Brain Research, 488 (1989) 121–125
Elsevier

BRE 14507

IG VS SC
REPEATED

94

Evidence that both intragastric and subcutaneous administration of methylenedioxymethylamphetamine (MDMA) produce serotonin neurotoxicity in rhesus monkeys

Mark S. Kleven[1], William L. Woolverton[1,2,3] and Lewis S. Seiden[1,2]

[1]*Department of Pharmacological and Physiological Sciences,* [2]*Department of Psychiatry and* [3]*Committee on Biopsychology, The University of Chicago, Chicago, IL (U.S.A.)*

(Accepted 1 November 1988)

Key words: Serotonin; Neurotoxicity; 3,4-Methylenedioxymethamphetamine; Primate

It has been demonstrated that repeated, subcutaneous administration of 3,4-methylenedioxymethamphetamine (MDMA) to rats, guinea pigs, and squirrel monkeys produces long-lasting depletions of serotonin (5-hydroxytryptamine; 5-HT) in several brain regions. Since evidence of degenerating 5-HT neurons has been observed in the rat brain following MDMA injections, it is likely that these depletions are due to neurotoxicity similar to that observed with other substituted amphetamines. The purpose of the present study was to determine if MDMA produces similar evidence of neurotoxicity in rhesus monkeys when administered by either the intragastric (i.g.) or subcutaneous (s.c.) route. Administration of MDMA (5.0 mg/kg/12 h × 4 days) by either i.g. or s.c. routes depleted 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in various brain regions 2 weeks after the last injection. Further, a significant decrease in [^{3}H]5-HT uptake sites in the hippocampus was observed in monkeys treated with MDMA by the i.g. route. Reductions in uptake sites did not achieve statistical significance when drug was administered s.c. The results suggest that repeated administration of MDMA produces long-lasting, potentially neurotoxic effects on central 5-HT neurons in primates and does so when given orally.

INTRODUCTION

3,4-Methylenedioxy-*N*-methylamphetamine (MDMA) is a ring-substituted amphetamine analog which possesses hallucinogenic activity and has been widely abused[5]. Other amphetamine-related drugs such as *N*-methylamphetamine and 3,4-methylenedioxyamphetamine (MDA) have been shown to have neurotoxic effects on brain dopamine (DA) and/or serotonin (5-HT)[8,9,13]. Recent biochemical and morphological evidence show that MDMA causes a selective degeneration of 5-HT neurons in rats, similar to that found previously with MDA. For example, levels of 5-HT are depleted as long as 8 weeks following repeated administration of MDMA[1,2,11,12,15,16]. Additionally, MDMA reduces numbers of 5-HT uptake sites[1,2,12], depresses tryptophan hydroxylase activity[15,16] and produces signs of neuronal degeneration[2]. It is clear that neurotoxicity is directed primarily toward the 5-HT systems since much higher doses are required to produce significant reductions in the levels of DA or metabolites.

The available data strongly suggest that amphetamine-related drugs may be neurotoxic in humans. However, few studies have addressed the fact that humans typically self-administer hallucinogenic amphetamines by the oral route. With regard to the potential of orally-administered MDMA to also cause neurotoxicity, long-lasting depletions of 5-HT have now been observed in squirrel monkeys and rats administered MDMA by this route[4,10], indicating that humans might also be at risk as a consequence of brief exposure. The purpose of the present

Correspondence: M.S. Kleven, The University of Chicago, Department of Pharmacological and Physiological Sciences, 947 E 58th St., Chicago, IL 60637, U.S.A.

0006-8993/89/$03.50 © 1989 Elsevier Science Publishers B.V. (Biomedical Division)

study was to examine and compare the neurochemical effects of MDMA in rhesus monkeys when administered by either subcutaneous or intragastric routes. The results suggest that both oral and subcutaneous administration of MDMA produce evidence of neurotoxicity to 5-HT neurons in the rhesus monkey.

MATERIALS AND METHODS

Subjects

Nine female rhesus monkeys weighing between 5.6 and 10 kg served as experimental subjects. Although all monkeys, including controls, had experimental histories of intravenous drug self-administration, none were exposed to doses (or regimens) of drugs known to deplete central 5-HT. All subjects had been drug-free for at least 30 days prior to the present experiment. They were housed in stainless steel cages in a room that was maintained at 24 °C and on a 14 h:10 h light:dark cycle (light 06.00–20.00 h). Water and food (Purine monkey chow) were continuously available.

Procedure

Monkeys were randomly assigned to experimental groups: 3 of the monkeys served as untreated experimental controls and the remaining 6 were administered MDMA (5.0 mg/kg) twice daily (08.00 h and 20.00 h) for 4 consecutive days. Monkeys (n = 3/group) received MDMA either subcutaneously (s.c.) or intragastrically (i.g.), in a volume of 1.0 ml/10 kg. During i.g. administration of MDMA, monkeys were seated in a plastic primate chair (Plas Labs, Lansing, MI) and a lubricated nasogastric feeding tube (Argyle, St. Louis, MO) was passed into the stomach. After injection of drug, the catheter was flushed with 5.0 ml of 0.9% saline and removed.

Two weeks following the last injection of MDMA, monkeys were anesthetized with sodium pentobarbital (30–50 mg/kg, i.v.) and exsanguinated. The brains were removed within 5 min and dissected in a cold-room (4 °C) using a procedure which has been described in detail elsewhere[8] into regions comprising somatosensory and frontal cortex, lateral and medial caudate, hippocampus, hypothalamus, midbrain, and pons-medulla. Each brain region was wrapped in aluminum foil and stored in liquid nitrogen until assayed for levels of monoamines and metabolites. Regions were assayed for norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) by use of high-performance liquid chromatography with electrochemical detection. The equipment, mobile phase (0.1 N sodium acetate/citrate), and elution of monoamines and metabolites are described in detail elsewhere[6].

For analysis of [^{3}H]5-HT uptake, fresh hippocampal samples were obtained during brain dissections and stored on ice at 4 °C until homogenized in 4 vols. (w/v) of ice-cold 0.32 M sucrose. The synaptosomal uptake of [^{3}H]5-HT was measured by a modification of the method of Snyder and Coyle[14], described previously[9].

Data analysis

Neurochemical data from left and right brain samples were averaged for each monkey and the resulting data for regions analyzed by one-way ANOVA. A posteriori comparisons with controls were made using Dunnett's procedure[17] with a $P < 0.05$ (one-tailed) deemed significant.

RESULTS

The 4-day regimen of either i.g. or s.c. administration of MDMA depleted 5-HT in hippocampus, somatosensory and frontal cortex, and midbrain (Table I). Levels of 5-HT following MDMA were decreased to between 10% and 55% of control. Significant decreases in 5-HT levels in the pons-medulla and hypothalamus were only observed after i.g. administration of MDMA. Levels of 5-HT in medial caudate were significantly reduced by s.c. administration of MDMA. Neither regimen decreased 5-HT in the lateral caudate. Levels of the 5-HT metabolite, 5-HIAA, exhibited a similar pattern of depletions following either i.g. or s.c. MDMA (data not shown). In addition, DA and NE levels in any of the regions examined were unaffected by MDMA regardless of the route of administration (Table I).

The 4-day regimen of i.g. MDMA produced a significant decrease in the V_{max} for 5-HT uptake (to

TABLE I

Monoamine levels in MDMA-treated monkeys

Values are mean ± S.E.M., ng/g tissue weight.

	5-HT			*NE*			*DA*		
	c.	*i.g.*	*s.c.*	*c.*	*i.g.*	*s.c.*	*c.*	*i.g.*	*s.c.*
Hippocampus	0.11±0.02	0.01±0.00**	0.04±0.01**	0.17±0.02	0.26±0.04	0.22±0.01	0.49±0.23	0.37±0.09	0.32±0.09
Sensory cortex	0.08±0.02	0.02±0.00*	0.03±0.01*	0.73±0.08	0.52±0.05	0.59±0.05	ND	ND	ND
Frontal cortex	0.06±0.01	0.01±0.00**	0.02±0.00**	0.17±0.04	0.17±0.06	0.18±0.05	0.02±0.00	0.02±0.01	0.02±0.01
Lat. caudate	0.61±0.06	0.43±0.05	0.53±0.08	ND	ND	ND	7.37±0.47	5.71±0.12	5.96±0.75
Med. caudate	0.72±0.04	0.63±0.04	0.58±0.02*	ND	ND	ND	7.08±0.30	6.34±0.50	5.67±0.40
Hypothalamus	0.41±0.08	0.20±0.04*	0.24±0.03	4.20±1.37	4.50±0.44	5.01±0.68	0.49±0.13	0.37±0.05	0.32±0.05
Midbrain	0.31±0.04	0.17±0.03*	0.20±0.03*	0.81±0.09	0.78±0.02	0.74±0.06	0.24±0.03	0.24±0.02	0.26±0.02
Pons-medulla	0.18±0.00	0.10±0.01**	0.15±0.02	0.46±0.06	0.38±0.01	0.42±0.04	0.02±0.05	0.02±0.01	0.01±0.01

ND = not detected.
*$P < 0.05$ vs control.
**$P < 0.01$ vs control.

36% of control) in the hippocampus with no change in affinity (Table II). The decrease in 5-HT uptake sites (to 70% of control) observed after 4 days of s.c. administration of MDMA failed to reach significance.

DISCUSSION

Either i.g. or s.c. administration of MDMA (5.0 mg/kg) twice daily for 4 days produced as much as a 90% decrease in 5-HT and 5-HIAA in hippocampus, somatosensory cortex and frontal cortex two weeks after the last dose was given. These results are similar to those observed in rats, guinea pigs, and squirrel monkeys[2,10]. The regional pattern of 5-HT depletions, in which the largest degree of 5-HT depletions were observed in hippocampal and cortical regions with smaller decreases seen in hypothalamus and caudate, was also similar to that found in rats[2], as was the lack of effect of MDMA on levels of DA or NE. These results indicate that orally administered MDMA depletes 5-HT neurons in a manner identical with that previously observed following s.c. administration.

The reduction in hippocampal 5-HT uptake following MDMA administration was also consistent with previous results in rats[1,2,12]. Oral administration of MDMA produced a 64% decrease in hippocampal 5-HT uptake sites. Although there was a 30% decrease in uptake sites following subcutaneous administration, these results were not statistically significant, perhaps because of the small number of monkeys examined. MDMA administered by either route did not affect the affinity of the 5-HT uptake site. A decrease in V_{max} of the 5-HT uptake carrier with no change in K_m indicates a loss of uptake sites with no change in the affinity of the remaining sites for 5-HT. This decrease in 5-HT uptake sites 2 weeks after the last injection of MDMA is similar to previous findings with methylamphetamine and MDA and is consistent with neurotoxicity. Since it has been reported that recovery of 5-HT depletions after a brief regimen of MDMA occurs in the rat[1,11,16], it is possible that neurotoxicity does not

TABLE II

Kinetic constants of [^{3}H]5-HT uptake into hippocampal synaptosomes 2 weeks after administration of 5.0 mg/kg of MDMA twice daily for 4 consecutive days

Values shown are mean ± S.E.M. (n = 3/group).

Treatment	K_m ($\times 10^{-7}$) *M*	V_{max} ($\times 10^{-10}$) *(mol/g tissue weight taken up by synaptosomes)*
Control	1.45 ± 0.05	12.3 ± 1.20
MDMA i.g.	1.39 ± 0.10	4.42 ± 1.33**
% of control	96%	36%
MDMA s.c.	1.45 ± 0.16	8.64 ± 1.78
% of control	100%	70%

**$P < 0.01$ vs control.

necessarily result from exposure to MDMA. However, since the number of 5-HT uptake sites was reduced, the present results strongly indicate that actual nerve terminal damage has occurred as a consequence of the 4-day exposure to MDMA. Further studies will be necessary to determine if recovery from MDMA-induced 5-HT neurotoxicity occurs in the rhesus monkey.

With regard to potential differences due to route of administration, our results suggest that oral administration of MDMA is more effective in reducing 5-HT levels than subcutaneous. In all regions examined except medial caudate, the degree of 5-HT depletion was greater following oral administration. In the case of the pons-medulla, subcutaneous doses were without effect while oral doses produced a 44% decrease in 5-HT. Differences between routes of administration were most apparent in the analysis of 5-HT uptake sites in the hippocampus. These results are inconsistent with a previous study of MDMA-induced 5-HT depletions in squirrel monkeys[10], where oral doses of MDMA were less effective than subcutaneous doses in depleting 5-HT in somatosensory cortex. However, administration of MDMA by either route produced the same degree of 5-HT depletion in the rat[4]. It is not clear why differences should be found between rhesus and squirrel monkeys.

Since differences in the neurotoxic effects of subcutaneous MDMA have been reported between mice and rats[15], it could be postulated that species differences in pharmacokinetics can account for the discrepancy between squirrel and rhesus monkeys. Although no data are available for MDMA, pharmacokinetic differences among primate species have been reported for several other drugs. For example, bioavailability of rolipram following oral doses varied from 0.1% in rhesus monkey, 3.7% in cynomolgus monkey and 35% in the rat[7]. Similarly, peak plasma drug concentrations and elimination rate of isosorbide dinitrate differed markedly between the rhesus monkey, cynomolgus monkey, and baboon[3]. These data indicate that it is possible for primate species to attain different brain levels of MDMA with the same route of administration. However, in the present case, further studies using larger numbers of animals would be required to demonstrate that there are, in fact, important differences between routes of administration within a given species.

Evidence of long-lasting depletions of brain 5-HT has been found with oral administration of MDMA to rats[4], squirrel monkeys[10] and, in the present study, rhesus monkeys. The additional finding that 5-HT uptake sites are reduced following a 4-day regimen strongly suggests that MDMA is neurotoxic in the rhesus monkey. These studies suggest that MDMA produces similar effects in a variety of species, regardless of the route of administration. The present studies further indicate that humans are potentially at risk, even with relatively brief exposures to MDMA.

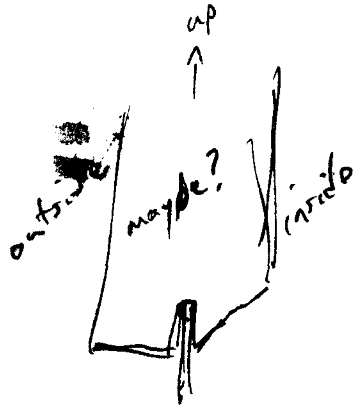
ACKNOWLEDGEMENTS

Research supported by NIDA Grant DA-00085. L.S.S. is supported by Research Service Award MH-10562. We wish to thank R. Corwin for her assistance with this study.

REFERENCES

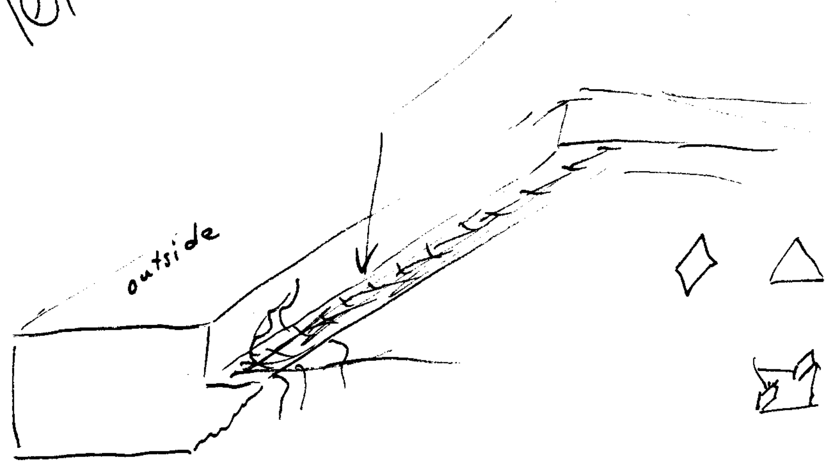
1 Battaglia, G., Yeh, S.Y. and De Souza, E.B., MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons, *Pharmacol. Biochem. Behav.*, 29 (1988) 269–274.
2 Commins, D.L., Vosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R. and Seiden, L.S., Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain, *J. Pharmacol. Exp. Ther.*, 241 (1987) 338–345.
3 Doyle, E. and Chasseaud, L.F., Pharmacokinetics of isosorbide dinitrate in rhesus monkey, cynomolgus monkey, and baboon, *J. Pharm. Sci.*, 70 (1981) 1270–1272.
4 Finnegan, K.T., Ricaurte, G.A., Ritchie, L.D., Irwin, I., Peroutka, S.J. and Langston, J.W., Oraly-administered MDMA causes a long-term depletion of serotonin in rat brain, *Brain Research*, 447 (1988) 141–144.
5 Gertz, K.R., The agony of ecstasy, *Science Digest*, February (1986) 27.
6 Kotake, C., Heffner, T., Vosmer, G. and Seiden, L., Determination of dopamine, norepinephrine, serotonin and their major metabolic products in rat brain by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection, *Pharmacol. Biochem. Behav.*, 22 (1985) 85–89.
7 Krause, W. and Kuhne, G., Pharmacokinetics of rolipram in the rhesus and cynomolgus monkeys, the rat and rabbit. Studies on species differences, *Xenobiotica*, 18 (1988) 561–571.
8 Preston, K.L., Wagner, G.C., Schuster, C.R. and Seiden, L.S., Long-term effects of repeated methylamphetamine administration on monoamine neurons in the rhesus monkey brain, *Brain Research*, 338 (1985) 243–248.

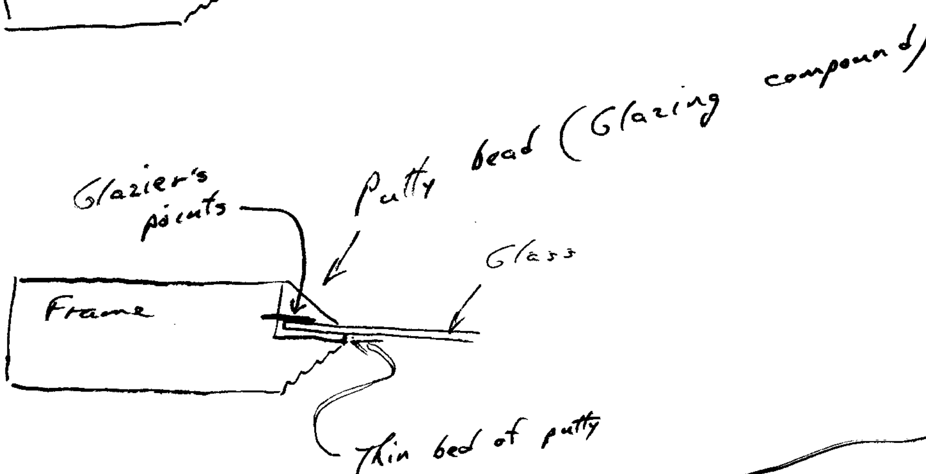
9 Ricaurte, G.A., Schuster, C.R. and Seiden, L.S., Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study, *Brain Research*, 193 (1980) 153–163.
10 Ricaurte, G.A., DeLanney, L.E., Irwin, I. and Langston, J.W., Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration, *Brain Research*, 446 (1988) 165–168.
11 Schmidt, C.J., Wu, L. and Lovenberg, W., Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue, *Eur. J. Pharmacol.*, 124 (1986) 175–178.
12 Schmidt, C.J., Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 240 (1987) 1–7.
13 Seiden, L.S. and Ricaurte, G.A., Neurotoxicity of methamphetamine and related drugs. In H.Y. Meltzer (Ed.), *Psychopharmacology: the Third Generation of Progress*, Raven, New York, 1987, pp. 359–366.
14 Snyder, S.H. and Coyle, J.T., Regional differences in ^{3}H-norepinephrine and ^{3}H-dopamine uptake in rat brain homogenates, *J. Pharmacol. Exp. Ther.*, 165 (1968) 78–86.
15 Stone, D.M., Hanson, G.R. and Gibb, J.W., Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats, *Neuropharmacology*, 26 (1987) 1657–1661.
16 Stone, D.M., Merchant, K.M., Hanson, G.R. and Gibb, J.W., Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat, *Neuropharmacology*, 26 (1987) 1677–1683.
17 Winer, B.J., *Statistical Principles in Experimental Design*, McGraw-Hill, New York, 1971, 201 pp.



~~Pete~~ Travis called for you call him

Tex →





4492-7146 beeper #07008
call with it

Elif's Place
493-0723

Matt's work
702-9650