurones recorded from the DRN and MRN of anaesthetized rats had basal firing rates of 10.6 ± 2.1 (8) and 6.1 ± 1.2 (6) spikes/10 sec, respectively. Acute administration of MDMA inhibited the firing activity of 5-HT neurones in the DRN in a dose-dependent (0.05–1.6 mg/kg i.v.) manner, and to the point where there was a complete cessation of firing. The selective 5-HT_{1A} receptor antagonist, WAY 100635 (0.1 mg/kg i.v.), reversed this inhibition in 4/4 DRN neurones tested. The inhibitory effect of MDMA on an individual DRN 5-HT neurone, and its reversal by WAY 100635, is shown in Fig. 1.

The firing activity of 5-HT neurones in the MRN was also inhibited by MDMA and this effect was dose-dependent (0.1–1.6 mg/kg i.v.) and complete. As with the DRN, the MDMA-induced inhibition of firing was reversed by 0.1 mg/kg i.v. WAY 100635 (5/6 MRN neurones tested). The effect of MDMA on an individual 5-HT neurone in the MRN is shown in Fig. 1.

The dose–reponse data (Fig. 2) show clearly that MDMA inhibited 5-HT cell firing in the DRN and MRN with equal potency. The ED_{50} values for the inhibitory effect of MDMA on 5-HT neurones in the DRN and MRN were 0.589 ± 0.151 (8) and 0.588 ± 0.207 (6) mg/kg i.v., respectively. Interestingly, however, individual 5-HT neurones in both the DRN and MRN had ED_{50} values which varied by up to one order of magnitude (Fig. 3).

Effect of MDMA on extracellular 5-HT in the frontal cortex and dorsal hippocampus

Microdialysis measurements of extracellular 5-HT in frontal cortex and dorsal hippocampus were made in the same animals. Basal levels of 5-HT in dialysates of the frontal cortex and dorsal hippocampus were not significantly different (10.1 ± 1.0 (10) and 13.9 ± 3.3 (10) fmol/20 min, respectively). Figure 4 shows that MDMA caused a dose-related increase in levels of 5-HT in dialysates of the frontal cortex. Doses of 1 mg/kg i.v. and 3 mg/kg i.v. MDMA increased 5-HT about 3- and 8-fold, respectively, and in each case the maximum effect occurred at 20 or 40 min post-injection.

MDMA (1 and 3 mg/kg i.v.) also induced a large and dose-related increase in 5-HT in dialysates of the dorsal hippocampus. This effect was similar in both magnitude and duration to that seen in the frontal cortex (Fig. 4). Analysis of variance of the area under the curve data

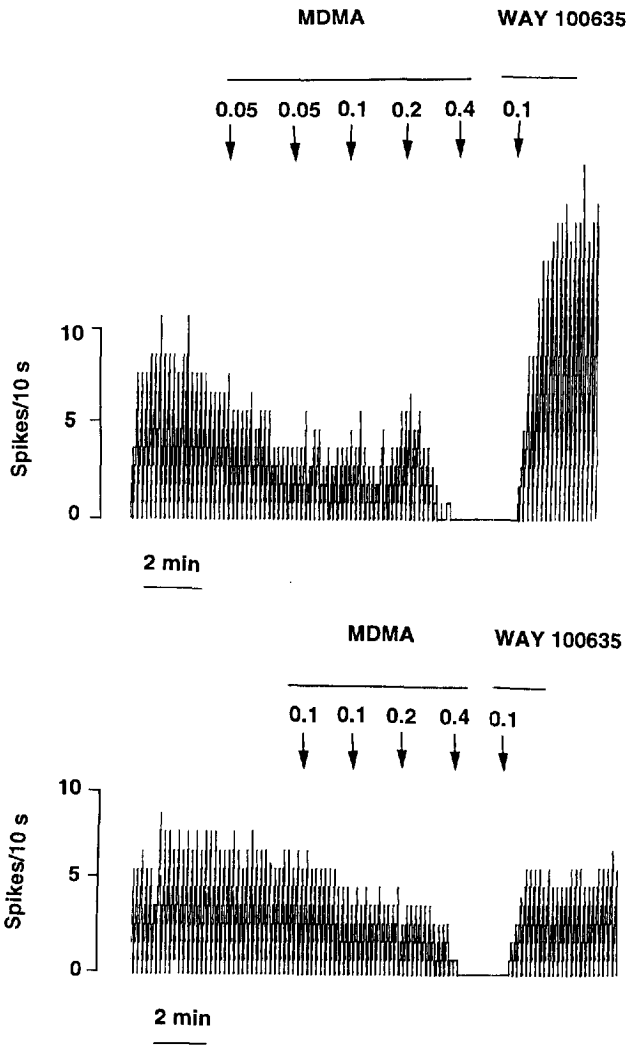

Fig. 1. Ratemeter recordings illustrating the inhibitory effect of MDMA on the firing rate of a spontaneously active 5-HT neurone in the DRN (top) and the MRN (bottom) of the anaesthetized rat. MDMA was administered intravenously at the dose (mg/kg) and time points indicated. Note the reversal of the effect of MDMA by the 5-HT_{1A} receptor antagonist, WAY 100635.

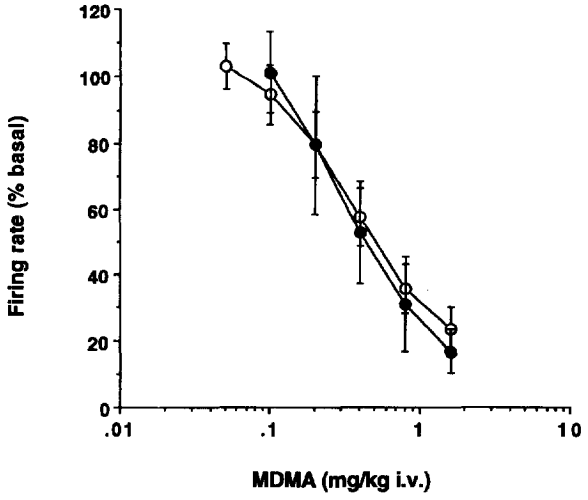

Fig. 2. Dose–response curve showing effect of i.v. administration of MDMA on firing rate of 5-HT neurones in the DRN (open circles) and MRN (closed circles). MDMA was given in doubling doses at approximately 2 min intervals. Data are mean ± SEM of 6 to 8 cells.

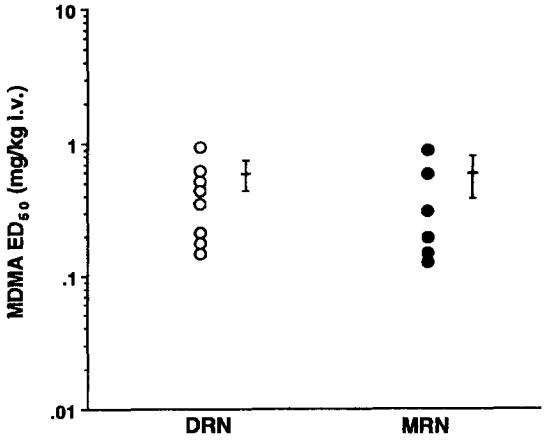

Fig. 3. Individual ED_{50} values for the inhibition of cell firing by MDMA in 5-HT cells from the DRN (open circles) and MRN (closed circles). Each individual value was calculated by interpolation on a semi-log plot. The horizontal bars refer to the mean ± SEM.

(t = 0–120 min post-drug) revealed no significant difference between the effect of MDMA in the frontal cortex and dorsal hippocampus at either dose (main effect of area $F_{1,1} = 1.2$, n.s., and interaction $F_{1,8} = 0.7$., n.s.), although the dose effect was highly significant ($F_{1,8} = 37$; $p < 0.001$).

DISCUSSION

MDMA may have a DRN-selective, 5-HT releasing action which could underlie the putative DRN-selective, 5-HT neurotoxic effects of the drug (see Introduction). In the present study we compared the acute effects of MDMA on DRN and MRN 5-HT pathways using *in vivo* electrophysiological and neurochemical techniques. We found that MDMA inhibited the firing of 5-HT neurones in both the DRN and MRN, and did so with equal potency and efficacy. MDMA was also equipotent in terms of its ability to increase extracellular 5-HT in the frontal cortex and dorsal hippocampus. These regions receive a DRN- and an MRN-selective 5-HT input, respectively (Azmitia and Segal, 1978; McQuade and Sharp, 1995).

The inhibition of 5-HT cell firing in the DRN and MRN by MDMA was used as a measure of the ability of the drug to release 5-HT in the somatodendritic region. It is well known that treatments which increase levels of 5-HT in the DRN will inhibit 5-HT cell firing in this nucleus by activation of somatodendritic 5-HT_{1A} autoreceptors. We found that MDMA inhibited the firing of 5-HT neurones in the DRN (in confirmation of previous *in vitro* data; Sprouse *et al.*, 1989, 1990). Furthermore, this effect was reversed by the selective 5-HT_{1A} receptor antagonist, WAY 100635. MDMA itself has little affinity for 5-HT_{1A} receptors (Lyon *et al.*, 1986; Battaglia *et al.*, 1988a; McKenna and Peroutka, 1990), but is known to be a 5-HT releasing agent, and has previously been shown to increase extracellular 5-HT in the DRN *in vitro* (Sprouse *et al.*, 1989, 1990). Taken together, these data indicate that the MDMA-induced inhibition of 5-HT cell firing in the DRN results from activation of somatodendritic 5-HT_{1A} autoreceptors following increased extracellular 5-HT in this nucleus.

In the present study, we found that MDMA also inhibited the firing of 5-HT neurones in the MRN. It is already established that 5-HT neurones in the MRN, like those in the DRN, are inhibited by 5-HT itself and treatments which increase extracellular 5-HT (Aghajanian *et al.*, 1968; Sheard *et al.*, 1972; Hajós *et al.*, 1995). As with the DRN, the inhibitory effect of MDMA on the MRN neurones was reversed by WAY 100635. Thus, it seems highly likely that the mechanism underlying the inhibitory effect of MDMA in the MRN is the same as in the DRN.

Interestingly, the potency of MDMA to inhibit 5-HT cell firing in the MRN and DRN was very similar, suggesting that MDMA releases similar amounts of 5-HT in these nuclei. This interpretation relies, however, on the assumption that the sensitivity of somatodendritic 5-HT_{1A} autoreceptors is equal in the MRN and DRN. Although earlier electrophysiological studies reported that 5-HT neurones in the MRN were much less sensitive than those in the DRN to systemically administered 5-HT_{1A} receptor agonists (Sinton and Fallon, 1988), we and others have been unable to confirm this difference (Lum and Piercey, 1989; VanderMaelen and Braselton, 1990; Hajós *et al.*, 1995, although see Blier *et al.*, 1990). Indeed, we have found that both the direct 5-HT_{1A} receptor agonist, 8-OHDPAT, and the selective 5-HT reuptake inhibitor, paroxetine, inhibit the firing of 5-HT neurones in the DRN and MRN with equal potency (Hajós *et al.*, 1995). Together, with the present evidence that the 5-HT neurones in the DRN and MRN are equally

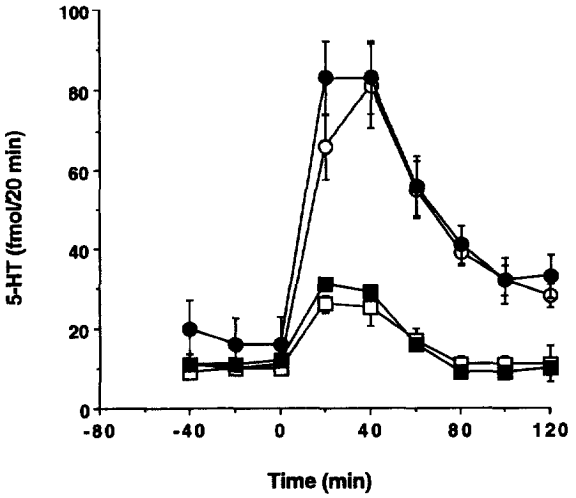

Fig. 4. Time-course of the effect of MDMA on 5-HT levels in dialysates of the frontal cortex (open symbols) and dorsal hippocampus (closed symbols) of the anaesthetized rat. Animals received MDMA in a dose of either 1 mg/kg i.v. (squares) or 3 mg/kg i.v. (circles) at time 0. Data are mean ± SEM of 5 rats per group.

sensitive to MDMA, these data support our conclusion that the ability of MDMA to release 5-HT is the same in the MRN and DRN.

In the second part of our study, we found that MDMA increased 5-HT in dialysates from both the frontal cortex and the dorsal hippocampus, and did so dose-dependently and with equal effect in the two regions. We have recently reported evidence that 5-HT released in the frontal cortex and dorsal hippocampus derives principally from the DRN and MRN 5-HT pathways, respectively (McQuade and Sharp, 1995). Thus, electrical stimulation of the DRN increased 5-HT in dialysates collected from the frontal cortex but not the dorsal hippocampus, whereas electrical stimulation of the MRN increased 5-HT in the dorsal hippocampus but not the frontal cortex. These functional data are in keeping with findings from earlier anatomical studies (Azmitia and Segal, 1978). The current finding that MDMA has a qualitatively and quantitatively similar effect on levels of 5-HT in the frontal cortex and dorsal hippocampus, strongly suggests that the drug releases 5-HT from neurones originating in the MRN as well as the DRN. Therefore, there appears to be no difference in the sensitivity of DRN- and MRN-derived 5-HT terminals to the acute 5-HT releasing actions of MDMA.

Taken together, our present electrophysiological and neurochemical data suggest that the acute 5-HT releasing action of MDMA is not selective to DRN neurones, at least in the brain regions that we have studied. Furthermore, the idea that the putative 5-HT neurotoxic action of repeated exposure to MDMA reflects a DRN-selective, 5-HT releasing action (see Introduction), is not borne out by our experiments.

Although the current study does not address directly the question of whether MDMA (and other substituted amphetamines) is selectively neurotoxic to DRN pathways (Molliver, 1987; Mamounas *et al.*, 1991; Molliver *et al.*, 1990), it is noteworthy that several recent studies have called this issue into question. Thus, Haring *et al.* (1992) presented histological evidence for a loss of MRN- as well as DRN-derived 5-HT axon terminals in rats treated with *p*-chloroamphetamine. Also, Hensler *et al.* (1994) reported that rats receiving *p*-chloroamphetamine showed evidence of 5-HT toxicity in regions receiving a significant MRN-derived 5-HT innervation as well as those receiving DRN-derived 5-HT innervation. These data concord with evidence from our microdialysis studies showing that *p*-chloroamphetamine is neurotoxic to MRN- as well as DRN-derived 5-HT terminals (McQuade and Sharp, 1995). In similar studies we found no evidence of DRN-selective neurotoxicity in rats receiving MDMA (Gartside *et al.*, 1996).

There is little dispute that certain 5-HT axons/pathways are more vulnerable than others to the neurotoxic effects of MDMA and other substituted amphetamines (Molliver, 1987; Battaglia *et al.*, 1991); however, further studies are necessary to establish the nature of this selectivity. Whilst the present study did not detect evidence of DRN-selectivity in the actions of MDMA, it is notable that there was large variability in the sensitivity of individual 5-HT neurones to MDMA. Thus, some 5-HT neurones needed 10 times higher doses of MDMA than others before complete inhibition of neuronal activity could be achieved. These MDMA-insensitive neurones were detected in both the DRN and MRN. If the long-term neurotoxic effects of MDMA are related to the acute release of 5-HT, then these MDMA-insensitive neurones may be the ones spared by the lesion.

In conclusion, the present data indicate that acute administration of MDMA causes the release of 5-HT from both the cell body and terminal region of the 5-HT neurone, and does so equally in the DRN and MRN pathways studied. These data do not support the hypothesis that MDMA has a DRN-selective acute 5-HT releasing effect which might underlie its putative DRN-selective neurotoxic action.

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