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DIFFERENCES IN THE CENTRAL SEROTONERGIC EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN MICE AND RATS

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Summary: The effects of subcutaneous injection of 3,4-methylenedioxymethamphetamine (MDMA), a psychoactive amphetamine congener, on mouse central monoaminergic systems were assessed and compared to effects in rats. Whereas neostriatal concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in mouse were transiently decreased after a single moderately high dose of MDMA (15 mg/kg), mouse neostriatal or hippocampal tryptophan hydroxylase activity was not significantly affected, even after a dose of 60 mg/kg. These results are in contrast to effects in rats, in which a single 10 mg/kg dose of MDMA induced immediate and prolonged decreases in both central tryptophan hydroxylase activity and 5-hydroxyindole concentrations. Decreases in mouse central tryptophan hydroxylase activity, and prolonged decreases in mouse 5-hydroxyindole concentrations, were observed only after multiple doses of MDMA, suggesting that the duration of exposure may be an important determinant of toxic effects. These results show mice to be less susceptible than rats to MDMA-induced neurotoxicity, and are discussed in terms of possible interspecies differences in MDMA pharmacokinetics.

Key words: methylenedioxymethamphetamine, tryptophan hydroxylase, serotonin, neurotoxicity, mouse, rat

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a ring-substituted amphetamine analog with reportedly unique psychoactive properties; its subjective effects in humans, which include euphoria, enhanced empathy and mild central stimulation (Adler, Abramson, Katz, and Hager, 1985), render MDMA a currently popular recreational drug. Similar to its N-desmethyl analog, MDA (Ricaurte, Bryan, Strauss, Seiden, and Schuster, 1985), MDMA causes both immediate and prolonged changes in rat central serotonergic systems (Schmidt, Wu and Lovenberg, 1986; Stone, Stahl, Hanson and Gibb, 1986); such changes include decreases in concentrations of 5-hydroxytryptamine (5HT) and its major metabolite, 5-hydroxyindoleacetic acid (5HIAA), as well as a decrease in the activity of the corresponding biosynthetic enzyme, tryptophan hydroxylase, and a long-term loss of central 5HT-uptake sites (Schmidt, 1987).

Decreases in rat central tryptophan hydroxylase activity persist for at least 110 days following multiple doses of MDMA (Stone, Merchant, Hanson and Gibb; unpublished observations), suggesting a neurotoxic action for this compound in the rat brain. Such long-term effects are disturbing in view of the widespread illicit human use of MDMA. Little is known, however, of the neurochemical effects of MDMA in species other than the rat. We have, therefore, attempted to characterize the central effects of MDMA in mice, a species which, in regard to amphetamine and structurally related compounds, exhibits a metabolic profile distinct from that of the rat (Caldwell, 1976).

As indicators of central neurochemical effects, neostriatal or hippocampal tryptophan hydroxylase activity, and neostriatal concentrations of 5HT, 5HIAA, dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were assessed at various time points after MDMA administration, in both the mouse and the rat. Percent changes in monoaminergic parameters induced by MDMA treatment were then calculated and contrasted between species.

METHODS

Male Sprague-Dawley rats (200-250 g) or male C57 white mice (20-25 g) were housed 5 or 7 per cage, respectively, in temperature-controlled rooms with 12-hour alternating light/dark cycles. Animals were

allowed free access to standard laboratory food and water. dl-MDMA hydrochloride was dissolved in 0.9% saline vehicle and administered subcutaneously to groups of rats or mice (n = 6-10 animals), as a single 10 mg/kg or 15 mg/kg dose (expressed as the free base), respectively. Control animals were injected with vehicle alone. In one experiment, a multiple dosing regimen was employed in which mice were injected with MDMA (15 mg/kg) once every 4 hours, for a total of 6 doses.

Animals were sacrificed by decapitation at various time points after drug administration (groups of rats or mice sacrificed at each time point consisted of both treated and control animals; time points are specified in Results), and the whole brain was removed and placed on ice. The hippocampi and neostriata were removed bilaterally and stored at -70° C until assayed. One of the paired brain structures from each animal was assayed for monoamine and monoamine metabolite content, while the contralateral tissue was analyzed for tryptophan hydroxylase activity.

Determination of monoamine and metabolite concentrations

Brain regions were analyzed for concentrations of monoamines and their major metabolites by high-performance liquid chromatography with electrochemical detection. Tissues were homogenized in 0.1-0.5 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), followed by centrifugation at 4800 x g for 15 min. The supernatant fraction was subsequently filtered through a 0.2-μm microfilter system (Bioanalytical Systems Inc., West Lafayette, IN), and aliquots of 50 μl were injected onto an 11-cm Partisphere C18 reversed-phase column equipped with a 1-cm RP guard column (Whatman Inc., Clifton, N.J.). The eluent was monitored with an amperometric electrochemical detector (model LC-4B; Bioanalytical Systems Inc.); the detector potential was set at +0.73 V. Tissue levels of 5HT, 5HIAA, dopamine, DOPAC, and HVA were quantitated by comparison with a calibration curve derived from standards of known concentration prepared in mobile phase buffer.

Tryptophan Hydroxylase assay

Contralateral brain 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 x g for 15 min, duplicate 7.5-μl aliquots of supernatant were removed and assayed for tryptophan hydroxylase activity by a $^{14}CO_2$-trapping procedure, as described by Hotchkiss, Morgan and Gibb (1979), using dl-6-methyl-5,6,7,8-tetrahydrobiopterin (Sigma Chemical Co., St. Louis, MO) as cofactor.

RESULTS

A time course of the neurochemical effects in mice of a single subcutaneous injection of MDMA (15 mg/kg) is presented in Fig. 1. For comparison, a similar time course is presented for the acute effects of MDMA (10 mg/kg) in rats. Although the dose administered to rats was only 2/3 that given to mice, rats exhibited a much more pronounced and longer-lasting serotonergic response to the drug. Between 6 and 24 hours after MDMA administration, rat neostriatal tryptophan hydroxylase activity and 5-hydroxyindole content were maximally decreased; in contrast, 5HT and 5HIAA in mouse brain were maximally decreased 3 hours after drug administration, and tryptophan hydroxylase activity in the mouse neostriatum (Fig. 1) or hippocampus (data not shown) was not significantly affected at any time after MDMA. Even a high single dose of either 40 or 60 mg/kg did not significantly decrease mouse regional tryptophan hydroxylase activity, as assessed 1 hour after treatment (40 mg/kg MDMA; enzyme activity in the neostriatum and hippocampus, respectively, was 93 and 71% of control; n = 8, control vs. treated: n.s.) or 3 hours after treatment (60 mg/kg MDMA; enzyme activity in the neostriatum and hippocampus, respectively, was 115 and 77% of control; n = 8, control vs. treated: n.s.). In addition, whereas all rat serotonergic parameters were still significantly lower than corresponding control values 2 weeks after MDMA administration, mouse neostriatal 5-hydroxyindole content was only transiently decreased by MDMA: at both 1 and 3 hours after drug treatment, mouse 5HT and 5HIAA concentrations were significantly lower than control values; at later time points, however, mouse neostriatal 5-hydroxyindole concentrations were either similar to, or actually higher than, corresponding controls (Fig. 1).

Of the mouse neostriatal dopaminergic parameters examined, only DOPAC was significantly affected: by 1 hour post-injection, DOPAC concentrations had declined to 48% of control ($p < 0.001$, data not shown); thereafter, DOPAC levels returned to normal. Although both dopamine and HVA concentrations were elevated at early times after MDMA, neither alteration was statistically significant (data not shown).

In an attempt to prolong tissue drug levels, multiple doses of MDMA (15 mg/kg, once every 4 hours for 6 doses) were administered to mice; animals were killed either 3 hours or 1 week after the final injection. Table 1 shows the effects of this treatment on mouse neostriatal and hippocampal serotonergic

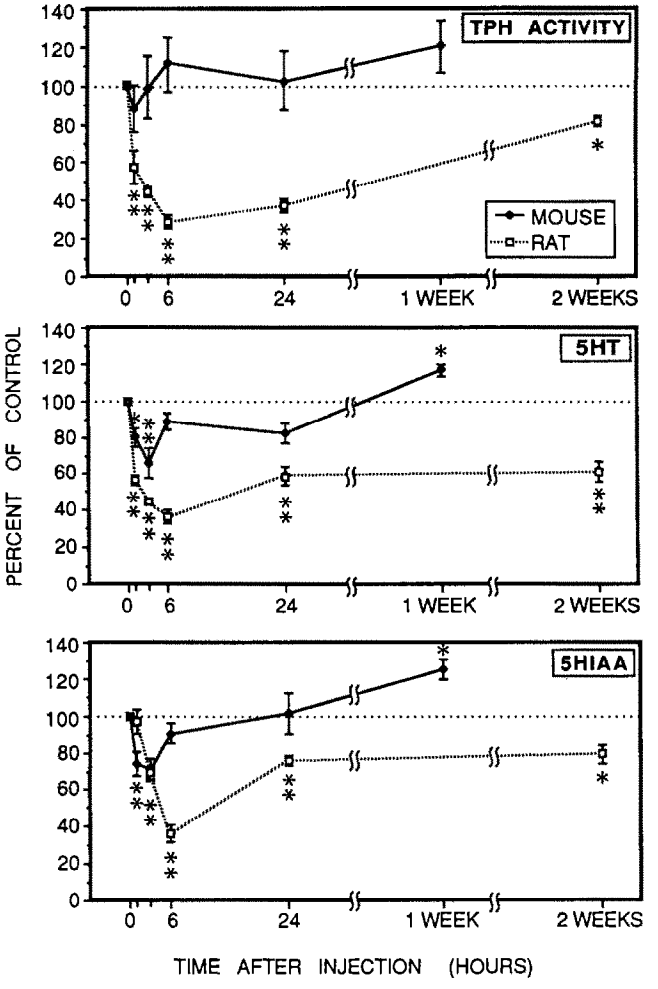

Figure 1. Time course of the neostriatal serotonergic effects of a single dose of MDMA in mouse and rat. MDMA was dissolved in saline and administered as a single subcutaneous injection to mice (15 mg/kg) or rats (10 mg/kg); animals were killed at specified time points thereafter. Points represent means ± S.E.M. for n= 6-10 animals, and are expressed as a percent of corresponding time-matched control (vehicle injected) animals. Representative control values (24 h time point) for mice and rats, respectively, were: tryptophan hydroxylase (TPH) activity (in nmol/g tissue/h): 27.0 ± 4.0 and 40.0 ± 2.5; 5-hydroxytryptamine (5HT) concentration (in μg/g tissue): 0.379 ± 0.021 and 0.503 ± 0.042; 5-hydroxyindoleacetic acid (5HIAA) concentration (in μg/g tissue): 0.228 ± 0.021 and 0.430 ± 0.028. Data were statistically analyzed by a two-way analysis of variance followed by the Student-Newman-Keuls multiple comparisons test. *$P < 0.05$, **$P < 0.01$ verses corresponding control.

parameters. Similar to effects in rats (Stone et al., unpublished observations), MDMA elicited a more pronounced serotonergic response in the hippocampal region than in the neostriatal region of the mouse brain. While tryptophan hydroxylase activity had significantly decreased in both regions 3 hours after treatment, only hippocampal enzyme activity remained significantly depressed 1 week later (69% of control; Table 1). Concentrations of 5HT and 5HIAA, however, remained significantly depressed in both regions 1 week after treatment (Table 1). At this 1 week time point, mouse neostriatal concentrations of dopamine, DOPAC or HVA were not affected (data not shown).

Table 1. Effect of repeated MDMA administration on mouse hippocampal and neostriatal serotonergic parameters. Mice were administered 6 doses of drug (15 mg/kg, s.c.; 4-h intervals) and killed either 3 h or 1 week after the final dose. Values are the means ± S.E.M., expressed as a percent of control; n represents the number of animals. Absolute values are indicated in parentheses, in nmol/g tissue/h (TPH activity) or µg/g tissue (5HT and 5HIAA). Data were statistically analyzed by a two-way analysis of variance followed by the Student-Newman-Keuls multiple comparisons test. *P < 0.05, **P < 0.01 versus corresponding saline control. [a]Tryptophan hydroxylase, [b]5-hydroxytryptamine, [c]5-hydroxyindoleacetic acid.

Treatment	n	TPH[a] activity		5HT[b]		5HIAA[c]	
		striatum	hippocampus	striatum	hippocampus	striatum	hippocampus
Saline 3 h	10	100 ± 7.6 (38.7 ± 3.0)	100 ± 8.7 (35.5 ± 3.1)	100 ± 5.5 (.486 ± .027)	100 ± 12.1 (.343 ± .041)	100 ± 8.0 (.160 ± .013)	100 ± 12.3 (.159 ± .020)
MDMA 3 h	12	60.1 ± 6.5*	35.3 ± 3.4**	55.4 ± 3.5**	31.6 ± 2.5**	46.3 ± 2.5**	34.1 ± 5.7**
Saline 1 wk	10	100 ± 15.9 (43.2 ± 6.9)	100 ± 8.6 (38.8 ± 3.3)	100 ± 8.5 (.416 ± .036)	100 ± 3.7 (.292 ± .011)	100 ± 4.7 (.173 ± .008)	100 ± 5.3 (.169 ± .009)
MDMA 1 wk	8	87.7 ± 13.1	68.6 ± 9.0**	78.5 ± 2.9*	77.5 ± 5.8**	81.0 ± 2.4*	74.2 ± 3.5**

DISCUSSION

The results of the present study clearly demonstrate species differences in the degree and duration of the central serotonergic effects of MDMA. Whereas MDMA has been implicated as a serotonergic neurotoxin in rats (Schmidt et al., 1986; Schmidt, 1987; Stone et al., 1986), due to the pronounced and long-term nature of the induced serotonergic deficits, this compound appears much less potent in its ability to affect central serotonin pathways in mice. Even a high single dose of 60 mg/kg did not significantly decrease mouse neostriatal or hippocampal tryptophan hydroxylase activity, whereas 10 mg/kg in rats resulted in significant enzyme depletion for up to 2 weeks. While short-term, transient decreases in neostriatal 5HT did occur in mice, long-term decreases were observed only after repeated exposure to MDMA, suggesting that the persistence of drug at the site of action is a necessary prerequisite to central toxic effects in this species.

Miller, Sanders-Bush and Dingell (1971), and Steranka and Sanders-Bush (1979) have demonstrated similar species differences for both para-chloroamphetamine (PCA) and fenfluramine (m-trifluoromethyl-N-ethyl amphetamine), 2 ring-substituted halogenated amphetamine derivatives with neurotoxic properties in rats. These investigators have correlated the higher neurotoxic potency of PCA and fenfluramine in rats (as compared to mice) with a prolonged drug half-life in brain, presumably resulting from an impaired metabolism of drug due to the presence of specific substituents on the amphetamine ring. In rat, ring para-hydroxylation is the major pathway for amphetamine metabolism, whereas in mice, side chain metabolism is of equal importance (Caldwell, 1976). In the case of PCA or fenfluramine, respectively, the para or bulky meta substituent on the amphetamine ring appears to inhibit normal ring para-hydroxylation, thus resulting in interspecies differences in drug pharmacokinetics. Due to the relatively short half-life of PCA in mice, long-term toxic effects are realized only after continuous exposure of animals to the drug (Steranka and Sanders-Bush, 1978).

The results from the present investigation suggest that, analogous to the ring substituents of PCA or fenfluramine, the methylenedioxy ring substituent of MDMA may interfere with normal metabolic pathways in the rat, thus prolonging the drug half-life and, hence, the drug-induced neurotoxic effects. In contrast, the serotonergic effects of MDMA in mouse, a species which is able to more readily metabolize this ring-substituted compound (presumably by deamination and subsequent side chain breakdown) thereby reducing its half-life in brain, are less pronounced and of shorter duration. In support of this hypothesis, prolonged exposure of mice to MDMA, by administration of multiple drug doses over a 24-hour period, resulted in long-term serotonergic changes in both the neostriatum and hippocampus; such findings demonstrate the quantitative rather than qualitative nature of the interspecies differences in neurochemical response. In addition, whereas comparable doses of MDMA in rats and mice induced similar initial behavioral and physiological responses (e.g., locomotion, grooming, and piloerection), such characteristic responses were sustained for several hours longer in rats than in mice (unpublished observations). Metabolic and pharmacokinetic profiles of MDMA in mouse and rat brain are necessary, however, to confirm such a hypothesis.

In summary, major interspecies differences were observed in the central neurochemical response to acutely administered MDMA. Whereas moderate doses of MDMA caused both immediate and long-term decreases in rat central serotonergic parameters, similar effects in mice were either absent or less pronounced and shorter-lived. The relative insensitivity of mice to MDMA and related analogs may be

due to the ability of this species to more rapidly metabolize such compounds, thereby resulting in a reduced half-life of drug (and possibly, related metabolites) in brain.

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