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SHORT COMMUNICATIONS

Determination and physiological disposition of *p*-methoxyphenylethylamine in the rat

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p-METHOXYPHENYLETHYLAMINE (PM) is the *O*-methylated analog of tyramine. It is structurally related to trimethoxyphenylethylamine or mescaline, a known hallucinogenic agent, and dimethoxyphenylethylamine, which is claimed by some investigators to be a specific compound found in the urine of schizophrenics.[1] PM has been reported to induce a catatonic state in mice[2] and to produce salivation, mydriasis, increased respiration, tremors and a hypokinetic syndrome in cats.[3, 4] In rabbits the compound evoked EEG arousal at the midbrain level similar to that produced by amphetamine.[5] Smythies *et al.*[6, 7] studied the effects of various methoxylated phenylethylamines on the behavior of rats and suggested that PM could be considered a "paradigm hallucinogenic compound". The compound has been detected by some[8] but not other investigators[9] in the urine of schizophrenic patients.

Due to the interest in this compound and a lack of data on its fate in animals, a rapid spectrophotofluorometric determination of PM was developed and its physiological disposition in the rat was studied.

The determination of PM is based on an extraction procedure followed by a reaction of PM with Dansyl chloride. Tissue (approximately 1 g) samples were homogenized in 3 vol. of 1 N HCl. To 3 ml homogenate or plasma, 1·5 ml of 5 N NaOH and 30 ml toluene were added. After shaking and centrifugation for 10 min, 25 ml of the toluene was transferred to tubes containing 1·5 ml of 0·5 M boric acid. Again, after shaking and centrifugation, the organic phase was discarded and 1 ml of the boric acid phase was boiled with 1 ml of 0·1 M borax solution and 0·02 ml Dansyl chloride solution (10 mg/l ml of acetone) for 15 min. The samples were cooled, extracted with 1·5 ml chloroform, and the organic phase was read in an Aminco–Bowman spectrophotofluorometer at 490 mμ (activation 338 mμ).

Readings of extracts of brain, heart, lung, liver, kidney, skeletal muscle and plasma obtained from uninjected animals were only slightly higher than readings of "water" blanks carried through this procedure. The addition of various amounts of PM to tissue samples produced readings which were linear in the range of 1–30 μg/tissue sample. The recovery with this procedure is between 70 and 85 per cent and the sensitivity is 1 μg/g of sample.

Verification of the specificity of the method was obtained by determining the quantities of PM in some tissue samples based on the fluorescence of this compound in the Aminco–Bowman spectrophotofluorometer at 640 mμ (activation 290 mμ) in 10% ethanolic KOH.[10] This procedure is less sensitive than the procedure described above. Nevertheless, similar results were obtained by both methods.

The intraperitoneal injection of 40 mg of PM-HCl/kg or higher in rats produced marked salivation and tearing, loss of control of the hindlegs, whole-body tremors and a hypokinetic syndrome. The effects usually started 5 min after injection and lasted for 15 min. Excessive salivation was probably not caused by inhibition of acetylcholinesterase, since it was found that PM (10^{-3}M) did not inhibit the enzyme *in vitro*. Pretreatment of the animals with atropine sulfate-H_2O (50 mg/kg) abolished only the excessive salivation. Injection of 100 mg/kg of *p*-methoxyphenylacetic acid, probably the main metabolite of PM,[11] produced no gross behavioral changes.

Peak levels of PM were observed in most tissues after 5 min (Table 1) and most of the compound was removed from these tissues in less than 30 min. In brain the highest concentrations were found after 15 min, at which time the ratio of the concentrations in brain and plasma was approximately 4. In agreement with earlier findings on dimethoxyphenylethylamine,[12] *O*-methylation of hydroxylated phenylethylamines seems to enhance their penetration through the blood–brain barrier. This is in

TABLE 1. TISSUE DISTRIBUTION OF *p*-METHOXYPHENYLETHYLAMINE IN THE RAT AFTER i.p. ADMINISTRATION OF 40 mg PM-HCl/kg*

Tissue	Time (min)		
	5 (μg/g or ml)	15 (μg/g or ml)	30 (μg/g or ml)
Brain	6·6 ± 0·8	17·0 ± 2·1	0·8 ± 0·4
Heart	14·4 ± 1·7	9·0 ± 2·1	ND
Lung	60·5 ± 7·3	29·2 ± 8·4	ND
Liver	49·1 ± 6·2	24·0 ± 6·1	ND
Kidney	38·4 ± 7·1	41·7 ± 12·1	9·9 ± 3·2
Skeletal muscle	1·4 ± 0·5	1·0 ± 0·4	ND
Plasma	6·9 ± 0·7	4·0 ± 0·9	ND

* Each value is the mean ± standard deviation of the results obtained from three to four animals. ND = not detectable.

TABLE 2. TISSUE DISTRIBUTION OF *p*-METHOXYPHENYLETHYLAMINE IN THE RAT 10 min AFTER i.p. ADMINISTRATION OF VARIOUS DOSES OF THE COMPOUND*

	Dose (mg/kg)		
	10 (μg/g or ml)	20 (μg/g or ml)	60 (μg/g or ml)
Brain	ND	0·7 ± 0·4	22·4 ± 4·7
Heart	ND	2·3 ± 1·0	32·9 ± 8·7
Lung	2·8 ± 0·8	3·1 ± 1·1	62·1 ± 10·9
Liver	1·5 ± 0·5	6·9 ± 1·9	68·5 ± 16·7
Kidney	7·7 ± 2·1	14·8 ± 3·2	88·6 ± 10·7
Skeletal muscle	ND	ND	3·9 ± 0·8
Plasma	ND	0·5 ± 0·3	7·3 ± 2·1

* Each value is the mean ± standard deviation of the results obtained from three to four animals. ND = not detectable.

contrast to serotonin, where *O*-methylation was found to be without effect on the penetration of this indolealkylamine.[13]

The physiological disposition of PM after the injection of various quantities of the compound is shown in Table 2. No tissue saturation was observed up to concentrations of 60 mg/kg of PM-HCl.

High speed centrifugation of 10% homogenates of liver or brain,[14] obtained from animals which had received 40 mg/kg of PM-HCl and which had been sacrificed 10 min after the injection, revealed approximately 80 per cent of the compound in the supernatant and 20 per cent in the pellet.

In summary, a method of determining *p*-methoxyphenylethylamine in biological samples is described. Peak levels of PM were observed in tissues 5 min after the injection of the compound except in the brain, which showed highest values (17 μg/g) after 15 min. Approximately 80 per cent of the compound was found in the supernatant of liver and brain homogenates. Most of the compound was lost from the tissues studied within 30 min.

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The epoxidation of aldrin by a modified Fenton's reagent and its inhibition by substituted 1,3-benzodioxoles

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FENTON'S reagent[1,2] and the Udenfriend system[3,4] have been widely employed as models in investigations of the mechanisms of enzymatic hydroxylation.[5,6] These systems often serve as useful models for the microsomal mixed-function oxidases, and the products resulting from aromatic hydroxylation are similar to those produced enzymatically.[7]

Although the epoxidation of double bonds is a well established property of microsomal enzymes,[8] there are no reports of epoxidation with either Fenton's reagent or the Udenfriend system. It has recently been reported that model systems involving OH-radicals are unable to hydroxylate aliphatic CH-bonds and do not form epoxides at a double bond.[9] Epoxidation is usually effected by peracid oxidation and is thought to occur by means of a mechanism involving the electrophilic OH^+ ion.[10] Thus, the epoxidation of 8- and 12-membered cyclic olefins has recently been reported to occur with hydrogen peroxide in the presence of metallic oxide catalysts and is considered to proceed through a highly active peracid intermediate.[11]

In the course of our continuing investigations of microsomal metabolism and of the mode of action of insecticide synergists, it was of interest to develop a model system capable of converting the cyclodiene insecticide, aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,3-endo-exo-5,8-dimethanonaphthalene), to its 6,7-epoxide, dieldrin. Initial attempts in this laboratory to epoxidize aldrin by means of either the Udenfriend or Fenton's system were unsuccessful. It was, however, established that the incorporation of bovine serum albumin (BSA) into Fenton's reagent resulted in the epoxidation of aldrin to dieldrin (Table 1). No similar initiation of dieldrin production was observed to occur, however, as a result of the inclusion of BSA in the Udenfriend system. In the presence of a similar concentration of egg albumin, aldrin epoxidation by the Fenton's reagent was approximately half that obtained with BSA. Although serum albumin and other sulfhydryl-containing proteins have been found to be necessary for some enzymatically catalyzed hydroxylation reactions,[12]