Communications

Identification of Metabolites of 3,4-(Methylenedioxy)methamphetamine in Human Urine

Sir: That 3,4-(methylenedioxy)methamphetamine (MDMA) is a serotonergic neurotoxin has been established in several animal models (*1–3*). Furthermore, it has been suggested that the neurotoxicity is due to a metabolite (*4*), on the basis primarily of the resemblance of MDMA's neurotoxicity to that of *p*-chloroamphetamine (*4*, *5*), and evidence that *p*-chloroamphetamine's neurotoxicity involves formation of a reactive metabolite (*6*, *7*).

Because MDMA continues to be a popular recreational drug, particularly among college students (*8*), there is a critical need to determine the potential hazards of MDMA use by humans. However, any assessment of risk in humans that is based on extrapolation of animals neurotoxicity data is complicated by the lack of information on human metabolism of MDMA, because metabolism of amphetamine-type drugs is species-dependent (*9*). Unfortunately, MDMA's status as a Schedule 1 drug prevents application of normal investigative procedures for studying the drug's metabolism in man.

At the beginning of our investigation, the only metabolic pathway that had been demonstrated for MDMA in rats (*10*) and humans (*11*) was N-dealkylation. Recently, we reported the identification of seven new metabolites of MDMA in the rat (*12*) and showed that the rat brain metabolizes MDMA in vitro. 3,4-(Methylenedioxy)-amphetamine (MDA), also a known neurotoxin, was one of the in vitro brain metabolites. Because little has been reported regarding the metabolism of MDMA in man, we report here our finding that all but one of the metabolites previously reported in the rat are also formed in man after ingestion of MDMA.

Materials and Methods. The reference metabolites of MDMA were synthesized in our laboratory as previously described (*12*). Because MDMA is a Schedule 1 drug, it is difficult to obtain approval to administer the drug to human subjects. However, a urine specimen from a fatally injured motorcyclist was made available to us after it was found to contain MDMA. The metabolites described here were all identified in this urine specimen. No information is available regarding the amount of MDMA ingested prior to the subject's death, nor the time of ingestion.

Enzymatic hydrolysis, extraction, and derivatization of MDMA and its metabolites were performed according to the procedures we described previously (*12*). All of the analyses were performed with a Finnigan MAT Model 800 ion-trap detector. MDMA and its metabolites were chromatographically separated on a fused-silica capillary column coated with 5% phenyl/95% methylsilicone (30 m × 0.2 mm i.d., 0.25-μm film thickness, J & W Scientific, Folsom, CA). The oven temperature was held at 100 °C for 0.5 min after injection and then temperature-programmed to 300 °C at 12 °C/min. Other chromatographic and mass spectrometric conditions were identical with those previously described (*12*).

Results and Discussion. The trifluoroacetylated extract of human urine was analyzed by capillary gas chromatography/mass spectrometry under both electron ionization (EI) and positive ion chemical ionization (CI). The total ion current chromatogram obtained from the trifluoroacetylated extract of the human urine specimen is shown in Figure 1; the locations of the peaks corresponding to metabolites of MDMA are indicated by arrows. Each of these peaks is clearly evident in the reconstructed ion current profile for the corresponding protonated molecule ion (not shown). Good quality EI and CI mass spectra were obtained for MDMA (I), 3-hydroxy-4-methoxymethamphetamine (II), 4-hydroxy-3-methoxymethamphetamine (III), 4-hydroxy-3-methoxyamphetamine (IV), 3,4-dihydroxymethamphetamine (V), and 3,4-(methylenedioxy)phenyl]acetone (VIII). The EI or CI mass spectra obtained for (4-hydroxy-3-methoxyphenyl)acetone (VII) contained interfering ions due to coeluting compounds, so that its identification was based primarily on its retention time relative to the internal standard, 3,4-(methylenedioxy)ethylamphetamine (MDE). The mass spectra of the other metabolites are in good agreement with those of the reference synthetic metabolites and with those previously published (*12*), and the relative retention times of the metabolites were identical with those of reference standards run after the sample.

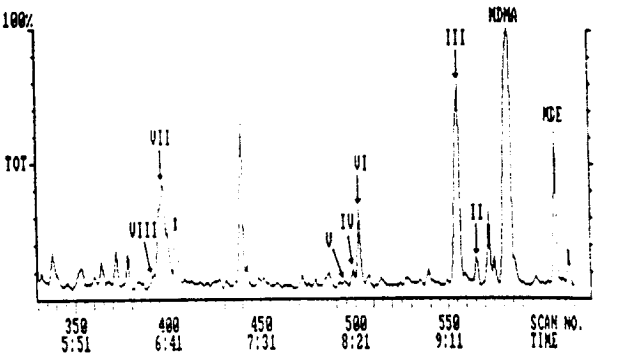

Figure 1. Total ion chromatogram (positive ion chemical ionization, reagent gas is methanol) of a trifluoroacetylated extract of human urine.

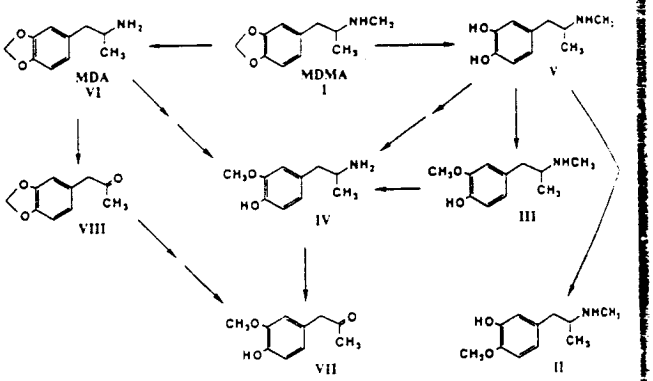

Figure 2. Proposed metabolic pathways of MDMA in human.

Although no quantitation was performed on the sample, the CI total ion chromatogram suggests that 4-hydroxy-3-methoxymethamphetamine (III) is the major metabolite in the basic extract of the human urine specimen.

Conclusions. Our data indicate that most of the metabolites identified in rat are also formed in man following MDMA ingestion. The metabolites that have been iden-

0893-228x/89/2702-0142$01.50/0

...fied include 3-hydroxy-4-methoxymethamphetamine, ...-hydroxy-3-methoxymethamphetamine, MDA, 4-...ydroxy-3-methoxyamphetamine, 3,4-dihydroxymeth...mphetamine, (4-hydroxy-3-methoxyphenyl)acetone, and ...3,4-methylenedioxy)phenyl]acetone. The human meta...olic pathways of MDMA that have been identified are N-demethylation, O-dealkylation, deamination, and O-...ethylation, as shown in Figure 2.

Acknowledgment. We thank Dr. Robert O. Bost at Southwestern Institute of Forensic Sciences (Dallas) for the urine specimen analyzed in this study.

References

(1) Ricaurte, G. A., Forno, L. S., Wilson, M. A., DeLanney, L. E., Irwin, I., Molliver, M. E., and Langston, J. W. (1988) (±)-3,4-Methylenedioxymethamphetamine selectively damages central serotonergic neurons in nonhuman primates. *JAMA, J. Am. Med. Assoc.* 260, 51–55.

(2) Logan, B. J., Laverty, R., Sanderson, W. D., and Yee, Y. B. (1988) Differences between rats and mice in MDMA (methylenedioxymethamphetamine) neurotoxicity. *Eur. J. Pharmacol.* 152, 227–234.

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

(4) Schmidt, C. J. (1987) Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. *J. Pharmacol. Exp. Ther.* 240, 1–6.

(5) Stone, D. M., Merchant, K. M., Hanson, G. R., and Gibb, J. W. (1987) Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. *Neuropharmacology* 26, 1677–1683.

(6) Sherman, A., Gal, E. M., Fuller, R. W., and Molloy, B. B. (1975) Effects of intraventricular p-chloroamphetamine and its analogues on cerebral 5-HT. *Neuropharmacology* 14, 733–737.

(7) Ames, M. M., Nelson, S. D., Lovenberg, W., and Sasame, H. A. (1977) Metabolic activation of para-chloroamphetamine to a chemically reactive-metabolite. *Commun. Psychopharmacol.* 1, 455–460.

(8) Peroutka, S. J. (1987) Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") on an undergraduate campus. *N. Engl. J. Med.* 317, 1542–1543.

(9) Caldwell, J. (1976) The metabolism of amphetamines in mammals. *Drug. Metab. Rev.* 5, 219–280.

(10) Gollamudi, R., Lopez, M., Leaky, J., Webb, P., and Slikker, W., Jr. (1988) Metabolism of 3,4-methylenedioxymethamphetamine (MDMA) by rat liver microsomes. *Toxicologist* 8, 200, Abstract 797.

(11) Verebey, K., Alrazi, J., and Jaffe, J. H. (1988) The complications of "Ecstasy" (MDMA). *JAMA, J. Am. Med. Assoc.* 259, 1649–1650.

(12) Lim, H. K., and Foltz, R. L. (1988) In vivo and in vitro metabolism of 3,4-methylenedioxymethamphetamine in the rat: Identification of metabolites using an ion trap detector. *Chem. Res. Toxicol.* 1, 370–378.

H. K. Lim, R. L. Foltz*
Center for Human Toxicology
Department of Pharmacology and Toxicology
University of Utah
Salt Lake City, Utah 84108
Received January 24, 1989