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Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration

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This study compared the toxic effects of oral versus subcutaneous and single versus multiple doses of 3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in non-human primates. Orally administered MDMA was approximately one-half as effective as subcutaneously administered drug. Multiple doses were more effective than single doses, but a single 5 mg/kg dose of MDMA given orally still produced a long-lasting depletion of serotonin in the monkey brain. These results indicate that when MDMA is given to monkeys in a manner similar to that employed by humans, it exerts toxic effects on central serotonergic neurons. This suggests that humans using MDMA may be at risk for incurring central serotonergic neuronal damage.

Over the fast few years, 3,4-methylenedioxymethamphetamine (MDMA) has become a very popular recreational drug[9,16]. Consequently, there is currently a great deal of interest in delineating the neurotoxic activity of this compound[1]. Recent studies in rats have indicated that MDMA destroys central serotonergic nerve fibers[4,8,10,14,17]. These findings have now been extended to non-human primates[11,12]. In monkeys, MDMA also appears to exert toxic effects on nerve cell bodies in the dorsal, but not median, raphe nucleus[12]. Moreover, monkeys have proved much more sensitive than rats to the toxic effects of MDMA[12], further increasing concern that humans may be at risk for incurring MDMA-induced neurotoxicity.

Although toxic doses of MDMA used in the primate study (2.5–5 mg/kg) approach those typically ingested by humans (1.7–2.7 mg/kg)[2], monkeys were given these doses *subcutaneously* and on *multiple* occasions. By contrast, humans generally self-administer MDMA *orally* and in *single* daily doses[15]. Thus, it is not clear to what extent findings in the monkey can be extrapolated to the human. For this reason, the present study was undertaken to assess the importance of route and frequency of drug administration as variables which might influence the toxic effects of MDMA on central serotonergic neurons in non-human primates.

Fifteen male squirrel monkeys (*Saimiri sciureus*) 500–700 g in weight and 2–3 years of age were used. They were housed individually in steel cages under controlled temperature (22 ± 2 °C) and lighting (12 h cycle) conditions. Animals were maintained on a diet of monkey chow, supplemented with fruit, and were given free access to water. MDMA hydrochloride, dissolved in sterile distilled water at a concentration of 5 mg/ml, was administered on a ml/kg basis to monkeys by either a subcutaneous or orogastric route. Subcutaneous injections were given in the dorsal intercapsular region. Intragastric administration was accomplished by means of oral intubation. Briefly, a pliable size 10 French catheter (cut to a length to reach the stomach) was passed to the stomach through a small hard plastic sheath that kept the jaw

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of the animal open. Location of the tip of the tube in the stomach was confirmed by auscultating a small bolus of air that was passed through the tube. Once this had been done, the appropriate volume of the MDMA hydrochloride solution was delivered. In Experiment 1, MDMA was given twice daily (09.00 and 17.00 h) for 4 consecutive days to 3 monkeys via the subcutaneous route and to 3 monkeys via the oral route. Three control animals were given an equivalent volume of vehicle subcutaneously. In Experiment 2, a single 5 mg/kg oral dose of MDMA was given to 3 monkeys at 09.00 h. Three control animals received an equivalent volume of vehicle. Two weeks after MDMA administration, monkeys were killed under deep ether anesthesia, the brain was removed and regionally dissected over ice. Dissected tissue was wrapped in aluminum foil and stored frozen in liquid nitrogen until assay. Tissue content of serotonin was determined by high-performance liquid chromatography coupled with electrochemical detection using a previously described method[13].

In accord with previous findings[11,12], multiple subcutaneous doses of MDMA produced a large depletion of serotonin in various regions of the monkey brain (Table I). The most severely affected brain regions were the somtosensory cortex, caudate nucleus, putamen and thalamus. Multiple oral doses of MDMA also depleted serotonin, but to a lesser extent (Table I). For example, in the caudate nucleus, oral doses depleted serotonin by 29%, whereas subcutaneous doses depleted it by 86%; in the somatosensory cortex, oral doses depleted serotonin by 58%, whereas subcutaneous doses lowered it by 86%. Thus, depending on brain region, oral doses were approximately one-third to two-thirds as effective as subcutaneous doses. That orally administered MDMA in the primate is less effective than subcutaneously administered drug contrasts with the recent observation that in the rodent, these two routes of administration produce comparable effects[5]. Why the oral route should be less effective in the monkey is not yet known, but may be related to differences in routes of amphetamine metabolism in the two species[3], or to differences in absorption of MDMA from the gastrointestinal tract of the monkey as compared to the rat.

Having determined that multiple oral doses of MDMA are in fact effective in the monkey, a second experiment was performed to explore the effects of a single dose, since humans typically ingest single daily doses, usually weeks apart[15]. Although the effects of a single dose were less pronounced and widespread than those of multiple doses, a single 5 mg/kg dose of MDMA still produced a definite depletion of serotonin in the thalamus and hypothalamus of the monkey two weeks later (Table II). This suggests that monkeys, when given MDMA in a manner closely resembling that used by humans, sustain central serotonergic neuronal damage.

The 5 mg/kg oral dose of MDMA used in the last experiment is only 2–3 times higher than that usually taken by humans (1.7–2.7 mg/kg)[2]. This could be taken to mean that humans still have a margin of safety, albeit a narrow one. However, this assumes

TABLE I

Oral versus subcutaneous MDMA in the primate

Treatment	*Serotonin concentrations in various brain regions*						
	Somatosensory cortex	*Frontal cortex*	*Caudate*	*Putamen*	*Hippocampus*	*Hypothalamus*	*Thalamus*
Control ($n = 3$)	0.14 ± 0.01	0.12 ± 0.03	0.21 ± 0.03	0.28 ± 0.02	0.13 ± 0.03	0.90 ± 0.06	0.73 ± 0.01
MDMA, s.c. ($n = 3$)	0.02 ± 0.01* (–86%)	0.03 ± 0.01* (–75%)	0.03 ± 0.01* (–86%)	0.03 ± 0.01* (–90%)	0.03 ± 0.01* (–77%)	0.23 ± 0.01* (–75%)	0.12 ± 0.01)* (–84%)
MDMA, p.o. ($n = 3$)	0.06 ± 0.01** (–58%)	0.07 ± 0.01** (–42%)	0.15 ± 0.01** (–29%)	0.19 ± 0.01** (–33%)	0.07 ± 0.01** (–47%)	0.56 ± 0.03** (–38%)	0.28 ± 0.07** (–62%)

*$P < 0.05$, determined by individual comparison to control after a simple one-way analysis of variance (ANOVA) showed F value $P < 0.05$.

**$P < 0.05$, determined by comparison to control and MDMA-s.c. after a simple ANOVA showed F value $P < 0.05$.

TABLE II

Single versus multi

Control ($n = 3$)

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TABLE II

Single versus multiple doses of MDMA in the primate

	Serotonin concentrations in various brain regions					
	Frontal cortex	*Hippocampus*	*Hypothalamus*	*Thalamus*	*Putamen*	*Caudate*
Control ($n = 3$)	0.12 ± 0.03	0.11 ± 0.01	0.85 ± 0.04	0.72 ± 0.02	0.28 ± 0.02	0.22 ± 0.02
MDMA Multiple ($n = 3$)	0.07 ± 0.01*	0.07 ± 0.01*	0.56 ± 0.03*	0.28 ± 0.07*	0.19 ± 0.01*	0.15 ± 0.01*
MDMA Single ($n = 3$)	0.12 ± 0.02	0.13 ± 0.02	0.71 ± 0.03**	0.57 ± 0.07**	0.20 ± 0.02	0.22 ± 0.02

*$P < 0.05$, determined by individual comparison to control after a simple one-way analysis of variance (ANOVA) showed F value $P < 0.05$.

**$P < 0.05$, determined by comparison to control and *MDMA multiple* after a simple ANOVA showed F value $P < 0.05$.

that humans and monkeys are equally sensitive to the toxic effects of MDMA. The validity of this assumption is questionable in light of the fact that humans are generally more sensitive than monkeys to the toxic effect of drugs. For example, in the case of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), humans are 3–5 times more sensitive than monkeys to the parkinsonogenic effects of this drug (cf. refs. 6 and 7). If a similar relationship holds for MDMA, it could mean that humans may be at risk for sustaining central serotonergic neuronal damage even when ingesting a typical 'moderate' oral dose of MDMA.

In view of these findings, we believe it is critical to determine if MDMA is toxic to central serotonergic neurons in humans. One way of addressing this important question is to first determine if 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) of monkeys can serve as marker for serotonergic neurotoxicity induced by MDMA. If 5-HIAA in the CSF proves to be an accurate marker, the current study suggests that CSF studies in humans previously exposed to MDMA are warranted. Such studies could have major public health implications.

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