Biochemical Pharmacology, 1963, Vol. 12, pp. 1399–1405. Pergamon Press Ltd., Printed in Great Britain.

METABOLISM OF DOPA-^{14}C IN THE NORMAL AND α-METHYLDOPA-TREATED MOUSE*

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(*Received* 29 *June* 1963; *accepted* 29 *July* 1963)

Abstract—Intraperitoneally injected DL-3, 4-dihydroxyphenylalanine (dopa)-α-^{14}C was rapidly metabolized in the intact mouse; the biological half-life was approximately 15 min. Decarboxylation constituted an important but not the only route of metabolism. One hour after the administration of dopa-^{14}C the following labeled catechols were found in whole mouse homogenates (concentrations expressed as a percentage of total recovered radioactivity): dopa (17·3%), dopamine (13·8%), norepinephrine (1·2%), epinephrine (0·5%), 3,4-dihydroxyphenylacetic acid (dopac) (0·8%). α-Methyldopa, injected intraperitoneally in a dose of 3·12 mg/kg, inhibited the decarboxylation *in vivo* of dopa-^{14}C. Larger doses resulted in progressively greater inhibition; maximal response was obtained with 50 mg/kg. In mice given α-methyldopa (25 mg/kg) 30 min prior to dopa-^{14}C and sacrificed 1 hr later, the dopa level was increased (30·6%), and the dopamine level was reduced (3·8%). The duration *in vivo* of the decarboxylase inhibitory activity of α-methyldopa in the mouse was 8 to 16 hr.

CONSIDERABLE evidence has been presented[1–4] in support of the dopa decarboxylase inhibitory action *in vitro* of α-methyldopa. However, of the methods for demonstrating this inhibitory activity, only the procedure recently reported by Hansson and Clark[5] involves direct measurement. In their procedure, ^{14}C-carboxyl-labeled dopa was administered to mice, and the expired $^{14}CO_2$ was measured in control and drug-treated animals. The authors[5] indicate that possible sources of error inherent in this method include the reutilization of radioactive CO_2 released by the decarboxylation of dopa, and the alterations in the amount of $^{14}CO_2$ expired as a result of drug-induced changes in the respiratory rate.

Other *in-vivo* methods involve indirect estimation of decarboxylase inhibitory activity and are nonspecific. For example, it has been reported that α-methyldopa decreased the accumulation of 5-HT[4,6–8] and dopamine[9,10] in various tissues after the administration of the corresponding precursor amino acids. However, Porter *et al.*[7] have found that certain other types of compounds, including the monoamine oxidase inhibitors, β-phenylisopropylhydrazine and iproniazid, and the hypotensive agent, hydralazine, also inhibited the 5-hydroxytryptophan-induced increase in the 5-HT concentration. These effects may be explained by an increased metabolism or excretion of the amines as well as by a decreased rate of synthesis. Similar explanations could be proposed for the decreased levels of 5-HT[6–8,11–16] and dopamine[8,9,15,17] found in tissues after the administration of α-methyldopa. The α-methyldopa-induced inhibition of the pharmacological responses to dopa[11,18] and 5-hydroxytryptophan[19] may be due to inhibition of decarboxylase activity, but this requires acceptance of the

* A preliminary report of this research appeared in *Fed. Proc.* **22**, 540 (1963).

assumption that the effects elicited by these amino acids are due to their respective decarboxylation products.

This report describes investigations of the qualitative and quantitative aspects of the disposition of exogenous dopa (utilizing ^{14}C-labeled dopa) in the intact mouse, and a more direct approach to the determination of the dopa decarboxylase inhibitory activity *in vivo* of α-methyldopa.

EXPERIMENTAL

Reagents. Merck Sharp and Dohme Research Laboratories donated l-α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa). The following chemicals were purchased: DL-3,4-dihydroxyphenylalanine-α-^{14}C (dopa-^{14}C), having a specific activity of 2·46 to 5·75 mc/mmole (Nuclear-Chicago Corp.); dopa, dopamine, norepinephrine, epinephrine (Nutritional Biochemicals Corp.); 3,4-dihydroxyphenylacetic acid (dopac) (California Corp. for Biochemical Research).

Dopa-^{14}C metabolism. Swiss Webster male mice weighing 20 to 25 g were injected intraperitoneally with 1 μc dopa-^{14}C. The mice were then deprived of food and water and confined to individual 250-ml beakers. Various groups were killed, by dislocation of the spinal cord, at 0, 7·5, 15, and 30 min and 1, 1·5, 2 and 4 hr after the administration of dopa-^{14}C.

α-Methyldopa. To determine dose–response relaiionships, various doses (3·12, 6·25, 12·5, 25, 50, 100, and 200 mg/kg) of α-methyldopa were injected intraperitoneally to groups of mice 30 min prior to the administration of dopa-^{14}C. To investigate the duration of action of α-methyldopa, groups of mice were sacrificed at 1·5, 4, 8, 16, and 24 hr after injection of 200mg of the compound /kg. Control animals received 0·25 ml of 0·9% NaCl solution, i.p. In all cases involving α-methyldopa, the mice were injected with 1 μc dopa-^{14}C 1 hr prior to sacrifice.

Regardless of the drug regimen, the remaining procedures were the same for all animals. For convenience they are described in the imperative.

Extraction and purification of catechols. Mince the mouse with scissors and homogenize for 2 min with 100 ml 15% trichloroacetic acid (TCA):0·1 N HCl (1:1) in a Waring Blendor to which has been added 1 mg each of carrier dopa, dopamine, norepinephrine, epinephrine, and dopac. Transfer the homogenate to a 250-ml centrifuge bottle containing 5 ml of 3% $Na_2S_2O_3$. Wash the blender and beaker that contained the mouse with 20 ml TCA:HCl and combine with the homogenate. Shake for 5 min and store overnight at 5°. Centrifuge at 1,500 × *g* for 10 min and transfer the supernatant to a 250-ml separatory funnel. Wash the precipitate with 50 ml TCA:HCl, shaking intermittently for 10 min and recentrifuge for 5 min.

Extract the combined supernatants with two 40-ml portions of anhydrous ether and discard the ether layer. Transfer the aqueous fraction to a 250-ml beaker and adjust to pH 8·1 ± 0·1 with 0·5 N NaOH, stirring constantly with a magnetic stirrer. Transfer to a centrifuge bottle, centrifuge at 1,500 × *g* for 2 min, and decant the supernatant into a 250-ml beaker containing 2 g of Woelm alumina, neutral, activity grade I for chromatography (Alupharm Chemicals, New Orleans, La.). Stirring constantly, adjust to pH 8·5 ± 0·05 with 0·5 N NaOH. Pour the alumina with approximately 30 ml of the extract into a 50-ml glass buret fitted with a plug of glass wool. Allow the extract to flow through the alumina column at a rate of 2 to 4 ml per min[20] while adding the remainder of the extract. Wash the column with 5 ml of cold distilled water.

The material not adsorbed on the alumina is retained for a determination of total radioactivity. Elute the column with 50 ml 0·1 N HCl in 95% ethanol. Collect the eluate in a 100-ml round-bottom flask fitted with a ground-glass stopper and containing 2 mg each of carrier dopa, dopamine, norepinephrine, epinephrine, and dopac. An aliquot of this solution is taken for determination of total radioactivity. Evaporate the eluate to approximately 1 ml *in vacuo* (Rinco rotary evaporator).

Separation, identification, and quantitation of catechols. Using approximately 25 μl of the concentrated catechol fraction, the individual catechols are separated by the solvent-reversal technique of paper chromatography[21,22] and identified on the basis of characteristics previously described.[21]

Cut the chromatographic sheet into strips separating the extracts and reference compound (control) areas, and scan with a radioactive strip scanner. Divide the strips into sections corresponding to the individual catechols and place each in a 20-ml potassium-free liquid scintillation spectrometer vial. Add 20 ml of fluorophor mixture[23] and measure radioactivity in a liquid scintillation spectrometer.[24] Calculate the percentage of the activity in each section as compared with the total activity on the chromatographic strip.

Recovery. From mice sacrificed immediately after the injection of ^{14}C-labeled catechols and extracted according to the procedure as outlined, the percentage recoveries (mean ± S.E.) of labeled dopa, dopamine, and norepinephrine were 65 ± 3%, 61 ± 4%, and 57 ± 4% respectively. Approximately 25 per cent of the administered radioactivity was found in the mouse precipitate. Small amounts of activity were found in the ether extract (less than 1%), in the material including the water wash not adsorbed on the alumina (3%) ,and retained on the alumina (3%).

RESULTS AND DISCUSSION

Metabolism of dopa-^{14}C

Results of the investigation of the metabolism of intraperitoneally administered DL-dopa-α-^{14}C in the intact mouse are presented in Table 1. A prominent finding was the rapid metabolism *in vivo* of dopa; the biological half-life was approximately 15 min. Other investigators have reported the biological half-life of intravenously administered tritiated norepinephrine[25] and epinephrine[26] to be approximately 5 min in the whole mouse. The somewhat slower rate of metabolism of intraperitoneally

TABLE 1. METABOLISM OF DOPA-^{14}C IN THE INTACT MOUSE

Time* (min)	No. of mice	Per cent recovered radioactivity†				
		Dopa	Dopamine	Norepinephrine	Epinephrine	Dopac
0	4	95·5 ± 1·1	0·9 ± 0·6	0·2 ± 0·2	0·1 ± 0·1	0·7 ± 0·5
7·5	4	66·1 ± 6·4	4·5 ± 1·0	1·0 ± 0·4	0·1 ± 0·1	0·4 ± 0·1
15	4	45·3 ± 1·2	9·0 ± 1·2	0·8 ± 0·2	0	1·0 ± 0·6
30	4	28·1 ± 3·2	12·0 ± 2·0	0·9 ± 0·5	0·2 ± 0·1	1·2 ± 0·9
60	27	17·3 ± 0·9	13·8 ± 0·8	1·2 ± 0·2	0·5 ± 0·1	0·8 ± 0·1
90	4	11·6 ± 0·9	18·3 ± 5·0	1·9 ± 1·0	1·7 ± 1·0	0·9 ± 0·9
120	4	14·0 ± 2·4	13·5 ± 4·0	1·6 ± 0·5	0·2 ± 0·2	1·0 ± 0·6
240	4	6·8 ± 0·3	11·7 ± 2·5	3·5 ± 0·8	0·3 ± 0·1	3·7 ± 1·0

* Time between i.p. injection of 1 μc dopa-^{14}C and sacrifice.
† Mean ± S.E. (standard deviation of the mean value) for all tables.

injected ^{14}C-labeled dopa in this study may be partially accounted for by the difference in the route of administration. That the half-life of dopa was shorter than the time required for loss of one-half the radioactivity in the total catechol fraction indicated the formation of both catechol and noncatechol metabolites.

Analysis of the catechol fraction demonstrated that dopamine was the predominant catechol metabolite. Significant amounts of dopamine were found 7·5 min after the administration of dopa; after 90 min the dopamine fraction accounted for approximately 20% of the total recovered activity. At subsequent intervals the dopamine concentration decreased. This suggests that the rate of metabolism exceeded the rate of formation, the precursor (dopa) having been reduced to very low levels. A rapid decarboxylation of dopa has been demonstrated by other investigators utilizing both *in-vitro*[27–29] and *in-vivo*[5] systems. For example, mice given ^{14}C-carboxyl-labeled DL-dopa expired 30% of the injected activity as $^{14}CO_2$ during the first 30 min.[5]

Decarboxylation normally constitutes a minor pathway of amino acid metabolism, expecially since dopa is quantitatively a minor intermediate in the metabolism of tyrosine. However, the relative importance of decarboxylation in the metabolism of exogenous dopa is indicated by the large amounts of dopamine that have been isolated (Table 1).

That both D-dopa and L-dopa were metabolized in the intact mouse was evidenced by the reduction of dopa levels to substantially less than 50 per cent of the recovered activity. Pellerin and D'Iorio[30] found 50 to 60 per cent of dopa unmetabolized after incubating DL-dopa-^{14}C with bovine adrenal homogenates for 2 hr. They presumed this to be the D-isomer. In an earlier study Pellerin and D'Iorio[31] found that, while 80 per cent of the activity of DL-dopa-^{14}C was recovered in the urine of rats, only 18 per cent was represented by dopa. Similarly, Holtz and Credner[32] have found that while the guinea pig and rat can decarboxylate only L-dopa, the rat, but not the guinea pig, is able to convert the D-form of dopa to the L-form and can therefore produce dopamine from either isomer. Sourkes *et al.*[33] also demonstrated dopamine in the urine of rats after the administration of D-dopa. It is conceivable that only the L-isomer would be metabolized by adrenal medullary homogenates, even when both isomers are metabolized by the whole animal. An explanation of this phenomenon would be that isomerization in some other tissue was affected by D-amino acid oxidation followed by a transamination, e.g., in liver.

Dopamine formed endogenously from dopa does not appear to be metabolized as rapidly as exogenous dopamine. In a pilot study, six mice were sacrificed 1 hr after the intraperitoneal injection of 1 μc dopamine-^{14}C. Only 5·5 per cent of the recovered activity was dopamine; the remainder consisted of noncatechol metabolites (92%), norepinephrine (1%), and dopac (1%). Since dopamine is bound intracellularly in granules,[34] it is conceivable that the amine formed *in vivo* is more effectively bound and is therefore metabolized less rapidly.

Werle and Jüntgen-Sell[35] proposed that 3,4-dihydroxyphenylserine (dops), the hydroxylated product of dopa, is an intermediate in the formation of norepinephrine. However, chromatographic analysis revealed no activity in the dops spot. No activity was found in the area corresponding to 3,4-dihydroxymandelic acid, another postulated catechol metabolite.[36] Although the location of dihydroxyphenylpyruvic acid on the chromatogram was not determined, approximately 99 per cent of the activity in the catechol fraction was accounted for in the other areas cited.

Effect of α-methyldopa on the metabolism of dopa-^{14}C

The effects of various doses of α-methyldopa on the metabolism *in vivo* of dopa^{14}-C in mice are presented in Table 2. An increase in the level of unmetabolized dopa-^{14}C and a decrease in the concentration of ^{14}C-labeled dopamine were obtained with doses of α-methyldopa ranging from 3·12 to 200 mg/kg. These data express a typical graded dose–response relationship; the extent of the increase in response diminished progressively with each dosage increment.

TABLE 2. EFFECT OF DOSE OF α-METHYLDOPA ON METABOLISM OF DOPA-^{14}C IN THE INTACT MOUSE

Dose* (mg/kg)	No. of mice	Per cent of recovered radioactivity		
		Dopa	Dopamine	Dopa/dopamine ratio
0	27	17·3 ± 0·9	13·8 ± 0·8	1·3
3·12	4	19·6 ± 1·5	8·4 ± 1·0	2·3
6·25	4	19·5 ± 2·0	5·1 ± 0·9	3·8
12·5	4	23·5 ± 1·2	4·7 ± 0·6	5·0
25	4	30·6 ± 3·6	3·8 ± 0·4	8·1
50	4	31·5 ± 2·8	3·0 ± 0·1	10·5
100	4	32·6 ± 1·4	3·0 ± 0·3	10·8
200	4	35·3 ± 1·3	3·0 ± 0·6	11·8

* α-Methyldopa injected i.p. 30 min prior to i.p. injection of 1 μc dopa-^{14}C. Animals sacrificed 1 hr after administration of dopa-^{14}C.

Several explanations for the action of α-methyldopa have been proposed. (a) α-Methyldopa may increase the urinary excretion of dopamine. This proposal does not appear valid, for in these experiments a decrease in total dopamine was observed, although the urine and feces were combined with the animal extract before analysis. (b) α-Methyldopa may accelerate the metabolism of dopamine. (c) α-Methyldopa may release bound amines from storage granules.[8,15,37] While the results of the present investigation do not negate them, these hypotheses do not explain the observed increase in the concentration of dopa. The data reported herein establish the effectiveness of α-methyldopa as an inhibitor *in vivo* of the decarboxylation of dopa but do not disqualify other possible mechanisms of action.

Quantitative aspects of the action of α-methyldopa on the metabolism of endogenous dopa may conceivably differ from the results of these experiments, which refer specifically to the effects of the decarboxylase inhibitor on the biotransformation of exogenous DL-dopa-α-^{14}C.

In this system α-methyldopa did not inhibit the total metabolism of dopa. Had this occurred, higher concentrations of dopa-^{14}C would have been anticipated. On the contrary, the total catechol fraction was similar for both saline- and α-methyldopa-treated mice. Although the decarboxylation of dopa was inhibited, dopa was apparently metabolized to a greater extent via other pathways.

The dopa/dopamine ratio (Table 2) provides a senstive index of dopa decarboxylase inhibition *in vivo*, as evidenced by the approximately twofold increase in the ratio obtained with a small dose (3·12 mg/kg) of α-methyldopa. This increase was due primarily to a decrease in dopamine concentration. With intermediate doses, it was due both to an increase in dopa and a decrease in dopamine. With larger doses, the

ratio did not change significantly, primarily because a further increase in dopa was prevented by its metabolism via other pathways.

It is interesting to compare the concentrations of α-methyldopa that inhibit the decarboxylation of dopa *in vitro* and *in vivo*. Seventy μg ($3{\cdot}3 \times 10^{-4}$ M) of α-methyldopa, corresponding to a dose level of 3·12 mg/kg, represents approximately $1{\cdot}5 \times 10^{-5}$ M per g mouse. Disregarding the selective distribution of α-methyl dopa (which has been reported[38] to concentrate in areas of high decarboxylase activity), a concentration of this order has been found to inhibit dopa decarboxylase *in vitro*.[1]

TABLE 3. DURATION OF EFFECT OF α-METHYLDOPA ON METABOLISM OF DOPA-^{14}C IN THE INTACT MOUSE

Time* (hr)	No. of mice	Per cent of recovered radioactivity: Dopa	Dopamine	Dopa/dopamine ratio
1·5	4	35·3 ± 1·3	3·0 ± 0·6	11·8
4	4	19·8 ± 5·0	3·3 ± 0·5	6·0
8	4	26·7 ± 2·0	6·6 ± 0·9	4·0
16	4	17·7 ± 1·6	18·4 ± 1·0	1·0
24	4	18·0 ± 1·1	11·8 ± 0·7	1·5

* Time between i.p. injection of 200 mg α-methyldopa/kg and sacrifice; 1 μc dopa-^{14}C injected i.p. 1 hr before sacrifice.

Results of investigations of the duration of action of α-methyl dopa are presented in Table 3. The duration of decarboxylase inhibitory activity *in vivo* induced by a single dose (200 mg/kg) of α-methyldopa was 8 to 16 hr. This agrees well with the results of other investigators[5,8,12,14,15,17] although various species and different methods of analysis were employed. The comparatively short duration of the inhibitory activity of α-methyldopa found in these studies adds support to the concept[7,8,15,37] that the relatively persistent reduction of the norepinephrine content of tissues is the result of mechanisms other than inhibition of decarboxylase activity.

To investigate the possibility of a cumulative action, repeated doses of α-methyldopa were given to mice (Table 4). An increase in the dopa/dopamine ratio would constitute

TABLE 4. EFFECT OF REPEATED DOSES OF α-METHYLDOPA ON METABOLISM OF DOPA-^{14}C IN THE INTACT MOUSE*

No. of injections	No. of mice	Per cent of recovered radioactivity: Dopa	Dopamine	Dopa/dopamine ratio
1	4	18·0 ± 1·1	11·8 ± 0·7	1·5
3†	6	21·7 ± 1·3	12·5 ± 0·7	1·7
13‡	6	16·7 ± 1·0	9·2 ± 0·8	1·8

* Animals sacrificed 24 hr after last injection of α-methyldopa (200 mg/kg, i.p.); 1 μc dopa-^{14}C injected i.p. 1 hr before sacrifice.

† α-Methyldopa once a day for 3 days.

‡ α-Methyldopa 3 times a day for a total of 13 doses.

evidence of cumulative activity since no inhibition was found 24 hr after administration of a single 200-mg dose of α-methyldopa/kg. Repeated administration of this dose resulted in a slight, but probably not significant, increase in the dopa/dopamine ratio, indicating that a cumulative action was not obtained in this system.

Acknowledgements—The authors wish to acknowledge the generous assistance of Dr. Louis A. Reber, Professor of Chemistry, and Dr. Grafton D. Chase, Director of the Radiochemistry Laboratories, Philadelphia College of Pharmacy and Science.

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