Metabolic Studies on Trimethoxyamphetamines[1] (34965)

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dl-2,4,5-Trimethoxyamphetamine (TMA-2) was reported by Shulgin (1) to be approximately 17 times more potent than mescaline as a psychotomimetic agent in man. An isomer of TMA-2, *dl*-2,3,4-trimethoxyamphetamine (TMA-3), however, was found to possess no psychotropic effects. The relative potency of these compounds as psychotomimetic agents was essentially confirmed in animal studies. Thus, the ED_{50} values (μmole/kg) for TMA-2 and TMA-3 in disrupting discrimination of different-sized disks by the trained squirrel monkey were 20.7 (95% confidence limits, 12.2–35.2) and 129.0 (95% confidence limits, 87.2–191.0), respectively (2). The ED_{50} values (μmole/kg) for TMA-2 and TMA-3 in disrupting performance in the Lashley III underwater maze by the trained rats were 12.8 (95% confidence limits, 7.5–21.8) and 52.1 (95% confidence limits, 27.6–98.5), respectively (3). The time of peak effect of these compounds in the rat was 30-min post-injection. The present studies were conducted to examine whether a difference in the metabolic handling of these two compounds by the rat is a factor in the observed relative potency of these compounds.

Materials and Methods. TMA-2 and TMA-3 labeled with ^{14}C on the β-carbon of the side-chain were synthesized[2] by a modified procedure of Shulgin (4) starting with the appropriate trimethoxybenzene and using $Zn^{14}CN$ as the formylating agent. The specific activities of TMA-2 and TMA-3 were 4.17 and 1.56 μCi/mg, respectively.

The labeled compounds were injected intraperitoneally to rats. Brain and liver were homogenized in approximately 5 vol of 0.1 *N* H_2SO_4. Plasma (1–1.5 ml) was brought to a final volume of 4 ml with 0.1 *N* H_2SO_4. To 4 ml of these tissue preparations, 0.5 ml of 4 *N* NaOH was added and they were extracted three times with 10 ml each of benzene. An aliquot of the combined benzene extracts from each organ was assayed for radioactivity and the counts were expressed as micrograms of unmetabolized TMA-2 or TMA-3.

Postmitochondrial supernatant fraction was prepared by centrifuging a 1:3 homogenate in isotonic KCl for 10 min at 10,000*g*. The microsomes were prepared by centrifuging the above supernatant for 30 min at 100,000*g*. Incubation mixture contained appropriate amounts of tissue fractions, 200 μmole of Tris buffer, pH 8.0; 10 μmole of TMA-2; 50 μmole of semicarbazide; 25 μmole of glucose-6-phosphate; 20 μmole of $MgCl_2$; 15 μmole of nicotinamide; 5 μmole of NAD, and 0.25 μmole of NADP in a final volume of 3.2 ml. Incubation was carried out in air for 2 hr at 37°. Formaldehyde was assayed by using the Nash reagent (5). Protein concentration was determined by the method of Lowry *et al.* (6).

Results and Discussion. After the administration of the labeled compounds, the distribution of these compounds in brain, liver, and plasma was examined as a function of time. As shown in Table I, the concentration of TMA-3 in the brain was higher than that of TMA-2 during the period examined. Since the peak effect of these compounds in behavioral studies was observed to be 30 min after injection, it can be concluded that the amount that enters the brain is not the factor that determines the psychotomimetic properties of these compounds.

The unmetabolized TMA compounds were

[1] This investigation was supported by US Public Health Service contract No. PH 43-65-1061 from the National Institute of General Medical Sciences.

[2] I am indebted to Morris A. Leaffer of the Pharmaceutical Chemistry Department for these syntheses.

TABLE I. Distribution of TMA-2 and TMA-3 in Rat Tissues.[a]

Exp. no.	Time after injection (min)	TMA-2 (μg/organ) Brain	TMA-2 (μg/organ) Liver	TMA-2 Plasma (μg/ml)	TMA-3 (μg/organ) Brain	TMA-3 (μg/organ) Liver	TMA-3 Plasma (μg/ml)
1	8	3.1	658.3	7.4	10.8	727.3	7.7
	30	5.3	214.1	6.1	17.0	143.6	2.4
	45	4.4	159.8	4.4	—	130.3	3.0
	60	2.4	75.3	1.4	9.3	77.8	1.5
2	8	—	—	—	9.1	422.2	5.1
	30	3.4	241.0	4.0	8.5	75.2	2.8
	45	3.2	140.0	3.3	11.1	100.2	1.8
	60	2.2	79.6	1.6	5.2	55.4	1.0

[a] Sprague-Dawley male rat (200 g) was injected with 12 mg/kg of TMA-2 (20,900,000 cpm or 1740 cpm/μg) or TMA-3 (7,810,000 cpm or 650 cpm/μg).

TABLE II. Site of *O*-Demethylation of TMA-2.[a]

	HCHO, μmole/g of: Liver	HCHO, μmole/g of: Protein
Supernatant, 10,000*g*	1.60	22.5
100,000*g*	Nil	Nil
+ microsomes	0.90	13.2
Microsomes + NADPH generating system[b]	1.22	90.6

[a] A New Zealand white rabbit (approx 2.5 kg) was used for tissue preparations.

[b] 3.5 units of glucose-6-phosphate dehydrogenase, 25 μmole of glucose-6-phosphate, and 0.25 μmole of NADP.

found to be quantitatively extracted into benzene from an alkaline pH. Most of the residual counts representing the metabolites were extracted into ethylacetate. Considerably higher ethylacetate extractable counts were found in the liver of rats injected with TMA-3 than with TMA-2.

In vitro studies with rat and rabbit liver homogenates indicated that TMA compounds were metabolized primarily by *O*-demethylation similarly to mescaline (7) but were not measurably deaminated as is the case with amphetamine (8). As shown in Table II, the TMA-demethylating enzyme is associated with liver microsomes. Rabbit liver preparation was used for this study because of its

TABLE III. *O*-Demethylation of Various Compounds.[a]

Enzyme source	Formaldehyde (μmoles/g of supernatant protein) *o*-Nitroanisole	Mescaline	TMA[b]	TMA-2	TMA-3
Rat	—	2.3	4.7	4.8	4.9
Rabbit	9.5	8.0	14.4	19.3	27.4
Rabbit (phenobarbital treated)	28.3	8.5	11.9	17.7	23.8

[a] Postmitochondrial supernatant fraction was prepared by centrifuging a 1:3 homogenate in isotonic KCl for 10 min at 10,000*g*. Phenobarbital (38 mg/kg) was given twice daily for 3 days and the rabbit was sacrificed on the fourth day. The data represent the average of two experiments.

[b] TMA is similar to mescaline in having 3,4,5-trimethoxy groups.

higher activity than that of the rat liver (Table III).

An interesting observation concerning the demethylase enzyme for TMA is that its activity is not elevated by pretreating the animals with phenobarbital. Under similar conditions, the demethylation of *o*-nitroanisole is stimulated by phenobarbital (Table III). Whether these data indicate different enzyme systems for the demethylation of *o*-nitroanisole and trimethoxy compounds is not clear. The position(s) of the methoxy group that is demethylated in TMA-2 has not been ascertained.

Summary. TMA-2 is a more potent psychotomimetic agent than TMA-3 but the latter is taken up by the rat brain to a greater extent than the former. These compounds are demethylated by the liver microsomal enzyme that does not respond to phenobarbital induction.

The technical assistance of Josephine Tagg and Theodore J. Sorich III, is gratefully acknowledged.

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Received Apr. 29, 1970. P.S.E.B.M., 1970, Vol. 134.