Studies of MDMA-Induced Neurotoxicity in Nonhuman Primates: A Basis for Evaluating Long-Term Effects in Humans

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INTRODUCTION

Studies of (±)3,4-methylenedioxymethamphetamine (MDMA) neurotoxicity in nonhuman primates are potentially of great importance to both basic science and public health. Scientifically, such studies could shed light on the functional role of serotonin in the primate central nervous system (CNS). In this regard, it is pertinent to recall that it was not until the effects of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were explored in the monkey that its profound dopaminergic neurotoxic effects were noted (Chiueh et al. 1983) and then utilized to develop the first complete animal model of Parkinson's disease (Burns et al. 1983). Given this precedent, it seems not unreasonable to speculate that studies of MDMA in the primate could similarly enhance our understanding of serotonergic function in higher animals. From a public health perspective, MDMA studies in monkeys are of value because they will help to define the risk that MDMA poses to humans. Additionally, these studies could help identify functional consequences of MDMA neurotoxicity in primates, and thus guide the clinical assessment of MDMA-exposed individuals.

This chapter will review some recently completed studies on the long-term effects of MDMA in nonhuman primates. The goals of these studies were to (1) determine if the neurotoxic effects of MDMA, which have been well documented in the rodent (see below), generalize to the primate; (2) compare the relative sensitivity of primates and rodents to the neurotoxic effects of MDMA; (3) ascertain if the toxic effects of MDMA in the monkey are restricted to nerve fibers (as they are in the rat), or if they involve cell bodies as well; (4) evaluate how closely toxic doses of MDMA in the monkey approximate those used by humans; and (5) examine whether 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) can be used to detect MDMA-induced serotonergic damage in the CNS of primates. Before presenting the results of these studies, previous results in the

rodent will be briefly summarized, so as to put findings in the primate in proper perspective.

PRIOR FINDINGS IN RODENTS

Rats given MDMA show prolonged reductions in the concentration of brain serotonin (Schmidt 1987; Commins et al. 1987; Stone et al. 1986; Battaglia et al. 1987; Mokler et al. 1987; Ricaurte et al. 1987), the number of serotonin uptake sites (Schmidt 1987; Battaglia et al. 1987; Commins et al. 1987), the level of 5-HIAA (Mokler et al. 1987; Stone et al. 1986), and the activity of tryptophan hydroxylase (TPH) (Stone et al. 1986). Correlative anatomical studies indicate that these neurochemical changes are due to damage of serotonergic axons (O'Hearn et al. 1988). Cell bodies in the brainstem of the rodent do not appear to be damaged by MDMA. Serotonin-containing perikarya in the raphe nuclei of rats have a normal cytological appearance and show no obvious reduction in number (Molliver 1987; O'Hearn et al. 1988). In guinea pigs, MDMA produces long-term neurochemical effects similar to those in rats (Commins et al. 1987). This is noteworthy because guinea pigs (like humans) metabolize amphetamine primarily by side-chain deamination, whereas rats do so mainly by ring hydroxylation (Caldwell et al. 1976). In contrast to guinea pigs and rats, mice do not develop long-term depletions of serotonin after MDMA, even after high doses (Stone et al. 1987). This provocative finding raises the important question of whether other animals might not also be resistant to MDMA's neurotoxic effects. In this regard, monkeys are of special interest because of their close phylogenetic relationship to humans and because they metabolize amphetamine in a manner similar to humans (Caldwell et al. 1976). For these reasons, as well as for those previously mentioned, studies were undertaken to evaluate the neurotoxic potential of MDMA in nonhuman primates.

OBSERVATIONS IN PRIMATES

Studies were performed in the squirrel monkey (*Saimiri sciureus*). This primate species was selected because of its size, availability, and prior use in neurotoxicity studies (Langston et al. 1984). Initial dose-response determinations were carried out using the following doses of MDMA: 2.50, 3.75, and 5.00 mg/kg. Each dose of MDMA was administered subcutaneously twice daily (at approximately 0800 and 1700 hours each day) for 4 consecutive days. This particular regimen of drug administration was employed because its prior extensive use in the rat (Commins et al. 1987; Battaglia et al. 1987; Ricaurte et al. 1987) would permit comparison of results in the monkey with those in the rat. Two weeks after drug treatment, the animals were killed, the brains were removed, dissected, and then analyzed for their regional content of serotonin, dopamine, and noradrenaline using the method of Kilpatrick et al. (1986), as previously described (Ricaurte et al. 1988a).

MDMA produced a dose-related depletion of serotonin without altering the concentration of either dopamine or norepinephrine in the monkey brain (table 1). Even the lowest dose of MDMA produced a substantial depletion

TABLE 1. *Selective dose-related depletion of serotonin in cerebral cortex of monkeys administered MDMA 2 weeks previously*

Treatment	5-HT	DA	NE
Saline	0.167 ± 0.015	10.4 ± 0.5	0.39 ± 0.03
MDMA - 2.50 mg/kg	0.093 ± 0.010* (-44%)	NT**	NT
MDMA - 3.75 mg/kg	0.037 ± 0.013* (-78%)	NT	NT
MDMA - 5.00 mg/kg	0.017 ± 0.003* (-90%)	9.7 ± 0.8 (ns)	0.41 ± 0.02 (ns)

*p<0.05, determined by individual comparison to control after one-way analysis of variance showed F value p<0.05.

**NT=not tested because higher dose was without effect.

NOTE: Values in μg/g represent the mean ± SEM (n=3).

of serotonin in the cerebral cortex of the monkey (-44 percent). MDMA also reduced the concentration of cortical 5-HIAA (table 2). Reduced levels of serotonin and 5-HIAA were evident not only in the cerebral cortex, but also in the caudate nucleus (-86 percent), hippocampus (-77 percent), hypothalamus (-77 percent), thalamus (-84 percent), and putamen (-90 percent) (table 3). Anatomical studies were subsequently carried out in collaboration with Dr. Mark Molliver and Marianne Wilson of the Johns Hopkins University School of Medicine to determine if there was a structural basis for the serotonin and 5-HIAA depletions induced by MDMA. These morphological studies showed that there was a marked reduction of serotonin-immunoreactive axons in the monkey forebrain (figure 1), and that, at high power, some of the remaining axons appeared swollen and misshapen (Wilson et al. 1988). Coupled with the biochemical observations, these morphological findings suggest that MDMA produces neurochemical deficits by damaging serotonergic axons. Further, they demonstrate that the long-term effects of MDMA originally documented in the rodent generalize to the primate.

TABLE 2. *Decreased concentration of 5-HIAA in the monkey brain 2 weeks after MDMA (5 mg/kg)*

	N	Neocortex	Caudate	Hippocampus	Hypothalamus
Control	3	0.250 ± 0.010	0.232 ± 0.005	0.245 ± 0.006	0.897 ± 0.042
MDMA	3	0.040 ± 0.006*	0.056 ± 0.002*	0.060 ± 0.001*	0.543 ± 0.084*

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± standard error of the mean.

TABLE 3. *Regional concentrations of serotonin in the monkey brain 2 weeks after MDMA (5 mg/kg)*

Somatosensory Cortex	Caudate	Putamen	Hippocampus	Hypothalamus	Thalamus
		CONTROL (n=3)			
0.14 ± 0.01	0.21 ± 0.03	0.28 ± 0.02	0.13 ± 0.03	0.90 ± 0.06	0.73 ± 0.01
		MDMA (n=3)			
0.02 ± 0.01*	0.03 ± 0.01*	0.03 ± 0.01*	0.03 ± 0.01*	0.21 ± 0.01*	0.12 ± 0.01*

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± standard error of the mean.

RELATIVE SENSITIVITY: PRIMATES VS. RODENTS

Next, the relative sensitivity of monkeys and rats to the serotonin-depleting effects of MDMA was evaluated. This was done by comparing dose-response data in these two experimental animals. In the monkey, a 2.5 mg/kg dose regimen of MDMA produced a 44 percent depletion of serotonin (figure 2). By contrast, in the rat, a 10 to 20 mg/kg dose regimen of MDMA was required to produce a comparable effect. Thus, monkeys are 4 to 8 times more sensitive than rats to the serotonin-depleting effects of MDMA. Inspection of the data in figure 2 also showed that the dose-effect curve of MDMA in the monkey is much steeper than in the rat. Consequently, small increments in dose cause large increases in serotonin depletion in the monkey but not in the rat. Clearly, this could have serious implications for humans experimenting with higher doses of MDMA, as it suggests that the margin of safety of MDMA in primates is narrow.

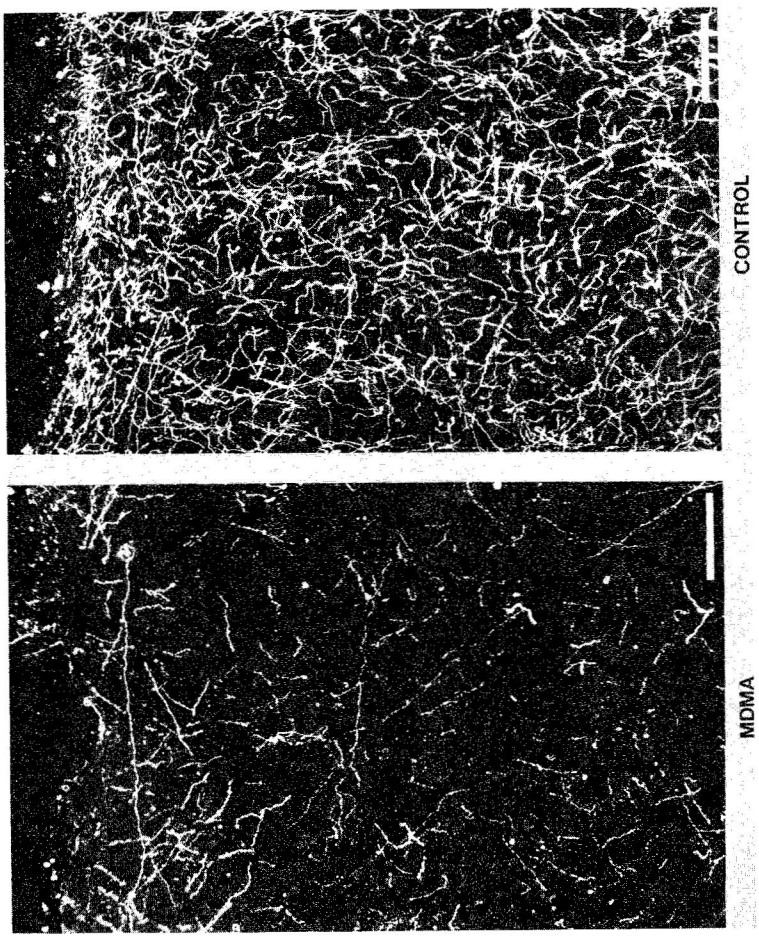

FIGURE 1. *Marked reduction of serotonin-immunoreactive axons in the somatosensory cortex of MDMA-treated monkey*

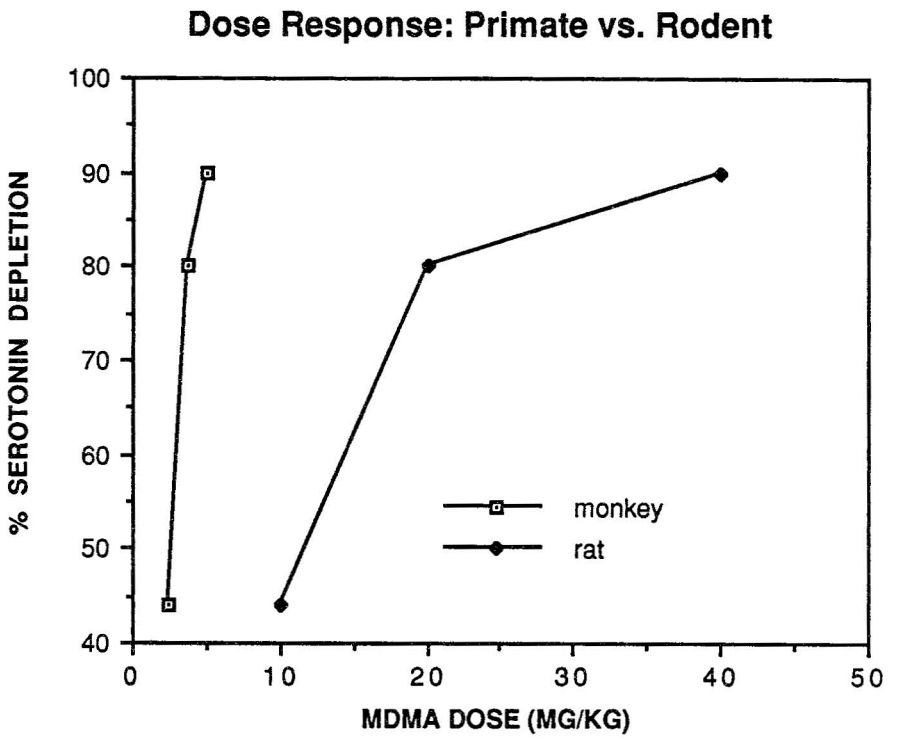

FIGURE 2. *Dose-response data in rats and monkeys administered MDMA 2 weeks previously*

INVOLVEMENT OF NERVE CELL BODIES

The severity of the axonal damage caused by MDMA in the forebrain of the monkey raised the question of whether cell bodies in this experimental animal might also be damaged by MDMA. As noted above, cell body damage does not occur in the rat (O'Hearn et al. 1988). However, it is to be recalled that in the case of MPTP, it was not until it was tested in the monkey that MPTP's toxic effects on cell bodies were appreciated (Chiueh et al. 1983; Langston et al. 1984). To determine if this was also the case for MDMA, the brainstem of monkeys treated with the high dose (5 mg/kg) regimen of the drug 2 weeks previously was examined histologically. MDMA-treated monkeys showed no obvious cell loss in either the dorsal or median raphe nuclei. However, there were clear cytopathologic changes in nerve cells of the dorsal (but not median) raphe nucleus. Specifically, nerve cells in the dorsal raphe nucleus appeared shrunken and contained brownish-red intracytoplasmic spherical inclusions, which were acid fast in Ziehl-Nielsen stain for lipofuscin, granular in LFB-PAS-stained sections, and

vividly PAS positive. While the significance of these inclusions remains unclear, their staining characteristics suggested the presence of an increased amount of lipofuscin. More recent studies investigating the fate of these inclusion-bearing nerve cells have suggested that they do not die, and that their survival is associated with partial recovery of serotonin in the monkey forebrain (Ricaurte et al. 1988c). Similar recovery of serotonin has been noted in MDMA-treated rats (Battaglia et al. 1988). It remains to be determined if recovery of serotonin is related to regeneration of serotonergic axons, and if the new axons innervate their original targets or form aberrant synaptic contacts.

RELEVANCE TO HUMANS

Clearly, much of the impetus for investigating the neurotoxic effects of MDMA in animals has come from concern that MDMA may produce similar toxic effects in humans. However, the extent to which findings in animals can be extrapolated to humans has been unclear, largely for three reasons: First, in most animal studies, MDMA has been administered subcutaneously or intraperitoneally (Schmidt 1987; Commins et al. 1987; Battaglia et al. 1987; Mokler et al. 1987), even though humans invariably take the drug orally (Seymour 1986). Second, most animal studies have used multiple doses of MDMA, and these have been given over relatively short periods of time (Commins et al. 1987; Battaglia et al. 1987; Mokler et al. 1987). By contrast, humans typically take single doses of MDMA, usually weeks apart (Seymour 1986). Third, doses of MDMA tested in animals have often far exceeded those used by humans.

In an effort to bridge the gap between studies of MDMA in animals and human MDMA use patterns, the following studies were performed. These studies tested the importance of dose, route, and schedule of drug administration as determinants of MDMA neurotoxicity. In the first experiment, one group of monkeys received MDMA (5 mg/kg twice daily for 4 days) subcutaneously; another group received an identical dosage regimen of the drug orally. The animals were killed 2 weeks later, and regional brain serotonin concentrations were determined. Monkeys given oral MDMA showed depletions of serotonin that, depending on brain region, ranged from one-third to two-thirds of those found in monkeys given the drug subcutaneously (table 4). Recently, similar results have been obtained in rhesus monkeys (Kleven et al. 1989). Taken together, the results of these studies indicate that the oral route of administration does not afford significant protection against the long-term effects of MDMA on serotonin neurons.

A second study compared the effects of single versus multiple doses. One group of monkeys received a single 5 mg/kg dose of MDMA orally; another group received the same dose by the same route, but on a twice daily basis for 4 days. As before, the multiple dose regimen produced a

large depletion of serotonin in all forebrain regions examined (table 5). By contrast, the single dose produced a depletion of serotonin only in the thalamus and hypothalamus. In both brain regions, the depletion was smaller than that produced by the multiple dose regimen, but achieved statistical significance. The long-term effects of even single doses of MDMA further attest to the high sensitivity of the primate to the serotonin-depleting effects of MDMA. Further, they raise the question of whether humans might be similarly affected, particularly since they take doses that are only 2 to 3 times lower than the dose that produce an effect in the monkey (1.7 to 2.7 vs. 5.0 mg/kg).

STUDIES OF CSF 5-HIAA

Detecting a depletion of serotonin in the brain of a living human poses a major challenge. To date, only two methods have been attempted. The first involve measurement of 5-HIAA in the CSF (Garelis et al. 1974; Moir et al. 1970); the second calls for neuroendocrine challenge with various serotonergic agents (Cowen and Anderson 1976; Heninger et al. 1984). Unfortunately, both of these methods are indirect, and neither has been fully validated. Accordingly, it was necessary to test the usefulness of CSF 5-HIAA as a marker of MDMA neurotoxicity in the monkey before attempting to use it on humans.

To validate the CSF 5-HIAA method in the monkey, three monkeys were given MDMA at a dose that produces extensive serotonergic damage (5 mg/kg twice daily for 4 days, SC); three other age- and sex-matched animals were given saline and served as controls. Two weeks later, all of the animals were lightly anesthetized with ether, and 200 to 300 µL of CSF were removed by cervical puncture. Later that same day, all animals were killed for determination of regional CNS and CSF serotonin and 5-HIAA levels. These measurements showed that MDMA lowered the concentration of 5-HIAA in the CSF but not that of homovanillic acid (HVA) or 3-methoxy-4-hydroxyphenylene-glycol (MHPG) (table 6). The reduction of CSF 5-HIAA was associated with a marked depletion of serotonin in the CNS (table 7). The decrease in 5-HIAA in cervical CSF was smaller than the depletion of serotonin in the forebrain (59 percent vs. 90 percent), but greater than the depletion of serotonin in the cervical spinal cord (45 percent vs. 59 percent) (Ricaurte et al. 1988b). Hence, cervical CSF 5-HIAA underestimates serotonin depletion in the forebrain, but overestimates serotonin depletion in the cervical spinal cord. These results indicated that while 5-HIAA in CSF does not fully reflect the depletion of serotonin in the forebrain, it can serve as a partial indicator of serotonergic damage induced by MDMA in the forebrain of primates.

TABLE 4. *Effect of oral vs. subcutaneous MDMA on regional brain serotonin in the primate 2 weeks later*

	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 - SC (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 - PO (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-SC after a simple ANOVA showed F value p<0.05.

TABLE 5. *Effect of single vs. multiple doses of MDMA on regional brain serotonin in the primate 2 weeks later*

	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*
DMA 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.

TABLE 6. *Selective reduction in 5-HIAA in CSF of monkeys administered MDMA 2 weeks previously*

Treatment	N	5-HIAA	HVA	MHPG
Saline	3	101 ± 7	255 ± 44	30 ± 4
MDMA	3	41 ± 7*	264 ± 20	40 ± 11

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± SEM (expressed in μg/mg of tissue).

TABLE 7. *Selective reduction in serotonin in the caudate nucleus of monkeys administered MDMA 2 weeks previously*

Treatment	N	Serotonin	Dopamine	Norepinephrine
Saline	3	0.218 ± 0.023	11.6 ± 0.9	2.59 ± 0.18
MDMA	3	0.021 ± 0.005*	9.7 ± 0.2	2.84 ± 0.91

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± SEM (expressed in μg/mg of tissue).

CSF 5-HIAA STUDIES IN HUMANS

In light of this, studies of CSF 5-HIAA have been initiated in a cohort of human volunteers with a history of extensive MDMA use. Most participants in the study are individuals who have recently learned of the neurotoxic properties of MDMA and have asked to be evaluated for possible serotonergic damage. To qualify for the study, subjects must (1) have used MDMA on at least 20 to 25 occasions, (2) be drug-free for at least 2 weeks prior to participating in the study, and (3) not have a history of neuropsychiatric illness thought to involve alterations in serotonin metabolism. To date, 34 individuals have participated in the study. The study is now in progress, and completion is anticipated by 1991. At this time, it would be premature to comment on the results.

NEUROENDOCRINE STUDIES

As noted earlier, the only other method presently available for detecting serotonergic dysfunction in living humans involves neuroendocrine challenge with serotonin-active drugs (Cowen and Anderson 1976). One such

neuroendocrine test is the L-tryptophan challenge test (Heninger et al. 1984). Briefly, this test calls for intravenous administration of L-tryptophan to human subjects with subsequent measurement of serum prolactin concentration. A rise in serum prolactin is taken as a measure of central serotonergic activity. Using the L-tryptophan challenge tests, serotonergic function was recently evaluated in nine MDMA subjects in collaboration with Drs. Price and Heninger of the Yale University School of Medicine. L-tryptophan induced a robust rise in serum prolactin in controls but not in MDMA subjects (figure 3). The peak change in serum prolactin concentration and the area under the prolactin response curve were diminished in MDMA subjects, but the difference was not statistically significant (Price et al. 1989). Additional studies are now in progress to assess the significance of these findings.

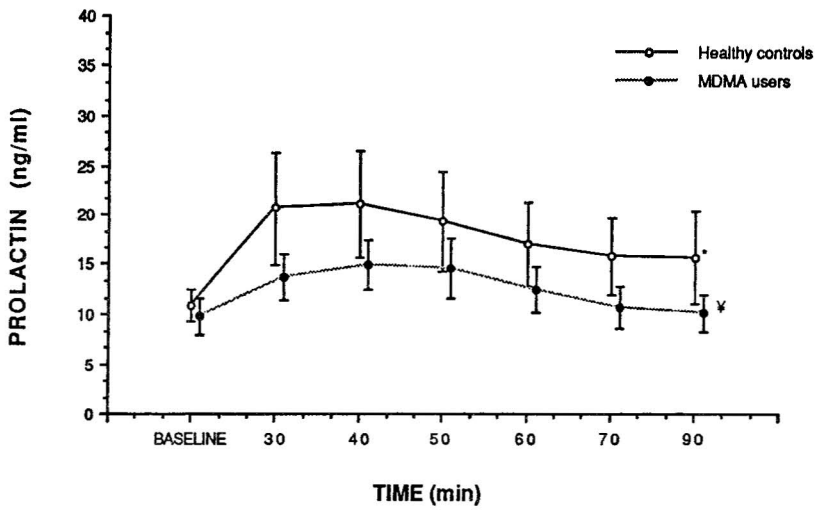

FIGURE 3. *Prolactin response to IV L-tryptophan in control and MDMA subjects (n=9)*

SUMMARY AND CONCLUSION

The results of the studies reviewed here show that the neurotoxic effects of MDMA generalize to the primate. Further, they indicate that monkeys are considerably more sensitive than rats to the serotonin-depleting effects of MDMA, and that the dose-response curve of MDMA in the monkey is much steeper than in the rat. Perhaps as a consequence of this, the toxic effects of MDMA in the monkey involve serotonergic nerve fibers as well as cell bodies, whereas in the rat, only nerve fibers are affected. The present studies also show that the toxic dose of MDMA in the monkey

(5 mg/kg) closely approaches the dose typically used by humans (1.7 to 2.7 mg/kg). This finding heightens concern that MDMA may be neurotoxic in humans, particularly since the steepness of the dose-response curve of MDMA in the primate suggests a narrow margin of safety. Finally, preclinical studies in monkeys have shown that CSF 5-HIAA can be used to detect MDMA-induced serotonergic damage in the primate CNS. Studies now underway in MDMA-exposed humans should help determine if MDMA exerts long-term toxic effects on serotonergic neurons in the human brain.

DISCUSSION

QUESTION: How long after the administration of the MDMA in your human subjects did you measure your parameter?

ANSWER: The request we made to the subjects was that they remain entirely drug-free for 2 weeks. We were trying to simulate the situation that we had in animals.

QUESTION: How long after the administration of the drug did you measure the 5-HIAA?

ANSWER: We did not administer MDMA. We were dealing with humans who had been previously exposed to MDMA. The request we made was that they not take the drug for at least 2 weeks. Some subjects had not taken the drug for over a year. Some had taken it as recently as 19 days.

QUESTION: Is there a decrease in serotonin metabolites, and does it show any relationship to age, cumulative dose, and so forth? Did you see anything there? You have that one person who had 42 grams. Was he any more or any less affected?

ANSWER: We are in the midst of analyzing the data. You will remember that the mean 5-HIAA level in control subjects is approximately 19 to 20. In that one individual, 5-HIAA level turned out to be 14 or 13. You might predict that he would have been the lowest one on the scale. I subsequently learned the importance of body height, and he happens to be a very short, stocky man. So we are now in the process of reanalyzing the data, trying to take in the appropriate variables into account. And, again, these are studies that have to be replicated in our own hands and extended by others.

QUESTION: Do you see a progressive decline as a function of age, and if you have someone who has been damaged, is there any age relationship in this depletion?

ANSWER: No. Only one individual was 70 or 71 years old. By and large, the individuals are younger than 40. The other side of the coin, of

course, is does 5-HIAA really change with age in the control population? That was one of the variables that I listed. I would emphasize that the data for that are actually very weak. My guess is that there are no age-related changes in 5-HIAA, at least out to 50 to 60 years of age. But that is something we are going to have to contend with as well.

QUESTION: You showed CSF levels in monkeys after the 4-day treatment regimen, but you also showed neurochemical data after a single oral administration. Did you have the opportunity to look at the CSF levels after a single oral administration?

ANSWER: No. That study was actually completed before the CSF studies. We did not get CSF data on the animals that received the single dose because that study was done before the CSF studies were undertaken.

COMMENT: With regard to the CSF levels, I want to emphasize that the decreases that you get are probably a gross underestimate, as was pointed out. Another reason is that the ventricular plexus of serotonin fibers is completely unaffected, and that is probably a very large source. I do not know to what extent that contributes to CSF levels of 5-HT and 5-HIAA, but those fibers are in the CSF and bathing in it, and they appear to be quite active. So they must be biasing against your seeing an effect. The fact that you are getting such a sizable effect must mean that there is a very profound depletion in the forebrain.

QUESTION: Does anything suggest that people who have taken these drugs or MDMA for a period of time are subject to episodes of depressive disorders or affective disorders?

ANSWER: Frankly, at this point, we have only anecdotal evidence. And as Dr. Schuster mentioned yesterday, people's responses can be very misleading. I could cite three individuals who attribute some mood disturbances to their prior MDMA use, but one wonders how much their reports are based on what you want to hear.

One individual was prescient enough to realize that his depression coincided with loss of his job so he did not know if his depression was related to losing his job or to MDMA ingestion. I think these people are going to need to be looked at by people who know what they are doing in terms of analyzing depression, and that has not been done.

COMMENT: It is interesting in that letter that George Greer wrote to me informally, off the record, that he had seen 10 patients in psychotherapy who had been treated extensively with fenfluramine for dieting. And, after several weeks on fenfluramine, they became very depressed, and two of them committed suicide. So that is a very serious consideration.

RESPONSE: Another interesting consideration is that a number of the subjects have participated with the intent of helping the rest of the country see that this is not such a harmful drug. A number of the proponents of MDMA use the paucity of behavioral abnormalities in MDMA users to point to the fact that literally thousands of subjects have used the drug, and that they are not walking around like zombies; they do not appear to be harmed.

My answer always is that no one has yet done a detailed neurobehavioral study of these individuals and the deficit that they may have. It may be very subtle in nature, and I am not sure that we have the methods available to detect and quantify those deficits. The fact that these people are not walking in with overt behavioral disturbances as the people with MPTP did, I think, is related to the fact that, one, they may not have the kind of neurotoxicity we are suspecting, and two, if they do, the kind of functional consequences that you may get from serotonergic dysfunction may be much more subtle than the kind of functional consequences you get with dopamine dysfunction, where it is very easy to recognize the parkinsonian patient.

QUESTION: Do you have any plans to study whether it would be possible to eliminate the large variation in dosage level and frequency and duration since the last dose by studying fenfluramine, where patients are receiving prescribed doses every day for finite periods of time? Perhaps one could set up a study where you sample CSF before and after the therapy so that you would avoid any concern about whether you had selected a group of patients who had low 5-HIAA levels.

ANSWER: Yes, that is one of the groups that we would hope to incorporate.

COMMENT: I am not an advocate of this view, but my colleague Efrain Azmitia from NYU has suggested that perhaps rather than seeing deficits, that pruning our serotonin projections now and then might be a very advantageous and beneficial thing to do.

RESPONSE: I am not going to try to follow up on that one.

QUESTION: We are used to seeing a lot of those big beaded axons which we, after methamphetamine, have interpreted as damage. Do you think it is what is left over rather than big axons that are damaged?

ANSWER: Yes. We have very carefully evaluated that question and Dr. O'Hearn is a great skeptic who is forcing us to look at it very closely. We are finding that the large varicosity fibers that are left are identical in distribution, morphology, and density to those present in the normal fibers. The damaged fibers are of a completely different nature; they are 10 times

as big, fragmented, and very, very damaged. They can be easily distinguished.

QUESTION: So you get both?

ANSWER: Yes.

REFERENCES

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* 29:269-274, 1988.

Battaglia, G.; Yeh, S.Y.; O'Hearn, E.; Molliver, M.E.; Kuhar, M.J.; and DeSouza, E.B. 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. *J Pharmacol Exp Ther* 242(3):911-916, 1987.

Burns, R.S.; Chieuh, C.C.; Markey, S.P.; Ebert, M.H.; Jacobowitz, D.M.; and Kopin, I.J. A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* 80:4546-4550, 1983.

Caldwell, J.; Dring, L.G.; and Williams, R.T. Metabolism of [14C]methamphetamine in man, the guinea pig and the rat. *Biochem J* 129:11-21, 1976.

Chiueh, C.C.; Markey, S.P.; Burns, R.S.; Johannessen, J.N.; Jacobowitz, D.M.; and Kopin, I.J. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a parkinsonian syndrome causing agent in man and monkey, produces different effects in the guinea pig and rat. *Pharmacologist* 25:131-138, 1983.

Commins, D.L.; Vosmer, G.; Virus, R.; Woolverton, W.; Schuster, C.; and Seiden, L. Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. *J Pharmacol Exp Ther* 241:338-345, 1987.

Cowen, P.J., and Anderson, I.M. 5HT Neuroendocrinology: Changes during depressive illness and antidepressant drug treatment. In: Deakin, J.F., and Freeman, H., eds. *Advances in the Biology of Depression.* London: Royal College of Psychiatry, 1976.

Garelis, E.; Young, S.N.; Lal, S.; and Sourkes, T.L. Monoamine metabolites in lumbar CSF: The question of their origin in relation to clinical studies. *Brain Res* 79:1-8, 1974.

Heninger, G.; Charney, D.S.; and Sternberg, D.E. Serotonergic function in depression: Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. *Arch Gen Psychiatry* 41:398-402, 1984.

Kilpatrick, I.; Jones, M.; and Phillipson, 0. A semiautomated analysis method for catecholamines, indoleamines, and some prominent metabolites in microdissected regions of the nervous system: An isocratic HPLC technique employing coulometric detection and minimal sample preparation. *J Neurochem* 46(6):1865-1876, 1986.

Kleven, M.S.; Woolverton, W.L.; and Seiden, L.S. Evidence that both intragastric and subcutaneously administered MDMA produce 5HT neurotoxicity in rhesus monkeys. *Brian Res* 488:121-125, 1989.

Langston, J.W.; Forno, L.S.; Rebert, C.S.; and Irwin, I. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) in the squirrel monkey. *Brain Res* 292:390-394, 1984.

Moir, A.T.; Ashcroft, G.W.; Crawford, T.B.; Eccleston, D.; and Guldbert, H.C. Cerebral metabolites in cerebrospinal fluid as a biochemical approach to the brain. *Brain* 93:357-368, 1970.

Mokler, D.J.; Robinson, S.E.; and Rosencranz, J.A. 3,4-Methylenedioxyamphetamine produces long-term reductions in brain 5-hydroxytryptamine in rats. *Eur J Pharmacol* 138:265-268, 1987.

Molliver, M.E. Serotonergic neural systems: What their anatomic organization tells us about function. *J Clin Psychopharmacol* 7:3-23, 1987.

O'Hearn, E.G.; Battaglia, G.; De Souza, E.B.; Kuhar, M.J.; and Molliver, M.E. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause ablation of serotonergic axon terminals in forebrain: Immunocytochemical evidence. *J Neurosci* 8:2788-2803, 1988.

Price, L.H.; Ricaurte, G.A.; Krystal, J.H.; and Heninger, G.R. Neuroendocrine and mood responses to intravenous L-tryptophan in 3,4-methylenedioxymethamphetamine (MDMA) users. *Arch Gen Psychiatry* 46:20-22, 1989.

Ricaurte, G.A.; Finnegan, K.T.; Nichols, D.E.; DeLanney, L.E.; Irwin, I.; and Langston, J.W. 3,4-Methylenedioxyethylamphetamine (MDE), a novel analog of MDMA, produces long-lasting depletion of serotonin in the rat brain. *Eur J Pharmacol* 137:265-268, 1987.

Ricaurte, G.A.; DeLanney, L.E.; Irwin, I.; and Langston, J.W. Toxic effects of 3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in the primate: Importance of route and frequency of drug administration. *Brain Res* 446:165-168, 1988a.

Ricaurte, G.A.; DeLanney, L.E.; Wiener, S.G.; Irwin, I.; and Langston, J.W. 5-Hydroxyindoleacetic acid in cerebrospinal fluid reflects serotonergic damage induced by 3,4-methylenedioxymethamphetamine in CNS of non-human primates. *Brain Res* 474:359-363, 1988b.

Ricaurte, G.A.; Forno, L.S.; Wiener, S.G.; DeLanney, L.E.; Irwin, I.; and Langston, J.W. Effects of MDMA on central serotonergic neurons in non-human primates: Permanent or transient? *Abstr Soc Neurosci* 14:558, 1988c.

Ricaurte, G.A.; Forno, L.S.; Wilson, M.A.; DeLanney, L.E.; Irwin, I.; Molliver, M.E.; and Langston, J.W. MDMA selectively damages central serotonergic neurons in the primate *JAMA* 260:51-55, 1988d.

Schmidt, C.J. Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. *J Pharmacol Exp Ther* 240:1-7, 1987.

Seymour, R.B. *MDMA*. San Francisco, CA.: Haight Ashbury Publications, 1986.

Stone, D.M.; Hanson, G.R.; and Gibb, J.W. Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. *Neuropharmacology* 26:1657-1661, 1987.

Stone, D.M.; Stahl, D.S.; Hanson, G.L.; and Gibb, J.W. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine on monoaminergic systems in the rat brain. *Eur J Pharmacol* 128:41-48, 1986.

Wilson, M.A.; Ricaurte, G.A.; and Molliver, M.E. Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine. *Neuroscience* 28:121-137, 1988.

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