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Inhibition of dopamine release by methylenedioxymethamphetamine is mediated by serotonin

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(±)-3,4-Methylenedioxymethamphetamine (MDMA), at doses of 0.1, 1 and 10 mg/kg, produced a long-lasting decrease in extracellular dopamine concentration in the neostriatum of anesthetized rats, as measured by in vivo voltammetry. Since MDMA has been shown to release serotonin from rat brain slices and synaptosomes, we examined the possibility that increased serotonin release might be the cause of the decrease in dopamine release. Rats were treated with d,l-p-chloroamphetamine seven days prior to acute MDMA administration. Rats pretreated with p-chloroamphetamine, which produced a marked decrease in serotonin content, showed no significant decrease in extracellular dopamine concentration when administered 10 mg/kg MDMA. These data suggest that MDMA produces a significant decrease in dopamine release when administered acutely, and that this decrease is an indirect effect mediated by an increase in serotonin release.

(±)-3,4-Methylenedioxymethamphetamine (MDMA); Dopamine; 5-Hydroxytryptamine (5-HT, serotonin); Voltammetry (in vivo); d,l-p-Chloroamphetamine; Amphetamine

1. Introduction

(±)-3,4-Methylenedioxymethamphetamine (MDMA), a psychoactive amphetamine analog that has been used as an adjunct to psychotherapy, has been classified as a Schedule I drug by the United States Food and Drug Administration because of its abuse potential and possible neurotoxic effects. Recent reports have documented the long-lasting (Schmidt, 1987b; Schmidt et al., 1987; Stone et al., 1987) and apparently neurotoxic (O'Hearn et al., 1988) effects of MDMA on central serotonergic neurons. In contrast, MDMA does not appear to produce a long-lasting effect on brain dopamine (DA) levels (Schmidt et al., 1987; Stone et al., 1987) except after a high dose of the (+) isomer (Schmidt et al., 1987), nor does it appear to be neurotoxic to catecholaminergic neurons (O'Hearn et al., 1988). In addition to these long-lasting effects, short-term acute effects on both the dopaminergic and serotonergic systems in vitro and in vivo have been reported. For example, MDMA has been shown to increase the release of serotonin (5-HT) from rat neostriatal slices (Schmidt, 1987a), hippocampal slices (Johnson et al., 1986) and whole-brain synaptosomes (Nichols et al., 1982); and to increase the release of DA from rat neostriatal brain slices in vitro (Johnson et al., 1986; Schmidt, 1987a; Schmidt et al., 1987) and from rat neostriatum and nucleus accumbens in vivo (Yamamoto and Spanos, 1988). Since the dopaminergic and serotonergic systems

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have both been implicated in the mechanism of action of psychoactive drugs, we decided to investigate the acute effects of MDMA on dopaminergic neurotransmission, and the dependence of these effects on an intact serotonergic system, using in vivo voltammetry.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing 250-300 g were used in these experiments. All animals were housed at constant room temperature (23° C) and maintained on a 12 h light-dark cycle. Food and water were available ad libitum.

2.2. Electrodes

Recording electrodes were constructed from Teflon-coated stainess steel wire with a tip diameter of 175 μm (Medwire, Mt. Vernon, NY). A small (0.3-0.5 mm) cylindrical well was formed at one end of the wire and tightly packed with graphite paste. The graphite paste was prepared by thoroughly mixing 1.5 g of graphite powder (UCP-1-M, Ultra Carbon, Bay City, MI) with a mixture of 100 mg of stearic acid (Sigma Chemical, St. Louis, MO; 99% purity) dissolved in 1.1 ml of mineral oil (Nujol) warmed to 40° C.

2.3. Surgical procedure

Rats were anesthetized with chloral hydrate (400 mg/kg i.p.), and supplementary doses were administered when necessary. Body temperature was monitored throughout the experiment with a rectal probe and tele-thermometer (Yellow Springs Instrument, Yellow Springs, OH) and maintained at 37 ± 0.2° C; heart rate was also monitored throughout the experiment. The animals were mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and two small craniotomies were made in the skull overlying the left neostriatum and the contralateral cortex. After incision of the dura, the recording electrode was lowered into the neostriatum at the rate of ca. 0.2 mm/min. Stereotaxic coordinates were: A 0.7 mm (measured from bregma); L 3.0 mm; V 3.5 mm (measured from the dura, Paxinos and Watson, 1982). The reference electrode-auxiliary electrode assembly was implanted on the surface of the cortex in order to make contact with the brain.

2.4. Electrochemical recording

Semidifferential voltammetry (Dalrymple-Alford et al., 1977; Lane et al., 1979) was performed by applying linear potential ramps (−150 to +250 mV vs. Ag/AgCl; scan rate 10 mV/s) to the working electrode and measuring the oxidation peak current centered at +120 mV. The oxidative peak current, using a stearate-modified electrode, is proportional to the concentration of DA or norepinephrine (NE) with no interference from catecholamine metabolites or ascorbic acid (Blaha and Lane, 1983; Blaha and Lane, 1984; Howard-Butcher et al., 1985), and thus a change in current reflects a change in the concentration of the catecholamines being measured (for a discussion of the in vivo selectivity of stearate-modified electrodes, see Gazzara et al., 1986). Although this technique cannot differentiate between DA and NE, the endogenous content of NE in the rat neostriatum is not more than 1% of the DA content (Brownstein et al., 1974; Oke et al., 1978; Palkovits, 1979). Thus NE contributes negligibly to the oxidation current.

Voltammetric scans were performed at 10 min intervals. Measurements of DA oxidation current were taken until stable baseline values were obtained and then continued for at least 60 min before drug administration. Drugs were then injected and changes in oxidation current were monitored for 3 h postinjection.

All time course data were expressed as a percentage of the preinjection baseline current. Baseline was calculated by averaging the three values recorded 30 min before drug administration. Each animal's data were calculated relative to its own baseline current, thus each animal served as its own internal control. All electrodes were tested in vitro after in vivo use in standard solutions of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and

ascorbic acid to verify selectivity to DA. In addition, a DA calibration curve was performed to determine whether the recording electrode had maintained a linear response to DA concentration.

After completion of the experiments, the animals were anesthetized deeply with sodium pentobarbital and perfused transcardialy with saline followed by 10% neutral-buffered formalin. The brain was removed, stored in 30% formal-sucrose for 48 h and recording sites were verified histologically from frozen sections stained with Cresyl violet.

2.5. Biochemical measurements

In a separate experiment, whole-tissue concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the neostriatum and the cerebral cortex were measured after pretreatment with d,l-p-chloroamphetamine. We also measured DA levels in the neostriatum to determine whether p-chloroamphetamine had altered DA content. Rats were injected with 10 mg/kg p-chloroamphetamine i.p., 7 days prior to the experiment; control rats were not injected. The animals were killed by decapitation, and the brains were removed rapidly and dissected on an ice-cold Petri dish. Samples of neostriatum and neocortex were prepared for 5-HT, 5-HIAA and DA analysis. Quantitation of 5-HT, 5-HIAA and DA was achieved by high-performance liquid chromatography with electrochemical detection (HPLC-EC). The analytes were separated on a 250 × 4.6 mm reverse phase column (C_{18}, 5.0 μm particle size) with a flow rate of 1.0 ml/min. 5-HT and 5-HIAA were analyzed using a mobile phase containing 10.5 g $NaH_2PO_4 \cdot H_2O$, 2.0 g citric acid, 40 mg EDTA, 26 mg OSS And 110 ml MeOH in 1 l, pH 3.30, with the electrode maintained at +0.7 V vs. Ag/AgCl. DA was analyzed using a mobile phase containing 10.5 g $NaH_2PO_4 \cdot H_2O$, 40 mg EDTA, 25 mg OSS and 110 ml MeOH in 1 l, pH 2.8, with the electrode maintained at +0.7 V vs. Ag/AgCl.

2.6. Drugs

All drugs were dissolved in saline and administered by either the s.c. or i.p. route in a volume of 1 ml/kg. Dosages were calculated based on the salt weight. (±)-MDMA · HCl was obtained from the National Institute of Drug Abuse; d,l-p-chloroamphetamine · HCl was obtained from Sigma Chemical Co. (St. Louis, MO) and d-amphetamine sulfate (AMPH) was obtained from the UCLA pharmacy.

2.7. Statistical analysis

The voltammetry data was analyzed statistically using a two-factor (treatment × time) analysis of variance with repeated measures on the time factor. Drug effects were determined by comparing each drug group separately with the appropriate saline control group. When the treatment × time interaction term was found to be statistically significant (defined as $P < 0.05$), a simple main effects test was performed at each time point to determine at which time points the treatment groups differed (Winer, 1971).

3. Results

3.1. Effect of MDMA on extracellular DA concentration in the neostriatum

MDMA, at doses of 0.1, 1.0 and 10.0 mg/kg i.p., produced a large decrease in neostriatal extracellular DA concentration when administered acutely in rats (fig. 1A). The 0.1 mg/kg dose of MDMA produced a significant decrease in extracellular DA concentration (160 min postinjection) to 70.8 ± 13.2% (Mean ± S.E.M.) of baseline compared with saline controls. This decrease reached 60.0 ± 12.7% of baseline at 3 h postinjection. The 1.0 mg/kg dose produced a significant decrease in extracellular DA concentration (70 min postinjection) to 85.0 ± 8.2% of baseline. At 3 h postinjection, the extracellular DA concentration was 37.2 ± 7.6% of baseline. The 10.0 mg/kg dose of MDMA produced a significant decrease in extracellular DA concentration (60 min postinjection) to 82.4 ± 7.9% of baseline. At 3 h after the injection, extracellular DA concentration was reduced to 33.3 ± 3.6% of baseline. An analysis of the three dose levels of MDMA showed that at

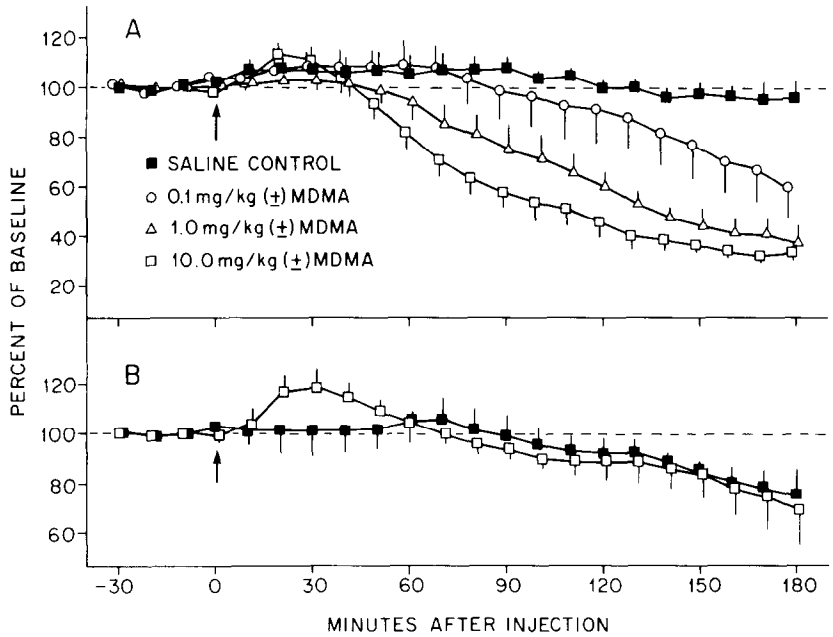

Fig. 1. (A) Effect of (±)-MDMA (0.1, 1 and 10 mg/kg i.p.) and saline (i.p.) treatment on extracellular DA concentration in the neostriatum of rats. (B) Effect of (±)-MDMA (10 mg/kg i.p.) and saline (i.p.) treatment on extracellular DA concentration in the neostriatum of p-chloroamphetamine-treated rats. Results are expressed as the mean percent of preinjection baseline (100%). Bars represent ±S.E.M.; n = 4 for each group.

160 min postinjection there was a significant difference between each of the three MDMA doses and the saline control, and between the 0.1 mg/kg dose and both the 1.0 and 10.0 mg/kg doses, however there was no difference between the 1.0 and 10.0 mg/kg doses. The initial response to each dose of MDMA was not significantly different from the saline control group. However, there was a slight, but non-significant, increase in extracellular DA concentration after the 10.0 mg/kg dose (20 min and 30 min postinjection; fig. 1A).

3.2. Effect of MDMA on DA release after 5-HT depletion by p-chloroamphetamine

MDMA has been shown to increase the release of 5-HT in rat brain synaptosomes and in brain slices (Nichols et al., 1982; Schmidt, 1987a; Schmidt et al., 1987). To determine whether the decrease in extracellular DA concentration was due to increased 5-HT release, we pretreated a group of rats with p-chloroamphetamine which has been shown to produce a long-lasting depletion of 5-HT (Harvey et al., 1977; Massari et al., 1978; Sanders-Bush et al., 1975). Seven days after treatment with p-chloroamphetamine (10.0 mg/kg i.p.), rats were prepared for in vivo voltammetry and injected with 10.0 mg/kg MDMA after baseline was established. In p-chloroamphetamine-treated rats, MDMA at 10/0 mg/kg produced no significant effect on extracellular DA concentration, compared with a p-chloroamphetamine-treated saline control group (fig. 1B). As in the experiment with the non-p-chloroamphetamine-treated animals, there was a slight increase in extracellular DA concentration during the initial response to 10.0 mg/kg MDMA (20-40 min postinjection; fig. 1B), however, as was the case with the non-p-chloroamphetamine-treated animals, this increase was not significantly different from the saline control group.

3.3. Effect of p-chloroamphetamine on 5-HT and DA content in the neocortex and neostriatum

In order to determine the extent of 5-HT depletion by p-chloroamphetamine and to assess its

TABLE 1

Effect of p-chloroamphetamine (PCA) treatment on content of 5-HT, 5-HIAA, and DA in the neocortex and neostriatum of the rat [a].

Tissue	5-HT (ng/g [b])	5-HIAA (ng/g)	DA (μg/g)
Neocortex			
Control	139.6 ± 52.5	69.7 ± 9.8	ND [c]
PCA	12.4 ± 3.9	9.8 ± 3.2	ND
Neostriatum			
Control	299.9 ± 18.9	417.7 ± 8.2	10.78 ± 2.13
PCA	58.1 ± 29.2	73.0 ± 33.0	10.35 ± 3.43

[a] Animals were either treated with 10 mg/kg p-chloroamphetamine (PCA group) or untreated (control group) and killed 7 days later. Results are expressed as means ± S.E.M.; n = 3 for each group. [b] Wet tissue. [c] Not determined.

effect on DA content, we examined levels of 5-HT, 5-HIAA and DA in samples of neostriatum and neocortex in a second group of rats treated with p-chloroamphetamine. p-Chloroamphetamine at a dose of 10.0 mg/kg resulted in the following mean changes in content: an 81% decrease in neostriatal 5-HT content; an 83% decrease in neostriatal 5-HIAA content; a 91% decrease in neocortical 5-HT content; and an 86% decrease in neocortical 5-HIAA content; compared with untreated controls. In contrast, p-chloroamphetamine treatment produced only a 4% decrease in neostriatal DA content compared with untreated controls (table 1).

3.4. Effect of p-chloroamphetamine on AMPH-induced DA release in the neostriatum

Even though p-chloroamphetamine treatment produced virtually no effect on DA content in the neostriatum, the possibility remained that the ability of the neurons to release DA was altered by p-chloroamphetamine. To test this possibility, we examined the effect of p-chloroamphetamine treatment on amphetamine (AMPH)-induced neostriatal DA release in a third group of rats treated with p-chloroamphetamine. AMPH, at a dose of 1.0 mg/kg s.c., produced a rapid and significant increase in extracellular DA concentration in rats treated with p-chloroamphetamine compared with p-chloroamphetamine-treated

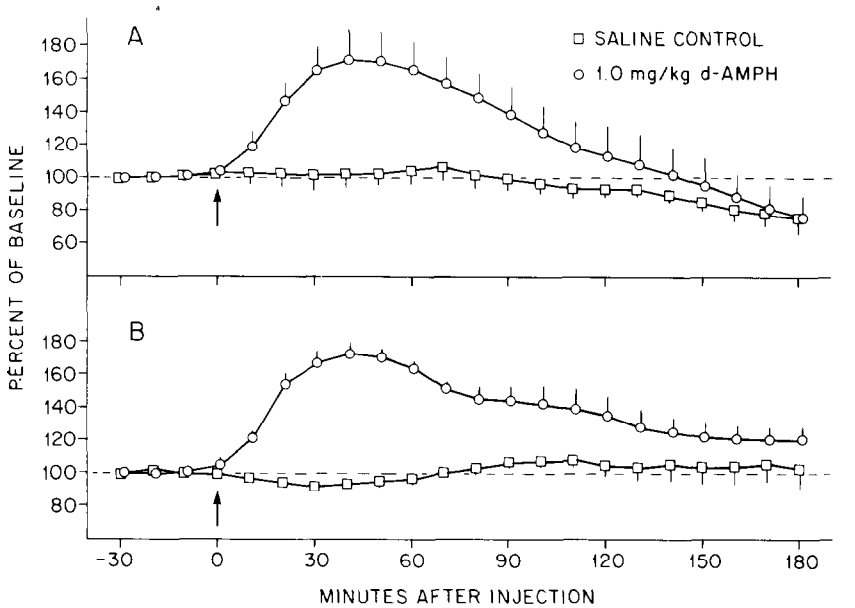

Fig. 2. (A) Effect of AMPH (1 mg/kg s.c.) and saline (s.c.) treatment on extracellular DA concentration in the neostriatum of p-chloroamphetamine-treated rats, n = 4 for each group. (B) Effect of AMPH (1 mg/kg s.c) and saline (s.c.) treatment on extracellular DA concentration in the neostriatum of non-p-chloroamphetamine-treated rats, n = 4 and 3 respectively. (Data in fig. 2B taken from Gazzara et al., 1986; for comparison only.) Results are expressed as the mean percent of preinjection baseline (100%). Bars represent ± S.E.M.

saline controls (fig. 2A). This AMPH-induced increase in DA release was very similar to that found in non-p-chloroamphetamine-treated rats (fig. 2B). In addition, the AMPH-induced increase in DA release peaked at exactly the same time point (40 min postinjection) and at virtually the same level in both the p-chloroamphetamine-treated and non-p-chloroamphetamine-treated groups (fig. 2).

4. Discussion

MDMA at doses of 0.1, 1.0 and 10.0 mg/kg produced a large decrease in DA release in the caudate-putamen of the anesthetized rat. While we did not see the large increase in DA release that has been reported in experiments using brain slices in vitro (Johnson et al., 1986; Schmidt, 1987a) or voltammetry in vivo (Yamamoto and Spanos, 1988), we did see a small but non-significant increase after the 10.0 mg/kg MDMA dose. The large MDMA-induced increase in DA release found in brain slices could be due to a direct effect on dopaminergic nerve terminals, whereas in the study reported here, the absence of a large MDMA-induced increase in DA release may have been a result of an interaction with neuronal circuitry that is not intact in the brain slice preparation.

It is more difficult to reconcile our results with those reported by Yamamoto and Spanos (1988). They reported a large MDMA-induced dose-dependent increase in DA release both in the neostriatum and the nucleus accumbens utilizing the same in vivo analytical technique, linear ramp semidifferential voltammetry using a stearate-modified carbon paste electrode, as we did. The only major differences between our two studies are the dose range (0.1, 1, 10 vs. 2.5, 5.0, 10.0 mg/kg) and the state of the animal (choral hydrate-anesthetized vs. awake). It is doubtful that the dose range of MDMA can explain the differences in our results since there is an overlap at 10.0 mg/kg. An alternate explanation is that the lack of a MDMA-induced increase in DA release seen in our study was a result of the chloral hydrate anesthesia. Although this explanation cannot be ruled out, it is considered unlikely since chloral hydrate anesthesia does not prevent the increase in DA release produced by AMPH as measured by voltammetry (Gazzara et al., 1986; Howard-Butcher et al., 1984; this study). However, studies employing microdialysis to examine extracellular DA and its metabolites have demonstrated differences in awake vs. anesthetized preparations (Howard, unpublished data).

It is not clear whether the increase in DA release reported by Yamamoto and Spanos (1988) is a direct effect of MDMA. DA release induced by AMPH and measured by such in vivo techniques as voltammetry (Gazzara et al., 1986; Howard-Butcher et al., 1984) and microdialysis (Butcher et al., 1988; Di Chiara and Imperato, 1988; Guan and McBride, 1988; Zetterström et al., 1983) has a very rapid onset that peaks at 40-60 min postinjection. Yamamoto and Spanos (1988) report a peak MDMA effect in the neostriatum (at 5.0 and 10 mg/kg) at 115 min postinjection. Unless the mechanism of action of MDMA is very different from that of AMPH, it seems reasonable to expect an MDMA-induced increase in DA release to peak at approximately the same time postinjection. Although differences in time of peak effect between AMPH and MDMA could possibly be due to differences in rate of adsorption or metabolism, an alternate explanation is that the increase in extracellular DA concentration reported by Yamamoto and Spanos (1988) is an indirect effect, perhaps induced by 5-HT.

In the study reported here, the possibility that MDMA reduced DA release indirectly by increasing 5-HT release was examined by determining whether the effect of MDMA on DA release is altered by depleting 5-HT. Rats were injected with 10.0 mg/kg p-chloroamphetamine 7 days prior to the voltammetry experiments in order to reduce brain 5-HT content. In these animals treated with p-chloroamphetamine, an injection of 10.0 mg/kg MDMA produced no significant change in extracellular DA concentration in the neostriatum when compared with a saline control group also treated with p-chloroamphetamine (fig. 1B). Thus, 5-HT depletion abolished the inhibitory effect of MDMA on DA release. One possible interpreta-

tion of these results is that since MDMA has been found to increase 5-HT release and 5-HT has been shown to inhibit the firing rate of neurons in the substantia nigra (Fibiger and Miller, 1977; Aghajanian and Bunney, 1975; Dray et al., 1976; Collingridge and Davies, 1980), then this depression of the firing rate of dopaminergic nigrostriatal neurons would in turn decrease DA release in the neostriatum. An alternate hypothesis is that increased 5-HT release in the neostriatum itself depressed DA release locally, as has been reported in rat neostriatal brain slices (Ennis et al., 1981; Muramatsu et al., 1988), although a stimulant effect of 5-HT on DA release from neostriatal synaptosomes has also been reported (De Belleroche and Bradford, 1980).

In order to establish that p-chloroamphetamine administration did deplete 5-HT content, we determined 5-HT and 5-HIAA levels in the neocortex and neostriatum in another group of rats injected with 10.0 mg/kg p-chloroamphetamine and allowed to survive for 7 days. This p-chloroamphetamine treatment produced a very large depletion in neocortical and neostriatal 5-HT and 5-HIAA (table 1), which is consistent with reports that p-chloroamphetamine is neurotoxic to serotonergic neurons (Harvey, 1978; Harvey et al., 1975; Hattori et al., 1976; Massari et al., 1978). At the same time, p-chloroamphetamine pretreatment did not significantly alter neostriatal DA content (table 1), which is in agreement with a report of no long-term effect of p-chloroamphetamine on brain DA content (Harvey, 1978). p-Chloroamphetamine has a short-duration effect on catecholamines, however this effect is over in 24 h (Sanders-Bush and Steranka, 1978).

Although p-chloroamphetamine treatment did not alter DA content in the neostriatum, the characteristics of DA release may have been altered. To investigate this possibility, we tested the effect of p-chloroamphetamine treatment on AMPH-induced DA release. AMPH, at a dose of 1.0 mg/kg s.c., produced a 72.4% increase in extracellular DA concentration at 40 min postinjection (Gazzara et al., 1986; fig. 2B, this study). p-Chloroamphetamine treatment did not significantly alter this AMPH-induced increase in extracellular DA concentration (fig. 2A), and thus we conclude that p-chloroamphetamine does not appear to have altered the characteristics of DA release.

In conclusion, the major effect of MDMA on the dopaminergic system in the neostriatum appears to be one of inhibition of release. This effect however is an indirect one probably mediated by 5-HT. We found little evidence of a direct effect of MDMA on DA release. The small increase in extracellular DA concentration that was found at 30-40 min after a high dose of MDMA in both the p-chloroamphetamine-treated and non-p-chloroamphetamine-treated groups, and thus was not mediated by 5-HT, was not statistically significant. However, in light of the study reported by Yamamoto and Spanos (1988), it is possible that this small increase in DA release is meaningful in that it represents a direct effect of MDMA on DA release that in our hands was too small to be statistically significant. It appears therefore, that MDMA differs greatly from other forms of amphetamine such as d-amphetamine and methamphetamine, both of which have very potent direct effects on dopamine release.

Since the MDMA that we used in this study is the racemic form, the possibility remains that one of the isomeric forms, either the (+) or the (−) form, may have different effects on dopamine release in vivo. It has already been shown that the (+) and (−) isomers have different potencies for DA (Schmidt et al., 1987) and 5-HT (Nichols et al., 1982; Schmidt et al., 1987) release in vitro and produce differential behavioral effects in mice (Glennon et al., 1987) and rats (Schechter, 1987). Further experiments are necessary to determine whether the differential behavioral effects of the (+) or (−) form of MDMA are caused by differential neurochemical effects on dopaminergic neurotransmission in vivo.

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References

Aghajanian, G.K. and B.S. Bunney, 1975, Dopaminergic and non-dopaminergic neurons of the substantia nigra: Dif-

ferential responses to putative transmitters, Exerpta Med. 359, 444.

Blaha, C.D. and R.F. Lane, 1983, Chemically modified electrode for in vivo monitoring of brain catecholamines, Brain Res. Bull. 10, 861.

Blaha, C.D. and R.F. Lane, 1984, Direct in vivo electrochemical monitoring of dopamine release in response to neuroleptic drugs, European J. Pharmacol. 98, 113.

Brownstein, M., J.M. Saavedra and M. Palkovits, 1974, Norepinephrine and dopamine in the limbic system of the rat, Brain Res. 79, 431.

Butcher, S.P., I.S. Fairbrother, J.S. Kelly and G.W. Arbuthnott, 1988, Amphetamine-induced dopamine release in the rat striatum: An in vivo microdialysis study, J. Neurochem. 50, 346.

Collingridge, G.L. and J. Davies, 1980, Responses of nigral compacta and reticulata neurones to putative inhibitory neurotransmitters and striatal stimulation, J. Physiol. (London) 301, 41.

Dalrymple-Alford, P., M. Goto and K.B. Oldham, 1977, Peak shapes in semidifferential electroanalysis, Anal. Chem. 49, 1390.

De Belleroche, J.S. and H.F. Bradford, 1980, Presynaptic control of the synthesis and release of dopamine from striatal synaptosomes: A comparison between the effects of 5-hydroxytryptamine, acetylcholine, and glutamate, J. Neurochem. 35, 1227.

Di Chiara, G. and A. Imperato, 1988, Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats, Proc. Natl. Acad. Sci. U.S.A. 85, 5274.

Dray, A., T.J. Gonye, N.R. Oakley and T. Tanner, 1976, Evidence for the existence of a raphe projection to the substantia nigra in rat, Brain Res. 113, 45.

Ennis, C., J.D. Kemp and B. Cox, 1981, Characterisation of inhibitory 5-hydroxytryptamine receptors that modulate dopamine release in the striatum, J. Neurochem. 36, 1515.

Fibiger, H.C. and J.J. Miller, 1977, An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat, Neuroscience 2, 975.

Gazzara, R.A., R.S. Fisher and S.G. Howard, 1986, The ontogeny of amphetamine-induced dopamine release in the caudate-putamen of the rat, Dev. Brain Res. 28, 213.

Glennon, R.A., P.J. Little, J.A. Rosecrans and M. Yousif, 1987, The effect of MDMA ('ecstacy') and its optical isomers on schedule-controlled responding in mice, Pharmacol. Biochem. Behav. 26, 425.

Guan, X.-M. and W.J. McBride, 1988, Amphetamine-induced in vivo release of dopamine in the striatum is influenced by local pH, Life Sci. 42, 2625.

Harvey, J.A., 1978, Neurotoxic action of halogenated amphetamines, in: Serotonergic Neurotoxins: Ann. N.Y. Acad. Sci., Vol. 305, eds. J.H. Jakoby and L.D. Lytle (N.Y. Acad. Sci., New York) p. 289.

Harvey, J.A., S.E. McMaster and R.W. Fuller, 1977, Comparison between the neurotoxic and serotonin-depleting effects of various halogenated derivatives of amphetamine in the rat, J. Pharmacol. Exp. Ther. 202, 581.

Harvey, J.A., S.E. McMaster and L.M. Yunger, 1975, p-Chloroamphetamine: Selective neurotoxic action in brain, Science 187, 841.

Hattori, T., P.L. McGeer and E.G. McGeer, 1976, Synaptic morphology in the neostriatum of the rat: Possible serotonergic synapse, Neurochem. Res. 1, 451.

Howard-Butcher, S., C.D. Blaha and R.F. Lane, 1984, A comparison of CNS stimulants with phencyclidine on dopamine release using in vivo voltammetry, Brain Res. Bull. 13, 497.

Howard-Butcher, S., C.D. Blaha and R.F. Lane, 1985, Differential effects of xylamine on extracellular concentrations of norepinephrine and dopamine in rat central nervous system: An in vivo electrochemical study, J. Pharmacol. Exp. Ther. 233, 58.

Johnson, M.P., A.J. Hoffman and D.E. Nichols, 1986, Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices, European J. Pharmacol. 132, 269.

Lane, R.F., A.T. Hubbard and C.D. Blaha, 1979, Application of semidifferential electroanalysis to studies of neurotransmitters in the central nervous system, J. Electroanal. Chem. 95, 117.

Massari, V.J., Y. Tizabi and E. Sanders-Bush, 1978, Evaluation of the neurotoxic effects of p-chloroamphetamine: A histological and biochemical study, Neuropharmacology 17, 541.

Muramatsu, M., J. Tamaki-Ohashi, C. Usuki, H. Araki, S. Chaki and H. Aihara, 1988, 5-HT$_2$ antagonists and minaprine block the 5-HT-induced inhibition of dopamine release from rat brain striatal slices, European J. Pharmacol. 153, 89.

Nichols, D.E., D.H. Lloyd, A.J. Hoffman, M.B. Nichols and K.W. Yim, 1982, Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes, J. Med. Chem. 25, 530.

O'Hearn, E., G. Battaglia, E.B. De Souza, M.J. Kuhar and M.E. Molliver, 1988, Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: Immunocytochemical evidence for neurotoxicity, J. Neurosci 8, 2788.

Oke, A., R. Keller and R.N. Adams, 1987, Dopamine and norepinephrine enhancement in discrete rat brain regions following neonatal 6-hydroxydopamine treatment, Brain Res. 148, 245.

Palkovits, M., 1979, Dopamine levels of individual brain regions: biochemical aspects of DA distribution in the central nervous system, in: The Neurobiology of Dopamine, eds. S.A. Horn, J. Korf and B.H.C. Westerink (Academic Press, New York).

Paxinos, G. and C. Watson, 1982, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York) p. 153.

Sanders-Bush, E., J.A. Bushing and F.J. Sulser, 1975, Long term effects of p-chloroamphetamine and related drugs on serotonergic mechanisms, J. Pharmacol. Exp. Ther. 192, 33.

Sanders-Bush, E. and L.R. Steranka, 1978, Immediate and long-term effects of p-chloroamphetamine on brain amines, in: Serotonergic Neurotoxins: Ann. N.Y. Acad. Sci., Vol. 305, eds. J.H. Jakoby and L.D. Lytle (N.Y. Acad. Sci., New York) p. 208.

Schechter, M.D., 1987, MDMA as a discriminative stimulus: isomeric comparisons, Pharmacol. Biochem. Behav. 27, 41.

Schmidt, C.J., 1987a, Acute administration of methylenedioxymethamphetamine: Comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs European J. Pharmacol. 136, 81.

Schmidt, C.J., 1987b, Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, J. Pharmacol. Exp. Ther. 240, 1.

Schmidt, C.J., J.A. Levin and W. Lovenberg, 1987, In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, Biochem. Pharmacol. 36, 747.

Stone, D.M., M. Johnson, G.R. Hanson and J.W. Gibb, 1987, A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its N-methylated and N-ethylated derivatives. European J. Pharmacol. 134, 245.

Winer, B.J., 1971, Statistical Principles in Experimental Design, 2nd ed. (McGraw Hill, New York) p. 514.

Yamamoto, B.K. and L.J. Spanos, 1988, The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat, European J. Pharmacol. 148, 195.

Zetterström, T., T. Sharp, C.A. Marsden and U. Ungerstedt, 1983, In vivo measurement of dopamine and its metabolites by intracerebral dialysis: Changes after d-amphetamine, J. Neurochem. 41, 1769.