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Orally administered MDMA causes a long-term depletion of serotonin in rat brain

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Recent studies suggest that 3,4-methylenedioxymethylamphetamine (MDMA), when administered subcutaneously, is toxic to central serotonergic neurons in rats. Because humans typically self-administer this drug orally, we compared this route to the s.c. route of administration. Orally administered MDMA produced a dose-related depletion of serotonin comparable to that produced by the s.c. route. These findings suggest that MDMA, when given orally, retains its neurotoxic activity and that humans using MDMA may be at risk for developing a persistent depletion of brain serotonin.

Using phenylisopropylamine as a template, clandestine chemists have synthesized a number of amphetamine-like analogs with high abuse liability[11]. Currently, one of the most popular of these controlled substance analogs is 3,4-methylenedioxymethylamphetamine (MDMA)[12]. The long-term consequences of MDMA use by humans are unknown, but biochemical[5,7,11] and morphologic[3] evidence is consistent with the proposal that MDMA has neurotoxic effects in rats. Both single[3,5] and multiple[7,11] dose administration of MDMA have been reported to induce a persistent depletion of serotonin in the rodent striatum, hippocampus, and frontal cortex. The depletion of brain serotonin is accompanied by a decline in striatal and hippocampal tryptophan hydroxylase activity[10] as well as the number of serotonergic uptake sites in the hippocampus[3]. Morphologic studies carried out in MDMA-treated rodents suggest that these biochemical changes can be explained on the basis of the ability of MDMA to damage serotonergic nerve terminals[3].

These findings have raised the concern that humans ingesting this drug might similarly be at risk. However, in all studies focusing on neurotoxicity to date, MDMA has been administered parenterally, while humans typically ingest MDMA orally[10]. Since different routes of administration may profoundly alter the absorption and biodisposition of a drug[8], it is quite possible that the neurotoxicity observed in animals after the parenteral injection of MDMA may be diminished or even absent after its oral administration. This question was examined directly in the present study by comparing the serotonin-depleting effects of orally and s.c. administered MDMA in rats. We now report that orally administered MDMA is as effective as parenterally administered MDMA in causing a persistent depletion of serotonin in the rodent brain.

Male Sprague–Dawley rats, weighing 200–220 g (Simonsen Laboratories, Gilroy, CA), were used as subjects. Animals were housed 5 to a cage under constant temperature (22 °C) and humidity conditions in a room illuminated for 12 h per day, with food and water freely available.

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A racemic mixture of MDMA hydrochloride, generously donated by D.E. Nichols, was administered either orally or s.c. to individual groups of rats (n = 5) at the following doses: 7.5 mg/kg/dose, 15 mg/kg/dose, or 30 mg/kg/dose. A seventh group received no treatment and served as the control group. All groups were administered MDMA twice daily (approximately 12 h apart) for 4 consecutive days (total doses = 8). This schedule of drug administration has been shown to be useful in detecting the toxicity of amphetamine and its derivatives[6,13]. The dose of MDMA was adjusted by varying the volume of the MDMA solution administered (15 mg/ml, calculated as the hydrochloride salt and dissolved in distilled water). Oral administration was accomplished by gavage, using a 16-gauge, curved cannula blunted at one end to minimize the chance of esophageal laceration.

Two weeks after the MDMA multiple-dose regimen, all rats were killed by decapitation and the striatum and hippocampus were rapidly dissected as previously described[7]. Tissue samples were immediately frozen and stored in liquid nitrogen until time of assay. Serotonin and norepinephrine in the hippocampus, and dopamine in the striatum, were assayed by reverse phase chromatography, coupled with electrochemical detection, as previously described[6]. The concentrations of these amines were determined by comparison to standard curves, constructed using commercially available dopamine, norepinephrine, or serotonin (Sigma Chemical Company, St. Louis, MO).

In agreement with previous results[3,11], s.c. administration of MDMA induced a dose-related, long-lasting depletion of serotonin in the hippocampus (Table I). Orally administered MDMA also produced a dose-related depletion of serotonin. The two routes of administration produced comparable effects, although a non-significant trend towards slightly larger depletions of serotonin was observed after s.c. injection (Table I).

The high dose (30 mg/kg) regimen of MDMA, given either orally or s.c. produced a long-term, but smaller, depletion of dopamine in the striatum (Table II). Route of administration did not influence the ability of MDMA to deplete dopamine. Norepinephrine levels were not altered on a long-term basis by MDMA when administered by either route (Table II). The effects of the lower MDMA dose regimens on the concentration of these amines were not determined.

The major finding of this study is that MDMA, when given orally, produces a long-term, dose-related depletion of serotonin in rat brain comparable to that obtained after s.c. administration. At first glance, this result seems surprising since oral administration typically exposes a drug to a number of factors that may diminish its efficacy[8]. For example, orally administered drugs must be absorbed via the gastrointestinal tract, a process that may be adversely affected by gastric acidity, as well as by intestinal enzymes and flora. Equally important, enterically absorbed agents are initially transported via the por-

TABLE I

Concentration of striatal serotonin after the oral or s.c. administration of MDMA

Values (ng/mg tissue) are expressed as the mean ± S.E.M. (n = 5). Values in parentheses represent percent depletion of serotonin. Statistical significance was assessed by a two-way ANOVA. Column effects: $F = 105.6$, $P < 0.001$; Row effects: $F = 2.48$, $P < 0.12$; Interaction effects: $F = 0.54$, $P < 0.65$.

Dose (mg/kg)	*Oral route*	*S.c. route*
Control	0.290 ± 0.020	0.290 ± 0.020
7.5	0.210 ± 0.010 (−27.6%)	0.200 ± 0.020 (−31.0%)
15.0	0.110 ± 0.020 (−62.0%)	0.075 ± 0.010 (−74.1%)
30.0	0.084 ± 0.004 (−71.0%)	0.067 ± 0.007 (−76.9%)

TABLE II

Concentration of dopamine (DA) and norepinephrine (NE) after the oral or subcutaneous administration of MDMA (30 mg/kg)

Values (ng/mg tissue) are expressed as the mean ± S.E.M. (n = 5). Values in parenthesis represent percent depletion of NE or DA. Individual sample means were compared using Student's t-test. *$P < 0.05$ (two-tailed test). ** Non-significant difference, compared to MDMA given by the oral route of administration.

	DA (striatum)	*NE (hippocampus)*
Control	12.10 ± 0.49	0.51 ± 0.03
Oral route	10.30 ± 0.30* (−14.9%)	0.52 ± 0.07 (+2.0%)
S.C. route	9.70 ± 0.54*,** (−19.8%)	0.45 ± 0.02 (−11.8%)

tal circulation to the liver where they may be metabolized prior to release into the systemic circulation. Some drugs (desipramine, propranolol) are extensively degraded by the liver prior to gaining access to the general circulation while others (diazepam, digitoxin) escape relatively unscathed[8]. Our results suggest that MDMA falls into the latter category.

Regarding the effects of MDMA on other neurotransmitter systems, there are conflicting reports as to whether or not MDMA alters the concentration of dopamine or norepinephrine on a long-term basis. Depending on the brain region examined, the dose, and time of sacrifice, the concentration of dopamine has been noted to be decreased[3], increased[5,9], or unaffected[10]. Our data indicate that multiple high doses (30 mg/kg) of MDMA, given by either route of administration, produce a small, but significant, depletion of dopamine in the striatum. Hence, dopaminergic neurons appear to be affected by MDMA, but are less vulnerable to its toxic effects than serotonergic neurons.

With respect to noradrenergic neurons, previous reports indicate that repeated high doses (40 mg/kg) of MDMA cause a slight reduction in the concentration of norepinephrine in the rat frontal cortex and hippocampus[3,5]. However, in the present study no significant alteration in the concentration of norepinephrine in the hippocampus was observed. These discrepant findings may be explained by the fact that a lower dose of MDMA was employed in the present study. Additional studies are needed to clarify the actions of MDMA on noradrenergic neurons.

Initial studies examining the effects of MDMA in experimental animals have employed conditions quite different from those under which humans typically ingest the drug (1.7–2.7 mg/kg in one or two doses)[1]. In particular, differences in doses, routes and schedules of drug administration, as well as variations between species in the pathways of amphetamine metabolism, have limited extrapolation of the findings in experimental animals to humans. However, the present study, as well as several other recent studies, have started addressing these differences bridging the gap between experimental investigations and human abuse patterns. For example, this study indicates that the neurotoxic effects of orally administered MDMA are comparable to those observed after its s.c. injection. Other investigators[3,5] have recently demonstrated that single parenteral doses of MDMA are also effective in producing long-term depletions of serotonin in rat brain. In addition, persistent MDMA-induced depletions of serotonin have now been observed in the guinea pig[3], a species that metabolizes amphetamines in a manner similar to humans[2]. Taken together, these studies bring the conditions under which toxicity is observed in animals closer to the patterns of self-administration typically employed by humans. Future studies will need to address the question of whether or not the toxicity of MDMA generalizes to primates and if it occurs at doses used by humans.

A number of mental health specialists have advocated the use of MDMA as an adjunct in psychotherapy, and have opposed the placement of this compound on Schedule I of Controlled Substances[4]. The results of the present and other recent studies indicate that the neurotoxic properties of this agent occur in animals under conditions which more closely parallel the pattern of use in humans, suggesting that humans using this psychoactive drug should be wary of its potential neurotoxic effects.

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