The Metabolism of 5-Methoxytryptophol

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5-Methoxytryptophol has been found to be present in the pineal gland and it has been reported that this compound, when administered to rats in microgram quantities, can reduce the incidence of estrus and retard the normal rate of ovarian growth (1).

This paper describes the synthesis of radioactive 5-methoxytryptophol, its distribution in various tissues and organs, and its metabolic fate, after administration to rats.

EXPERIMENTAL PROCEDURE

Compounds—Attention was initially given to the synthesis of tryptophols employing "cold" compounds prior to deciding on a suitable method for the radioactive material. An attempt was made to obtain a pure sample of 5-methoxytryptophol by use of the method found applicable for 5-benzoxyltryptophol and 5-hydroxytryptophol (2). This consisted of permitting oxalyl chloride and the required indole to react, yielding a substituted indole-3-glyoxyloyl chloride that was in turn reduced with lithium aluminum hydride. The structure of 5-hydroxytryptophol as reported in the literature was confirmed by synthesizing the compound by reduction of 5-(benzoxyl)indole-3-acetic acid with lithium aluminum hydride, an alternate and structurally unequivocal synthesis. Subsequent debenzylation yielded 5-hydroxytryptophol that was identical to the compound made by the other method.

Various attempts to prepare pure 5-methoxytryptophol by the lithium aluminum hydride reduction of 5-methoxyindole-3-glyoxyloyl chloride always yielded a mixture of at least two major products. However, when 5-methoxyindole-3-acetic acid was prepared and reduced with lithium aluminum hydride, 5-methoxytryptophol was synthesized in a way permitting radioactive labeling of the α carbon atom. Since 5-methoxytryptophol is a viscous liquid, it was found convenient to prepare a picrate and use that derivative in subsequent studies.

5-Methoxytryptophol-α-14C picrate with a specific activity of 256 μc per g was synthesized according to the method outlined in Fig. 1. Thus, treatment of 5-methoxyindole with 37% aqueous formaldehyde and 25% aqueous dimethylamine (3) gave 5-methoxyxymine. The latter was reacted with dimethyl sulfate (4) to give 5-methoxyxymine quaternary methosulfate, which was converted to 5-methoxyindole-3-acetonitrile-α-14C by treatment with sodium cyanide-14C (5). Saponification of the nitrile with aqueous potassium hydroxide (5) gave 5-methoxyindole-3-acetic acid α-14C, which was reduced with lithium aluminum hydride (6, 7) to 5-methoxytryptophol-α-14C. The 5-methoxytryptophol-α-14C was dissolved in the least amount of chloroform, and an equimolar amount of picric acid, dissolved in the least amount of boiling chloroform, was added to give 5-methoxytryptophol-α-14C picrate, m.p. 116-116.5° (literature, m.p. 117-118°) (8). Elementary analysis showed

C13H19NO6

Calculated: C 48.57, H 3.84, N 13.33

Found: C 48.66, H 3.88, N 13.30

Animals—Female Sprague-Dawley rats weighing 200 to 240 g were used for metabolic studies. Solutions of 5-methoxytryptophol α-14C picrate were prepared in 23% aqueous propylene glycol. The solution was administered by intraperitoneal injection. Animals were fed on a standard diet, but were deprived of food, although not water, on the experimental day.

Chromatographic Method—Descending paper chromatography was employed for the detection of metabolites in urine extracts. The solvents, Rf values, and color reactions of reference compounds are given in Table I. Radioactive chromatograms were scanned with a Scanogram RSC-5 (Atomic Accessories, Inc.).

Measurement of Radioactivity—Measurements were carried out with solid samples of "infinite thickness" on nickel planchets with an end window counter tube, the background of which was 22 c.p.m. The specific activities were determined by comparison with a stable polymer reference. A sample of 4 cm2, containing 0.1 μc of 14C per g of substance, gave approximately 255 c.p.m.

Urine and Tissues—The estimation of radioactivity in urine and tissues was done by first drying the tissue homogenate under a heat lamp until it was brittle enough to grind. The dried tissue was then ground into a fine powder in a mortar and pestle and deposited in layers onto a planchet with n-hexane until a constant number of counts was obtained. In the case of tissues which yielded an insufficient amount of dried material to give a sample of "infinite thickness" (i.e. adrenals, thyroid, ovaries), the estimation of radioactivity was done by dilution of the tissue with an inactive substance.

Estimation of 5-Methoxyindole-3-acetic acid-α-14C by Isotope Dilution—Urine samples were collected from each rat for a 24-hour period. Inactive 5-methoxyindole-3-acetic acid (200 mg) was dissolved in an aliquot of urine and allowed to stand for 2 hours to equilibrate. The solution was then adjusted to pH 3 with dilute HCl. The resulting erucoid 5-methoxyindole-3-acetic acid was recrystallized from toluene to constant specific activity.

RESULTS

Amount of Total Radioactivity Recovered and Distribution in Tissues of Administered 5-Methoxytryptophol-α-14C Picrate—An
Fig. 1. Synthetic scheme for 5-methoxytryptophol-\(\alpha\)-14C

TABLE I

Paper chromatography of metabolites

Descending paper chromatography was done on Whatman No. 1 paper. The solvent systems used were: Solvent A, 1-propanol-NH\(_3\) (8:2); and B, 1-butanol-acetic acid-water (4:1:5). The sprays used for detecting compounds on paper were: Reagent 1, Ehrlich's reagent (p-dimethylaminobenzaldehyde, 0.5% solution in acetone plus a few drops of concentrated HCl); 2, xanthydrol reagent (2% solution in ethanol plus a few drops of concentrated HCl); and 3, Gibb's reagent (2,6-dichloroquinonechlorimide, 0.05% solution in ethanol, followed by saturated aqueous NaHCO\(_3\) solution).

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R_F) values in Solvent</th>
<th>Color of spots on paper with Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>5-Methoxyindole-3-acetic acid</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>5-Methoxytryptophol</td>
<td>0.88</td>
<td>0.86</td>
</tr>
<tr>
<td>5-Hydroxyindole-3-acetic acid</td>
<td>0.23</td>
<td>0.80</td>
</tr>
</tbody>
</table>

average of 91.0% of the administered activity was found in the 24-hour urine. The specific activities and percentage distribution of the radioactivity in the tissues 1, 2, and 6 hours after administration are given in Table II.

Identification of Metabolites—Paper chromatography of the 24-hour urine of rats given 5-methoxytryptophol-\(\alpha\)-14C picrate showed the presence of one major metabolite which had the same \(R_F\) and color reactions in Solvents A and B (Table I) as 5-methoxyindole-3-acetic acid. No unchanged 5-methoxytryptophol-\(\alpha\)-14C picrate was found, with one exception, where about 50% of the radioactivity was associated with it as determined by scanning. The presence of a minor metabolite was noted on one chromatogram. This compound had an \(R_F\) of 0.12 in Solvent A, and gave a positive Ehrlich, negative naphthoresorcinol, and positive p-dimethylaminobenzaldehyde in acetic anhydride reaction, indicating that it was probably the glycine conjugate of 5-methoxyindole-3-acetic acid.

Determination of 5-Methoxyindole-3-acetic Acid-\(\alpha\)-14C—5-Methoxytryptophol-\(\alpha\)-14C picrate (3.0 mg) was administered to each of three rats, and the 24-hour urines collected and used for determination of 5-methoxyindole-3-acetic acid by the isotope dilution method. 5-Methoxyindole-3-acetic acid was found in all samples with a specific activity of 0.014 \(\mu\)curies/mg.

TABLE II

Tissue distribution of 5-methoxytryptophol-\(\alpha\)-14C in rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Radioactivity distribution after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour, given 2.9 mg</td>
</tr>
<tr>
<td></td>
<td>Rat 1</td>
</tr>
<tr>
<td></td>
<td>(\mu)curies/g</td>
</tr>
<tr>
<td>Urine</td>
<td>6,260</td>
</tr>
<tr>
<td>Blood</td>
<td>5.9</td>
</tr>
<tr>
<td>Lung</td>
<td>2.5</td>
</tr>
<tr>
<td>Liver</td>
<td>1.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.0</td>
</tr>
<tr>
<td>Brain</td>
<td>0.68</td>
</tr>
<tr>
<td>Heart</td>
<td>0.88</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.93</td>
</tr>
<tr>
<td>Ovary</td>
<td>1.5</td>
</tr>
<tr>
<td>Uterus</td>
<td>2.7</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.98</td>
</tr>
<tr>
<td>Total</td>
<td>71.59</td>
</tr>
</tbody>
</table>

\(^a\) Combined tissue from rats 5 and 6.
\(^b\) Average percentage for rats 5 and 6.
\(^c\) Combined tissue from rats 1 and 2.
\(^d\) Average percentage for rats 1 and 2.
\(^e\) Combined tissue from rats 3 and 4.
\(^f\) Average percentage for rats 3 and 4.
in an amount which represented an average of 93.3% of the administered 5-methoxytryptophol picrate.

**DISCUSSION**

5-Methoxytryptophol is rapidly metabolized, an average of 91% of the administered activity appearing in the urine within 24 hours. In some animals as much as 65% and 92% of the activity was found in the urine within 1 hour and 2 hours, respectively.

The major metabolite was identified as 5-methoxyindole-3-acetic acid and found, by isotope dilution technique, to represent 93.3% of the administered 5-methoxytryptophol. Excretion of unchanged 5-methoxytryptophol in the urine, though observed, does not appear to represent a normal major metabolic pathway.

The distribution of radioactivity in tissue 2 hours after administration of 5-methoxytryptophol showed high levels in thyroid, adrenals, ovaries, and uterus.

The presence of 5-methoxyindole-3-acetic acid in pineal tissue has been reported by Lerner, Case, and Takahashi (9), who suggested that it might be a metabolite of melatonin. Kveder and McIsaac (10), however, found less than 2% of radioactive melatonin excreted as the acid whereas 5-methoxytryptamine was 96% converted into 5-methoxyindole-3-acetic acid. Although the presence of 5-methoxytryptamine in the urine of rheumatic patients has been reported (11), to this time no evidence has been presented for its presence in pineal tissue. Axelrod and Weissbach (12) found that 5-hydroxyindole-3-acetic acid, though a poor substrate, could be converted into 5-methoxyindole-3-acetic acid by pineal hydroxyindole O-methyl transferase and suggested that this might account for the presence of the latter compound. Another more likely explanation now would appear to be that 5-methoxyindole-3-acetic acid present in pineal tissue is a metabolite of 5-methoxytryptophol.

**SUMMARY**

5-Methoxytryptophol-α-14C picrate was prepared. Since 5-methoxytryptophol is found in the pineal gland and it inhibits sexual development, its metabolism and tissue distribution were studied. After administration of the radioactive compound 91% of the activity was excreted in the urine within 24 hours. The major metabolite was 5-methoxyindole-3-acetic acid, representing 93.3% of the injected methoxytryptophol-α-14C picrate. Tissue distribution studies demonstrated that the radioactivity was higher in the thyroid, adrenals, ovaries, and uterus than other tissues.

**REFERENCES**

The Metabolism of 5-Methoxytryptophol
Peter Delvigs, William M. McIsaac and Robert G. Taborsky


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