European Journal of Pharmacology, 138 (1987) 265–268
Elsevier

EJP 00794

(±)3,4-Methylenedioxymethamphetamine (MDMA) produces long-term reductions in brain 5-hydroxytryptamine in rats

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Received 24 March 1987, accepted 31 March 1987

(±)3,4-Methylenedioxymethamphetamine (MDMA) was administered to rats as a single 40 mg/kg injection s.c. or 40 mg/kg s.c. every second day for 4 injections. Sixteen days following the last injection rats were killed. MDMA produced significant depletions of 5-HT and its metabolite 5-HIAA in the hippocampus and the frontal cortex. 5-HT was depleted to 30% of control value in the hippocampus following a single dose. 5-HT levels were not affected in the hypothalamus, suggesting differential effects on brain 5-HT systems. DA levels in the hypothalamus were significantly increased while NE levels in the frontal cortex were decreased to 73% of control following 4 doses of MDMA. MDMA, therefore, produces long-term depletions in 5-HT which suggests that it may act as a neurotoxin at 5-HT neurons in the brain of rats.

Methylenedioxymethamphetamine; 5-Hydroxytryptamine; Dopamine; Norepinephrine; Brain areas

1. Introduction

Much recent attention has been given to the drug (±)3,4-methylenedioxymethamphetamine (MDMA). MDMA is structurally related to amphetamine and the hallucinogen methylenedioxyamphethamine (MDA). In addition to its abuse on the streets MDMA has been hailed as a valuable adjunct to psychotherapy. Its precise mechanism of action has not been elucidated although it has been reported to have some properties similar to amphetamine as well as hallucinogenic properties.

MDMA has been shown to interact with both 5-hydroxytryptaminergic (5-HT) and dopaminergic (DA) systems in the brain (Glennon et al., 1982; Glennon and Young, 1984). Some reports have suggested that MDMA is a neurotoxin of 5-HT neuronal systems (O'Hearn et al., 1986; Virus et al., 1986; Schmidt et al., 1987). The purpose of the present investigation was to examine the long-term effects of a single or multiple injections of MDMA on the levels of 5-HT, DA and norepinephrine (NE) as well as 5-HT and DA turnover in the frontal cortex, hippocampus and hypothalamus of the rat.

2. Materials and methods

Twenty-four male Sprague-Dawley rats (Charles River) were housed individually in stainless steel cages in a temperature- and humidity-controlled vivarium with a 12-12 h light-dark cycle (lights on 07:00-19:00 h). Animals had free access to food and water. One week after arrival animals were divided into 3 groups. One group (4 ×) received a

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dose of 40 mg/kg MDMA s.c. every second day for 4 doses. A second group (1 ×) received injections of vehicle s.c. every second day for 3 doses and a single injection of 40 mg/kg MDMA s.c. on day 8. A third group (CON) served as a control and received only saline injections s.c. every second day for 4 injections.

Sixteen days following the last injection animals were killed by rapid decapitation and the frontal cortex, hippocampus and hypothalamus dissected free on ice and frozen immediately on dry ice. Samples were stored at −70°C until assayed. Levels of 5-HT, NE, DA and metabolites were determined using high performance liquid chromatography with electrochemical detection. The column was a 150 × 4.6 mm Rainin Microsorb C18 column with a pump speed of 0.7 ml/min at a pressure of 1200 psi. The buffer consisted of 0.1 M sodium phosphate, 0.1 mM EDTA, 4.75 mM 1-heptanesulfonic acid (Fisher), 0.25 mM octyl sodium sulphate (Eastman Chemicals) with 10% methanol, pH 4.0. The electrochemical detector was set at +0.8 V versus a Ag/AgCl reference electrode. Protein levels were determined using the method of Lowry et al. (1951).

Turnover rates of DA and 5-HT were estimated by the ratio of metabolite to amine. Although changes in these ratios may reflect changes in degradative pathways, the use of this method has been shown to be accurate and equivalent to other methods in the determination of turnover rates of monoamines in the CNS (Shannon et al., 1986).

The effects of MDMA on monoamine and metabolite content were analyzed using one-way analysis of variance with drug treatment as the factor. The ratios of metabolites to transmitters were analyzed using two-way analysis of variance with drug treatment and brain area as factors. Individual comparisons were made using a least significant differences test (Steel and Torrie, 1980).

3. Results

Administration of 40 mg/kg MDMA s.c. for 1 or 4 times did not produce changes in body weight at the end of the experiment ($F(2,22) = 2.575$; data not shown). The administration of MDMA

TABLE 1

Effects of administration of 40 mg/kg s.c MDMA on 5-HT, 5-HIAA and 5-HT turnover (5-HIAA/5-HT). Each value is the mean with the S.E.M. for 8 animals. Values are pmol/mg protein. Values in parentheses are the percent of control. [a] Significantly different from control value, [b] significantly different from 1×MDMA value, $P < 0.05$, least significant differences, test, one-way analysis of variance, [c] significantly different from hippocampal value, $P < 0.05$, least significant differences test, two-way analysis of variance.

Area	Control	1×MDMA	4×MDMA
Frontal cortex			
5-HT	16.1 ±1.9	15.1±2.5 (94)	7.5±1.2 [a,b] (47)
5-HIAA	17.9 ±1.2	12.8±1.8 [a] (72)	9.3±0.9 [a] (52)
5-HIAA/5-HT	1.44±0.37	0.84±0.11 [c]	1.43±0.23
Hippocampus			
5-HT	14.5 ±1.7	4.4±1.8 [a] (30)	7.3±1.8 [a] (50)
5-HIAA	23.4 ±1.8	13.7±2.0 [a] (59)	17.3±1.2 [a] (74)
5-HIAA/5-HT	1.64±0.13	2.34±0.61	2.45±0.66
Hypothalamus			
5-HT	43.6 ±6.0	47.1±5.1 (108)	35.0±7.0 (80)
5-HIAA	32.0 ±6.0	33.7±2.2 (105)	42.6±4.9 (133)
5-HIAA/5-HT	0.92±0.09	0.81±0.10 [c]	1.01±0.09 [c]

TABLE 2

Effects of administration of 40 mg/kg s.c MDMA on DA, DOPAC and DA turnover (DOPAC/DA). Each value is the mean with the S.E.M. for 8 animals Values are pmol/mg protein. Values in parentheses are the percent of control. [a] Significantly different from control value, $P < 0.05$, least significant differences test, one-way ANOVA.

Area	Control	1×MDMA	4×MDMA
Frontal cortex			
DA	30.2 ±5.5	32.4±8.6 (107)	28.1±7.1 (93)
DOPAC	2.42±0.77	2.36±1.15 (98)	3.99±1.90 (165)
DOPAC/DA	0.10±0.02	0.08±0.01	0.12±0.03
Hypothalamus			
DA	15.9 ±2.1	22.3±2.1 (140)	27.8±3.3 [a] (175)
DOPAC	4.52±1.00	6.49−.01 (144)	6.93±1.53 (153)
DOPAC/DA	0.23±0.06	0.29±0.06	0.27±0.07

TABLE 3

Effects of administration of 40 mg/kg s.c. MDMA on NE. Each value is the mean with the S.E.M. for 8 animals. Values are pmol/mg protein. Values in parentheses are the percent of control. [a] Significantly different from control value, [b] significantly different from 1×MDMA value, $P < 0.05$, least significant differences test, one-way analysis of variance.

Area	Control	1×MDMA	4×MDMA
Frontal cortex	17.4±0.8	17.2±1.7 (99)	12.7±0.9 [a,b] (73)
Hippocampus	20.1±1.3	20.5±0.8 (102)	20.9±0.9 (104)
Hypothalamus	85.9±9.3	77.7±6.0 (90)	79.6±6.6 (93)

either 1 or 4 times produced a decrease in 5-HT and 5-HIAA levels in the frontal cortex and hippocampus whereas the hypothalamus was spared (table 1). There was a significant difference in the turnover rates by area ($F(2,59) = 4.179$, $P < 0.05$) as estimated by 5-HIAA/5-HT ratios. Turnover rates of 5-HT in all areas were unaffected by administration of MDMA ($F(2,59) = 1.211$).

The DA level in the hypothalamus was increased to 175% of control in the group receiving 4 doses of MDMA (table 2), whereas levels of DA and DOPAC were unchanged in the frontal cortex. Levels of DA and DOPAC in the hippocampus were blow the level of sensitivity of the assay (50 fmol/μl). NE levels were unchanged in the frontal cortex, hippocampus and hypothalamus, with the exception of a significant decrease in NE in the frontal cortex following 4 × MDMA (table 3).

4. Discussion

MDMA, when administered once or 4 times at a dose level of 40 mg/kg, produces a depletion of 5-HT in some areas of the brain 16 days after administration. In the present study depletions of 5-HT and 5-HIAA were seen in the frontal cortex and hippocampus with no changes seen in the hypothalamus. The decrease in content with no change in 5-HT turnover suggests the possibility that an MDMA-induced decrease in the number of 5-HT nerve terminals may be responsible for the observed changes in 5-HT and 5-HIAA content. In support of this contention, recent histological evidence indicates that MDMA can produce a profound reduction in 5-HT nerve terminals (O'Hearn et al., 1986; Virus et al., 1986; Schmidt, 1987). However, it has been established that MDMA can produce an acute inhibition of tryptophan hydroxylase (Stone et al., 1986), thus the possibility that MDMA has a long-lasting inhibitory effect on tryptophan hydroxylase cannot be excluded. Histological experiments are necessary to determine if the changes in 5-HT content observed in our experiments are, in fact, a result of MDMA-induced neuronal degeneration.

Differences are apparent in the sensitivity of sites for depletion of 5-HT. The hippocampus is most sensitive to the effects of 1 and 4 doses of 40 mg/kg MDMA with no statistical significance between the depletions produced by the 2 dosing regimens. The prefrontal cortex showed depletions only after 4 doses of MDMA, while the hypothalamus was not affected by the neurotoxic effects of MDMA. This differential sensitivity to MDMA may be related to differences to 5-HT turnover in these areas. In the present study the level of 5-HT depletion appears to correlate with the 5-HIAA/5-HT ratio; the value for 5-HIAA/5-HT and depletion of 5-HT being highest in the hippocampus and lowest in the hypothalamus.

Schmidt et al. (1986) reported that MDMA acutely decreases 5-HT in the striatum. In their study, doses of MDMA in excess of 10 mg/kg depleted 5-HT to less than 50% of control 3 h after administration. A time course showed that 5-HT levels remained at 60% of control up to 7 days following a single dose of 10 mg/kg MDMA. Virus et al. (1986) have shown that MDMA (40 mg/kg twice daily for 4 days) produces substantial depletions of 5-HT in the hippocampus, hypothalamus, striatum, somatosensory cortex, as well as other cortical areas, while 20 mg/kg of MDMA twice daily for 4 days produced a decrease in hippocampal [^{3}H]5-HT uptake sites without an apparent change in affinity. Thus, higher doses of MDMA are effective in depleting 5-HT in the hypothalamus, an area unaffected by the doses used in the present experiment.

MDA, another hallucinogenic amphetamine,

also produces long-term depletions of 5-HT in the brain. Ricaurte et al. (1985) showed that MDA decreased 5-HT levels in striatum, hippocampus and the rest of the rat brain without affecting DA or NE. Furthermore, they showed that the changes induced by MDA in 5-HT levels in the striatum were due to a loss of nerve terminals in this area.

The present study found that 4 × MDMA increased DA in the hypothalamus, but a statistically non-significant increase in DOPAC prevented a change in the ratio of DOPAC/DA. This may suggest an increase in synthesis of DA in the hypothalamus. Furthermore, the effects of MDMA on DA in the brain may be area-specific. Schmidt et al. (1986) have also reported an increase in DA in the striatum following acute (3 h) administration of MDMA; Virus et al. (1986) reported that multiple injections of MDMA produce a long-term depletion of DA in the striatum 2 weeks following the last injection. MDMA has been shown to be a potent releaser of both 5-HT and DA (Nichols et al., 1982; Levin et al., 1986). Stone et al. (1986) have also reported that, although MDMA reduces the activity of tryptophan hydroxylase following acute administration, MDMA has no effect on tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis. These results and those of the present experiment suggest long-term effects of MDMA on DA systems. That these effects may result from actions on other neurotransmitter systems cannot be ruled out.

The findings of the present study suggest that MDMA produces a long-term effect on brain 5-HT systems. Many of these effects are similar to the effects of other drugs of similar structure which inhibit 5-HT synthesis, such as p-chloroamphetamine (Sanders-Bush et al., 1974). Although p-chloroamphetamine (Harvey et al., 1977), the related agent MDA (Ricaurte et al., 1985) and MDMA at high doses (Virus et al., 1986; O'Hearn et al., 1986) destroy 5-HT neurons, histological studies need to be performed to verify that MDMA does so at the 5-HT-depleting doses in the present experiments.

Acknowledgements

D.J. Mokler was supported, in part, by NIDA Individual Post-Doctoral Fellowship DA 05260 This grant was also supported by a Virginia Commonwealth University-grant-in-aid to J.A.R

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