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Short communication

A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its N-methylated and N-ethylated derivatives

Donna M. Stone, Michel Johnson, Glen R. Hanson and James W. Gibb *

Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, U.S.A.

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Three psychoactive amphetamine congeners were evaluated for their ability to cause long-term changes in several neurochemical parameters indicative of central serotonergic function. Two weeks after multiple doses (10 mg/kg) of 3,4-methylenedioxyamphetamine (MDA) or its N-methylated derivative, 3,4-methylenedioxymethamphetamine (MDMA), selective and dramatic decreases were observed in regional brain tryptophan hydroxylase (TPH) activities, and in corresponding concentrations of 5-hydroxytryptamine (5-HT) and its primary metabolite, 5-hydroxyindoleacetic acid (5-HIAA). However, the N-ethylated derivative of MDA, N-ethyl-3,4-methylenedioxyamphetamine (MDE), was much less potent in its ability to lower brain hydroxyindoles, and in most regions examined did not significantly affect TPH activity. The neurotoxic implications of these results are discussed.

Methylenedioxyamphetamine; Neurotoxicity; Amphetamines; Serotonin

1. Introduction

Recent reports have implicated two illicit amphetamine analogs, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), as potential serotonergic neurotoxins (Ricaurte et al., 1985; Schmidt et al., 1986; Stone et al., 1986). These findings are of particular interest and concern in view of the widespread recreational use of these and other closely related compounds (Taylor et al., 1986). MDA has been described as a hallucinogenic amphetamine; its N-methylated analog, MDMA ('ecstasy'), elicits qualitatively different effects, reportedly enhancing insight and awareness without causing perceptual distortion or disturbance of normal thought processes (Shulgin, 1978).

A third member of this class of compounds, the N-ethylated derivative of MDA, N-ethyl-3,4-methylenedioxyamphetamine (MDE), has been identified in a street-drug sample submitted to the Drug Enforcement Administration (Vallejo, 1982). While the potency and psychopharmacological activity of MDE are very similar to that of MDMA (Shulgin, 1978), its effects occur more rapidly following ingestion, and are shorter-lived than those of either MDMA or MDA (Shulgin, 1978).

Ricaurte and coworkers (1985) examined the persisting effects of multiple 10 mg/kg doses of MDA on central monoaminergic transmitter concentrations. These investigators reported dramatic decreases in brain serotonin (5-HT) content two weeks after treatment, as well as histological evidence indicative of serotonergic neurotoxicity. No accompanying changes occurred in brain catecholamine levels. It was of interest to determine the long-term effects of the two related compounds, MDMA and MDE, on central monoaminergic systems; such information would allow compari-

* To whom all correspondence should be addressed: Department of Biochemical Pharmacology and Toxicology, 113 Skaggs Hall, University of Utah, Salt Lake City, UT 84112, U.S.A.

son of the neurotoxic potential of these three substituted amphetamine analogs. To facilitate comparison with previously reported investigations, a multiple 10 mg/kg dosing regimen was employed. Two weeks after treatment, regional brain concentrations of dopamine and serotonin as well as their respective metabolites, were measured. The enzymatic activities of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), the rate-limiting biosynthetic enzymes for dopamine and 5-HT synthesis, respectively, were assessed as additional indicators of neurotoxicity.

2. Materials and methods

Male Sprague-Dawley rats (200-250 g) were housed five per cage in a temperature controlled room (26°C) with a 12 h alternating light-dark cycle. They were allowed free access to food and water. Drugs were dissolved in 0.9% saline and administered subcutaneously at 6 h intervals, for a total of five doses. Treatment groups were injected with 10 mg/kg (expressed as the free base) of either dl-MDA, dl-MDMA, or dl-MDE, or with vehicle alone. Rats were killed two weeks after the initiation of treatment; brain regions were immediately dissected free (on ice), and stored at −80°C until assayed.

Individual tissues were weighed and homogenized in 50 mM HEPES buffer (pH 7.4) containing 0.2% Triton X-100 and 5 mM dithiothreitol. Following centrifugation at 27000 × *g* for 15 min, duplicate 7.5 μl aliquots of the supernatant were removed and assayed for TPH activity by a modified CO_2-trapping procedure as described by Hotchkiss et al. (1979). Similar 7.5 μl aliquots were diluted to 50 μl with glass-distilled water and analyzed for TH activity according to the method of Nagatsu et al. (1964). In the enzyme assays, dl-6-methyl-5,6,7,8-tetrahydrobiopterin (Sigma chemical Co., St. Louis, MO) was utilized as cofactor.

The contralateral brain tissues were used to measure concentrations of monoamines and their respective metabolites by high-performance liquid chromatography. Tissues were homogenized in 0.3-0.5 ml mobile phase buffer (0.15 M monochloroacetic acid, 2.0 mM EDTA, 0.1 mM 1-octanesulfonic acid, and 12.5% methanol, pH 2.9), the supernatant fraction filtered through a 0.2 μm microfilter system (Bioanalytical Systems Inc., West Lafayette, IN), and 50 μl aliquots injected onto a 10 cm Microsorb reverse-phase column. The eluent was monitored with an amperometric electrochemical detector (model LC-4B; Bioanalytical Systems, Inc., West Lafayette, IN), with the potential set at +0.73 V. Tissue levels were quantitated by comparison with standards of known concentration.

3. Results

Two weeks after treatment with MDA or MDMA, TPH activity was dramatically de-

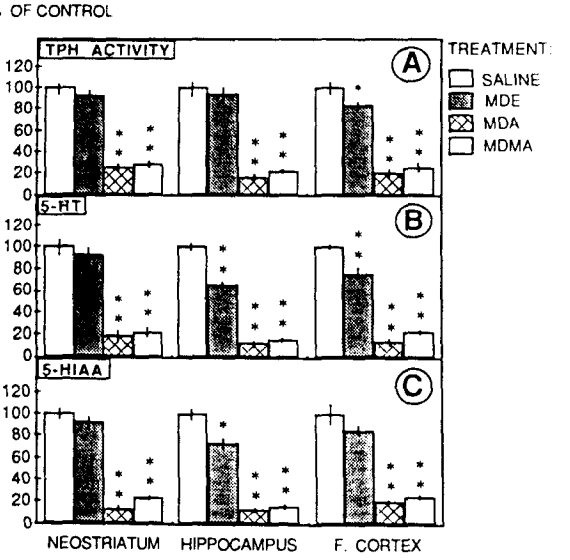

Fig. 1. (A) Effect on regional rat brain tryptophan hydroxylase (TPH) activity two weeks after multiple doses of MDA, MDMA or MDE. Treatment consisted of five doses of drug (10 mg/kg); doses were administered at 6 h intervals. Values are the means ± S.E.M., represented as percent of control, for n = 5-9 animals. Control values (in nmol/g tissue per h) were: neostriatum, 34.7 ± 1.7; hippocampus, 48.8 ± 3.0; frontal cortex, 56.6 ± 3.7. (B) Effect on regional serotonin (5-HT) concentration and (C) 5-hydroxyindoleacetic acid (5-HIAA) concentration two weeks after drug treatment. Values are the means ± S.E.M., expressed as percent of control. Control values (in μg/g tissue) for 5-HT and 5-HIAA concentrations, respectively, were: neostriatum, 0.53 ± 0.04 and 0.55 ± 0.03; hippocampus, 0.32 ± 0.01 and 0.37 ± 0.02; frontal cortex, 0.60 ± 0.01 and 0.20 ± 0.02. * $P < 0.05$, ** $P < 0.001$ versus corresponding control, by the two-tailed Student's t-test.

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4. Discussion

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TABLE 1

Effect on neostriatal dopaminergic parameters two weeks after drug treatment. Rats were administered five doses of drug (10 mg/kg; 6 h intervals) and killed two weeks later. Values are the means ± S.E.M., expressed as percent of control. Absolute values are indicated in parentheses, in nmol tyrosine oxidized/g tissue per h (TH activity) or μg/g tissue (dopamine, DOPAC, HVA). [a] $P < 0.05$, [b] $P < 0.005$ versus saline, by the two-tailed Student's t-test. [c] Tyrosine hydroxylase, [d] dihydroxyphenylacetic acid, [e] homovanillic acid.

Treatment	(n)	TH [c] activity	Dopamine	DOPAC [d]	HVA [e]
Saline	6	100.0 ± 4.9	100.0 ± 4.5	100.0 ± 3.4	100.0 ± 3.2
		(3121 ± 154)	(11.1 ± 0.5)	(0.87 ± 0.03)	(0.62 ± 0.02)
MDE	9	98.9 ± 3.1	100.5 ± 4.3	102.3 ± 6.9	100.0 ± 4.8
MDA	6	99.4 ± 3.9	91.2 ± 9.8	92.0 ± 10.3	91.9 ± 9.7
MDMA	7	110.0 ± 6.1	94.7 ± 4.9	87.4 ± 4.6 [a]	83.9 ± 3.2 [b]

pressed, to less than 30% of control, in the neostriatum, hippocampus and frontal cortex regions of the rat brain (fig. 1A). In contrast, treatment with MDE had no effect on TPH in the neostriatum or hippocampus; in the frontal cortex, however, MDE caused a significant reduction in enzyme activity, to 83% of control (fig. 1A).

Figure 1B and C illustrate the corresponding regional 5-HT and 5-HIAA concentrations, respectively, two weeks after treatment. Comparison of fig. 1A, B and C reveals a correlation, in most areas and with most treatments, between the depletion of 5-hydroxyindoles and that of TPH. The response of the hippocampus to MDE was an exception; in this area TPH activity was not affected, yet 5-HT and 5-HIAA concentrations were both significantly reduced, to 66 and 73% of control, respectively.

The effects of MDA, MDMA and MDE on markers of dopaminergic function two weeks after treatment, are presented in table 1. While none of the three drugs affected neostriatal TH activity or dopamine concentration, MDMA caused a significant reduction in the concentrations of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

4. Discussion

The data presented here allow for comparison of the neurotoxic potential of three amphetamine analogs, MDA, MDMA and MDE, on the rat central serotonergic system. Multiple doses of either MDA or MDMA caused dramatic, long-term decreases in brain TPH activities. At an earlier time point (18 h after treatment), regional TPH activity was decreased to < 15% of control in response to MDA and < 25% of control in response to MDMA (Stone et al., 1986). In addition, regional 5-HT levels at this time were decreased to less than 20% of controls in response to either drug. Two weeks after treatment all serotonergic parameters were still dramatically depressed (fig. 1); no significant recovery had occurred in regional 5-HT concentrations, whereas TPH activities showed a modest trend toward recovery (fig. 1A). At the 18 h time point, MDE-treated rats also exhibited decreased regional TPH activities (< 80% of control, unpublished observations), as well as significantly decreased levels of brain 5-HT (regional concentrations ranged from 60-85% of control, unpublished observations). Two weeks after treatment, however, TPH activity had completely recovered in the neostriatum and hippocampus, and significantly recovered in the frontal cortex; concurrently, 5-HT levels had returned to control values in the neostriatum, while remaining depressed in the two other areas (fig. 1B). These results demonstrate that, while N-methylation of MDA has little effect on the ability of this compound to adversely affect the serotonergic system, N-ethylation of MDA (to MDE) appears to reduce dramatically its neurotoxic potency. Interestingly, N-ethylation also markedly reduces the psychostimulant properties of amphetamine (Biel and Bopp, 1978), as measured by alterations in the spontaneous locomotor activity of mice, yet N-

methylation of amphetamine (to methamphetamine) enhances these characteristic effects (Biel and Bopp, 1978).

The lack of a persistent effect of these drugs on central dopamine concentrations agrees with the data presented by Ricaurte et al. (1985) on the two week effects of MDA, as well as with the results of a shorter-term study (Stone et al., 1986). However, the MDMA-induced depression of dopamine metabolite concentrations contrasts with the elevated neostriatal HVA levels observed at an earlier time point (18 h after treatment; Stone et al., 1986) or 3 h after a single injection of MDMA (Schmidt et al., 1986; Stone et al., 1986). Since, acutely, MDMA induces dopamine release (Levin et al., 1986) and may enhance dopamine turnover (Schmidt et al., 1986; Stone et al., 1986), the decreased metabolite levels two weeks after treatment could be explained by a subsequent compensatory decrease in the release and turnover of this transmitter.

In summary, the two amphetamine analogs, MDA and MDMA, appear to be nearly equipotent in their ability to cause persistent and dramatic depletions of central serotonergic neuronal markers; in contrast, the lack of long-term effects of multiple doses of MDE on brain serotonergic systems suggests this congener is less apt to cause irreversible neuronal damage. Although the doses of MDA and MDMA used in these experiments were several-fold higher than the effective human dose (1-3 mg/kg; Shulgin, 1978), cumulative effects from repeated exposure, different rates or pathways of metabolism between human and rat, or increased human sensitivity to the drug (Ricaurte et al., 1985) could present a serious toxicological threat to human abusers of these compounds.

Acknowledgements

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