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A pharmacokinetic analysis of 3,4-methylenedioxymethamphetamine effects on monoamine concentrations in brain dialysates

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Interpretation of the in vivo actions of 3,4-methylenedioxymethamphetamine (MDMA) is complicated by the formation of the active metabolite, 3,4-methylenedioxyamphetamine (MDA). This study evaluates the role of MDA in the dopamine releasing actions of (+)-MDMA. In the study, rats were given subcutaneous doses of (+)-MDMA and concentrations of monoamines and their metabolites in striatal dialysate were measured at 15 min intervals. In parallel experiments, plasma concentrations of (+)- and (−)-MDMA and MDA were determined by GC/MS procedures. The time course of MDMA levels was comparable for the two isomers as were their bioavailabilities. In contrast, the plasma levels of MDA were about three times higher after (+)-MDMA. (+)-MDMA caused a rapid increase in striatal dialysate levels of dopamine and decreased extracellular levels of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). There was a significant correlation between dopamine concentration in striatal dialysate and plasma MDMA concentration, but not with plasma MDA. These results indicate that MDMA itself has stereoselective actions on dopamine neurons. However, the higher plasma MDA levels after (+)-MDMA may account for part of the enantiomeric differences in the behavioral and neurotoxicological effects of MDMA.

MDMA (3,4-methylenedioxymethamphetamine); Dopamine release; Microdialysis (in vivo); Enantiomers; Pharmacokinetics; Pharmacodynamics

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), a ring-substituted derivative of methamphetamine, has received a great deal of recent attention as one of a number of 'designer drugs' being abused. MDMA has been reported to produce both stimulant- and hallucinogen-like effects in man (Shulgin, 1986). In experimental animals, MDMA produces a variety of effects such as stereotypy, hyperactivity, disruption of operant behavior, stimulus generalization with amphetamine and hyperthermia (Anderson et al., 1978; Glennon et al., 1987; Hiramatsu et al., 1989; Schechter, 1987; Glennon et al., 1988). Neurochemical studies in rodents following in vivo administration of MDMA (Battaglia et al., 1987; Johnson et al., 1988; Schmidt, 1987; Stone et al., 1986) have demonstrated effects on brain serotonin (5-HT) systems including a decrease of uptake and in levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), and a reduction in tryptophan hydroxylase activity.

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The optical isomers of MDMA affect brain 5-HT and dopamine systems with different potency. Comparison of the effects of enantiomers of MDMA on [^{3}H]5-HT and [^{3}H]dopamine release and uptake in vitro showed that the (+) isomer of MDMA was a more potent agent (Johnson et al., 1986; Kalix et al., 1988; Nichols et al., 1982; Schmidt et al., 1987; Steele et al., 1987). An in vivo microdialysis study showed that (+)-MDMA, but not (−)-MDMA, also caused dopamine increases in striatal dialysate (Hiramatsu and Cho, 1990). Behavioral studies with rodents have shown that (+)-MDMA is more potent than (−)-MDMA in causing stereotypy in rats (Hiramatsu et al., 1989), the disruption of operant responding in mice (Rosecrans and Glennon, 1987) and in mimicking the discriminative stimulus produced by racemic MDMA (Battaglia et al., 1988; Schechter, 1987). On the other hand, (−)-MDMA is more potent than (+)-MDMA in binding to central 5-HT binding sites (Lyon et al., 1986), suggesting that the (−) isomer is more active at post synaptic sites.

In a previous study, we have demonstrated that overall MDMA elimination in rats after intravenous (i.v.) dosage for both isomers was essentially the same, but that 3,4-methylenedioxyamphetamine (MDA) formation from the (+) isomer was much higher (Cho et

al., 1990). This observation raised the possibility that the dopamine-releasing action of (+)-MDMA could be due to the (+)-MDA formed. This possibility was evaluated in this study by determining the time course of dopamine release and the plasma concentrations of MDMA and MDA after subcutaneous (s.c.) doses of MDMA.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–350 g) were housed in a room with controlled lighting (12 h light/dark cycle) and temperature (23 °C) and access to food and water ad libitum.

2.2. Drugs

The drugs were dissolved in saline and injected s.c. Each rat received only one drug injection. (+)- and (−)-MDMA were kindly supplied by National Institute on Drug Abuse (Rockville, MD). 3,4-Dihydroxyhydrocinnamic acid (as an internal standard) was obtained from Aldrich Chemical Co. (Milwaukee, WI). All HPLC assay reagents were of analytical grade.

2.3. Surgical procedure

Rats were anesthetized with pentobarbital (40 mg/kg i.p.), and placed in a stereotaxic frame. Using coordinates chosen according to the stereotaxic atlas of Paxinos and Watson (1986), guide cannulas were implanted so that the tips were just above the striatum (A: 1.0, L: 3.0, V: 4.0 mm relative to bregma).

Dialysis probes

After surgery, the cannulated rats were allowed a 5–7 day recovery period before the experimental session. The dialysis probes were constructed in the laboratory as described by Hiramatsu and Cho (1990). The recoveries of the measured components from dialysis membranes (cellulose acetate, 5000 molecular cut off · inner diametre 230 μm, wall thickness 10 μm, Bioresearch Center, Nagoya, Japan) were between 9 and 15% at 37 °C. The experimental data are presented as a percent of the baseline concentrations for each animal to correct for the differences in recovery that might occur.

2.5. Sampling procedure

The dialysis tube was perfused with physiological Ringer solution (composition in mM: NaCl 147; KCl 4; $CaCl_2$ 2.3) at 2 μl/min using microbore tubing connected to a microinfusion pump (Syringe Infusion Pump 22, Harvard Apparatus) via a single-channel liquid swivel. The infusion and effluent cannulas were passed through and attached to a tether which was attached to the animal by a rodent jacket. This arrangement allowed the animal to move freely within the cage. The rats were placed in individual cages (18 × 29 × 31 cm) and the dummy cannulas replaced with dialysis probes which were fixed to the guide cannulas with wax. Upon insertion of the dialysis probe, a 4 mm length of dialysis membrane was exposed to the extracellular space of the striatum. The animals were allowed to adapt for at least 60 min before the experiment was started. The dialysate was collected in a small microcentrifuge tube which was secured to the middle of the tether. The collecting tube contained 20 μl of a 0.4 N perchloric acid solution containing internal standard (3,4-dihydroxyhydrocinnamic acid, 1.2 ng). The total dead volume from the probe tip to the collection tube was about 20 μl but was always measured in order to adjust the delay in sample collection after drug administration. Samples (60 μl) were collected at 15 min intervals and at least four control samples were taken before drug administration. Brain dialysate samples were taken up to 240 min after (+)-MDMA injection.

2.6. Neurochemical analysis

The dialysates were assayed for dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-HIAA by high performance liquid chromatography (HPLC) with electrochemical detection. These compounds were separated by reverse phase chromatography using Biophase ODS 5 μm (4.6 × 250 mm, Bioanalytical Systems, Inc., IN) and 0.075 M citrate buffer, pH 3.5 (adjust pH 3.5 using acetic acid), containing 10% methanol, 1% tetrahydrofuran and 50 mg/l EDTA, 55 mg/l sodium octylsulfate at 0.7 ml/min flow rate. Electrochemical measurements were made using a glassy carbon working electrode set at +0.7 V vs. Ag/AgCl reference electrode (LC-4, Bioanalytical Systems, Inc.). The dialysate samples were injected directly onto the HPLC column. Signals were recorded with a Hewlett Packard 3390A recording integrator, and the peak height ratio (compound/internal standard) of each sample compared with the control sample from each animal before treatment.

Under the HPLC conditions employed, the retention times were: dopamine, 5.3 min; DOPAC, 6.3 min; 5-HT, 7.8 min; 5-HIAA, 8.8 min; internal standard, 9.9 min; and HVA, 11.5 min. Baseline resolution of all compounds was achieved and a chromatographic run consumed 14 min. The basal levels of the amines and metabolites were stable over the 6 h sampling period.

2.7. Cannulas implantation

Vascular-Access-Ports (Model SLA; Norfolk Medical Products, Stokie, IL) were implanted 24 h before blood sampling. Prior to insertion, the port was filled with sterile heparinized saline (20 units/ml). After pretreatment with atropine methylbromide (1.0 mg/kg s.c.) the rats were anesthetized with diethyl ether. The catheter attached to the port was tunneled under the skin from the back to the neck area, inserted into the jugular vein and advanced into the atrium, The rats were injected i.m. with 60000 units of penicillin G procaine suspension.

2.8. Blood sampling

On the morning of an experiment, the ports were flushed with 0.6 ml of heparinized saline (20 units/ml). Because of difficulties in handling rats after injection of (+)-MDMA, animals were anesthetized with α-chloralose (55 mg/kg i.p.) 30 min before MDMA administration. Preliminary experiments had shown no significant changes in plasma pharmacokinetics of MDMA or MDA after the anesthetic.

(+)- or (−)-MDMA (10 mg/kg) was dissolved in 0.9% saline and injected s.c. in a volume of 1 ml/kg. Blood samples were taken 5, 10, 20, 30, 60, 120, 180, 300, 420 and 600 min after drug injection as described before (Cho et al., 1990). The blood samples were centrifuged at $15000 \times g$ for 3 min, and the plasma frozen and stored at −80 °C until assayed.

2.9. MDMA and MDA analysis by GC/MS (Cho et al., 1990)

Volumes of 0.2 to 0.5 ml of plasma were treated with 3% $HClO_4$ containing internal standards deutero MDMA (d2-MDMA) and deutero MDA (d2-MDA). After extraction with CH_2Cl_2, samples were evaporated and derivatized with trifluoroacetic anhydride for injection into the GC/MS system. Standard curves were prepared from blank plasma for in vivo samples covering the range 125–25000 pmol/ml and carried through each assay.

2.10. Data analysis

Time intervals for plasma sample collection were chosen to allow optimal estimation of pharmacokinetic parameters and differed from the regular, 15 min intervals used in the microdialysis experiments. To estimate the plasma concentrations at the times of dialysate collection, the plasma concentration values were fitted to a model that describes the first order absorption and elimination of MDMA and the first order formation and elimination of MDA (fig. 1). In the model, q_1 is

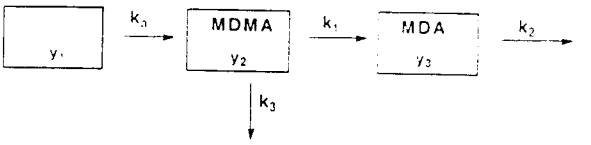

Fig. 1. Pharmacokinetic model used to analyze plasma MDMA and MDA concentrations.

the quantity of MDMA at the absorption site, q_2 is the quantity of MDMA and q_3 is the quantity of MDA. The corresponding concentrations y_1, y_2 and y_3 were assumed to be proportional to the volume of distribution (assumed to be the same for MDMA and MDA). This compartment system is described by the following equations:

$$\frac{dq_1}{dt} = -k_a q_1$$

$$\frac{dq_2}{dt} = k_a q_1 - (k_1 + k_3) q_2$$

$$\frac{dq_3}{dt} = k_1 q_2 - k_2 q_3$$

$$q_1(0) = q_0$$
$$q_2(1) = 0$$
$$q_3(0) = 0$$

where q_0 is the dose, 43.6 μmol/kg. In solving this system, we constrained $k_a = k_1 + k_3$. The solution is:

$$y_1 = \frac{q_0}{V} e^{-k_a t}$$

$$y_2 = \frac{q_0}{V} k_a t\, e^{-k_a t}$$

$$y_3 = \frac{q_0 k_a k_1}{V(k_2 - k_a)^2} [(t(k_2 - k_a) - 1) e^{-k_a t} + e^{-k_2 t}]$$

Since the S.D.s were linearly related to the means for the plasma concentrations, a log transformation was employed.

3. Results

Concentrations of dopamine, DOPAC, HVA, 5-HT and 5-HIAA in striatal dialysate were measured at 15 min intervals over a period of 4 h after s.c. injection of MDMA (10 mg/kg). (+)-MDMA caused a 400–500% increase in dialysate dopamine levels in the striatum (fig. 2A). The effects were maximal between 60–90 min after dosage and returned to control level by about 4 h. In contrast to the amine, DOPAC and HVA levels in striatum dropped (fig. 2B). 5-HT levels did not change significantly, but tended to decrease gradually 3–4 h after MDMA administration (fig. 2A).

Plots of plasma MDMA and MDA concentrations after s.c. administration of (+)- or (−)-MDMA are shown in fig. 3A and B. The MDMA concentrations were maximal about 70 min after injection and dropped

TABLE 1

Pharmacokinetic parameters after injection of (+)- or (−)-MDMA.

Data from all animals were fitted for the first order absorption and elimination of MDMA and the first order formation and elimination of MDA, as described in Methods. V_d is the apparent value of distribution of the central compartment and AUC is the area under the plasma concentration–time curve, estimated by Simpson's Rule. The values are shown ± asymptotic S.D.s and were estimated from plasma samples collected from five animals.

	V_d (l/kg)	k_a^a	k_1^a	k_2^a	AUC ($\times 10^3$) [b]
(+)-MDMA	3.09	13.0 ± 0.3	3.8 ± 0.4		1056.9 ± 61.2
(+)-MDA				6.5 ± 0.6	586.3 ± 53.5
(−)-MDMA	2.67	13.5 ± 0.4	1.5 + 0.2		1033.6 ± 60.5
(−)-MDA				10.1 ± 1.1	113.3 ± 4.8

[a] ($min^{-1} \times 10^3$), [b] nmol/min per l.

below detectable limits by 10 h. The MDMA and MDA concentrations were fitted simultaneously to the model described in the Methods section. The kinetic constants for the models and other factors derived from the data estimated from the pooled data of five rats are summarized in table 1. The solid lines in fig. 3 reflect the calculated values from the model and match the observed concentrations with good agreement. MDMA enantiomers had similar rates of absorption and elimination and comparable bioavailabilities. Plasma MDA levels, which were maximal at 120–240 min, decayed with longer half-lives than MDMA. The formation of MDA, however, exhibited a marked stereoselectivity and levels of MDA after the (+) isomer gave a bioavailability that was about five times greater than the (−). This difference is accounted for by a larger k_1 and smaller k_2 for the (+) enantiomer.

Figure 4 shows the relationship between concentrations of amines in striatal dialysate and plasma MDMA concentration. Plasma concentrations at which analyzed levels were not available were estimated as described in Methods. There was good correlation between dialysate dopamine levels and MDMA concentrations, but not with MDA concentrations (fig. 5).

4. Discussion

One of the problems in interpreting in vivo effects of drugs is the possible involvement of active metabolites. MDMA is an example of such a drug as it is

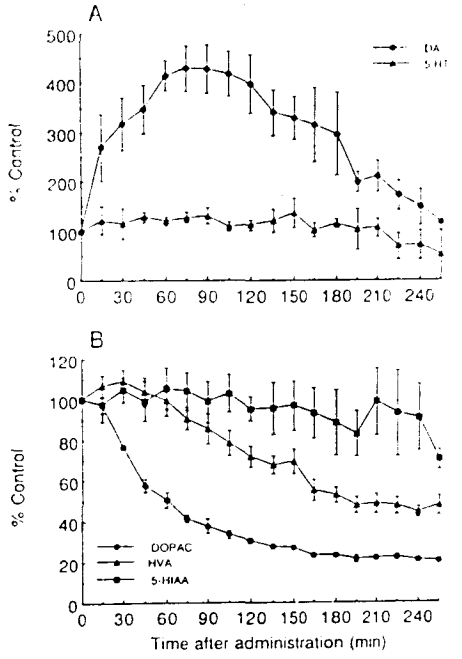

Fig. 2. Effects of (+)-MDMA on the extracellular concentrations of dopamine, 5-HT and their metabolites in dialysates collected from rat striatum. Results are expressed as a percent of control for each rat. Forty microliters of each sample of dialysate was injected into the HPLC-EC and the results are the mean of five independent experiments.

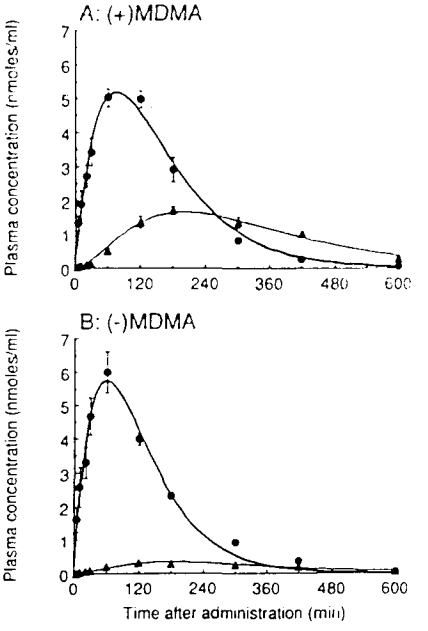

Fig. 3. Time course of plasma MDMA and MDA concentration after s.c. injection of (+)- or (−)-MDMA. Data were fitted to a first order absorption and elimination model as described in Methods. The overlaid curve is the function described which was used to estimate the plasma concentrations for fig. 4. (●) MDMA concentration, (▲) MDA concentration.

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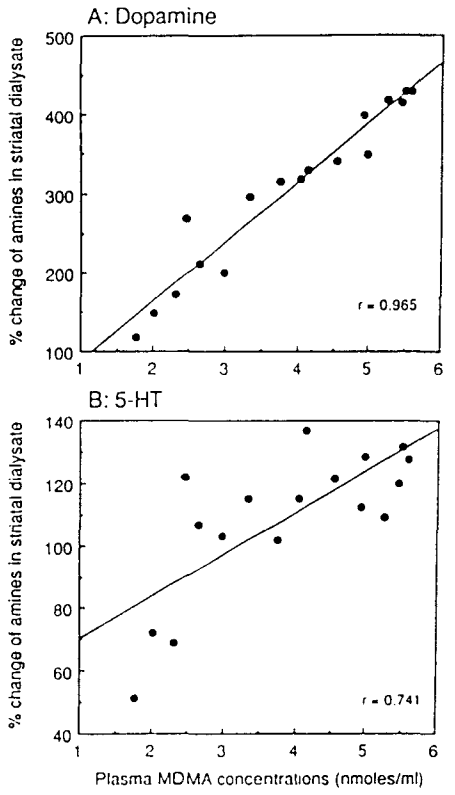

Fig. 4. Relationship between changes in amine efflux in striatal dialysate and plasma MDMA concentration.

converted to an active desmethyl metabolite, MDA (Glennon et al., 1988; Johnson et al., 1986). This problem is further confounded in this case because of the stereoselectivity of MDMA effects (Johnson et al., Kalix et al., 1988) and of its metabolism (Cho et al., 1990). The more extensive formation of MDA from (+)- than from (−)-MDMA together with the greater effect of the (+) isomer on some actions raises the possibility that these reflect the higher levels of the active metabolite. One of the actions of this group of amphetamine derivatives that is amenable to time related analysis is the increase in extracellular dopamine that is observed after (+) but not (−)-MDMA (Hiramatsu and Cho, 1990). The temporal nature of the

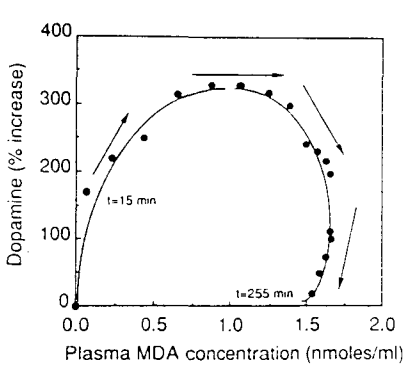

Fig. 5. Relationship between changes in dopamine efflux in striatal dialysate and plasma MDA concentration.

effect and the ease with which it can be monitored over time made it an useful parameter with which to compare plasma drug levels. A more precise comparison would be the corresponding brain concentrations, but recoveries from dialysate were too low to allow analysis. Prior studies examining behavior responses to MDMA and MDA showed that the initiation of response occurred with comparable time courses so that the rate of brain entry of the two compounds must be comparable (Hiramatsu et al., 1989).

The data presented in fig. 4 show correlation between MDMA levels and dopamine efflux, but not MDA indicating that the dopamine effects are due to the parent compound with little contribution from the metabolite. Since the bioavailabilities and the volumes of distribution of the MDMA enantiomers are comparable, the difference in action observed for the enantiomers (Hiramatsu and Cho, 1990) must reflect different potencies. This analysis does assume, however, that most of the metabolic formation of MDA occurs peripherally and then enters the brain with comparable rates to MDMA. Other actions of MDMA, including its neurotoxicity may involve this metabolite and need to be evaluated.

Acknowledgements

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