Studies on the Metabolism and Mechanism of Action of Methyldopa

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The decarboxylase-inhibiting, sedative, and antihypertensive properties of \(\alpha\)-methyl-3,4-dihydroxy-\(L\)-phenylalanine (\(DL\)-\(\alpha\)-methyl-dopa) in man were first reported from this department in 1960. It was shown subsequently that these three properties of the compound reside solely in the L isomer (methyldopa, Aldomet). Numerous favorable reports on the use of methyldopa in the treatment of hypertension have appeared; prominent among these are the articles by Cannon et al., Dollery and Harrington, Irvine et al. Smirk, and Hamilton and Kopelman.

It is worth recalling that the original finding of blood pressure-lowering effects in man was not predicted. The compound had been shown to have no significant cardiovascular hemodynamic effects in animals and was used solely as an investigational tool for inhibiting the decarboxylation of aromatic-L-amino acids added exogenously to in vitro or in vivo systems. It seemed doubtful that the synthesis of endogenous norepinephrine could be decreased (and blood pressure thereby lowered) even with a more potent decarboxylase inhibitor for the following reasons: formation of 3,4-dihydroxyphenylalanine (dopa) from tyrosine is the rate-limiting step in synthesis, the amounts of dopa in tissues are so small as to be generally undetectable normally and the decarboxylation of dopa to dopamine (the immediate precursor of norepinephrine) is a highly efficient process. Indeed, it was the decrease in blood pressure in man that first suggested an effect on the sympathetic nervous system and afforded the major impetus to detailed studies which showed in animals that \(\alpha\)-methyl-dopa produces a long-lasting depletion of tissue norepinephrine. Several investigators have reported evidence that the norepinephrine depletion produced by \(\alpha\)-methyl-dopa and a close analogue, \(\alpha\)-methyl-\(L\)-tyrosine, is not due to decarboxylase inhibition per se but is produced by amine metabolites of the drugs.

As a working hypothesis, it has been assumed that the mechanism of norepinephrine depletion in animals is also responsible for the blood pressure effect in man. It was decided to carry out a more detailed investigation of the over-all metabolism of the drug, since this knowledge would be fundamental to further correlations with clinical response. Previous studies using \(DL\)-\(\alpha\)-methyl-dopa were indicative of poor absorption, though in studies with \(C^{14}\)-labeled methyldopa in two patients Dollery and Harrington noted about 60 per cent recovery of radioactivity in urine following single oral doses. Furthermore, while decarboxylation to urinary \(\alpha\)-methyl-dopamine was noted in previous studies with \(DL\)-\(\alpha\)-methyl-dopa, it seemed important to demonstrate that this was occurring only with the L isomer and not with the inert \(D\) isomer. In some of these studies, urinary vanilmandelic acid excretion was measured as an overall index of daily synthesis and release of norepinephrine. Finally, another decarboxylase inhibitor, the hydrazino analog of \(\alpha\)-methyl-dopa was administered to patients in an attempt to block the decarboxylation of...
methyldopa and thereby possibly antagonize its effects on blood pressure. The results of these studies are discussed in relationship to the mechanism of action of methyldopa and to the effects of other decarboxylase inhibitors in man.

Materials and Methods

The subjects were adult patients hospitalized at the Clinical Center with uncomplicated labile or persistent essential hypertension. All the \(\alpha\)-methyl-substituted amino acids and amines used in this study were obtained from the Merck, Sharp & Dohme Laboratories. The \(L\) and \(D\) isomers of \(\alpha\)-methyl-dopa, and \(\alpha\)-methyl-dopa hydrazine (MK-485) were administered orally in capsules, and methyldopa was infused intravenously as a fresh 0.5 per cent solution in 0.9 per cent NaCl solution.

Fluorometric Assays of \(\alpha\)-Methyl-Dopa and \(\alpha\)-Methyl-Dopamine

These compounds can be oxidized to highly fluorescent derivatives essentially according to the trihydroxyindole procedure of Bertler, Carlsson, and Rosengren,17 principally based on the method of Euler and Flodin.18 After adjustment of pH to about 6.0 to 6.5 with 5 N potassium carbonate, a sample containing 0.15 to 1.5 \(\mu\)g. of \(\alpha\)-methyl-compound in 0.5 to 3.0 ml. was transferred to a tube for assay. The following were then added: 0.5 ml. of 0.1 M phosphate buffer, pH 6.5, 0.05 ml. of 0.5 per cent zinc sulfate, and deionized distilled water to a final volume of 5 ml. Oxidation was then carried out by addition of 0.05 ml. of 0.25 per cent potassium ferriyanide solution. After 5 minutes, 0.5 ml. of a freshly prepared mixture of 5 N sodium hydroxide and 2 per cent ascorbic acid (9:1, v/v) were added, and the solution was allowed to stand in room light for 40 minutes. Faded blanks were used, consisting of samples in which the fluorescent product was allowed to develop and fade. This was accomplished by addition of sodium hydroxide after the 5-minute oxidation period, followed in 5 minutes by separate addition of the ascorbic acid. In addition to internal standards, 0.5 to 2.0 \(\mu\)g. standards of \(\alpha\)-methyl-dopa and \(\alpha\)-methyl-dopamine as well as reagent blanks were run parallel with the samples. The intensity of fluorescence was measured with an Aminco-Bowman spectrophotofluorometer. The fluorescent derivatives of \(\alpha\)-methyl-dopa and \(\alpha\)-methyl-dopamine are identical in their activation and emission maxima (400 and 515 m\(\mu\), respectively) and have a similar fluorescence intensity, about one fifth that of the fluorescent derivative of norepinephrine.

Procedures for Plasma

Heparinized blood samples (5 to 10 ml.) were centrifuged immediately at 0 C. and the plasma was removed. Cold 1 N perchloric acid (0.3 ml. per ml. plasma) was added, and the precipitate formed after standing for 30 minutes was centrifuged. Appropriate volumes of the supernatant (0.02 to 1.0 ml.) were assayed fluorometrically. Recovery of \(\alpha\)-methyl-dopa carried through the procedure was 95 ± 5 per cent. For assay of \(\alpha\)-methyl-dopamine alone, 2.0 ml. of the perchloric acid extract was brought to pH 6.5 with potassium carbonate and passed over a Dowex-50 column as described below for urine. Recovery of \(\alpha\)-methyl-dopamine added to blood was satisfactory (95 ± 5 per cent).

Procedure for Urine

Timed urine samples were collected in glass bottles containing sufficient 6 N HCl to give a final pH less than 3.5. Alpha-methyl-dopa plus \(\alpha\)-methyl-dopamine, presumably largely unconjugated, was determined by direct fluorometric assay on highly diluted urine (usually 1:50 to 1:400 with 0.01 N HCl). Urine collected at a time when the patient was receiving no medications served as a blank; at the dilutions used control samples gave readings approximating the reagent blank. To correct for moderate variations in fluorescence intensity from sample to sample, internal standards of 1 to 2 \(\mu\)g. \(\alpha\)-methyl-dopa were also added to diluted samples before oxidation. All samples were assayed in duplicate and the results averaged.

Assay of \(\alpha\)-methyl-dopamine was accomplished after separation from \(\alpha\)-methyl-dopa on a strong cationic exchange resin, Dowex-50-X4, 200-400 mesh. One to 2 ml. of urine were adjusted to pH 6.5, diluted to 10 ml. with water, and passed over a 13 mm.\(^2\) x 35 mm. column of the resin previously buffered at pH 6.5 with 0.1 M phosphate buffer. The column of the resin was washed with 40 ml. of water followed by 7 ml. of 1 N HCl. The \(\alpha\)-methyl-dopamine was then eluted with 10 ml. of 2 N HCl. The purity of this fraction was confirmed by paper chromatography. The recovery of \(\alpha\)-methyl-dopamine through the procedure was 95 ± 5 per cent.

The excretion of \(\alpha\)-methyl-dopa was taken as the difference between the total excretion of the two compounds determined directly on diluted urine and that of \(\alpha\)-methyl-dopamine.

Fate of Radioactive Methyldopa

Single 10 \(\mu\)C. doses of 2-C\(^14\)-methyldopa (sp. activity 5.2 \(\mu\)C./mg.) along with 250 mg. of nonradioactive methyldopa were administered orally to fasting subjects in a vehicle of 0.01 M citric acid.
acid flavored with sucrose. Urine was collected fractionally for the next 3 days and feces were collected for 4 days. All counting was done in a Packard Tri-Carb liquid scintillation spectrometer equipped with an automatic changer. One-milliliter samples of urine (undiluted or diluted up to 1:5) were added to 10 ml. of a liquid scintillant and counted, as described by Bray.\textsuperscript{19} Absolute efficiency of the system for counting benzoic acid-\textsuperscript{14}C in 1 ml. of water was in the range of 53 to 60 per cent. Carbon-14 was added to duplicate samples of the urine to correct for quenching, which varied from 0 to 30 per cent.

Single or pooled specimens of feces were homogenized in water to a final volume of 0.7 to 2 liters, with 0.5 ml. of octyl alcohol added to prevent foaming. One-milliliter portions of the homogenates were dried in small bags made from dialysis tubing and assayed for carbon-14 with essentially the combustion-liquid scintillation technique described by Kelly et al.\textsuperscript{20} The only modification was that a Thomas Safety Oxygen Flask Igniter was used to comb the samples, whose dry weights were 10 to 70 mg. In this method \textsuperscript{14}O\textsubscript{2} is absorbed with Hyamine hydroxide, which is then dissolved in a toluene phosphor. Numerous recoveries of carbon-14 (as cholesterol-4-\textsuperscript{14}C) added to fecal homogenates were in the range of 90 to 100 per cent. In the medium used, \textsuperscript{14}C\textsubscript{2}-toluene was counted with an efficiency of 42 to 45 per cent.

### Chromatographic Identification of \(\alpha\)-Methyl-Dopa and \(\alpha\)-Methyl-Dopamine in Urine

Filter paper chromatography of untreated urine and urine extracts was performed overnight with Whatman \#1 paper. Systems employed were: n-butanol: acetic acid: water 12:3:5, ascending; n-butanol saturated with 1 N HCl, descending; and isopropanol: ammonia: water 20:1:2, ascending. The chromatograms were sprayed with 0.01 N iodine in 5 per cent aqueous ethylene diamine, 5 per cent potassium ferricyanide in 0.01 M phosphate buffer, pH 7, diazotized p-nitroaniline, Gibb's reagent, and Folin's reagent. The presence of \(\alpha\)-methyl-dopa was shown repeatedly by chromatography of untreated urine from patients receiving either \(\alpha\)-methyl-DL-dopa or methyldopa.

Other identifications were carried out on various fractions of urine prepared by column chromatography. These were concentrated to near dryness in vacuo, and the residues were extracted with 1 ml. of ethanol and 10 ml. of acetone. The salts were centrifuged away and the extracts were evaporated to 0.5 to 1.0 ml. under nitrogen and 0.01 to 0.1 ml. volumes were applied to paper for chromatography.

In earlier studies by Dr. J. R. Crout in this department, the results of which have been reported elsewhere,\textsuperscript{2} systematic identification of metabolites was conducted on urine from two patients receiving 1.0 Gm. per day of DL-\(\alpha\)-methyl-dopa, as follows: 20 ml. of urine were adjusted to pH 6.0 and passed over a 1.2 by 5 cm. column of Amberlite CG-50 (type 2, 200 mesh and over), buffered at pH 6.0 as described by Pisano.\textsuperscript{21} The column was eluted with 5 ml. of 2 N acetic acid and 5 ml. of water. This eluate, designated “total bases” was found to contain \(\alpha\)-methyl-dopamine. A small spot, which was not identified, was also detected with Gibb's reagent in this fraction. If the urine was first subjected to acid hydrolysis (100 C. for 20 minutes at pH 1), approximately equal amounts of 3-methoxy-\(\alpha\)-methyl-dopamine could also be identified in this fraction. The effluent from this column was brought to pH 8.4 with 1 N ammonium hydroxide and passed over a 1.2 by 4 cm. column of alumina and eluted with 10 ml. of 0.2 N acetic acid to give a fraction designated “neutral and acid catechols.” This fraction was found to contain large amounts of \(\alpha\)-methyl-dopa. The effluent from the alumina column was brought to pH 6.0 with 1 N HCl, passed over a 1.2 by 5 cm. column of Dowex-1 chloride to remove organic acids and the effluent designated “neutral non-catechols.” A small spot, possibly 3-methoxy-\(\alpha\)-methyl-dopa, was detected in this fraction with Gibb's reagent.

In the present study, methyldopa and \(\alpha\)-methyl-dopamine were also identified in the urine of two patients receiving 1-2 Gm. per day of methyldopa. Aliquots of urine (up to 10 per cent of a 24-hr. collection) were in turn applied to Dowex-50 columns as were used for \(\alpha\)-methyl-dopamine assays (see above) and to columns of Amberlite CG-50, pH 6.0. A single fluorescent spot (ferricyanide spray) representing methyldopa was found in the effluents from the two columns, and another single fluorophor representing \(\alpha\)-methyl-dopamine was found in the acid eluates from these columns. In addition, the purity of the \(\alpha\)-methyl-dopamine fraction obtained from the Dowex-50 columns utilized in the method of assay for this substance has been verified repeatedly by paper chromatography. In the course of these studies, several attempts were made to isolate and identify \(\alpha\)-methyl-norepinephrine. In one case, tentative identification of this compound was made but more studies are required.

### Results

**Plasma Levels of Methyldopa**

Three subjects were given 500 mg. of methyldopa intravenously and orally on dif-
different days. Plasma levels were similar in all three cases; those observed in two of the subjects are shown in figure 1. After oral administration, peak plasma levels were obtained in 2 hours but failed to rise to the concentrations that followed intravenous administration. Alpha-methyl-dopamine was not detected in plasma (<0.3 µg./ml.) These results indicate that while absorption from the gastrointestinal tract is relatively rapid, it is incomplete. The plasma half-life of the drug was in the range of 1.4 to 1.8 hours.

Plasma levels of methyldopa were studied in the same three subjects at another time during treatment with 250 mg. or 375 mg. of methyldopa every 8 hours for 5 days. No significant accumulation of drug was found (fig. 2). Variations in the levels obtained 3 hours after the first dose each day are suggestive of variable absorption. Each patient exhibited a decrease in blood pressure that was consistent and not correlated with variations in plasma levels.

**Urinary Excretion of Methyldopa and α-Methyl-Dopamine**

The recovery of unchanged drug plus free α-methyl-dopamine in the first 24 hours following the single oral and intravenous doses in the same three subjects is shown in table 1. Recovery of the oral dose ranged from 12 to 17 per cent, again indicating incomplete absorption, while 74 to 96 per cent of the intravenous dose appeared in the urine as unconjugated drug plus its amine metabolite. The latter accounted for 1.5 to 3.5 per cent of the doses administered. In other studies (see, for example, table 3 subsequently), it was found that the recovery of drug in urine following oral administration could be increased con-
were not determined in five of these patients, so that no correlation with per cent absorption could be made. Patient F.D., who exhibited rather poor absorption, has been found to require as much as 4.0 Gm. of the drug per day for a significant pharmacologic effect.

**Absence of Decarboxylation of D Isomer; Effect of L Isomer on Urinary Vanillmandelic Acid.**

The key to the mechanism of action of \(\alpha\)-methyl-dopa on blood pressure may lie in the differences between the \(n\) and \(L\) isomers. Though the pharmacologic, decarboxylase-inhibiting and norepinephrine-depleting properties were shown previously to reside solely in the \(L\) isomer,\(^2,3,10\) decarboxylation of the individual isomers themselves was not studied. If the action of methyldopa on blood pressure is related to decarboxylation to \(\alpha\)-methyl-dopamine, the latter compound should not appear in urine during treatment with the \(n\) isomer. Summary of studies in three patients is presented in table 3. Each isomer was given on a 6-hour schedule in the total daily dosage indicated, for 4 days. At least 3 days were allowed to elapse between treat-

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### Table 1

**Urinary Excretion of Methyldopa and \(\alpha\)-Methyl-Dopamine after Single Oral and Intravenous 500-Mg. Doses of Methyldopa**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Route</th>
<th>Methyldopa</th>
<th>(\alpha)-Methyl-dopamine</th>
<th>% Recovery of dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I oral</td>
<td>72</td>
<td>6.1</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>I.V.</td>
<td>333</td>
<td>10.2</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>II oral</td>
<td>56.3</td>
<td>5.6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>I.V.</td>
<td>475</td>
<td>6.8</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>III oral</td>
<td>74.7</td>
<td>11.9</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>I.V.</td>
<td>355</td>
<td>17</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

*Values presented represent unconjugated material; see text.

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### Table 2

**Excretion of Radioactivity after Oral Administration of 2-C\(^{14}\)-Methyldopa**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Per-cent of administered dose</th>
<th>4-Day feces</th>
<th>3-Day urine</th>
<th>Total % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.W.</td>
<td>30</td>
<td>74</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>J.M.</td>
<td>53</td>
<td>50</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>F.D.</td>
<td>74</td>
<td>31</td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

*\(^{14}\)C dose = 10 \(\mu\)C. See Methods section for details.

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### Fate of 2-C\(^{14}\)-Methyldopa

A more precise evaluation of the over-all fate of methyldopa was obtained by measuring the urinary and fecal excretion of radioactivity following single 10 \(\mu\)C. doses of the radioactive drug in three patients. Total recovery of radioactivity was essentially quantitative (table 2). Since excretion in the urine appeared to represent the entire amount absorbed, only urinary radioactivity was determined in three additional subjects. The cumulative urinary excretion of \(^{14}\)C in all six subjects is shown in figure 3. It is apparent that the absorption is highly variable between individuals, with the total percentage appearing in the urine ranging from 26 to 74 per cent in this group. More than half of the total \(^{14}\)C excreted appeared within 6 hours, and excretion was essentially complete within 24 hours though small amounts of radioactivity could be detected for up to 72 hours. The blood pressure-lowering effects of methyldopa
MENTS. It may be seen that no a-methyl-dopamine (<0.1 mg./day) was found in the urine during treatment with the n isomer, whereas easily measured amounts were found in the same patients when receiving L isomer.

In some cases the urinary excretion of the catecholamine metabolite, vanilmandelic acid (VMA) was also determined as shown in table 3. In confirmation of the findings of Cannon et al. as well as numerous unpublished observations in our laboratory, methyldopa apparently has no significant inhibitory action on the synthesis of endogenous norepinephrine as indicated by urinary VMA in man.

Inhibition of Methyldopa Decarboxylation by a-Methyl-Dopa Hydrazine (MK-485) without Effect on Blood Pressure

It was of interest to learn whether inhibition of the decarboxylation of methyldopa to a-methyl-dopamine with another decarboxylase inhibitor would alter the blood pressure response to methyldopa. Alpha-methyl-dopa hydrazine, the compound made available for this purpose, has been shown to be a much more potent decarboxylase inhibitor than methyldopa. Unlike methyldopa and a-methyl-m-tyrosine, the hydrazine compound does not readily penetrate the brain, nor does it produce significant depletion of tissue norepinephrine levels in animals except in extremely high dosage (>300 mg./Kgm.).

Three patients were selected for study on the basis of demonstrable blood pressure responsiveness to methyldopa in a dose of 500 mg. every 8 hours. The hydrazine compound was administered in a similar dosage schedule, each dose being given 1 hour before a dose of methyldopa when the two compounds were being given in combination. In two patients (W.G. and C.R., table 4), MK-485 was added for 5 days to existing therapy with methyldopa. In the third patient (C.W.), methyldopa was added to maintenance treatment with MK-485; after 6 days of combination therapy MK-485 placebo capsules were substituted for active drug. As seen in table 4, much less a-methyl-dopamine was excreted in the urine during combination therapy than during treatment with methyldopa alone. There was no discernible alteration, however, in the sedative or hypotensive effects of methyldopa. This is illustrated by the findings in patient C.W., which are graphed in figure 4. As shown, MK-485 alone had no significant effect on the blood pressure. When methyldopa was added, a prompt decrease in blood pressure occurred, which continued in the presence of low urinary levels of a-methyl-dopamine. Levels of amine metabolite increased following change to MK-485 placebo but the blood pressure lowering effect of methyldopa was essentially unchanged. While superficially these experiments would appear to rule out decarboxylation of the drug as being a factor in the hypotensive effect, they

Table 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isomer (Gm./day)</th>
<th>a-Methyl-dopa* mg./day</th>
<th>a-Methyl-dopamine* mg./day</th>
<th>VMA† mg./day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.T.</td>
<td>D (1.0)</td>
<td>111</td>
<td>..</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>L (1.0)</td>
<td>127</td>
<td>645</td>
<td>12.8</td>
</tr>
<tr>
<td>R.B.</td>
<td>D (1.0)</td>
<td>69</td>
<td>..</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>L (1.0)</td>
<td>116</td>
<td>241</td>
<td>4.6</td>
</tr>
<tr>
<td>F.D.</td>
<td>D (4.0)</td>
<td>225</td>
<td>..</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>L (4.0)</td>
<td>487</td>
<td>690</td>
<td>13.0</td>
</tr>
</tbody>
</table>

*Each value is average of results obtained for at least 2 days. Total refers to the value obtained if the urine sample was subjected to acid hydrolysis, i.e., adjusted to pH 0-1 and heated at 100 C. for 20 minutes.

†Values in parentheses represent controls.
Table 4
Excretion of $\alpha$-Methyl-Dopamine during Treatment with Methyldopa Alone and in Combination with $\alpha$-Methyl-Dopa Hydrazine (MK-485)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Drugs-dose (Gm./day)</th>
<th>Urinary $\alpha$-methyl-dopamine (mg./day)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.G.</td>
<td>a. Methyldopa (1.5)</td>
<td>9.0-13.9</td>
</tr>
<tr>
<td></td>
<td>b. Methyldopa (1.5) plus MK-485 (1.2)</td>
<td>2.0-3.2</td>
</tr>
<tr>
<td>C.R.</td>
<td>a. Methyldopa (1.5)</td>
<td>5.6-13.9</td>
</tr>
<tr>
<td></td>
<td>b. Methyldopa (1.5) plus MK-485 (1.2)</td>
<td>0.1-0.7</td>
</tr>
<tr>
<td>C.W.</td>
<td>a. Methyldopa (1.5)</td>
<td>0.9-2.4</td>
</tr>
<tr>
<td></td>
<td>b. Methyldopa (1.5) plus MK-485 (0.4-1.05)</td>
<td>0.1-0.6</td>
</tr>
</tbody>
</table>

*Figures shown represent range of values obtained during at least 5 consecutive days of treatment.

Discussion

As shown by the studies on urine, the pathways for metabolism of methyldopa in man are as depicted in figure 5. Following intravenous administration, most of the drug is excreted unchanged in the urine. After oral administration, that portion which is absorbed is excreted in the urine chiefly as unchanged drug plus acid-labile conjugates. In extensive studies on the fate of $C^{14}$-labeled methyldopa Trenner has shown the conjugated product to be the ethereal sulfate, 3-0-sulfate-methyldopa. A small percentage of the drug is metabolized by catechol-$\alpha$-methyl-transferase to the 3-0-methyl derivative and a significant fraction is metabolized also by decarboxylation to $\alpha$-methyl-dopamine. The latter compound becomes the predominant amine in the urine in a patient under treatment with clinically effective doses of methyldopa and has been present in every case we have studied.

There is considerable species variation in decarboxylation of the drug. Porter and Titus have found that about 50 per cent of the radioactivity injected as 1-$C^{14}$-methyldopa in rats appears in the expired CO$_2$ in 24 hours. In contrast, probably no more than 5 to 10 per cent of a given dose of methyldopa is decarboxylated in man. Since $\alpha$-methyl-dopamine is not a substrate for monoamine ox-
METHYLDOPA

dase, probably most of the amine formed would be excreted as such or as conjugates. Presumably some of the amine is 0-methylated, and theoretically β-hydroxylation to α-methyl-norepinephrine might also occur but the latter compound has been identified only tentatively in human urine. Carlsson and Lindqvist\textsuperscript{12} reported the presence of this amine in the brains of mice and rabbits given large doses of α-methyl-dopa but this could not be confirmed by Porter and Titus.\textsuperscript{24}

The finding of considerable individual variation in the percentage of drug absorbed from the intestinal tract probably accounts in part for the widely varying doses required to obtain a therapeutic response in different patients. In any given patient, however, it is generally true that the blood pressure response is a relatively smooth one on a 6- or even 8-hour schedule of dosage. In view of the rapid disappearance of drug from the plasma and its quick appearance in the urine, the evenness of blood pressure control with methyldopa in responsive patients is all the more striking. Obviously, the effect on blood pressure is not a function of blood level of the compound at a given moment. Thus, methyldopa itself may be looked upon as a "hit and run" drug, the major question being which aspect of its action or metabolism accounts for a persistence of clinical effect.

The weight of available evidence suggests that the mechanism of action of methyldopa on blood pressure is not via decarboxylase inhibition, though that it does produce significant enzyme inhibition in man is unquestionable.\textsuperscript{1, 3, 25} To be effective in lowering blood pressure, decarboxylase inhibition would have to be sufficient to block endogenous synthesis of the pressor mediator, norepinephrine. The fact that urinary vanilmandelic acid levels are unchanged during treatment with methyldopa provides strong evidence against the existence of any significant alteration in norepinephrine synthesis. Furthermore, norepinephrine depletion in animal tissues produced by methyldopa and α-methyl-m-tyrosine appears to be unrelated to decarboxylase inhibition alone, and other potent decarboxylase inhibitors have been found to lack norepinephrine-depleting activity (table 5).

Currently, we favor the hypothesis that blood pressure lowering is mediated by the amine metabolites, α-methyl-dopamine and possibly α-methyl-norepinephrine (cobefrine) formed following decarboxylation of the drug itself. The data reported here, showing that the inactive D isomer is not decarboxylated, are consistent with this hypothesis and also afford validation of the concept that the decarboxylating enzyme is specific for aromatic-L-amino acids.\textsuperscript{26} While the amine compounds are pressor substances (albeit weaker than norepinephrine) when injected directly into the circulation, it is well known that the effects of amines may be quite different when introduced into the body via their precursor amino acids.\textsuperscript{13, 14} It is known also that the amine metabolites of methyldopa and α-methyl-m-tyrosine are much more potent than the parent drugs in producing catecholamine depletion,\textsuperscript{10} that far less than stoichiometric amounts in tissues are required to elicit and maintain depletion and that α-methyl-m-tyrosine must be decarboxylated to bring about norepinephrine release.\textsuperscript{13} The present experiments showed inhibition of the decarboxyla-

\textit{Pathways of methyldopa metabolism. Solid arrows indicate reactions proved to occur in man.}

\textbf{Figure 5}
tion of methyldopa by α-methyl-dopa hydrazine without blockade of the blood pressure effect might at first seem to negate the hypothesis. However, Udenfriend et al.13, 27 found that amounts of the hydrazine sufficient to reduce by over 90 per cent the urinary excretion of α-methyl-m-tyramines from α-methyl-m-tyrosine had little effect on amine formation in brain and heart. When larger doses of the hydrazine were used, it was possible to prevent formation of the amines in tissues and to antagonize the norepinephrine-depleting effect of the precursor amino acid. In comparison, the doses of α-methyl-dopa hydrazine employed in our clinical trials are minute. There is need for a potent decarboxylase inhibitor that readily penetrates the central nervous system (unlike MK-485), does not deplete norepinephrine, and that may be given safely in large doses to man.

Finally, added insight may be gained from studies in animals and man of a variety of decarboxylase inhibitors. Pertinent observations on five inhibitors, four of which we have administered to patients, are listed in table 5. It is apparent that the decarboxylase-inhibiting and norepinephrine-depleting properties are clearly dissociable. The mechanism by which compound no. 5 depletes norepinephrine is unknown but may be nonspecific, since many agents have slight amine-depleting activity. In terms of hypotensive effect, α-methyl-m-tyrosine was a critical compound. Numerous studies in animals had failed to elicit any hypotensive activity. This presented a dilemma, since the general pattern of biochemical activity of this compound is similar to that of methyldopa. In recent studies, however, we have observed significant decreases in blood pressure following intravenous infusion of α-methyl-m-tyrosine to hypertensive patients.33 More research is required, particularly along the lines of the precise chemical and pharmacologic actions of the α-methyl-amines at sympathetic nerve synapses in blood vessels and in the brain.

Summary

The metabolism and particularly the decarboxylation of methyldopa were studied in hospitalized patients with uncomplicated hypertension. Both unlabeled and radioactive drugs were employed in these studies. Absorption from the intestine, as indicated by plasma levels of the drug and recovery of radioactivity in urine, was shown to be incomplete. The drug disappeared from the plasma with a half-time of less than 2 hours. On the average, about half of the radioactivity administered orally as 2-C14-methyldopa could be recovered in the urine. That portion not excreted in the urine appeared in the feces. Urinary excretion of radioactivity was rapid and essentially complete within 24 hours.

Confirming previous studies with DL-α-methyl-dopa, the major urinary excretory products of L-α-methyl-dopa (methyldopa, Aldomet) were found to be the drug itself and conjugates thereof. Small fractions appear as other metabolites, the most significant of which is the decarboxylated derivative, α-methyl-dopamine. The pharmacologically

Table 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>Norepinephrine depletion</th>
<th>Hypotensive effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. α-methyl-dopa</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>2. α-methyl-2,3-dopa</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. α-methyl-dopa hydrazine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. α-methyl-m-tyrosine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5. Serine-N7-(2,3,4-OH-benzyl)-hydrazide</td>
<td>+ (weak)</td>
<td>±</td>
</tr>
</tbody>
</table>

*See references 9, 10, 11, 13, 16, 28, 29, and 30 for chemical and pharmacologic studies in animals of compounds 1 to 4. Basic studies on compound no. 5 have been presented by Burkard et al.31 and Pletscher and Gey.32
inert p-isomer of a-methyl-dopa was found not to be decarboxylated. Alpha-methyl-dopa hydrazine was shown to reduce significantly the excretion of a-methyl-dopamine during treatment with methyldopa but the effects of the latter drug on blood pressure were not altered. On the basis of unchanged urinary excretions of vanilmandelic acid, decarboxylation inhibition during treatment with methyldopa was judged to be inadequate to inhibit endogenous synthesis of norepinephrine.

The relationships between metabolism of the drug and clinical response are discussed. The hypothesis is presented and defended that the hypotensive effects of methyldopa are mediated by amine metabolites of the compound, which are formed following its decarboxylation. Probably norepinephrine depletion is the final denominator but other possibilities exist.

Addendum

In a recent paper, Davis et al.\textsuperscript{14} report that the hypotensive effects of methyldopa in the renal hypertensive rat can be almost abolished by prior treatment with a potent decarboxylase inhibitor (NSD-1059).

References


Circulation, Volume XXVIII, October 1963

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*METHYLDOPA*

23. TRENNER, N. R.: Personal communication.


Discovery

Whether or not it is true that the age of the adventurer in the physical world is now closed, adventure in the world of thought is still open to every soul that is not wholly tamed and in love with the cage. It has always been more difficult than adventure in the ordinary sense, and it is becoming more difficult every day. Uniformity of thought is increasingly the apparent goal and demand of civilization; education has no use for the fires of rebellion, and even science itself is not above lending an occasional hand at the fire-engine. Still there burns on in most of us a small wild spark. I advise you to nourish it as a precious possession. Do not, however, be under any misapprehension. Really to think for oneself is as strange, difficult, and dangerous as any adventure, and, as the wise ones say, 'it will do you no good'; but, like virtue—which it does not otherwise greatly resemble—it will be its own reward.—The Collected Papers of Wilfred Trotter, F.R.S. London, Oxford University Press, 1946, p. 88.
Studies on the Metabolism and Mechanism of Action of Methyldopa
ALBERT SJOERDSMA, AADO VENDSALU, KARL ENGELMAN and Miss Doris Watts

_Circulation_. 1963;28:492-502
doi: 10.1161/01.CIR.28.4.492

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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