Plasma Dopa and Feeding (42933)

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Abstract. **Dopa is a normal constituent of plasma in man and experimental animals. Dopa concentrations in plasma have been used to reflect the activity of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines. However, it is not known to what extent plasma dopa is affected by feeding, since several foods contain dopa or tyrosine, the immediate precursor of dopa. In this study dopa and its major metabolites (3-*O*-methyldopa and free and conjugated catecholamines) were measured in plasma of dogs during 5 hr after feeding. Plasma dopa did not change significantly after feeding. This finding increases the value of plasma dopa as an index of tyrosine hydroxylase activity. Plasma norepinephrine decreased, and conjugated dopamine increased, after feeding; the other analytes did not change significantly.**

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Dihydroxyphenylaline (dopa) has been shown to be a normal constituent of plasma in man and experimental animals (1, 2). Changes in plasma concentration of dopa have been taken to reflect the activity of tyrosine hydroxylase (3). Tyrosine hydroxylase catalyzes the conversion of tyrosine into dopa in sympathetic nerves and in the adrenal medulla and is the rate-limiting enzyme in the biosynthesis of catecholamines. However, dopa is present in certain foods (4, 5), and dopa glucoside can be extracted from the broad bean, *Vicia faba* (6). Therefore, fluctuations in plasma concentrations of dopa might also occur after the consumption of foods containing either dopa or perhaps its precursor, tyrosine. Such fluctuations would limit the use of plasma dopa as an indicator of tyrosine hydroxylase activity. The aim of this study was to determine how plasma dopa is affected by feeding. Levels of 3-methoxy-4-hydroxyphenylalanine (3-*O*-methyldopa [3-OMD]), dopamine (DA), norepinephrine (NE), and epinephrine (E), all major metabolites of dopa, were also measured in plasma of the dogs before and after they had consumed a can of dog food.

Dopa and its metabolites were also measured in the food itself. Hoeldtke *et al.* (4) showed that dopa was contained in proteins in rat food. Therefore, dopa and its metabolites were measured in the dog food with and without an incubation period with a proteolytic enzyme or acid boiling to hydrolyze proteins. Dogs were used in this study so that analyses could be done on several samples of blood drawn from the same animal.

Materials and Methods

Materials. The canned dog food used in this study was Alpo Beef Chunks Dinner (397 g). The enzyme pronase (from *Streptomyces griseus*) was obtained from Calbiochem and β-glucosidase (from almonds) and arylsulfatase (from *Enterobacter aerogenes*) were obtained from Sigma Chemical Co.

Subjects. Experiments were done on five male hound dogs (average weight, 22.8 kg; range, 22–24 kg) in the morning, after a 24-hr fasting period with *ad libitum* access to water. Blood samples were drawn before and at hourly intervals for 5 hr after the dogs ate 397 g of canned dog food. The dogs were not perturbed by the blood withdrawal and remained calm during the experiments.

Collection of Plasma. Approximately 10 ml of blood were drawn from the jugular vein into a heparinized syringe. After immediate centrifugation at 4°C and 3000 rpm for 30 min, plasma (4–5 ml) was transferred into acid-washed tubes containing 40–50 μl of freshly prepared 5% sodium metabisulfite. Samples were frozen until analyses were done.

Separation of Dopa. Plasma samples were shaken for 15 min with 50 μl of 5% sodium metabisulfite, 100 mg of alumina, and 1.0 ml of 2.0 *M* Tris buffer (pH 8.6) containing 5% disodium EDTA. The mixtures were quantitatively transferred into columns (internal diameter, 0.3 cm) and the alumina was washed with 3 ml of water. Effluents and washes were saved for analy-

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sis of 3-OMD and conjugated catecholamines. Dopa was eluted from the alumina with 1.1 ml of 0.2 *N* perchloric acid. The first 0.1 ml was discarded and the remaining 1.0 ml was collected in acid-washed tubes.

Separation of Free Catecholamines. Fractions (900 μl) of the dopa eluates were diluted to 6.0 ml with water, followed by an addition of 0.2 ml of 5% disodium EDTA and 0.2 ml of freshly made 0.1% ascorbate. Samples were adjusted to pH 6.0–6.2 with 0.2 *N* sodium carbonate and passed through 3.0-cm × 3-mm columns containing Bio-Rex 70 (Bio-Rad; 100–200 mesh in 0.4 *M* sodium phosphate buffer, pH 6.5). The resin was washed with 5 ml of water and the catecholamines were eluted with 1.0 ml of 0.67 *M* boric acid.

Separation of 3-OMD. 3-OMD, present in the effluent from the alumina used for the dopa separation, was separated on columns containing 500 mg of Dowex 50 × 4 (200–400 mesh). Before using, the resin was washed with 20 ml of 2 *N* HCl, 5 ml of water, 10 ml of 1 *N* sodium acetate buffer (pH 6.5), and 5 ml of water. (After use the resin was reused up to four times by washing as described above.) Proteins were removed from 1.0-ml aliquots of the effluents from the dopa separation by adding 0.1 sample volume of 7.5 *M* perchloric acid and centrifuging at 4°C and 3000 rpm for 30 min. Perchlorates were removed from supernatants by adjusting extracts to pH 5.0–5.5 with 2, 0.2, and 0.02 *N* potassium hydroxide, setting on ice for 20 min, and centrifuging at 4°C and 3000 rpm for 15 min. Samples were adjusted to pH 2 with 1 *N* HCl and passed directly through the Dowex columns. The resin was washed with 5- and 10-ml samples of water, and 3-OMD was eluted with 8 ml of 0.2 *M* potassium acetate buffer (pH 6.5).

Separation of Conjugated Catecholamines. Conjugated catecholamines present in the alumina effluent from the dopa separation were hydrolyzed by acid boiling. Proteins were removed from the remainder of the dopa effluents and washes as described in the 3-OMD determination. Supernatants containing 0.6 ml of 1% dithiotreitol were heated in a boiling water bath for 30 min and cooled on ice for 15 min. One milliliter 0.2 *M* phosphate buffer with 5% EDTA (pH 6.1) was added to the samples, and the perchlorates were removed as described in the 3-OMD determination. Catecholamines released by boiling were then separated as described for free catecholamines.

Determination of Dopa and Metabolites in Dog Food. Samples of the canned dog food were homogenized in 10 volumes of 0.4 *M* perchloric acid. Proteins and perchlorates were removed from 5-ml aliquots of the homogenate before assays were done as described for separation of analytes in plasma.

Extracts of dog food were also incubated with the proteolytic enzyme pronase as described earlier (4). Briefly, the dog food was homogenized in 40 volumes of water at 4°C. To 5 ml of this homogenate were added 5 ml of 0.05 *M* Tris buffer containing 0.01 *M* calcium chloride (pH 7.5), pronase (1 mg/ml), and three drops of ethanol. The mixtures were incubated at 37°C for 16 hr before assays were done as described for separation of analytes in plasma. In other experiments, aliquots (2 ml) of the aqueous homogenates of dog food were incubated with β-glucosidase, 10 units in 0.5 ml of phosphate buffer at pH 6.2 (7) for 20 hr. After incubation, proteins were precipitated with perchloric acid and dopa was isolated as described for plasma separation. In an additional two experiments, aliquots (2 ml) of aqueous homogenates of dog food were subjected to hydrolysis in 6 *N* HCl under nitrogen for 24 hr at 100°C to hydrolyze proteins. After hydrolysis, the solutions were evaporated to dryness. The residue was dissolved in 5 ml of 0.01 *N* HCl, and dopa was isolated as described for the separation from plasma.

An attempt was also made to ascertain whether dog food contained dopa sulfate. After homogenizing dog food in 20 volumes of water, proteins were precipitated by addition of a 0.1 volume of 4 *M* perchloric acid and centrifugation. After removal of perchlorates by adjustment to pH 5 with 5 and 0.5 *M* KOH, aliquots (5 ml) of the supernatant were either incubated with sulfatase (1 unit) at 37°C for 2 hr or adjusted to pH 1 and heated to 100°C for 30 min as described for hydrolysis of conjugated catecholamines. Dopa was separated from these mixtures as described for separation of dopa from plasma.

Chromatographic Determination of Analytes. Measurement of all analytes was done by high-pressure liquid chromatography with electrochemical detection. A Biophase column (ODS, 5-μm spherical particles, C-18, 250 × 4.6 mm) was used and the mobile phase, with the exception of dopa, was composed of 0.07 *M* NaH_2PO_4, 0.2 m*M* EDTA, 2.0 m*M* heptane sulfonate, and 5% acetonitrile (pH 3.3). The mobile phase used for dopa was 0.07 *M* NaH_2PO_4, 0.2 m*M* EDTA, 1.0 m*M* heptane sulfonate, and 0.15% trifluoroacetic acid (pH 2.8). The flow rate was 0.6–1.0 ml/min, and the electrode potential was maintained at 0.65 V vs a silver:silver chloride reference electrode.

Recoveries of Analytes. The following mean (±SD) recoveries of 20 ng of each analyte, except 3-OMD, were obtained: 87.5 ± 3.5% for dopa; 91.9 ± 3.8%, 94.6 ± 2.1%, and 89.2 ± 2.0% for free NE, E, and DA, respectively; 61.9 ± 5.5% and 91.0 ± 4.6% for sulfated NE and DA through boiling, respectively. The mean (±SD) recovery of 500 ng of 3-OMD was 78.5 ± 1.0%. Data have been corrected for these recoveries.

Analysis of Data. The data were analyzed in two ways to determine if there was a significant change in plasma concentration of the analytes. The first way (Table I) was to compare the individual values obtained at each time interval and also to compare the mean of all postfeeding values to the value at time 0. The second

Table I. Dopa, 3-OMD, and Free and Conjugated Catecholamines

Analyte ($n = 5$)	Time after feeding						Mean of 1–5 hr
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	
Dopa	2.27 ± 0.88	2.21 ± 0.49	2.36 ± 0.91	2.46 ± 0.95	2.81 ± 1.16	2.54 ± 0.97	2.48 ± 0.88
3-OMD	161.8 ± 61.9	163.4 ± 62.0	155.5 ± 57.2	170.2 ± 60.5	159.8 ± 56.6	168.1 ± 58.0	163.4 ± 57.3
DA							
Free	0.29 ± 0.59	0.17 ± 0.29	0.05 ± 0.07	0.04 ± 0.03	0.05 ± 0.04	0.06 ± 0.05	0.07 ± 0.09
Conjugated	2.48 ± 2.08	2.76 ± 1.76	3.52 ± 1.77	3.62 ± 1.52	3.55 ± 1.35	3.51 ± 1.44	3.39 ± 1.55
NE							
Free	0.34 ± 0.11	0.36 ± 0.11	0.28 ± 0.10	0.26 ± 0.11	0.31 ± 0.15	0.34 ± 0.13	0.31 ± 0.11
Conjugated	0.48 ± 0.39	0.45 ± 0.35	0.39 ± 0.31	0.37 ± 0.29	0.32 ± 0.27	0.29 ± 0.22	0.36 ± 0.29
E							
Free	0.19 ± 0.05	0.19 ± 0.10	0.18 ± 0.17	0.11 ± 0.03	0.10 ± 0.06	0.12 ± 0.06	0.14 ± 0.06
Conjugated[a]	0.05, 0.08	0.05, 0.06	0.05, 0.06	0.04, 0.06	0.04, 0.05	0.04, 0.05	

Note. Data are mean values ± SD and are expressed as ng/ml plasma.
[a] Conjugated E was not detected in three dogs; single values are shown for the other two dogs.

way (Table II) was to determine (by graphing concentration of analyte in plasma against time) to which time interval there was an increase or decrease and then to calculate the slope of the lines of best fit up to that point. It was then determined whether the slope of that line was significantly different from a 0 slope (Table II). The Wilcoxon rank sum test (8) or Student's t test was used to determine significances of differences.

Results

There were no differences for dopa or for any other for the analytes measured in individual values at the different time intervals after feeding and the values at time 0 (Table I). Nor were there any differences between the mean values of the five time intervals after feeding compared with the value at time 0. Conjugated DA appeared to increase in plasma after feeding, but the differences from the time 0 value did not reach significance by Student's t test or by Wilcoxon rank sum test (8). However, one of the dogs had a much higher time 0 level of conjugated DA (6.1 ng/ml) than the other four dogs (mean value, 1.6 ± 0.3 ng/ml). The dog with the high fasting level of conjugated DA showed little change after feeding (maximum of 6.4 ng/ml at 2 hr after feeding), while the other four dogs showed clear increases. The mean value of the 1- to 5-hr values for the four dogs was 3.0 ± 0.4 ng/ml, which was significantly higher ($P < 0.05$ by the Wilcoxon rank sum test) than the time 0 value.

The mean of slopes of the lines of best fit for conjugated DA for values from 0 to 3 hr was significantly different from a 0 slope (Table II). The mean rate of increase of conjugated DA for the first 3 hr after feeding was 0.42 ng/ml/hr. Likewise, the mean of the slopes of lines of best fit for NE from 0 to 3 hr after feeding was significantly different from a 0 slope. Free NE decreased at a mean rate of 0.03 ng/ml/hr after feeding. None of the other analytes showed slopes of lines of best fit which were significantly different from 0, although the slope for conjugated NE showed a t value of 2.187 for difference against a 0 slope which approached the 2.776 needed for significance at a $P < 0.05$ level.

Table II. Slopes of the Lines Showing a Linear Change in Analyte

Analyte ($n = 5$)	Time period (hr)	Slope	SE	t
Dopa	0–4	0.133 ± 0.254	0.113	1.173
3-OMD	0–3	1.724 ± 5.865	2.623	0.657
DA				
Free	0–3	−0.088 ± 0.192	0.086	1.020
Conjugated	0–3	0.418 ± 0.252	0.113	3.700[a]
NE				
Free	0–3	−0.029 ± 0.019	0.009	3.478[a]
Conjugated	0–5	−0.069 ± 0.071	0.032	2.187
E				
Free	0–5	−0.020 ± 0.024	0.011	1.830

Note. Data are mean values ± SD. t = slope/SE of the slope.
[a] $P < 0.05$ for difference from a slope of zero.

Only traces of free dopa or 3-*O*-methyldopa were detected in the dog food (Table III). Free NE, E, and DA had the highest concentrations in dog food (4.06, 2.41, and 0.77 ng/g, respectively) (Table III). Smaller amounts of the conjugated catecholamines were present.

Incubation of dog food with pronase or boiling the food in 6 *N* HCl released considerable amounts of dopa. The mean amounts (±SD) of dopa released were 78.6 ± 7.2 ng/g ($n = 3$) after incubation with pronase and 1132 and 4414 ng/g after boiling in 6 *N* HCl ($n = 2$, from samples from the same can of dog food). No dopa was detected after incubation of dog food with β-glucosidase. The identity of the dopa produced after acid boiling was confirmed by comparing the signal produced to that of authentic dopa standard when oxidations were done at different potentials between 0.5 and 0.8 V and also by establishing that the putative dopa was isographic with authentic dopa in different chromatographic solvents obtained by varying the pH from

Table III. Dopa and Metabolites in Dog Food[a]

Analyte	Concentration (ng/g) in dog food
Dopa	ND[b]
3-*O*-methyldopa	ND
DA	
Free	0.77 ± 0.10
Conjugated	0.39 ± 0.17
NE	
Free	4.06 ± 0.57
Conjugated	0.23 ± 0.14
E	
Free	2.41 ± 0.53
Conjugated	0.19 ± 0.12

[a] Values are means (±SD), $n = 5$.
[b] ND, not detectable, i.e., < 0.2 ng/g of dopa and < 30 ng/g of 3-*O*-methyldopa.

2.8 to 3.3 or by varying the heptane sulfonate from 0.1 to 3 m*M*. However, added standard dopa (and free catecholamines) was not recovered through the acid boil or through the enzyme incubation, although it was recovered if added *after* acid boiling or incubation with enzymes. Thus, dopa was not stable through the long acid boil or through the enzyme incubation. The more stable 3-*O*-methyldopa was recovered essentially quantitatively through enzyme incubations and boiling. The losses of the added catechol standards were not surprising in view of the long enzyme incubation times and the long acid boil. Thus, these experiments indicate that considerable amounts of dopa were released from the dog food under conditions under which proteins were hydrolyzed, but the actual amounts of dopa that were formed could not be quantitated.

After incubation with sulfatase, 202 ng/g of free dopa was detected in dog food and 14 ng/g, 15 ng/g, and 61 ng/g dopa was detected in three samples of food after 30-min heating at 100°C at pH 1. The recovery of 50 ng of dopa through incubation with sulfatase and separation on alumina was 73.5%, and through 30 min of acid boiling and separation on alumina, the mean (±SD) recovery was 72.1 (±11.8) ($n = 3$).

Discussion

This study showed that dopa was present in plasma in dogs, which is consistent with previous studies (1, 2), and also that plasma levels of dopa were not affected by feeding. The lack of effect of feeding on plasma dopa was surprising, since the dog food contained considerable amounts of dopa. However, it appears that when dopa is given orally, it is rapidly metabolized by oxidative deamination and conjugation, probably before absorption from the gastrointestinal tract (4). Thus, after oral administration of [^{3}H]dopa to patients with Parkinson's disease, the major urinary metabolite was a conjugate of [^{3}H]dopamine and only traces of [^{3}H]dopa were excreted. After intravenous administration of [^{3}H]dopa, much less [^{3}H]dopamine conjugate and much more [^{3}H]dopa were excreted in the urine (9).

The dopa present in dog food was not free but was most likely bound to proteins. Hoeldtke *et al.* (4) have previously shown that considerable amounts of L-dopa were liberated from rat food after incubation with pronase, but there are to our knowledge no previous studies analyzing dopa in dog food. The dopa in dog food did not appear to be a glucoside conjugate, although dopa glucosides have been detected in several plants (6). Small amounts of dopa sulfate were also detected in the dog food. Previous studies (4, 10) have emphasized that rat diets that contain cereals had a high content of dopa. The canned dog food used in the study did not list cereals as ingredients, the only plant products listed (as minor ingredients) being soy flour and vegetable gum.

Although plasma levels of dopa did not change after the dogs had eaten food containing considerable amounts of dopa, plasma levels of this compound clearly depend not only on the rate of entry but also on the rate of clearance of this compound from plasma. Clearance of dopa from plasma occurs principally in kidney (11) with the formation of urinary free dopamine. The possibility must be considered that metabolism of dopa to dopamine in kidney occurs at a more rapid rate after feeding. Certainly after a protein meal, the excretion of dopamine is markedly increased without any changes in plasma dopa levels (11).

Other investigators (5, 10, 12) have shown that in rats, consuming foods rich in cereals increases urinary excretion of DA and its metabolites. Conjugated metabolites (especially conjugated dihydroxyphenylacetic acid) were most affected by diet. These investigators did not measure plasma concentrations of catecholamines or their metabolites after feeding. Cottet-Emard *et al.* (12) also measured high levels of free and conjugated DA in urine of rats fed an animal protein, i.e., milk. Catecholamines or dopa in plasma or in urine could, of course, be increased after feeding not only because of the presence of dopa in the food but also because of the increased availability of the remote precursors, tyrosine (5) or phenylalanine.

Conjugated DA showed definite increases in plasma after feeding, but very little free or conjugated DA was present in the food. A total of only 2 nmol of free and 1 nmol of conjugated DA was present in the 397 g of dog food that was consumed by the animals. A mean increase of 1.14 ng/ml of conjugated DA was noted in plasma of the dogs from time 0 value to the maximum value at 3 hr after feeding (Table I). In the total plasma volume of the dogs (51.5 ml of plasma/kg body weight [13] or 1174 ml), this increase would total 1339 ng or 9 nmol of conjugated DA. If conjugated DA in plasma is in equilibrium with conjugated DA in extracellular fluid (assumed to be 27.6% of the body

weight [13] or 6.3 liters), then there would be an increase of 46.4 nmol of conjugated DA in this compartment after feeding. This conjugated DA was considerably more than the small amounts of free or conjugated DA in dog food and most likely was derived from dopa in the diet, since it would appear that the dogs consumed more than enough dopa to account for the rise in conjugated DA in the total extracellular fluid. If dopa in the diet is the source of the conjugated DA in plasma, then the conversions dopa → DA → DA sulfate may have occurred in the gastrointestinal tract, perhaps in liver immediately after absorption, thus attenuating changes of dopa and dopamine in plasma.

Although the dogs consumed a considerable amount of free and conjugated NE (a total of 10 nmol), the amounts in plasma actually decreased after feeding. Presumably, the NE in the food was metabolized and did not reach the circulation.

As pointed out by Cottet-Emard *et al.* (12), changes in free and conjugated catecholamines as a result of feeding could result from changes in availability of the precursors in the diet or could be secondary to adrenergic activation induced by feeding or by fasting, and presumably the same factors could influence plasma dopa. It was not possible to distinguish between the effects of these two factors in the present study. However, this study did show that in dogs plasma dopa was not affected by feeding. This finding is in agreement with the suggestion of Goldstein *et al.* (3) that dopa in plasma originates principally from neurons and provides support for the proposal (1, 3) that changes in plasma dopa can be used as an index of the activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines.

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