Synthesis of DOPA in Rat Stomach Following Ingestion of Cereals

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The cereal-containing chow routinely consumed by our laboratory rats contains both free dihydroxyphenylalanine (DOPA) and an enzyme that catalyzes the conversion of tyrosine to DOPA. When tyrosine-^{3}H (1.8 μCi) was added to this food (0.5 g) and incubated for 2 hr, 1.26% of the tyrosine-^{3}H was converted to DOPA-^{3}H. When the tyrosine-^{3}H was incubated with the gastric contents from an animal that had ingested the rat food, 0.8% of the tyrosine-^{3}H was converted to DOPA. If the gastric contents were boiled prior to the incubation, or if the animals consumed a diet lacking cereals, little or no DOPA-^{3}H was synthesized. The 24-hr excretion of free and conjugated dihydroxyphenylacetic acid was much higher among animals ingesting the cereal-containing rat food than among rats whose diet contained casein as the sole protein source. These findings suggest that the tyrosine-hydroxylating enzyme in cereals continues to catalyze DOPA synthesis within the lumen of the rat stomach.

THE BIOSYNTHESIS of catecholamines in mammalian cells begins with the uptake of circulating L-tyrosine and its enzymatic meta-hydroxylation to L-dihydroxyphenylalanine (L-dopa).[1,2] Tyrosine hydroxylase has, to date, been found in mammals only in cells that contain catecholamines (i.e., the dopaminergic and noradrenergic neurons in the central nervous system, postganglionic sympathetic neurons, and adrenomedullary chromaffin cells).[3] This has naturally led to the assumption that all of the catecholamines and catecholamine metabolites present in tissues and body fluids are derived from tyrosine hydroxylated within these cells.

Exogenous L-dopa is taken up and decarboxylated to dopamine (DA) by many cells that do not normally synthesize DA from tyrosine. Evidence for the ubiquity of DA synthesis from exogenous L-dopa is provided by the recent demonstration that the destruction of most of the catecholamine nerve terminals in the brain and in peripheral organs (by the intracisternal or systemic administration of 6-hydroxydopamine) does not significantly decrease the accumulation of DA within those organs following L-dopa administration.[4] Hence, if significant quantities of L-dopa were to enter the body from the gastrointestinal tract, DA and its metabolites could be formed in vivo by cells other than those that normally contain DA or tyrosine-hydroxylase activity.

We recently observed small quantities of DOPA (0.33 μg/g) in rat foods containing wheat and oats.[5] We now have evidence that a tyrosinase, also present in these foods, synthesizes DOPA from tyrosine and continues to do so even within the lumen of the

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stomach; this DOPA, moreover, is the source of most of the dihydroxyphenylacetic acid (DOPAC) excreted by the rat.

MATERIALS AND METHODS

DOPA Synthesis by Pure Cereals

One gram of cereal (see Table 1 for kinds of cereals) was suspended in 10 ml of 0.1% ethanol and incubated overnight with pronase 0.1% (w/v) and 1-mg tyrosine. Diethyldithiocarbamate (5×10^{-4} *M*), dihydroxymaleic acid (5×10^{-4} *M*), or catalase (beef liver, final concentration 2300 units/ml, Calbiochem) were added to some of the cereals prior to incubation. DOPA was then extracted from the incubation medium and assayed fluorimetrically as described previously.[5]

Animals and Diets

Male Sprague-Dawley rats (Charles River Laboratories) weighing 200 g were given a diet containing cereal (Big Red Laboratory Animal Chow, Agway Co.) or a cereal-free, casein diet containing the same percentage of protein (24%, Test Diet #71373, General Biochemicals Co.). Both diets were in the form of solid pellets.

DOPA-^3H Synthesis by Gastric Contents

^3H-Tyrosine (L-tyrosine 3,5-H^3, 240.4 mc/g, New England Nuclear Corp., Boston, Mass.) was purified prior to use by passage over alumina at pH 8.6 to remove any contaminating DOPA-^3H. Gastric contents were obtained from rats fed normally and from rats that had been fasted for 24 hr and then fed a single pellet of rat food weighing 5 g. One hour later the animals were killed, and their gastric contents removed. Tyrosine-^3H (1.8 μCi) was added to 0.5 g of rat food or to gastric contents suspended in 0.5 ml of 0.1% ethanol. Incubation lasted for 2 hr (unless otherwise indicated) at 37°. The reaction was terminated by adding 10 ml of 10% trichloroacetic acid (TCA). The resulting mixture was centrifuged at 27,000 × g for 15 min, and the TCA supernatant was filtered through paper. DOPA-^3H was extracted from the resulting solution by passage over alumina at pH 8.6 and elution with 0.2 *N* acetic acid.[6] Aliquots of the alumina eluates were used for the estimation of DOPA-^3H content by liquid-scintillation spectrophotometry.

The identity of the DOPA-^3H in some of the alumina eluates was confirmed by passing the eluate over Dowex 50W-4X at pH 2.0, and eluting the DOPA with 5.0-ml phosphate buffer, pH 6.5. This procedure separates DOPA from dopamine and norepinephrine.[7] DOPA was then extracted from the Dowex eluate by passage over a second alumina column, and the final alumina eluate was lyophilized. This material was taken up in ethanol and subjected to paper chromatography (butanol, acetic acid, H_2O; 4:1:1). All of the radioactivity was located at the same R_f (0.16) as authentic DOPA. Insignificant radioactivity was eluted from the Dowex in the fractions that would have contained catecholamines.

Table 1. DOPA Synthesis by Cereals

Cereal	Color After Incubation	μg DOPA Formed
Wheat germ (Kretchmer's)	Dark brown	282
Macro Flake Wheat	Black	291
Hard Red Spring Wheat	Red brown	115
Whole oats	Brown	20
Soft white wheat	Light brown	32
Rolled oats	Yellow	5
Durum wheat	Yellow	2
Wheat germ (Erewhon)	Yellow	0

One gram of cereal was suspended in 10 ml of 0.1% ethanol. One milligram tyrosine and 10 mg pronase were added and a 16-hr incubation was performed at 37°C.

DOPA-^{3}H Synthesis in Vivo

Rats were fasted 24 hr and then given 5 g of rat food to whcih 3.91 μCi of purified tyrosine-^{3}H was added. In order to minimize DOPA-^{3}H synthesis by the rat food prior to its ingestion, the tyrosine-^{3}H was added just after the animal started to eat. Since the tyrosine-^{3}H was distributed as evenly as possible along the surface of the food pellet, it was ingested by most of the animals in less than 15 min. Thirty minutes after all of the food had been eaten, the animals were killed. The gastric contents and the stomach wall were dissected out as a unit for DOPA-^{3}H assay.

Assay of DA and DOPAC

The animals were given the test diets for three days; on the last day urine was collected for assay of DA and DOPAC. The rats were then killed, and brain DA was assayed according to the technique of Carlsson and Waldeck.[8] During urine collection the animals were placed in metabolic cages with funnels placed in plastic bottles containing 1 ml of 6 *N* HCl. The bottles were kept in foam rubber containers packed in dry ice, so that the urine remained frozen for most of the collection period. Urinary DA was assayed by a procedure described previously.[9]

Enzymatic hydrolysis of the conjugated DOPAC was performed with 2.5 ml of urine, according to the method of Smith and Weil-Malherbe.[10] Proteins were then precipitated by the addition of 0.5-ml 40% TCA. DOPAC was first extracted onto anionic Dowex, chloride form, 1-X4.[11] One milliliter of 5% EDTA was added to the Dowex eluate, which was then passed over alumina at pH 8.6. The 0.2 *N* acetic acid eluate was discarded, and the DOPAC was eluted with 2 ml of 1 *N* H_2SO_4.[12] Recovery of added standard through the two-column procedure averaged 42%. DOPAC was extracted from the alumina eluate into ether, and then separated from dihydroxymandelic acid and assayed according to the procedure of Spano and Neff.[13]

RESULTS

The amount of authentic DOPA synthesized by the cereals could readily be correlated with the color of the incubated suspension (Table 1). Little color developed in those cereal suspensions to which no tyrosine or pronase were added. When authentic DOPA (1 mg) was added to cereals with high tyrosinase activity, a dark brown pigment was produced in several hours. Inclusion of a copper chelating agent, diethyldithiocarbamate, partially blocked both the DOPA synthesis (Table 2) and color production. Addition of catalase slightly decreased the DOPA synthesis by the wheat germ and the Macro Flake Wheat, but had the opposite effect on the Durum wheat. Dihydroxymaleic acid had no effect on DOPA synthesis.

The time course of DOPA-^{3}H synthesis by the cereal-containing rat food and by the gastric contents is shown in Fig. 1. Each point represents the mean of five separate experiments. After 2 hr, approximately 0.8% of the tyrosine-^{3}H incubated with the gastric contents, and 1.26% of the tyrosine-^{3}H incubated with the rat food, were present as

Table 2. The Effects of Enzyme Inhibitors on DOPA Synthesis by Cereals. Data Represent μg of DOPA Synthesized

Cereal	No Cofactor	Diethyldithiocarbamate	Catalase
Wheat germ (Kretchmer's)	294	72	256
Macro Flake Wheat	487	20	274
Durum wheat	2.0	0	9.6
Whole oats	19.7	7.4	

One gram of cereal was suspended in 10 ml of 0.1% ethanol with or without potential inhibitors of tyrosine: diethyldithiocarbamate, 5×10^{-4} *M*; catalase, 2300 U/ml. One milligram of tyrosine and 10-mg pronase were then added, and a 16-hr incubation was performed at 37°C.

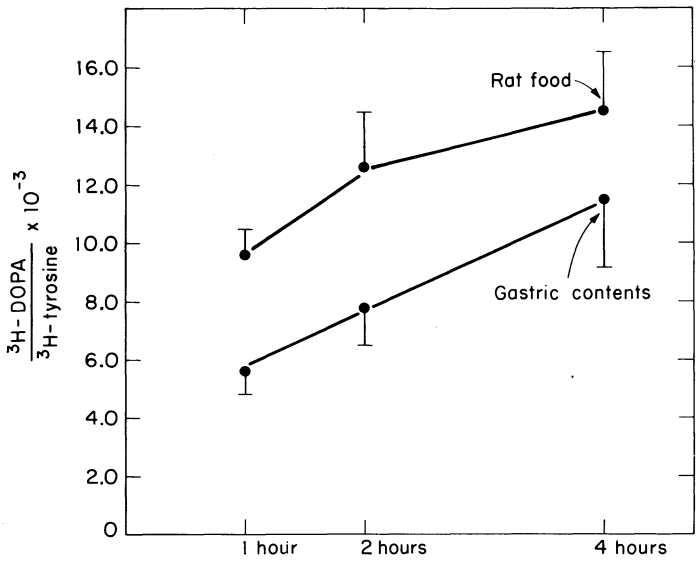

Fig. 1. 1.80 μCi of tyrosine-^{3}H was added to the cereal-containing rat food or to the gastric contents obtained postmortem. Significantly more DOPA-^{3}H was synthesized by the rat food than by the gastric contents ($p < 0.01$).

DOPA-^{3}H. A one-way analysis of variance was performed. The between-conditions variance was partitioned by an orthogonal contrast in which DOPA-^{3}H synthesis by the rat food (+1,+1,+1) was compared with that by the gastric contents (−1,−1,−1).[14] The synthesis of DOPA-^{3}H by the rat food was significantly greater ($p < 0.01$) than the synthesis by the gastric contents. Boiling of the food or the gastric contents for 15 min almost completely blocked its capacity to catalyze synthesis of DOPA-^{3}H. When cereals were excluded from the rat food, the gastric contents synthesized negligible quantities of DOPA-^{3}H (Table 3). DOPA-^{3}H synthesis could be more readily demonstrated in the gastric contents obtained from animals that had been fasted for 24 hr prior to sacrifice than in gastric contents from normally fed animals.

The results of the in vivo experiment (Table 4) closely resemble those of the in vitro study (Table 3). In both experiments, the DOPA-^{3}H synthesis by the cereal-containing gastric contents was approximately eight times that of the gastric contents from rats

Table 3. Appearance of DOPA-^{3}H after Addition of Tyrosine-^{3}H to Gastric Contents Obtained Postmortem

Protein Source	Treatment of Gastric Contents	Number of Rats	dpm in Alumina Eluate ($\times 10^3$)	DOPA-^{3}H/ Tyrosine-^{3}H ($\times 10^{-3}$)
Cereals and casein	None	5	19.45 ± 2.3	10.22 ± 1.2*
Cereals and casein	Boiled	4	2.07 ± 0.10	1.08 ± 0.48
Casein	None	4	3.09 ± 0.37	1.62 ± 0.19

*$p < 0.01$ different from other groups.

1.80 μCi of tyrosine-^{3}H was added to 0.5 g of gastric contents and a 2-hr incubation was performed.

Data are given as mean ± SEM.

Groups were compared by the nonpaired t test.

Table 4. Appearance of DOPA-^{3}H in Gastric Contents of Rats Following Ingestion of Foods Containing Tyrosine-^{3}H

Protein Source	Number of Rats	dpm/Gastric Contents: Total ($\times 10^3$)	dpm/Gastric Contents: Alumina Eluate ($\times 10^3$)	DOPA-^{3}H/ Tyrosine-^{3}H ($\times 10^{-3}$)
Cereals and Casein	8	5010 ± 104	25.6 ± 7.1*	10.04 ± 1.6†
Casein	6	6420 ± 90	3.6 ± 0.90	1.48 ± 0.72

*$p < 0.05$ differs from casein diet.

†$p < 0.01$ differs from casein diet.

Each rat consumed 5 g of food containing 3.91 μCi of tyrosine-^{3}H.

Data are given as means ± SEM. There was no difference in the total radioactivity present in the gastric contents of the animals in the two dietary groups. The nonpaired t test was used to compare dpm in alumina eluate and DOPA-^{3}H/tyrosine-^{3}H in the two groups.

consuming a casein diet. In the in vivo experiment, the rats spilled or refused to eat some of the food, and some of the radioactivity was probably absorbed into the general circulation during the 30-min waiting period after food ingestion. Thus only about 65% of the administered radioactivity (3.91 μCi) could be recovered in the homogenate of the gastric contents and stomach wall (Table 4).

The ingestion of the cereal-based diet dramatically increased the excretion of free and conjugated DOPAC, and caused a small but significant increase in urine volume (Table 5). The free DA excretion was not significantly altered.

DISCUSSION

These studies suggest that cereals in rat food catalyze DOPA synthesis in the lumen of the rat stomach, and that sufficient DOPA is formed to increase dramatically the excretion of free and conjugated DOPAC. Boiling of the cereals blocks their capacity to catalyze DOPA synthesis (Table 3); this suggests that the synthesis is enzymatically mediated. Cereals are known to contain peroxidases[15] and tyrosinases;[16] the correlation between pigment formation and DOPA synthesis (Table 1) suggests that the DOPA-synthesizing enzyme is a tyrosinase. The partial inhibition of tyrosine hydroxylation

Table 5. Brain DA and Urinary DA Metabolites in Rats Consuming Casein and Cereal Diets

	Number of Rats	Casein	Cereals and Casein	Significance
Food intake (g/24 hr)	10	13.7 ± 2.0	16.5 ± 1.5	NS
Urine volume (ml/24 hr)	10	9.11 ± 1.2	13.8 ± 1.2	$p < 0.02$
Brain DA (μg/g)	9	0.51 ± 0.05	0.56 ± 0.04	NS
Urinary metabolites (μg/24 hr)				
Free DA	8	2.36 ± 0.27	3.34 ± 0.72	NS
Free DOPAC	6	6.58 ± 0.24	23.4 ± 3.3	$p < 0.001$
Conjugated DOPAC	6	7.27 ± 3.3	47.9 ± 12.6	$p < 0.01$
Total DOPAC	6	13.9 ± 3.2	66.4 ± 12.8	$p < 0.01$

Data are given as means ± SEM.

Groups compared by nonpaired t test.

by the copper chelating atent, diethyldithiocarbamate, also is compatible with tyrosinase activity, although some peroxidases respond similarly.[17] The failure of catalase or dihydroxymaleic acid to effectively inhibit DOPA synthesis indicates further that the enzyme involved is not a peroxidase.[18]

It is not clear how the cereal tyrosinase retains partial activity within the lumen of the rat stomach. When the rat food was incubated below pH 4.0 with tyrosine and/or pronase, negligible DOPA synthesis occurred. Perhaps the gastric contents were not well acidified early after food ingestion. The gastric contents from starved and refed rats were nearly solid, and the gastric juices may not have been well distributed. This may explain why these gastric contents synthesized more DOPA-^{3}H than did the softer, probably more acidified, stomach contents from normally fed rats.

From the urinary DOPAC data, we can roughly estimate the amount of DOPA consumed by the rat over a 24-hr period. The mean DOPAC excretion among the rats ingesting the diet containing cereal was 0.395 μmoles, as opposed to 0.083 μmoles among the animals consuming casein. The difference (0.312 μmoles) probably reflects exogenous DOPA. If it is assumed that 40% of exogenous DOPA is excreted as DOPAC in the rat[19] as in man,[20] it can be inferred that the rat consumes approximately 0.78 μmoles of DOPA, or 154 μg/24 hr. This is probably an underestimate, since no correction was made for the urine lost during the collection period.

No physiologic action of DOPA has been shown to result from doses of this magnitude. Moreover, this DOPA presumably gains only slow access to the general circulation during the process of digestion and absorption. Only a small fraction of exogenous DOPA is taken up into the brain;[7] hence, it is not surprising that brain DA levels were no different in rats consuming a casein diet. Exogenous DOPA has been shown to be taken up and decarboxylated within many tissues,[21] including the stomach. Thus, the small amount of DA present in the rabbit stomach may be derived from dietary DOPA.[22] The amount of DA in the peripheral tissues of the rat is low[23] since only a small amount of DOPA is derived from the diet, perhaps because food passes so rapidly (2–4 hr) through the rat stomach. In ruminants, cereals remain in the stomach for much longer periods of time, giving the tyrosinases a much greater opportunity to synthesize DOPA. This may explain the presence of measureable quantities of DA in ruminant lung and spleen,[24] liver and intestine,[25] mast cells,[26] and blood.[27]

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