Biochemical Pharmacology, Vol. 27, pp. 1503–1505.
© Pergamon Press Limited, 1978. Printed in Great Britain.
0006-2952/78/0501-1503/$02.00/0

SHORT COMMUNICATIONS

Metabolism of β-3,4-methylenedioxyamphetamine in the rat*

(*Received* 31 *May* 1977; *accepted* 3 *October* 1977)

Racemic, (S)- and (R)-methylenedioxyamphetamine (MDA) have been shown to produce differential pharmacological effects both peripherally and centrally [1, 2]. Casida *et al.* [3] have demonstrated the conversion of many methylenedioxyphenyl compounds (e.g. safrole) to the corresponding catechols by hepatic microsomal enzymes. α-Methyldopamine (α-MeDA) is the only microsomal metabolite of MDA *in vitro* [4]. Further, α-MeDA isomers have been implicated in some of the differential pharmacological and toxicological effects of MDA isomers [2]. The present study was conducted to determine the metabolites of MDA enantiomers in the urine and brains of treated rats.

[6-^{3}H(N)]-3,4-Methylenedioxyamphetamine ([^{3}H](±)-MDA), 306 Ci/m-mole, was generously provided by Dr. Robert Willette of the National Institute of Drug Abuse. Male Sprague–Dawley rats (150–200 g) were given either [^{3}H](±)-MDA (5 mg/kg, i.p.) or distilled water (1.0 ml/kg, i.p.) and placed in metabolic cages (Maryland Plastics Inc., model 1100). Animals were given a water load (15 ml/kg, p.o.) 30 min before drug treatment to facilitate urine collection. Two ml of 2.0 N acetic acid containing 0.05% $Na_2S_2O_5$ was added to the container before urine collection to minimize oxidation of any catecholamine metabolites. At the end of each experiment, urine samples were transferred to volumetric flasks and diluted with distilled water.

The total tritium content of diluted urine samples was estimated by determining the total radioactivity of 500-μl aliquots. Other aliquots (500 μl) were incubated with 6250 units of β-glucuronidase in 0.2 M sodium acetate buffer, pH 5.0 (total incubation volume, 2.0 ml), in 15-ml screw-capped, glass culture tubes for 24 hr. Incubations were terminated by chilling the samples. The total radioactivity of aliquots (500 μl) was determined in 10 ml of Bray's solution [5].

Unchanged [^{3}H](±)-MDA in hydrolyzed and unhydrolyzed urine samples was isolated by column chromatography in XAD-2 resin. The addition of [^{3}H]MDA to the urine collected from untreated animals allowed the estimation of recovery.

Animals were decapitated, and the whole brain was removed and divided in half sagittally. One half was homogenized in 0.3 N acetic acid, 4 ml/g (with 0.05% $Na_2S_2O_5$); the other half was homogenized in 0.4 N $HClO_4$, 4 ml/g (with 0.05% $Na_2S_2O_5$), using a Polytron homogenizer (Brinkman Instruments). Aliquots of the acetic acid homogenate were transferred to glass scintillation vials and digested with 1 ml of NCS tissue solubilizer. The radioactivity of the digests was determined in 10 ml of Bray's solution. The remainder of the acetic acid homogenate was centrifuged at 30,000 *g* for 60 min to sediment the cellular debris. The supernatant fraction was decanted and 500-μl aliquots were counted in 10 ml of Bray's solution.

The radioactivity present as unchanged [^{3}H]MDA and its metabolites was determined by paper chromatography. Aliquots (5 μl) of diluted, untreated urine, hydrolyzed urine, the XAD-2 eluate, the supernatant fraction of the acetic acid homogenate of brain tissue, the acetic acid eluate of compounds adsorbed onto alumina from the $HClO_4$ homogenates of brain tissue, and aliquots of the [^{3}H]MDA injection solution were chromatographed on SG 81 paper in solvent systems A (ethyl acetate–methanol–ammonium hydroxide, 17:2:1), B (*n*-butanol–acetic acid–water, 12:3:5) and C (methanol–ammonium hydroxide, 100:1.5).

Aliquots (5 μl) of 0.1% solutions of authentic MDA, 3-*O*-methyl-α-dopamine (3-*O*-Me-α-MeDA) and α-methyldopamine (α-MeDA) were chromatographed with each sample to determine their identities in the urine and brain. Further confirmation of α-MeDA was accomplished by alumina adsorption [6] and subsequent chromatography. The paper chromatograms of each sample were cut horizontally into 0.5 to 1.0 cm segments. The radioactivity of each segment was eluted with methanol and determined in 10 ml of Bray's solution. Background radioactivity was determined by similar treatment of 0.5 to 1.0 cm segments taken 1.0 to 2.0 cm above the solvent front from each chromatogram. Results were plotted as percentages of the total radioactivity in the chromatogram segment vs the R_f value. Authentic samples of MDA, 3-*O*-Me-α-MeDA and α-MeDA were used to verify their position on chromatograms of urine and brain.

The total urine and brain radioactivity 0.5, 1, 2, 6, 16 and 24 hr after MDA administration is presented in Table 1. The glucuronide of 3-*O*-Me-α-MeDA was identified by chromatography of untreated urine and urine that had been hydrolyzed with β-glucuronidase (Fig. 1). Enzymatic hydrolysis resulted in a loss of radioactivity at R_f values

Table 1. Tritium content of urine and brain in rats at various times after treatment with [^{3}H](±)-MDA (5 mg/kg, i.p.)*

Time after dosing (hr)†	Tritium content‡	
	Urine	Brain
0.5	ND§	9.22 ± 1.719
1	ND	4.00 ± 0.350
2	6.01 ± 0.4	1.91 ± 0.045
6	14.20 ± 10.6	0.20 ± 0.017
16	50.20 ± 6.2	0.08 ± 0.001
24	63.70 ± 15.4	0.03 ± 0.001

* Rats were given a water load (15 ml/kg) 30 min before drug treatment.

† Animals were sacrificed at various times after dosing and the radioactivity in the brain and urine of each animal was determined.

‡ Mean per cent of administered radioactivity ± S. E. M.; N = 4.

§ Not determined.

* Supported in part by Grants GM-00032 and GM-15190 from USPHS.

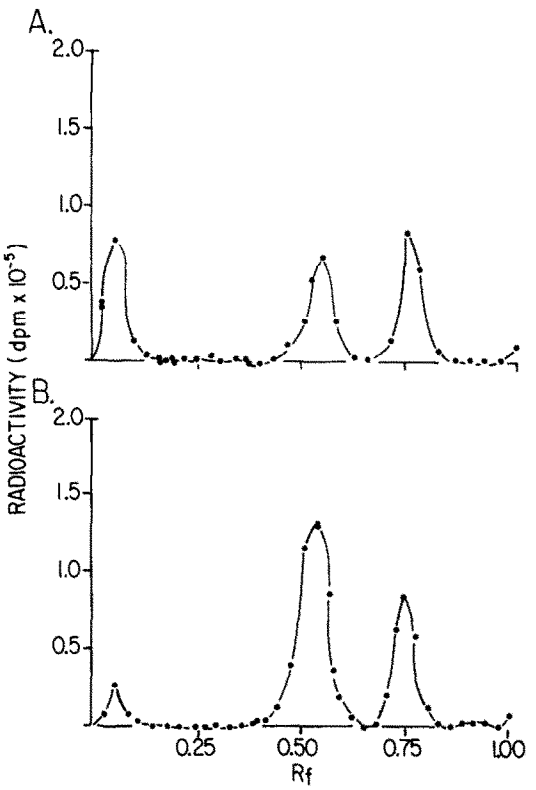


Fig. 1. Localization of radioactivity on a chromatograph of untreated urine (A) and hydrolyzed urine (B) from rats treated with [^{3}H](±)-MDA (5 mg/kg, i.p.) 24 hr before chromatographic analysis. Urine was enzymatically hydrolyzed with β-glucuronidase (6250 units), as described in the text. Urine samples (5-μl aliquots) were chromatographed on SG 81 paper in solvent system A (ethyl acetate–methyl alcohol–NH_4OH, 17:2:1). Chromatography of reference compounds allowed identification of the peaks as 3-*O*-Me-α-MeDA glucuronide (R_f value of 0.07), 3-*O*-Me-α-MeDA (R_f value of 0.54) and MDA (R_f value of 0.75).

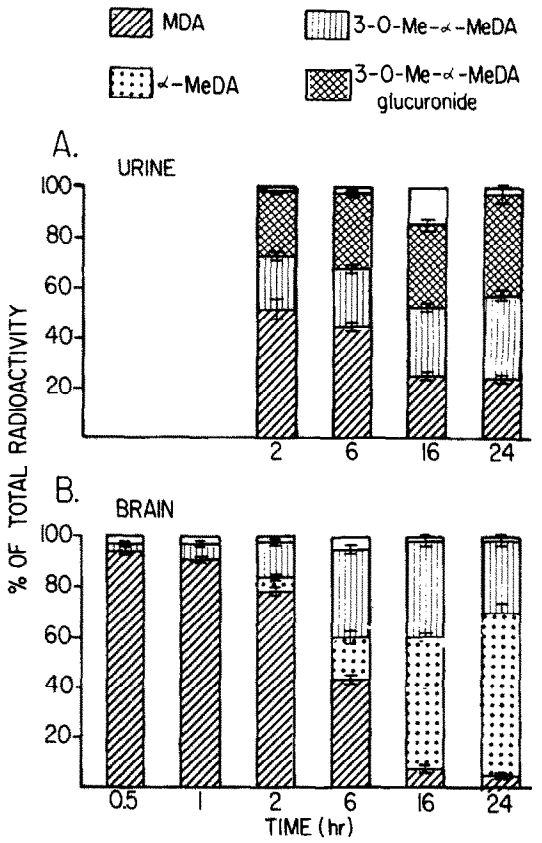


Fig. 2. Distribution of radioactivity among unchanged [^{3}H](±)-MDA and its identified metabolites in urine (A) and whole brain tissue (B) of rats at various times after drug treatment. Results are expressed as the percentage of total radioactivity in the urine or brain and represent the mean ± S.E.M. (N = 4). The blank spaces represent radioactivity which could not be accounted for by MDA, α-MeDA, 3-*O*-Me-α-MeDA or the glucuronide of 3-*O*-Me-α-MeDA.

of 0.07, 0.12 and 0.10 in solvent systems A, B and C respectively. A corresponding increase in the radioactivity of 3-*O*-Me-α-MeDA was noted in each system.

Figure 2 illustrates the fraction of total urine tritium present as MDA and its metabolites at various times after drug treatment. Unchanged [^{3}H]MDA represented the majority of urine tritium 2 hr after drug treatment. This fraction decreased markedly with time until it accounted for only about 20 per cent of the total radioactivity in the urine of animals 24 hr after dosing.

Analysis of urine samples collected 6, 16 and 24 hr after drug treatment indicated that 3-*O*-Me-α-MeDA and its glucuronide conjugate accounted for the majority of urine radioactivity. This indicates that the majority of the administered [^{3}H]MDA is metabolized to α-MeDA, which is then conjugated. Free α-MeDA was not detected in the urine of rats at any time after dosing.

Brain tritium was present as unchanged [^{3}H]MDA, α-MeDA, 3-*O*-Me-α-MeDA and as an unidentified metabolite (Fig. 2B). These results indicate that unchanged [^{3}H]MDA accounted for more than 90 per cent of the brain radioactivity 30 and 60 min after dosing. α-MeDA could not be detected in brain tissue at this time. Total brain radioactivity declined rapidly; 24 hr after dosing, the brain radioactivity was less than 1 per cent of the administered radioactivity (Table 1). α-MeDA represented the majority of brain tritium; unchanged [^{3}H]MDA represented less than 10 per cent of the brain tritium at 24 hr (Fig. 2B).

The MDA metabolism studies carried out *in vitro* indicate that the sole metabolite is α-MeDA [4]. This result suggests that α-MeDA and/or its conjugates may be important metabolites of MDA *in vivo*. In fact, Foreman and Mandlik [7], in a preliminary report, showed that 3-*O*-Me-α-MeDA and its glucuronide were the principal urinary metabolites of MDA in the rat.

No unconjugated α-MeDA could be detected in the urine at any time after drug treatment. However, 3-*O*-Me-α-MeDA and its glucuronide accounted for over 50 per cent of urine radioactivity; unchanged MDA accounted for practically all of the remainder.

In the brain, the majority of the radioactivity 30–60 min after dosing was in the form of MDA. Analysis of brain samples 6–24 hr after dosing, however, revealed that free α-MeDA accounted for the majority of brain radioactivity. This indicates that MDA is either metabolized to α-MeDA in the brain or that α-MeDA formed as a peripheral metabolite of MDA passes the blood–brain barrier. The latter is unlikely since neither α-methyl-norepinephrine [8] nor dopamine [9], which are close structural analogues of α-MeDA, crosses the blood–brain barrier. The possibility that 3-*O*-Me-α-MeDA is *O*-demethylated to α-MeDA in the brain must also be considered as an explanation for the α-MeDA observed in brain tissue. The fact that the peak

brain levels of [^{3}H]α-MeDA occurred at approximately the same time as the non-amphetamine-like behavior (greatly reduced response to external stimuli, decreased motor activity, and signs of fatigue 6–18 hr after MDA administration to mice[1, *]) supports the hypothesis that α-MeDA may be the mediator.

Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, U.S.A.

GERALD M. MARQUARDT†
VICTOR DISTEFANO
LYDIA L. LING

* G. M. Marquardt, V. DiStefano and L. L. Ling, *Toxic. appl. Pharmac.* In Press.

† Present address: Environmental Protection Agency, Metabolic Effects Branch, OPP/CED, 401 M Street S.W., Washington, DC 20460.

REFERENCES

1. G. M. Marquardt, V. DiStefano and L. L. Ling, in *The Psychopharmacology of Hallucinogens* (Eds. R. Willette and R. Stillman), Chap. 7. Pergamon Press, New York (1977).
2. G. M. Marquardt, V. DiStefano and L. L. Ling, *Biochem. Pharmac.* **27**, 1497 (1978).
3. J. Casida, J. Engel, E. Essac, F. Kamienski and S. Kuwatsuka, *Science, N.Y.* **153**, 1130 (1966).
4. G. M. Marquardt and V. DiStefano, *Life Sci.* **15**, 1603 (1974).
5. G. A. Bray, *Analyt. Biochem.* **1**, 279 (1960).
6. J. R. Crout, in *Standard Methods of Clinical Chemistry* (Ed. Seligson), Vol. III, p. 62. Academic Press, New York (1961).
7. R. L. Foreman and J. V. Mandlik, *Pharmacologist* **16**, 218 (1974).
8. F. P. Nijkamp and W. DeJong, *Eur. J. Pharmac.* **32**, 361 (1975).
9. M. Winn, R. Rasmussen, F. Minard, J. Kyncl and N. Plotnikoff, *J. med. Chem.* **18**, 434 (1975).

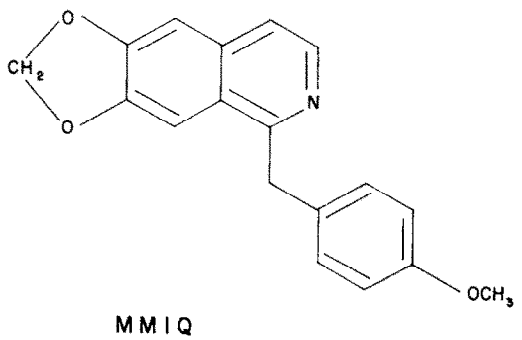
Biochemical Pharmacology, Vol. 27, pp. 1505–1507.
© Pergamon Press Limited, 1978. Printed in Great Britain.
0006-2952/78/0515-1505/$02.00/0

Effect of 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline on mitochondrial respiration

(*Received* 9 *May* 1977; *accepted* 22 *July* 1977)

The isolation of an alkaloid extracted from a species of *Ocotea* (*lauracea*) which grows in the Amazon region, Brazil, has recently been reported. Its chemical structure is 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline (MMIQ) (Fig. 1)[1]. The compound inhibits the conversion of cyclic 3′,5-AMP to 5′-AMP[1] and influences the contraction–relaxation cycle of smooth muscle. MMIQ is hydrophobic and possesses a positive charge at a pH around 7. Since certain hydrophobic compounds with a positive charge inhibit mitochondrial oxidative phosphorylation[2–4], the effect of MMIQ on the respiration and phosphorylation processes of mitochondria was explored. The results show that MMIQ inhibits the electron-transfer process of mitochondria.

Rat liver mitochondria were isolated in 0.25 M mannitol, 2 mM Hepes and 0.1% bovine serum albumin (pH 7.4). EDTA submitochondrial particles were prepared as described elsewhere[3]. Oxygen uptake was measured either with a Gilson or a YSI apparatus adapted with a Clark electrode. The ATPase activity of submitochondrial particles was measured in a mixture that contained 0.24 M mannitol, 10 mM Tris–HCl (pH 7.4), 11 mM ATP and 4 mM $MgCl_2$ in a final volume of 1.0 ml. The incubation time was 10 min at a temperature of 30°. The reaction was stopped with 6% trichloroacetic acid (final concentration). Inorganic phosphate was determined in the supernatant



MMIQ

Fig. 1. Chemical structure of MMIQ.

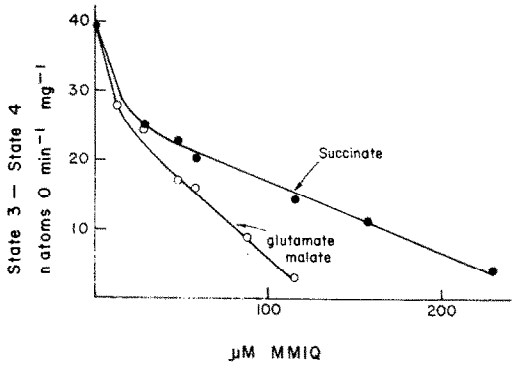


Fig. 2. Effect of MMIQ on the State 3–State 4 respiration of rat liver mitochondria. The incubation mixture contained, in a final volume of 2.0 ml, 10 mM glutamate, 10 mM malate, 5 mM phosphate (pH 7.4), 0.25 M mannitol, 1 mg of bovine serum albumin, 1.5 mg of mitochondrial protein and the indicated concentrations of MMIQ. State 3 was induced by the addition of 1 μmole ADP. Where indicated the mixture contained 10 mM succinate and 3 μg rotenone instead of glutamate-malate. Temperature was 27°. The results show enhancement of the respiratory rate attained by the addition of ADP to mitochondria incubated with the indicated concentration of MMIQ. For the effect of MMIQ on State 4, see text.