

Neuroendocrine and Mood Responses to Intravenous L-Tryptophan in 3,4-Methylenedioxymethamphetamine (MDMA) Users

Preliminary Observations

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• 3,4-Methylenedioxymethamphetamine (MDMA; "ecstasy") is a selective serotonin (5-HT) neurotoxin in laboratory animals. To assess its effects on 5-HT function in humans, serum prolactin (PRL) and mood responses to intravenous L-tryptophan were measured in nine recreational users of MDMA and compared with findings from nine matched healthy controls. L-Tryptophan induced a rise in the PRL concentration in controls, but not in MDMA users. Peak change and the area under the curve of the PRL response appeared to be blunted in MDMA users, but the difference from controls did not reach statistical significance. This study provides suggestive evidence of altered 5-HT function in MDMA users, but more definitive studies clearly are needed.

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A ring-substituted amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") has serotonin (5-HT)-selective neurotoxic effects in the brains of rats^{1,5} and nonhuman primates.^{6,7} Although classified on Schedule I by the Drug Enforcement Agency since July 1985, MDMA has become popular in some settings as a recreational drug. In an informal survey, up to 40% of undergraduates at a major university reported having used it at least once.⁸ Some clinicians have claimed therapeutic utility for MDMA as an adjunct to psychotherapy, stating that it facilitates interpersonal communication, enhances insight, and increases self-esteem.⁹

We are aware of only one published report on the effects of

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MDMA on 5-HT function in humans. Peroutka et al¹⁰ measured cerebrospinal fluid levels of the 5-HT metabolite 5-hydroxyindoleacetic acid in five recreational MDMA users. They found no significant difference from mean levels in historical control subjects. It is possible, of course, that this sample was too small to detect a difference, or that lumbar cerebrospinal fluid does not sensitively reflect MDMA-induced changes in central 5-HT function in humans.

The neuroendocrine challenge strategy offers a more dynamic means of assessing central 5-HT function. Intravenous infusion of the 5-HT precursor L-tryptophan increases the serum prolactin (PRL) concentration, probably via enhanced synthesis and release of 5-HT from hypothalamic 5-HT neurons.¹¹ The PRL response to L-tryptophan is blunted in depressed patients compared with healthy controls,¹² consistent with other evidence of abnormal 5-HT function in depression.¹³ Many antidepressant drugs, particularly those with demonstrable effects on 5-HT function, enhance the PRL response.¹⁴ Depletion of dietary L-tryptophan also enhances the PRL response, perhaps by a mechanism analogous to denervation supersensitivity.¹⁵ In a pilot study, we compared neuroendocrine and behavioral responses to L-tryptophan in nine heavy users of MDMA with those of matched healthy controls.

SUBJECTS AND METHODS

Nine subjects (seven male, two female; mean [$\pm$ SD] age, 34 ± 7 years; age range, 22 to 47 years) with a current or recent history of substantial MDMA use volunteered to participate. They had been using what they believed to be MDMA for a mean of 5.1 ± 2.3 years (range, two to seven years) at a rate of 1.9 ± 1.7 times per month (range, 0.33 to 5.0 times per month). The average "usual" dose used was 135 ± 44 mg (range, 50 to 200 mg), corresponding to a mean dose of 1.8 ± 0.4 mg/kg (range, 1.1 to 2.3 mg/kg). Many subjects reported the occasional use of much higher doses (up to 500 mg, or 6 mg/kg). The mean cumulative total dose of MDMA was estimated at 13.3 ± 13.4 g (range, 2.5 to 44.2 g). Nine healthy controls (seven male, two female; mean age, 33 ± 8 years; age range, 22 to 48 years),

matched to the MDMA-using subjects for sex and age, were selected from a larger sample of normal volunteers who had undergone testing. Controls were screened for mental disorder and substance abuse by a research psychiatrist using a structured review.

All subjects gave voluntary informed consent and were found to be free of serious medical illness after physical, neurologic, and laboratory evaluations. Among MDMA-using subjects, the last reported use of MDMA was a mean of 66 ± 50 days before testing (range, 20 to 180 days). Both control and MDMA-using subjects were instructed to remain free of psychoactive drugs for at least three weeks before testing, although three MDMA-using subjects admitted to infrequent marijuana use during that time. Testing was conducted on an outpatient basis at the Clinical Neuroscience Research Unit, New Haven, Conn. Control subjects were recruited locally, but MDMA-using subjects flew to New Haven from their previous residences the day before testing.

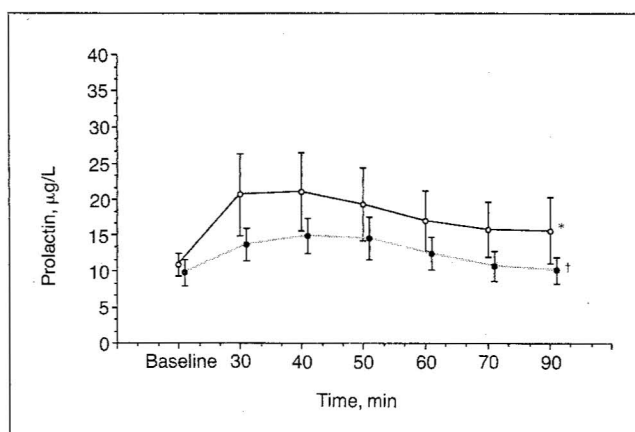
Subjects fasted overnight and throughout the three-hour L-tryptophan test, which began at 9 AM. The test dose consisted of 7 g of L-tryptophan diluted in 500 mL of 0.45% saline solution that was infused through an antecubital vein catheter over 20 minutes. Subjects were awake and supine with the head elevated during the test. Blood for PRL measurement was obtained through the indwelling catheter, which was kept patent by the slow infusion of saline solution. Starting at least 60 minutes after catheter insertion, samples were obtained at 15 and 0.5 minutes before, and at 30, 40, 50, 60, 70, and 90 minutes after the start of the L-tryptophan infusion. Visual analog scales (0 indicates "not at all"; 100 indicates "most ever") and 11 different mood states (happy, sad, drowsy, nervous, calm, depressed, anxious, energetic, fearful, mellow, high) were scored by subjects at these times.

The L-tryptophan infusions were prepared by dissolving 8.4 g of L-tryptophan in 600 mL of a 0.45% saline solution, with 50% sodium hydroxide added to bring the solution to a pH of 7.4. Each 600-mL aliquot was sterilized by passage through a 0.22-mm filter and tested for pyrogenicity and sterility before use. Serum was assayed for PRL in control subjects using a radioimmunoassay (RIA) kit (Serono Diagnostics Inc, Randolph, Mass) with intra-assay and interassay coefficients of variation of 3% and 7%, respectively. Because manufacture of this kit was discontinued, all serum from MDMA-using subjects was assayed for PRL with a radioimmunoassay kit (Clinical Assays, Cambridge, Mass), with intra-assay and interassay coefficients of variation of 6% and 11%, respectively. Values obtained with the Serono assay were converted to values comparable with those obtained with the Clinical Assay kit using a formula ($y = 1.99x + 14.343$; $r = .93$) that we derived from the testing of 59 specimens with both kits.

Data from the -15-minute and -0.5-minute time points were averaged to obtain a single baseline value for each variable. The peak change in the PRL level was determined by subtracting the baseline from the highest PRL value after L-tryptophan infusion. The area under the curve (AUC) was calculated for PRL responses using the trapezoidal rule. Because of nonnormal distributions, comparisons of PRL data within and between subjects used the Wilcoxon signed-rank and Wilcoxon rank-sum tests, respectively. Mood ratings were subjected to analysis of variance (ANOVA) with repeated measures. Correlations were determined using Spearman's ρ . All tests were two-tailed, with significance set at $P < .05$.

RESULTS

The mean ($\pm$ SD) baseline PRL concentration did not differ between MDMA-using subjects (9.8 ± 5.4 μ g/L) and controls (10.8 ± 4.8 μ g/L). After L-tryptophan infusion the peak increase in the PRL level over baseline was robustly significant in the controls (11.0 ± 13.1 μ g/L; $P < .008$), but failed to reach statistical significance in the MDMA users (5.9 ± 8.5 μ g/L; $P < .07$). However, the difference in peak change in the PRL concentration between the two groups was not statistically significant. There was no correlation between the baseline PRL concentration and peak change in the PRL concentration in the MDMA group ($\rho = -0.12$; not significant), whereas these variables were significantly correlated in the controls ($\rho = 0.72$; $P < .03$). The AUC PRL response was also significantly greater than baseline in the controls (568.8 ± 762.5 μ g-min/L; $P < .02$), without reaching statistical significance in the MDMA users (224.8 ± 491.9 μ g-min/L; $P < .09$) (Figure). Again, the difference between groups was not significant. Within the MDMA group, baseline PRL and peak PRL concentrations and the AUC PRL did not correlate with total



Mean ($\pm$ SEM) prolactin (PRL) response over time to intravenous L-tryptophan in nine 3,4-methylenedioxymethamphetamine users (broken line) and nine healthy controls (solid line). Asterisk indicates $P < .02$ for area under PRL response curve compared with baseline (Wilcoxon signed-rank test, two-tailed); dagger, $P < .09$ for area under PRL response curve compared with baseline (Wilcoxon signed-rank test, two-tailed).

duration of MDMA use, frequency of monthly use, "usual" dose, or estimated cumulative dose.

As in previous studies, L-tryptophan caused significant decreases in ratings of energy ($F = 4.7$; $df = 5, 80$; $P < .001$) and happiness ($F = 3.2$; $df = 5, 80$; $P < .02$), and increases in ratings of drowsiness ($F = 5.2$; $df = 5, 80$; $P < .0005$). However, there were no significant differences between diagnostic groups nor were there differences in group responses to L-tryptophan.

COMMENT

Results of this exploratory study have suggested some intriguing differences between MDMA users and healthy controls. The peak change in the PRL concentration after L-tryptophan administration was 46% lower and the AUC in the PRL response was 60% lower in MDMA-using subjects than in controls. Although neither of these differences between groups was statistically significant, PRL response measures within the control group were significantly greater than baseline, while those within the MDMA group were not. Most subjects in both groups had relatively modest increases in their PRL concentration after administration of L-tryptophan, as would be expected in samples composed primarily of men.¹² However, the MDMA users seemed less likely to manifest the very marked PRL responses demonstrated by some healthy subjects, suggesting a degree of blunting in the responsiveness of those subjects ordinarily most sensitive to the effects of L-tryptophan.

This evidence suggesting altered 5-HT function in MDMA users is consistent with preclinical studies in laboratory animals that have found MDMA to have highly toxic effects on 5-HT neurons. Such studies have reported MDMA to cause decreased brain levels of 5-HT and 5-hydroxyindoleacetic acid,^{1,7} decreased tryptophan hydroxylase activity,¹ loss of 5-HT uptake sites,^{2,5} and degeneration of 5-HT axons and cell bodies.^{3,6} While large doses of MDMA (10 to 20 mg/kg) have been required to demonstrate these effects in rodents, neurotoxicity in monkeys has been observed at doses comparable with those used by our subjects (2.5 to 5.0 mg/kg).^{6,7}

The present findings obviously must be interpreted cautiously. The suggested attenuation in the PRL response to L-tryptophan in MDMA users must be considered in light of the multiple factors known to affect PRL secretion.¹⁶ It is also possible that our findings could reflect the nonspecific stress experienced by MDMA subjects in flying to New Haven on

the day before testing, although our extensive experience with PRL in neuropsychiatric assessment does not support this hypothesis.

Our failure to demonstrate more statistically significant effects of MDMA probably reflects the small sample size of this study. Even assuming a large effect of MDMA (standardized difference between group means = 0.8), ruling out a type II error ($\alpha = 0.05$; $1 - \beta = 0.80$) would require 26 subjects in each group. In addition to larger samples and more rigorous methodology, other approaches to assessing 5-HT function in humans might prove more sensitive to MDMA effects. Such approaches include modification of the standard L-tryptophan test (eg, use of lower or higher doses of L-tryptophan to determine if the "threshold" for an increase in PRL is altered), use of tryptophan depletion techniques, and use of direct 5-HT

agonists (eg, *m*-chlorophenylpiperazine). At present, the nature of MDMA's effects on 5-HT function in humans is unknown and the alteration in function suggested by the results of this study cannot be considered established. The potential for 5-HT neurotoxicity in humans is a pressing concern, however, and the development of sensitive and reliable tests for assessing this remains a challenge.

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