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L-3,4-DIHYDROXYPHENYLALANINE METABOLISM BY THE GUT *IN VITRO**†

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Abstract—The metabolism of L-3,4-dihydroxyphenylalanine (L-dopa) by rat gastric and intestinal mucosa was studied by incubating everted sacs with ^{14}C-L-dopa, 10^{-4} M. Metabolites in the tissue and in the mucosal and serosal fluids were separated by ion-exchange chromatography and the radioactivity was determined by liquid scintillation counting. The data on the metabolites, expressed as per cent of the total radioactivity of the respective compartments, were 20·1 ± 1·4 per cent for the mucosal fluid, 41·8 ± 2·4 per cent for the tissue, and 65·3 ± 1·6 per cent for the serosal fluids of the stomach preparation. Values for the metabolites from the intestines were 39·6 ± 1·1, 31·0 ± 1·2, and 46·1 ± 1·8 per cent for mucosal, tissue, and serosal fluids respectively. The metabolites in order of decreasing concentration were phenylcarboxylic acids, dopamine, and other catecholamines in both stomach and intestines. There was significant inhibition of metabolism when ^{14}C-L-dopa was incubated with tissues from rats pretreated with a decarboxylase inhibitor, α-methyl hydrazine (MK 485). The presence of boiled tissue was shown to inhibit the auto-oxidation of L-dopa ordinarily seen at neutral pH under conditions of oxygenation and incubation at 37°. The drug-metabolizing capacity of the gut could theoretically decrease serum concentrations of an affected drug, produce metabolites capable of inducing local or systemic toxicity, enhance or block absorption of a second drug, sensitize the gut to other substances, or produce morphologic changes in the gut.

L-3,4-HYDROXYPHENYLALANINE (L-dopa) in relatively large oral doses has been shown to produce significant clinical improvement in Parkinsonian patients.[1,2] Despite the magnitude of the dose, very low serum levels of L-dopa are achieved in treated patients.‡ In preliminary work we compared the tissue distribution of radioactive L-dopa in rats after oral and intravenous administration of drug. The total radioactivity recovered in the serum and brain of the animals after intravenous injection was significantly greater than after oral administration, and the radioactivity in the metabolite fractions in the gastric wall of the latter group was much higher than that in the former group. If the metabolites of L-dopa found in the stomach were derived only through the systemic circulation from other metabolizing organs, this difference should not exist. These findings led us to the hypothesis that oral L-dopa could be significantly metabolized in the gastric mucosa prior to absorption. The results of studies *in vitro* of L-dopa metabolism by everted rat stomach and intestine support the above hypothesis.

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‡ M. J. T. Peaston and J. R. Bianchine, personal communication.

MATERIALS AND METHODS

Technique in vitro

Everted rat stomach and segments of intestine were incubated in buffer solutions containing ^{14}C-L-dopa labeled at the β carbon (Amersham-Searle Laboratories), and the radioactivity of the chromatographic fractions from the mucosal, tissue, and serosal fluids was determined so as to quantify the metabolites of L-dopa. Eight Sprague–Dawley rats, weighing 295–330 g, were fasted, with access to water *ad lib.*, for 15 hr before the experiment. Immediately after stunning and decapitation, the stomach and 5 cm of proximal small intestine were excised, tied at one end, everted, as described by Wilson and Wiseman,[3] and filled with 0·5–1·0 ml Krebs–Ringer phosphate buffer and then securely closed by ligation. The tissues were separately incubated with ^{14}C-L-dopa, 10^{-4} M (1·2 μc), in 5 ml Krebs–Ringer phosphate buffer. The L-dopa solution was always added after the tissues had been placed in the medium.

Each flask was gassed with 95% O_2 and 5% CO_2 for 5 min, then sealed with Parafilm. Incubation for 90 min was done in a Dubnoff metabolic incubator at 37°. The above procedure was repeated in another group of eight Sprague–Dawley rats, with incubation under nitrogen atmosphere without preliminary gassing with oxygen. Boiled tissues were used as controls.

After incubation, the everted sacs were blotted on tissue paper, weighed and the serosal fluid was recovered by needle aspiration. The sacs were then opened and washed three times with 0·1 N HCl. After washing, the tissues were blotted, weighed again, and homogenized in 7 ml of 1 N HCl. The homogenate was centrifuged and the supernatant deproteinized with 1 ml of 70% perchloric acid. The pH of the deproteinized supernatants and the mucosal and serosal fluids was brought to 2. These samples were then fractionated by the ion-exchange column chromatographic technique of Bartholini and Pletscher[4] as modified by Peaston and Bianchine* to fraction PCA† [phenylcarboxylic acids; especially vanillmandelic acid (VMA), homovanillic acid (HVA); and dihydroxyphenylacetic acid (DOPAC)]; fraction B (unchanged L-dopa and *O*-methyl dopa); fraction CA [catecholamines; norepinephrine (NE), epinephrine (E), metanephrines (ME) and normetanephrines (N-ME)] and fraction DA (dopamine). One-ml aliquots of the mucosal, serosal, and tissue homogenates and the separated fractions were added to 10 ml Triton X-100–toluene scintillation liquid[5] and assayed for radioactivity in a Packard Tri-Carb scintillation spectrometer. Data were corrected for the control values.

Non-enzymatic metabolism of L-dopa in the absence and presence of tissue. Autooxidation of L-dopa has been shown to occur in neutral and alkaline medium and is accelerated by oxygen, light, and increased temperature.[6] Since our tissue incubation involved buffer medium (pH 7 to 7·2), oxygenation, and a temperature of 37°, we examined the possible nonenzymatic breakdown of L-dopa.

Keeping the composition, volume, and pH (7·2) of the buffer medium and the concentration of ^{14}C-L-dopa constant, the mixture was subjected to different conditions to study the effect of the pH of the buffer, oxygen, and incubation at 37°: (1) the mixture without tissue was allowed to stand at room temperature without oxygenation; (2) the mixture without tissue was gassed with 95% O_2 and CO_2 for 5 min, after

* M. J. T. Peaston and J. R. Bianchine, personal communication.

† It is conceivable that this fraction also contains such compounds as dopamine, 5,6-dihydroxyindole, and other melanin precursors.

which the flask was sealed with parafilm and allowed to stand at room temperature for 90 min; (3) the mixture without tissue was oxygenated as described above and incubated for 90 min at 37° in a Dubnoff metabolic shaker; (4) the mixture with boiled tissue was oxygenated and incubated for 90 min at 37°; (5) the pH of the mixture was reduced to pH 5 and was oxygenated for 90 min at 37°.

Enzymatic metabolism of L-dopa. Decarboxylase activity of the gastric and intestinal tissues was further investigated by the use of a decarboxylase inhibitor, α-methyl hydrazine (MK 485). The rats were pretreated with MK 485, 100 mg/kg intraperitoneally, 1 hr before decapitation. The everted stomach and intestine were incubated with ^{14}C-L-dopa plus 2·5 mg MK 485. The same incubation procedure and assay of radioactivity described above in the methods were followed. This investigation was done under nitrogen and oxygen atmosphere to determine whether significant autooxidation of L-dopa occurred under oxygen.

L-dopa metabolites were fractionated and assayed by the methods described in the text.

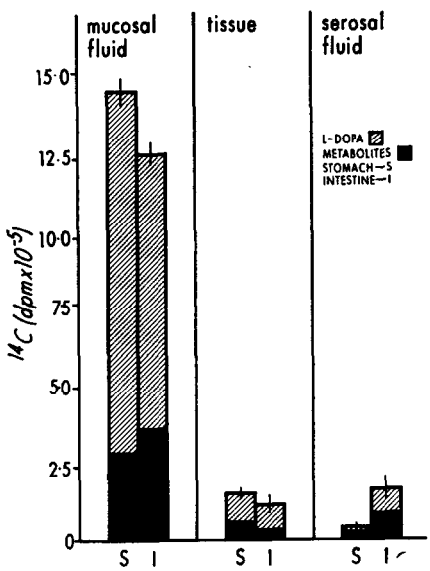

FIG. 1. Distribution of the total radioactivity and the radioactivity found in the form of metabolites in the mucosal, tissue, and serosal compartments of the everted gut after incubation with ^{14}C-L-dopa, 10^{-4} M(1·2 μc). Values are expressed as the means ± S.E.M. (N = 8). Mean weights of the stomach and intestines were 1·6 and 0·6 g respectively.

RESULTS

Figure 1 shows the distribution of the total radioactivity and the radioactivity found in the form of metabolites in the mucosal, tissue, and serosal compartments of the everted gut after the incubation with ^{14}C-L-dopa. The metabolites expressed as per cent of the total radioactivity of the different compartments were 20·1 per cent for the mucosal fluid, 41·8 per cent for the tissue, and 65·3 per cent for the serosal fluids of the stomach preparation. Values for the metabolites from the intestines were 39·6, 31·0 and 46·1 per cent for mucosal, tissue, and serosal fluids respectively. The meta-

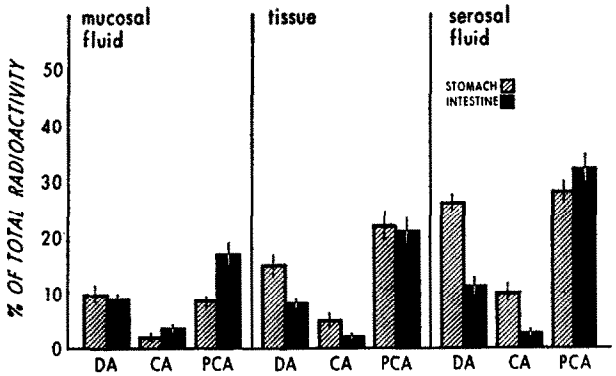

FIG. 2. ^{14}C-L-dopa metabolites expressed as mean ± S.E.M. per cent of the total radioactivity of the respective compartments shown in Fig. 1 ($N = 8$).

bolites in the order of decreasing concentration were PCA, DA, and CA in both stomach and intestines, as shown in Fig. 2.

Nonenzymatic oxidation of ^{14}C-L dopa

Table 1 summarizes the values obtained from the fractionation of the buffer medium subjected to different conditions. It is evident that neutral pH, oxygenation and incubation at 37° accelerate nonenzymatic metabolism of L-dopa, when mixed with incubation medium without tissue. However, under the same conditions, insignificant amounts of metabolites were recovered when the ^{14}C-L-dopa was added to the medium with boiled tissue. The degradation of ^{14}C-L-dopa incubated with buffer at pH 7 was significantly inhibited when the pH of the buffer was reduced to pH 5, even in the absence of tissue. There was little difference between the amounts of metabolites recovered from the fractions of the media containing viable tissue when subjected to nitrogen or oxygen atmosphere, as shown in Table 2.

TABLE 1. EFFECT OF pH, OXYGEN, INCUBATION AND TISSUE ON AUTO-OXIDATION OF L-DOPA*

Experiment	PCA	L-dopa	CA	DA
Buffer (pH 7) with oxygenation without incubation ($N = 4$)	11·4 (10·8–12·2)	85·6 (84·0–86·2)	3·50 (2·7–4·7)	0·44 (0·21–0·7)
Buffer (pH 7) with incubation without oxygenation ($N = 4$)	37·17 (32·0–44·2)	58·77 (49·0–63·0)	3·67 (3·2–4·6)	0·78 (0·1–4·6)
Buffer (pH 7) with oxygenation with incubation ($N = 6$)	76·3 (65·0–80·4)	19·2 (14·0–31·2)	3·3 (2·5–3·7)	1·7 (0·2–2·4)
Presence of boiled tissue in medium No. 3 ($N = 5$)	1·55 (1·4–1·8)	92·77 (89·0–95·0)	4·8 (2·8–8·6)	0·4 (0·18–0·24)
Medium No. 3 at pH 5 ($N = 6$)	1·8 (1·2–2·7)	96·5 (95·6–97·6)	0·74 (0·18–2·9)	0·15 (0·1–0·25)

* Values are expressed as mean per cent of total dose 10^{-4} M ^{14}C-L-dopa (1·2 μc).

TABLE 2. METABOLITES IN THE MEDIUM AFTER INCUBATION OF THE STOMACH AND INTESTINES WITH ^{14}C-L-DOPA*

	PCA	Dopa *O*-methyldopa	CA	DA
Stomach				
Under oxygen	8·48 ± 1·1	78·9 ± 3·7	1·7 ± 0·2	10·0 ± 1·9
Under nitrogen	14·1 ± 4·8	79·0 ± 4·8	3·4 ± 0·4	3·3 ± 0·7
Intestine				
Under oxygen	17·4 ± 2·0	69·8 ± 2·3	3·32 ± 4·7	9·3 ± 0·9
Under nitrogen	20·8 ± 2·3	59·3 ± 4·0	7·7 ± 1·3	14·0 ± 3·4

* Values are expressed as mean ± S.E.M. per cent of the dose of ^{14}C-L-dopa, 10^{-4} M (1·2 μc; $N = 8$). Statistical difference between values obtained under oxygen and under nitrogen is $P > 0·5$.

Effect of decarboxylase inhibitor MK 485. Figure 3 shows the concentration of dopamine in the mucosal, tissue and serosal fluids, after incubating the everted stomach and intestines with ^{14}C-L-dopa, 10^{-4} M, with and without a decarboxylase inhibitor. Without decarboxylase inhibitor, the dopamine fraction constitutes 10–26 and 8–11 per cent of the total radioactivity recovered from the stomach and the intestinal preparations respectively. In the presence of decarboxylase inhibitor, dopamine constitutes a negligible fraction, whether the incubation was done under oxygen or nitrogen as shown in Table 3.

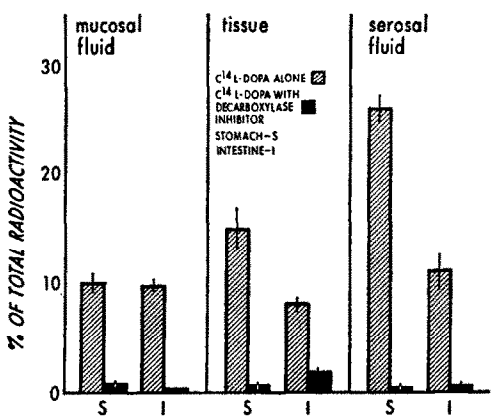

FIG. 3. Effect of a decarboxylase inhibitor, MK 485 (α-methyl hydrazine), on L-dopa conversion to dopamine. Values are expressed as mean ± S.E.M. per cent of the total radioactivity recovered as dopamine from the 3 compartments.

DISCUSSION

The therapeutic value of L-dopa in Parkinson's disease presumably depends upon its concentration in the brain, where it is decarboxylated to dopamine, the supposedly deficient neurotransmitter. The consistently low serum concentrations of L-dopa (0·2–1·1 μg/ml) and the high serum concentrations of L-dopa metabolites in the form of phenylcarboxylic acids, catecholamines and dopamine, after oral administration

TABLE 3. EFFECT OF DECARBOXYLASE INHIBITOR MK 485 ON ^{14}C-L-DOPA CONVERSION TO DOPAMINE*

	Under oxygen		Under nitrogen	
	With MK 485	Without MK 485	With MK 485	Without MK 485
Mucosal				
Stomach	0·11 ± 0·03	3·3 ± 0·7	0·07 ± 0·01	10·0 ± 1·9
Intestine	0·05 ± 0·01	14·0 ± 3·4	0·11 ± 0·02	9·3 ± 0·9
Tissue				
Stomach	2·2 ± 0·05	12·54 ± 1·6	1·33 ± 0·03	15·0 ± 2·2
Intestine	2·3 ± 0·03	16·0 ± 1·18	1·69 ± 0·36	8·0 ± 1·2
Serosal				
Stomach	0·7 ± 0·3	8·8 ± 3·9	0·27 ± 0·06	10·0 ± 3·0
Intestine	0·8 ± 0·3	10·0 ± 3·0	0·5 ± 0·16	11·1 ± 1·8

* Values are expressed as the mean ± S.E.M. per cent of the total radioactivity in each compartment after incubation of the everted gut with ^{14}C-L-dopa, 10^{-4} M (1·2 μc), with and without decarboxylase inhibitor. The differences are significant at $P < 0·001$ whether the incubation was done under oxygen or nitrogen.

of several grams of the drug, show that extracerebral metabolism of L-dopa is significant. Since oral administration is the conventional route for L-dopa therapy, we studied the fate of L-dopa incubated with everted rat stomach and intestine. The high recovery of radioactivity in the metabolite fractions indicates metabolic activity in the gastric and intestinal tissues. Of interest is the higher concentration of dopamine and catecholamine fractions in all compartments of the stomach preparation, compared to that of the intestine. This could be due to a greater activity of hydroxylases and decarboxylases in the gastric tissues than in the intestine, or to the fact that the intestine has higher levels of monoamine oxidase, which rapidly metabolizes the dopamine or other catecholamines formed into inactive phenylcarboxylic acids. This latter possibility would explain the higher concentration of phenylcarboxylic acids in the intestinal preparations.

The possibility that the data could reflect a nonenzymatic auto-oxidation has been evaluated. Previous experience suggests that incubation of tissue homogenates with L-dopa should be carried out under nitrogen, because of the instability of L-dopa under oxygen.

Although the data (Table 1) show that alkalinity and oxygenation accelerate auto-oxidation of ^{14}C-L-dopa incubated in the buffer medium, the incorporation of boiled tissue in the system prevented the process. It should be noted that in this procedure, ^{14}C-L-dopa was added when the tissues were already in the medium.

The data in Tables 2 and 3 illustrate the insignificant difference between the concentration of metabolites obtained from incubating the tissues under nitrogen or oxygen atmosphere. This permits metabolic studies *in vitro* in the absence of oxygen.

The results described above with normal tissues and with boiled tissue controls suggested enzymatic degradation of L-dopa by the rat stomach and intestine. Additional evidence was provided by the finding of significant inhibition of degradation of L-dopa *in vitro* when the rats were pretreated with the decarboxylase inhibitor MK 485.

The most common side effect of L-dopa therapy is nausea and vomiting, and antacids are used by some clinicians to alleviate the symptoms. The gastric pH after treatment with available antacids ranges from pH 3 to pH 9 depending upon the kind, dose and frequency of administration of these agents. Since the stability of L-dopa may be decreased as the pH rises, it is advisable to use antacids that do not raise the pH to greater than pH 5.

This demonstration of the drug-metabolizing capacity of the gut suggests an interesting area for investigation, i.e. the possible metabolism of orally administered drugs by the gut. Such metabolism could obviously lower the serum concentrations of such drugs. It is also conceivable that metabolites formed in the gut could produce a malabsorption syndrome, enhance or block absorption of a second drug, sensitize the gut to other substances, or produce morphologic changes in the gut.

The everted sac preparation is simple and gives reproducible results. It not only allows the simultaneous study of drug metabolism and drug transport across gastric and intestinal mucosa, but can also provide information on the effect of drug metabolites on membrane transport of the drug.

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