A Metabolic Study of α-Methyl-β-(3,4-dihydroxyphenyl)-DL-alanine in Man

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α-Methyl-(3,4-dihydroxyphenyl)-DL-alanine (*alpha*-methyldopa) was administered to ten hospitalized patients in amounts ranging from 0.75–10.0 g./24 hr. After intravenous administration it appeared to be cleared rapidly from the plasma. Very little of the material passed into the erythrocytes. The percentage of the daily oral dose excreted in the urine was 9.9% (standard deviation, 5.7%; number of observations, 22). Small amounts of the drug were still measurable in the urine on the second day after its withdrawal from these subjects. Studies in three healthy males ingesting 0.25 g. of the drug showed that excretion of *alpha*-methyldopa reaches a peak in 3–4.5 hr. *alpha*-Methyldopamine, the decarboxylation product of *alpha*-methyldopa, was detected in certain urines.

Anti-decarboxylases represent a new category of pharmacological agent whose name is derived from their inhibitory action upon decarboxylases of L-amino acids. The best known representatives[1] are α-methyl-β-(3,4-dihydroxyphenyl)-alanine[2] and α-methyl-β-(3-hydroxyphenyl)-alanine. Their inhibitory properties *in vitro* were first described in 1954[3]; it was later shown that *alpha*-methyldopa is also active *in vivo*, inhibiting the decarboxylation of dopa[2,4,5] and 5-hydroxytryptophan.[6] The anti-decarboxylase properties of *alpha*-methyldopa are also exhibited in humans. In demonstrating this, Oates *et al.* have shown that this compound lowers the blood pressure of hypertensive individuals.[7] An incidental finding in their

(1) T. L. Sourkes and A. D'Iorio, in "Metabolic Inhibitors," R. M. Hochster and J. H. Quastel, eds., Academic Press, Inc., New York, in press.

(2) These short names are used: dopa = β-(3,4-dihydroxyphenyl)-alanine; *alpha*-methyldopa = α-methyl-β-(3,4-dihydroxyphenyl)-alanine; *alpha*-methyldopamine = 3,4-dihydroxyamphetamine = 1-(3,4-dihydroxyphenyl)-2-aminopropane.

(3) T. L. Sourkes, *Arch. Biochem. Biophys.*, **51**, 444 (1954).

(4) H. Dengler and G. Reichel, *Arch. exptl. Pathol. Pharmakol.*, **234**, 275 (1958).

(5) G. F. Murphy and T. L. Sourkes, *Rev. canad. biol.*, **18**, 379 (1959).

(6) E. Westermann, H. Balzer, and J. Knell, *Arch. exptl. Pathol. Pharmakol.*, **234**, 194 (1958).

(7) J. A. Oates, L. G. Gillespie, S. Udenfriend, and A. Sjoerdsma, *Science*, **131**, 1890 (1960).

study was that *alpha*-methyldopa induces a certain measure of sedation.

There is little information available on the metabolism of *alpha*-methyldopa at the present time. This compound has been shown to follow certain of the metabolic pathways already known for its natural analog, 3,4-dopa: combination with pyridoxal phosphate,[3] formation of melanin,[8] and decarboxylation.[9] It does not undergo transamination.[10] Following its intraperitoneal injection into rats in a dose of 100 mg./kg. *alpha*-methyldopa has been found in the brain, liver, spleen, and kidneys.[5] Its concentration in the first three organs rises rapidly to a peak and then gradually falls over a period of 2.5 hr. to barely detectable levels. In the kidneys *alpha*-methyldopa is maintained at a constant level for at least 1.5 hr., after which its concentration falls rapidly. With larger doses of the drug the concentration in the brain may be kept at over 20 μg./g. for 2 hr., and some *alpha*-methyldopa is still measurable there 6 hr. following the injection.[11] Hogans and Porter have tested the individual optical isomers in the mouse and dog, and have observed a much more rapid excretion of D-*alpha*-methyldopa than of the L-isomer.[12] The recently described clinical actions of *alpha*-methyldopa now indicate the need for more information about the metabolism of this compound in man. The results of a study on the levels of *alpha*-methyldopa found in the blood and urine of subjects receiving the drug form the subject of the present paper.[13]

Because *alpha*-methyldopa is slowly decarboxylated enzymatically,[9] attempts were made to measure the amine formed. This substance, *alpha*-methyldopamine, belongs to the family of amphetamines.[2]

Methods

alpha-Methyldopa and *alpha*-methyldopamine were extracted from urine and from deproteinized blood filtrates by adsorption on alumina at pH 8–9 (phenolphthalein end-point), and were eluted with 0.5 *M* acetic acid. Fluorophores were

(8) T. L. Sourkes, *Rev. canad. biol.*, **14**, 49 (1955).

(9) H. Weissbach, W. Lovenberg, and S. Udenfriend, *Biochem. Biophys. Res. Communications*, **3**, 225 (1960).

(10) W. W. Umbreit, in "Amino Acid Metabolism," W. D. McElroy and B. Glass, eds., The Johns Hopkins Press, Baltimore, 1955.

(11) T. L. Sourkes, G. F. Murphy, B. Chavez, and M. Zielinska, *J. Neurochem.*, in press.

(12) A. F. Hogans and C. C. Porter, *Federation Proc.*, **20**, 113 (1961).

(13) This study was conducted with the cooperation of Drs. H. Azima, T. Farkas, and B. K. Tan, and Miss Dorothy Arthurs, R. N. Their primary aim was to assess the efficacy of *alpha*-methyldopa as a central nervous system depressant.

formed from these compounds by oxidation (iodine) under controlled conditions, and by subsequent alkalinization with sodium hydroxide to which ascorbic acid has been added. When the oxidation proceeds in a buffer adjusted to pH 3.0 *alpha*-methyldopa yields about 10 times as much fluorescence as an equivalent amount of *alpha*-methyldopamine; at pH 6.0 the two compounds yield approximately equal fluorescence. Fluorescence was determined at 510 mμ, with activation at 410 mμ (uncorrected instrumental values), using the Aminco–Bowman spectrophotofluorometer. The concentrations of the α-methyl compounds in the fluids examined were usually many hundred-fold greater than the level of endogenous catecholamines. The detailed procedures used routinely in this laboratory have been described elsewhere.[5,14] Because the method for determining *alpha*-methyldopamine depends upon applying a correction for the *alpha*-methyldopa which is present and because urinary *alpha*-methyldopa, in subjects administered this compound, often greatly exceeds in concentration its decarboxylation product, suitable estimates of the amine could not always be obtained. *alpha*-Methyldopa was also measured by the fluorescence evoked when its solutions are irradiated by ultraviolet light; this method is, of course, less specific than the oxidative procedure.

The DL-*alpha*-methyldopa used in this work was generously supplied by Merck Sharp and Dohme, Inc., Rahway, New Jersey. Ten adult patients (Table I) and three healthy adults were studied.

TABLE I

DIAGNOSTIC CATEGORY OF PATIENTS RECEIVING *alpha*-METHYLDOPA

Case	Sex	Diagnosis
1	F	Psychoneurotic reaction, hysterical type with phobias
2	F	Character neurosis with reactive depression, with chronic alcoholism
3	F	Paranoid schizophrenia
4	M	Simple schizophrenia
5	M	Schizo-affective disorder
6	F	Imbecillitas
7	M	Paranoid schizophrenia
8	F	Incipient paranoid schizophrenia
9	M	Schizophrenic reaction in a homosexual
10	M	Paranoid schizophrenia

Results

Intravenous Administration of *alpha*-Methyldopa.—Six patients were given *alpha*-methyldopa by intravenous infusion of 1 g. in 50 ml. of saline over a period of 10 min. Samples of blood (10 ml.) were withdrawn from a vein in the opposite arm at 5 min. and at two or three later times after the completion of the transfusion. Heparin was used as anticoagulant. The erythrocytes were separated

(14) T. L. Sourkes and G. F. Murphy, in "Methods in Medical Research," J. H. Quastel, ed., Vol. IX, Year Book Publishers, Chicago, Illinois, 1961.

from the plasma by centrifugation; they were washed twice with saline before being frozen for storage. They were later thawed and analyzed for *alpha*-methyldopa and *alpha*-methyldopamine. Because the amounts of *alpha*-methyldopa in the erythrocytes were consistently small relative to the plasma concentrations, only a few typical data are shown in Table II. The lowest concentration of *alpha*-

TABLE II

CONCENTRATION OF *alpha*-METHYLDOPA IN BLOOD, μg./ml.

Case no.		Fluorescence method used[a]									
		Intrinsic					After oxidation				
		5[b]	15	30	60	120	5	15	30	60	120
5	Plasma	51	42	39			49	44	31		
	Plasma						(7)[c]	(3)	(1)		
	Erythrocytes	0.4	2.1	1.0			0	0.2	0		
6	Plasma	109		66	52	25	120		55	33	22
7	Plasma	37		25	22	11	40		29	25	13
8	Plasma	75		57	42	29	193		115	45	23
9	Plasma	62	35	31			49	34	40		
	Plasma						(28)	(1)	(1)		
	Erythrocytes	0.6	0.4	1.6			0.7	0.3	0.2		
10	Plasma	65		40	22		63		26	13	

[a] See "Methods." [b] Minutes after completion of the infusion. [c] Figures in parentheses represent concentration of *alpha*-methyldopamine.

methyldopa found in the initial analysis, *i.e.*, 5 min. after the end of the infusion, was 37 μg./ml.; the highest value was 193 μg./ml If the plasma volume is estimated at 2–2.5 l., these values would indicate that at the time the sample is taken, 50–90% of the infused amino acid already has been withdrawn from the plasma.

Urinary Excretion of *alpha*-Methyldopa.—Urines were collected for several 24-hr. periods from each of 7 patients receiving *alpha*-methyldopa in daily dosage of 0.75–10.0 g. Except on the first day, the drug was given by mouth. The results are set out in Table III. The percentage of the dose excreted as the amino acid varied considerably from day to day in given patients and did not bear any obvious relationship to the dose of *alpha*-methyldopa taken. In a small proportion of the urine samples it was possible to detect *alpha*-methyldopamine, but the amounts of this amine were small relative to its precursor.

Three normal adult males were each given 0.25 g. of *alpha*-methyldopa by mouth, and urines were collected at 1.5-hr. intervals for several hours. In these cases, because of the smaller quantity of the drug taken, it was possible to determine both *alpha*-methyldopa and *alpha*-methyldopamine in the urine. The results are shown in Table IV.

Discussion

alpha-Methyldopa is known to give rise to at least one metabolite, *viz.*, *alpha*-methyldopamine. Whether it undergoes *O-meta*-methyla-

TABLE III

EXCRETION OF *alpha*-METHYLDOPA IN THE URINE ON SELECTED DAYS OF TREATMENT

Case no.	Day	Dose of *alpha*-methyldopa (g./day)	—Urinary *alpha*-methyldopa— % of daily dose	Concentration (μg./ml.)
1	6	0.75	5.5	32
	17	1.75	4.4	43
	18	0	..	35
	19	0	..	9
2	19	1.5	15.8	272
	20	0	..	79
	21	0	..	35
3	1	1.0	[a]	38
	3	1.0	8.6	101
	4	1.5	[a]	190
	11	1.5	[a]	131
	12	1.5	3.7	57
	19	1.5	12.3	240
	20	0	..	12
	21	0	..	6
4	1	2.0[b]	5.0	100
	3	1.0	[a]	82
	4	1.0	16.4	255
	12	1.5	18.3	659
	20	1.5	20.3	307
	21	0	..	22
	22	0	..	6
5	2	1.0	8.9	57
	8	1.5	10.0	110
	9	1.5	0.9	18
6	2	4.0	13.1	325
	3	5.0	7.0	175
	7	10.0	12.5	525
	8	10.0	15.7	700
	18	1.0	1.6	20
	19	0	..	10
	20	0	..	5
7	3	4.0	9.9	185
	4	5.0	10.2	160
	8	10.0	16.0	650
	9	10.0	2.0	185

[a] Incomplete collection of urine. [b] In addition to the intravenous administration, 1 g. was given by mouth.

TABLE IV

METABOLISM OF *alpha*-METHYLDOPA IN NORMAL MALE ADULTS

Time period, hr.	1		2		3	
	AMDP[a]	AMD[a]	AMDP	AMD	AMDP	AMD
0 – 1.5	10[b]	3	30	1	24	2
1.5– 3.0	51	13	88	0	58	4
3.0– 4.5	60	10	92	0	77	1
4.5– 6.0			29	1	49	0
6.0– 7.5			28	0	19	0
4.5–24.0	12	3				
7.5–24.0			5	0	3	0
% Recovery[c]	11.1		11.6		10.1	

[a] AMDP = *alpha*-methyldopa; AMD = *alpha*-methyldopamine. [b] μg./min. [c] Percentage of administered dose of DL-*alpha*-methyldopa (250 mg.) accounted for as [*alpha*-methyldopa + *alpha*-methyldopamine] in the 24-hr. urine.

tion like dopa[15] is not yet known. This derivative, α-methyl-β-(3-methoxy-4-hydroxyphenyl)-alanine, has been available through chemical synthesis, and has been shown to be virtually devoid of antidecarboxylase activity *in vitro*[8] and *in vivo*.[16] In the present study, *alpha*-methyldopa has been measured by two methods applied to the purified extracts of pyrocatecholic compounds, prepared by adsorption on alumina. The fact that both methods give essentially the same values for *alpha*-methyldopa in plasma (Table II) indicates that this amino acid is responsible for most of the fluorescence observed under the specified conditions. The outstanding discrepancies occurred in Case 8 in the two early periods of blood collection. Smaller discrepancies also occurred in Cases 6 and 9 in the 5 min. sample. The reason for these differences occurring in the samples collected shortly after the conclusion of the intravenous infusion is not clear.

For the urinary studies, 7 patients who were on varying daily dosages of *alpha*-methyldopa for periods of up to 3 weeks were examined. Urines collected for several 24-hr. periods during the period of treatment with *alpha*-methyldopa were analyzed for the compound and it was found that the amount excreted on a given day varied from 1–20% of the actual amount administered on that particular day. For 22 determinations this gave a mean (± standard deviation) of 9.9% (±5.7). These experiments, it should be noted, are not balance studies, and the variable carry-over of drug from one day to

(15) W. von Studnitz, *Scand. J. Clin. Lab. Invest.*, **12**, Supplement 48 (1960).
(16) G. F. Murphy and T. L. Sourkes, *Arch. Biochem. Biophys.*, **93**, 338 (1961).

the next in terms of metabolic disposition undoubtedly has increased the variability of the mean excretion rate.

When smaller amounts of *alpha*-methyldopa were administered, as in the case of 3 normal adults, both the amino acid and its amine could be determined in the urine. From the data in Table IV it can be seen that the rate of excretion of *alpha*-methyldopa reaches its peak in the period 3–4.5 hr. after the ingestion of the drug. Because only the L-isomer of the racemic *alpha*-methyldopa used would undergo decarboxylation it can be expected that the *alpha*-methyldopa measured in the urine, at least, in the earlier periods, is enriched with respect to the D-amino acid. This would be in accord with the finding which Hogans and Porter[12] have made in dogs given *alpha*-methyldopa intravenously. They found that the concentration of the L-isomer rises higher in plasma than that of the D-amino acid, and that the latter is more rapidly excreted in the urine. The recovery of 10–11% of the administered *alpha*-methyldopa as the amino acid and its amine in the three normal subjects agrees with the mean recovery of 9.9% in the patient group, but it is much less variable. The fate of the remainder of the drug is not known.

In spite of the fact that *alpha*-methyldopa is rapidly removed from the plasma, it is only slowly lost from the body. This is shown by the continued excretion of small amounts of the compound in the urine even on the second day after it has been withdrawn from medication (Table III).

It should, finally, be added that *alpha*-methyldopa was without significant action as a tranquillizer in the patients tested.[17]

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(17) H. Azima, personal communication.