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SHORT COMMUNICATIONS

The relationship between the metabolic fate and pharmacological action of 5-methoxy-N-methyltryptamine *

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THE grass *Phalaris tuberosa* has been known to cause 'staggers' in sheep grazing on it. In studies to find the causative agents two compounds, hordenine and 5-methoxy-N-methyltryptamine, were isolated and characterized by Wilkinson.[1] The latter compound is of particular interest because of its close relationship to serotonin and other substituted tryptamines which have been described as psychotomimetic agents capable of disrupting conditioned behavior in animals.

In order to continue our studies of the metabolism and pharmacological actions of compounds related to serotonin, radioactive 5-methoxy-N-methyltryptamine was synthesized. This paper describes the characterization of 5-methoxyindoleacetic acid as the major urinary metabolite when 5-methoxy-N-methyltryptamine was given intraperitoneally to rats and also reports observations on oxytocic activity and behavioral reactions to the compound.

METHODS

Compounds

5-Methoxy-N-methyltryptamine-α-^{14}C, with a specific activity of 247 μc/g, was synthesized according to the procedure initially reported[1] and modified to give the free base in good yield.[2] Thus, 5-methoxytryptamine-α-^{14}C with a specific activity of 800 μc/g was converted to the tosyl derivative with *para*-toluenesulfonic acid. This intermediate with one hydrogen temporarily replaced was monomethylated with methyl iodide. The tosyl group was then removed in liquid ammonia to yield the required 5-methoxy-N-methyltryptamine-α-^{14}C.

5-Methoxyindole-3-acetic acid was obtained by saponification of 5-methoxyindoleacetonitrile with sodium hydroxide.

Measurements of radioactivity in urine and tissue were carried out on samples of 'infinite' thickness in an end-window G.M. counter.

Oxytocic activity was measured in the isolated estrus rat uterus preparation. Behavioral effects were measured on male and female Sprague-Dawley rats, which had reached stable basal rates for bar pressing in a Skinner box, on a schedule of reinforcement which provided a food pellet for the first emitted response after an average interval of 90 sec.[3]

RESULTS AND DISCUSSION

An average of 89·5% of the administered activity was found in the 24-hr urine. The specific activities, total activities, and percentage distribution of the radioactivity were obtained for a 10-min and a 2-hr period and are presented in Table 1.

Rats were dosed with 3 mg (0·74 μc) of 5-methoxy-N-methyltryptamine-α-^{14}C and the 24-hr urines collected. Chromatography of the urine in solvents A and B (see Table 2) showed the major metabolite to have properties identical with 5 methoxyindoleacetic acid (negative reaction with Gibb's reagent. R_f 0·14 and 0·82 in solvents A and B respectively). No unchanged 5-methoxy-N-methyltryptamine (R_f 0·86 and 0·68 in solvents A and B respectively) was found. The presence of a minor metabolite was noted on some chromatograms. This compound had an R_f of 0·33 in solvent B and gave a positive

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Ehrlich, negative Gibb's, and positive naphthoresorcinol reaction indicating that it was probably an O-glucuronide of 5-methoxy-N-methyltryptamine.

Scanning the radioactive chromatograms showed a peak of activity indicating that 5-methoxyindoleacetic acid accounted for 95% of the activity in the urine. In some chromatograms a few per cent of the activity was associated with what appeared to be the O-glucuronide.

TABLE 1. TISSUE DISTRIBUTION OF THE RADIOACTIVITY FROM N-METHYL-5-METHOXYTRYPTAMINE-α-^{14}C INJECTION IN RATS

Tissue	Rat 1 Tissue distribution after 10 min, 10-mg dose			Rat 2 Tissue distribution after 2 hr, 3-mg dose		
	(μc/g)*	(μc)†	(%)‡	(μc/g)*	(μc)†	(%)‡
Blood	$1{\cdot}38 \times 10^{-1}$	0·4	16·2	$1{\cdot}6 \times 10^{-3}$	$4{\cdot}2 \times 10^{-3}$	0·57
Heart	$1{\cdot}1 \times 10^{-1}$	$1{\cdot}4 \times 10^{-2}$	0·59	$1{\cdot}6 \times 10^{-3}$	$1{\cdot}6 \times 10^{-4}$	0·022
Lungs	$1{\cdot}2 \times 10^{-1}$	$2{\cdot}8 \times 10^{-2}$	1·16	$3{\cdot}4 \times 10^{-3}$	$8{\cdot}2 \times 10^{-4}$	0·11
Liver	$1{\cdot}9 \times 10^{-1}$	$3{\cdot}03 \times 10^{-1}$	12·3	$5{\cdot}6 \times 10^{-3}$	$8{\cdot}2 \times 10^{-3}$	1·1
Spleen	$1{\cdot}32 \times 10^{-1}$	$3{\cdot}04 \times 10^{-2}$	1·25	$9{\cdot}6 \times 10^{-3}$	$1{\cdot}2 \times 10^{-3}$	0·16
Kidney	$1{\cdot}92 \times 10^{-1}$	$8{\cdot}3 \times 10^{-2}$	3·4	$1{\cdot}0 \times 10^{-2}$	$2{\cdot}8 \times 10^{-3}$	0·38
Brain	$1{\cdot}34 \times 10^{-2}$	$4{\cdot}2 \times 10^{-3}$	0·17	$1{\cdot}5 \times 10^{-3}$	$3{\cdot}3 \times 10^{-4}$	0·45

* Specific activity (μc/g).

† Total activity (μc).

‡ Percentage of total activity recovered from the total radioactivity administered to the rats.

TABLE 2. R_f VALUES AND COLOR REACTIONS OF COMPOUNDS RELATED TO 5-METHOXY-N-METHYLTRYPTAMINE

	R_f values in solvent		Color of spots on paper with		
	A	B	Ehrlich	Gibbs	Naphtho-resorcinol
Serotonin	0·48	0·54	Blue	Blue	
5-Methoxytryptamine	0·76	0·64	Blue	None	
5-Methoxy-N-methyltryptamine	0·86	0·68	Blue	None	
5-Hydroxyindoleacetic acid	0·15	0·80	Blue	Blue	
5-Methoxyindoleacetic acid	0·14	0·82	Blue	None	
5-Methoxy-N-methyltryptamine-O-glucuronide		0·33	Blue	None	Purple

Solvent systems used were: (A) *n*-propanol:NH_3, 8:3; (B) *n*-butanol:acetic acid:water, 4:1:5. Sprays used for detection were Ehrlich's reagent (*p*-dimethylaminobenzaldehyde), 0·5% solution in 1·5 N HCl; Gibb's reagent, 2% ethanolic solution of 2,6-dichloroquinoneimide followed by saturated aqueous $NaHCO_3$; naphthoresorcinol, 1% in 10% solution of trichloroacetic acid in *n*-butanol (heated at 100° for 10 min).

5-Methoxy-N-methyltryptamine-α^{14}C (3 mg) was given to each of four rats, the 24-hr urines were collected and used for determination of 5-methoxyindoleacetic acid by the isotope dilution method.

It was found that 89·5% of administered 5-methoxy-N-methyltryptamine was excreted as 5-methoxyindoleacetic acid within 24 hr. The distribution of activity in the various tissues (see Table 1) showed, 10 min after administration, all specific activities to be similar with the exception of brain which had approximately one tenth the specific activity. Rapid metabolism of 5-methoxy-N-methyltryptamine was indicated by the fact that all tissue levels were reduced 10- or 100-fold within 2 hr.

5-Methoxy-N-methyltryptamine possessed only one tenth the oxytocic potency of serotonin, and was less active than either bufotenine or 5-methoxytryptamine. It was slightly more active than 5-methoxy-N,N-dimethyltryptamine (see Table 3).

5-Methoxy-N-methyltryptamine proved to have a moderately disruptive effect on conditioned behavior, a dose of 0·5 mg/kg reducing response rates to 50% of normal. It was not so potent as bufotenine or 5-methoxy-N,N-dimethyltryptamine and, whereas these compounds acted for more than an hour, it was noted that the response rates of rats dosed with 5-methoxy-N-methyltryptamine were depressed for 45 min and then returned toward normal, indicating a short-acting effect (see Table 3).

TABLE 3. A COMPARISON OF EFFECT ON CONDITIONED BEHAVIOR AND OXYTOCIC ACTIVITY OF COMPOUNDS RELATED TO 5-METHOXY-N-METHYLTRYPTAMINE

	Oxytocic activity*	Effect on VI behavior†
Serotonin	1	—
Bufotenine	0·49	—
5-Methoxytryptamine	0·7	—
5-Methoxy-N-methyltryptamine	0·1	—
5-Methoxy-N,N-dimethyltryptamine	0·08	—

* Potency of equimolar concentration of the base.

† Depression of response rates of rats on a variable-interval (VI) schedule after i.p. dosage at 0·5 mg/kg. Each + represents a 25% decrement in rate.

It has been postulated that the duration of action of psychotomimetic derivatives of tryptamine may be related to the rate at which they are metabolized.[4] It was observed that serotonin and N-monomethyl serotonin are good substrates for monoamine oxidase, whereas bufotenine is not.[5, 6] 5-Methoxytryptamine has been shown to be rapidly and almost quantitatively metabolized to 5-methoxyindoleacetic acid.[7] The N,N-dimethylated tryptamines bufotenine and psilocybin were found not to be readily metabolized to the corresponding acids.[4, 8] The rapid and nearly quantitative metabolic degradation of 5-methoxy-N-methyltryptamine to 5-methoxyindoleacetic acid found in the present study is in accord with the general concept that tryptamines and N-monomethyl derivatives are substrates for amine oxidase, whereas N,N-dimethylation protects the molecule from degradation by this means

N,N-Dimethyl and diethyltryptamine have been shown to be metabolized by hydroxylation in the 6-position.[8, 9] There was evidence that a minor metabolite of 5-methoxy-N-methyltryptamine was a glucuronide, presumably of a 6-hydroxy derivative, which may be significant since it has been shown that 6-hydroxylation of the N,N-disubstituted tryptamines increases potency in effecting conditioned behavior.[9] Since it has also been reported that 5-methoxylation of tryptamines, although decreasing oxytocic potency, increases their potency in disrupting conditioned behavior,[10] it is not surprising that 5-methoxy-N-methyltryptamine was found to have one tenth the oxytocic activity of serotonin but to have a moderate potency in disrupting the behavior of rats conditioned to a variable-interval schedule. This ability to affect behavior proved to be transient, as would be expected from the rapid metabolism of the compound. The present study failed to show any direct correlation between the radioactive level in the brain and the effect on behavior. A more detailed account of the behavioral effects of this compound will be presented elsewhere.

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Preliminary observations of the effect of DDT ingestion on the soluble proteins of rat liver

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IT HAS been observed that the ingestion of DDT results in a marked decrease in liver glucose-6-phosphate dehydrogenase,[1] an enzyme found in the soluble fraction. This observation has prompted a study of the distribution of soluble liver proteins on modified cellulose columns. Studies have been made with preparations from animals raised on control rations or rations containing 200 ppm DDT.

Male rats weighing 100 g were raised for 4 weeks on a semisynthetic ration[1] or a ration containing 200 ppm DDT. At the end of this period the animals were sacrificed, the livers excised, and 10% homogenates prepared in 0·25 M sucrose buffered to pH 7·4 with 0·005 M phosphate. Mitochondria, nuclei, and cell debris were removed by centrifuging at 10,000 *g* for 10 min. Centrifuging at 100,000 *g* for 30 min sedimented the microsomal fraction, and the soluble fraction remaining was used for these studies.

The soluble fraction was then treated with solid ammonium sulfate and the protein fraction recovered between 40% and 80% saturation was used for cellulose chromatography. One could expect a large proportion of the enzyme protein to be recovered in this fraction. The protein was redissolved in 0·005 M phosphate buffer at pH 7·0. The residual ammonium sulfate was removed by applying to a Sephadex G-25 column and eluting with buffer.

After this preliminary treatment the protein solution was applied to a DEAE-cellulose column (2·0 × 40 cm), prepared as described by Seal & Gutman.[2] The protein was eluted with 0·005 M phosphate buffer, pH 7·0 with a linear sodium chloride gradient. The effluent concentration of sodium chloride increased from 0·015 M at tube 35 to 0·46 M at tube 260. In the different phases of these experiments protein concentration was monitored by observing the absorbance at 280 mμ. Protein recoveries from both the Sephadex and cellulose columns ranged from 95–98%.

This level of DDT in the ration for the feeding period does not cause a change in growth rate but does result in a 35% increase in liver size. Also, the proportions of protein recovered at the different levels of ammonium sulfate saturation were not modified to any extent by DDT ingestion; approximately 32–38% of the soluble protein was recovered in the fraction from 40–80% saturation. The data illustrated in Fig. 1 show that DDT ingestion markedly affects the distribution of the particular protein fraction when chromatographed on DEAE cellulose. With the preparation from the control rat a major portion of the protein is eluted in tubes 40–60. Preparations from DDT-fed rats do not give this fraction, but a series of smaller peaks is eluted at higher sodium chloride concentrations. These experiments have been repeated with three rats from each dietary group, and distribution comparable to those given in Fig. 1 have been obtained. From the 260/280-mμ ratio it appears that the last fraction to be eluted is nucleoprotein.

The observed change of protein distribution could result from the direct interaction of DDT or its metabolites with the soluble proteins, or it is possible that different proteins are synthesized under this stress. In the latter case DDT might interact in some fashion with the reactions of protein synthesis. If DDT or its metabolites were adsorbed on a protein surface, a change in tertiary structure could produce a changed charge distribution and consequent changes in elution pattern on a cellulose