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BRIEF COMMUNICATION

The Neurochemical and Stimulatory Effects of Putative Metabolites of 3,4-Methylenedioxyamphetamine and 3,4-Methylenedioxymethamphetamine in Rats[1]

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YEH, S. Y. AND F.-L. HSU. *The neurochemical and stimulatory effects of putative metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in rats.* PHARMACOL BIOCHEM BEHAV **39**(3) 787–790, 1991.—Rats were injected SC with a dose of 10 mg/kg (as base) of 3,4-methylenedioxyamphetamine (MDA), or 3,4-methylenedioxymethamphetamine (MDMA), 4-hydroxy-3-methoxyamphetamine, α-methyldopamine and α-methylnorepinephrine, metabolites of MDA, and α-methylepinephrine, a putative metabolite of MDMA, twice daily for either 5 or 7 consecutive doses. The rats were killed 24 h after the last injection and monoamines in discrete brain regions were assayed. MDA, MDMA, 4-hydroxy-3-methoxyamphetamine and α-methyldopamine, but not α-methylepinephrine, decreased the concentration of serotonin (5-HT) in the frontal cortex. MDA and MDMA, but not 4-hydroxy-3-methoxyamphetamine, α-methyldopamine and α-methylepinephrine, also decreased the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortexes. In stimulatory studies, MDA and MDMA, but not their metabolites except α-methylepinephrine, which increased activity at 15 and 30 min, increased locomotor activity from 15 to 180 min following the drug administration.

Neurotoxicity Locomotor activity MDA MDMA 5-HT 5-HIAA α-Methyldopamine α-Methylnorepinephrine α-Methylepinephrine 4-Hydroxy-3-methoxyamphetamine

MDA and MDMA, the ring substituted derivatives of amphetamine and methamphetamine, respectively, are potent neurotoxins and have been abused in the USA. When administered systemically, MDA and MDMA deplete 5-HT and 5-HIAA concentration and destroy 5-HT axon terminals in certain brain regions of rats and guinea pigs with little effect on the levels of NE, DA, DOPAC and HVA (1–3, 11, 14, 16–22). MDMA did not alter concentrations of 5-HT and 5-HIAA in the frontal cortex in the mice (2). Interestingly, MDA and MDMA were without effect on 5-HT axon terminals (12) when administered intracerebrally. This suggests that the neurotoxicity of MDA and MDMA may be due to their metabolite(s) formed in the periphery.

3-Hydroxy-4-methoxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine, α-methyl-N-methyldopamine (3,4-dihydroxymethamphetamine), 4-hydroxy-3-methoxyamphetamine, and MDA have been identified as metabolites of MDMA in rats both in vivo and in vitro (5, 6, 25) and in the urine of humans (25). 4-Hydroxy-3-methoxyamphetamine and α-methyldopamine have been identified as metabolites of MDA in the brains of rats and in the urine of various species (8, 9, 25). α-Methyl-N-methyldopamine and α-methyldopamine, like dopamine, may undergo beta-hydroxylation in the body to form α-methylepinephrine and α-methylnorepinephrine, respectively.

The present study was designed to compare the neurochemical and stimulatory effects of MDA with its metabolites, 4-hydroxy-3-methoxyamphetamine, α-methyldopamine and α-methylnorepinephrine, and of MDMA with its metabolites, MDA and α-methylepinephrine, in rats.

METHOD

MDA and MDMA were obtained from the National Institute on Drug Abuse. α-Methylnorepinephrine HCl, α-methylepinephrine HCl and α-methyldopamine HCl were obtained as gifts from Sterling-Winthrop Res. Inst. (Rensselaer, NY) and Merck Sharp

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& Dohme Res. Inst. (West Point, PA). 4-Hydroxy-3-methoxyamphetamine was synthesized by condensation of vanillin with nitroethane to form β-nitrostyrene and reduction with lithium aluminum hydride according to an earlier procedure (15). The purity of the product was confirmed by GC/MS, NMR, melting point (261–262°C) and chemical analysis. Calculation for $C_{10}H_{15}NO_2HCl$: C 55.17, H 7.14, N 6.43%; found C 54.80, H 7.26, N 6.57%. Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Male Sprague-Dawley rats (200–225 g) were housed in an air conditioned room (22°C) with 12 h light-dark cycle and food and water provided ad lib. Drug dissolved in saline was injected 10 mg/kg (as base) SC twice daily (at 0800 and 1800 h), for either 5 (MDA and its metabolites) or 7 (MDMA and its metabolite and saline) consecutive doses. Six rats were used for each group. Twenty-four h after the last injection, the rats were killed, the brains removed and placed on ice, the frontal cortexes dissected, frozen in liquid nitrogen immediately and stored at −70°C until assayed. Measurement of DA, NE, 5-HT and their metabolites by HPLC with electrochemical detection and assay of uptake sites' density of NE, DA and 5-HT by ligand binding were described previously (1,2).

Locomotor activity was monitored 30 min before and every 15 min for a total of 180 min after drug administration (Digiscan Optical Animal Activity Monitor, model RXY, Omnitech Electronics, Inc., Columbus, OH). Area under curve (AUC) for locomotor activity from 0 to 180 min was calculated according to the trapezoidal rule.

Data were analyzed for statistical significance using a one-way analysis of variance followed by comparisons between means obtained from drug- and saline-treated rats using Duncan's multiple range *t*-test.

RESULTS

As compared to the saline controls, MDA decreased the concentration of 5-HT and 5-HIAA in the frontal cortex by 86% and 73%, respectively, confirming previous results (Fig. 1) [cited in (1,2)]. 4-Hydroxy-3-methoxyamphetamine and α-methyldopamine also reduced the concentration of 5-HT, by 35% and 16%, respectively, but did not alter the 5-HIAA level. The differences in the depletion of 5-HT and 5-HIAA concentration in the frontal cortex of rats treated with MDA and that with the metabolites were highly significant. Depletion of 5-HT concentration in the frontal cortex of rats treated by 4-hydroxy-3-methoxyamphetamine was significantly different from that by α-methyldopamine (Fig. 1).

Ten to 15 h after administration of α-methylnorepinephrine, the rats, in general, showed piloerection and hypothermia after the first and 2nd injection. Two and three rats died after the 2nd and 3rd injection, respectively, and the rectal temperature of the one surviving rat (15 h after the 4th injection) was 27.6°C. The rat was killed 16 h after the 4th injection and the levels of 5-HT and 5-HIAA in the frontal cortex were 71% and 158% of saline control, respectively.

MDMA decreased the levels of 5-HT and 5-HIAA in the frontal cortex of rats by 56% and 55%, respectively, again confirming previous results [cited in (1,2)]. α-Methylepinephrine, a putative metabolite of MDMA, had no effect on 5-HT and 5-HIAA (Fig. 1).

Depletion of 5-HT and 5-HIAA by MDA was more significant, even with fewer injections, than that by MDMA. MDA, MDMA and their metabolites did not alter the levels of NE, DA, DOPAC and HVA in the frontal cortex, again comfirming previous results [cited in (1,2)].

MDA and MDMA decreased the density of 5-HT uptake sites

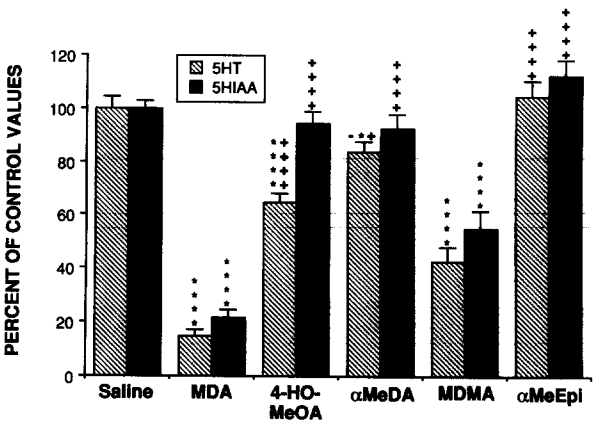

FIG. 1. The effects of MDA, MDMA and their metabolites on 5-HT and 5-HIAA levels in the frontal cortex of rats. All values are the mean ± S.E.M. (n = 6) expressed as a percent of saline control. Control levels of 5-HT and 5-HIAA were 420 ± 8, and 361 ± 17, pg/mg tissue, respectively. Data were analyzed by one way ANOVA and Duncan's multiple range *t*-test. * and **** indicate significant difference at $p<0.05$, and $p<0.001$, respectively, from the saline control rats. + and + + + + indicate significant difference between MDA- and 4-hydroxy-3-methoxyamphetamine (4-HO-MeOH)-, MDA- and α-methyldopamine (α-MeDA)-treated rats or between MDMA- and α-methylepinephrine (α-MeEpi)- treated rats at $p<0.5$ and $p<0.001$, respectively, - indicates significant difference between 4-HO-MeOH- and α-MeDA-treated rats at $p<0.05$.

in the cerebral cortex by 55% and 57%, respectively, but not those of NE and DA, also confirming previous results [cited in (1,2)]. 4-Hydroxy-3-methoxyamphetamine, α-methyldopamine and α-methylepinephrine did not significantly alter the uptake sites density of 5-HT, NE and DA (data not shown).

As compared to the saline controls, both MDA- and MDMA-induced locomotor activity was significantly increased over a period of 15 to 180 min, whereas metabolites of MDA had no effect (Fig. 2, panel A). α-Methylepinephrine, a putative metabolite of MDMA, slightly increased locomotor activity at 15 and 30 min (Fig. 2, panel B).

MDA-induced locomotor activity peaked at 45 min and then declined rapidly, whereas the MDMA-induced locomotor activity increased more slowly, peaked at 105 min and then declined slowly. The MDA- and MDMA-induced locomotor activity at the time points from 75 to 135 min and of the AUC from 0 to 180 min are significantly different.

DISCUSSION

The neurochemical and stimulatory effects of the putative metabolites of MDA and MDMA were much less than those of their parent compounds when administered systemically. This suggests that the neurotoxic effect of MDA and MDMA is not due to their metabolite(s) formed in periphery, but the results do not rule out neurotoxicity due to metabolites formed in the brain (see below). Intracerebral injection of MDA and MDMA did not induce neurotoxic effects (12). This may be using a single small dose (12 μg) of MDA and MDMA administered intracerebrally since the neurotoxic effects of MDA and MDMA are dependent on the dose and dosage administered systemically (2, 3, 17). A single large dose of MDMA (20 mg/kg) did not induce the neurotoxic effect when administered systemically (2).

The present results showed that when administered systemically and as compared to their parent compound, MDA or

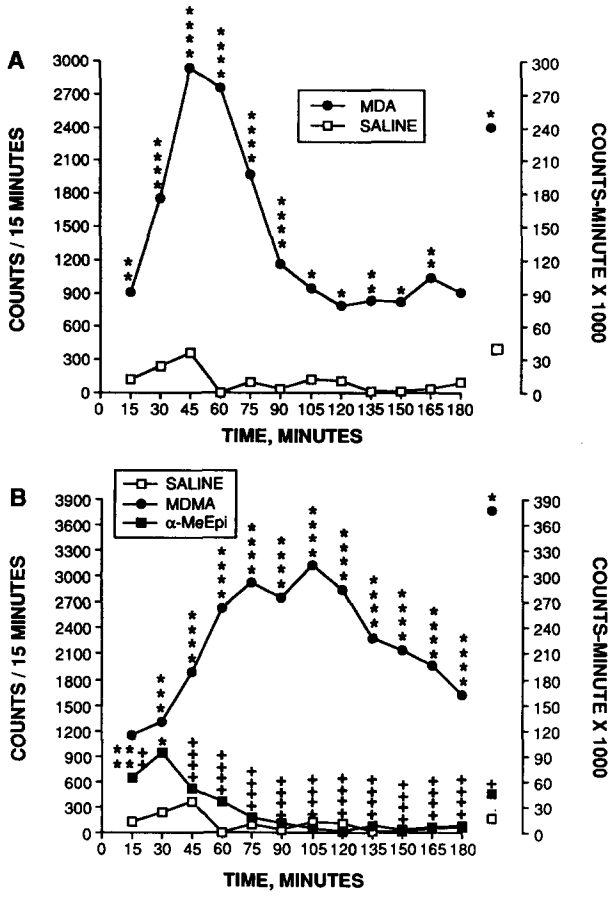

FIG. 2. Locomotor activity induced by MDA and saline (panel A) and by MDMA, α-methylepinephrine and saline (panel B). Values are means for 4 rats. *, ** and **** indicate significant difference from saline-treated rats and + indicate significant difference between MDMA- and α-methylepinephrine (α-MeEpi)- treated rats, at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

MDMA, 4-hydroxy-3-methoxyamphetamine and α-methyldopamine, metabolites of MDA, and α-methylepinephrine, metabolites of MDMA, are less potent in depletion of 5-HT and 5-HT in the frontal cortex. This is probably due to these metabolites being more polar and less able to cross the blood-brain barrier than their parents.

4-Hydroxy-3-methoxyamphetamine decreased 5-HT concentration by 35% in the frontal cortex. This suggests that 4-hydroxy-3-methoxyamphetamine, and 4-hydroxy-3-methoxymethamphetamine and their metabolites, α-methyldopamine and α-methyl-N-methyldopamine, respectively, formed in the brain after injection of MDA and MDMA may play a role in the neurotoxicity of MDA and MDMA. This assumption was supported by 1) the presence of 4-hydroxy-3-methoxyamphetamine and α-methyldopamine in the brain of rats after MDA and of 4-hydroxy-3-methoxymethamphetamine, 3-hydroxy-4-methoxymethamphetamine and α-methyl-N-methyldopamine after MDMA (6,7), 2) the slower egress of the polar metabolites of MDA from the brain than that of MDA (7), and 3) identification of α-methyl-N-methyldopamine glutathione conjugate in vitro (5), suggesting α-methyl-N-methyldopamine and α-methyldopamine may bind thiols in the brain tissue. Pharmacokinetic studies of these metabolites via intracerebral injection will provide valuable information on the neurochemical effects of these metabolites.

The amount of 5-HT and 5-HIAA depleted by MDA was about 1.6 times that by MDMA. We (unpublished data) and others have reported that 10 mg/kg of 3,4-methylenedioxyethylamphetamine (MDE), analogue of MDA and MDMA, did not alter the amount of 5-HT and 5-HIAA in the frontal cortex (17,18). These data suggest that depletion of 5-HT and 5-HIAA by MDMA may be due to the N-dealkylated metabolite, MDA, since the rate of N-demethylation was greater than N-deethylation (4). Pharmacokinetic studies of MDA, MDMA, and MDE in the brain of rats will provide information on the relationship of the neurotoxicity between these compounds.

The duration of the locomotor activity and the AUC of the locomotor activity induced by MDA was shorter and smaller than that induced by MDMA. This may be due to the fact that MDMA is more polar than MDA and resulted in a faster egress of MDA than MDMA from the brain. Lack of neurotoxic and stimulatory effects of 4-hydroxy-3-methoxyamphetamine, α-methyldopamine, and α-methylepinephrine, metabolites of MDA and MDMA, may be due to these compounds not crossing the blood-brain barrier, or crossing the blood-brain barrier to a lesser extent. α-Methylnorepinephrine (13) and dopamine, with structural similarity to α-methyldopamine, do not cross the blood-brain barrier (24). The cause of death by α-methylnorepinephrine may be due to hypotension and bradycardia (13).

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