Cereal Ingestion and Catecholamine Excretion

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We have identified a group of cereal foodstuffs that contain an enzyme, presumably a tyrosinase, which converts dietary tyrosine to dihydroxyphenylalanine (DOPA). Nabisco Wheat Thins and Kretchmer's Wheat Germ are good sources of this enzyme. Urinary catecholamines and their metabolites were measured in subjects ingesting either a cereal-rich diet or a control diet containing milk or eggs as their only protein source. Consumption of the cereal diet was associated with a fourfold increase in the excretion of conjugated dopamine (DA), and a twofold increase in conjugated dihydroxyphenylacetic acid (DOPAC). Homovanillic acid (HVA) excretion was not significantly influenced by diet. These findings suggest that DOPA is synthesized in the human gastrointestinal tract following the consumption of a cereal-containing diet. Cereal ingestion is thus a potential source of error in clinical studies of catecholamine metabolism.

STANDARD LABORATORY RAT CHOW and several varieties of wheat contain small amounts of free DOPA and an enzyme, probably a tyrosinase, that catalyzes the conversion of tyrosine to DOPA.[1] Following cereal consumption, sufficient DOPA is formed within the lumen of the rat gastrointestinal tract to cause a fourfold increase in the excretion of DOPAC, a major DA metabolite, in this species.[2] Since varieties of wheat germ consumed by humans possess considerable tyrosine-hydroxylating activity, it was of interest to determine whether consumption of this and related foods caused DOPA to be formed within the human gut. We found that the urinary excretion of conjugated DA and conjugated DOPAC is markedly elevated in subjects ingesting large quantities of wheat germ and other cereals with tyrosine-hydroxylating activity.

MATERIALS AND METHODS

Cereal Analysis

The products containing cereal were purchased at local supermarkets. Portions of the cereal weighing 0.2 g were suspended in 5 ml of 0.1% ethanol, to which 2 mg of tyrosine was added. Incubation was performed at 37°C for 3 hr, then authentic DOPA was extracted from the suspension by column chromatography and assayed fluorimetrically by techniques described previously.[1]

Experimental Design

Our subjects, healthy young men associated with the Massachusetts Institute of Technology, did not have their daily activities in any way disrupted by the experiment. Eight- to ten-hour timed urine collections were obtained from morning awakening until the end of the working day. Urine was collected in plastic bottles containing 3 ml of 4 *N* HCl, and frozen immediately in dry ice. Foods known to contain catecholamine-like compounds[3] (bananas, cheese, avocados, oranges, and tomatoes) were excluded from the diets of all subjects.

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Received for publication April 10, 1973.

Supported by USPHS Grants AM-14228 and NS-10459 and by USPHS Training Grant AM-05371 to Dr. Hoeldtke.

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Table 1. Design of Clinical Study 1: Ad-lib Cereals vs. All-milk Diet

	Phase 1	Phase 2
	Monday–Tuesday	Wednesday–Thursday (Urine Collection Period)
Diet 1 High cereal	General diet and cereal	Milk products and cereal
Diet 2 No cereal	General diet no cereal	Milk products only

Bananas, cheese, avocados, oranges, vanilla, and tomatoes were excluded from the diet throughout the experiment.[3] Coffee was excluded from the diet during phase 2.

Clinical Study 1 (Ad-lib. Cereals vs. All-milk Diet)

Four subjects were included in the study, each serving as his own control. Each volunteer was subjected to two dietary treatments, each lasting four days and divided into two phases (Table 1). During phase 1 (days 1 and 2) the dietary restrictions were less stringent than in phase 2 (days 3 and 4) when the urine was collected. During one experimental period the subjects ate milk products and large amounts of cereals known to contain tyrosinase activity (Table 2). No attempt was made to control the quantity of food ingested. Most of the subjects ate considerably more cereal (e.g., 25–50 g of wheat germ and 100–200 g of wheat thins) during the collection period than they would under normal circumstances. During the second experimental period, cereals were excluded from the diet, and the subjects ingested only milk and unflavored ice cream.

Clinical Study 2 (Controlled Cereal Intake vs. Cereal-like Formula Diet)

The second clinical study followed the same general format as the first (Table 3), except that we controlled the quantity of food ingested, and the nutrient composition of the diet during phase 2

Table 2. DOPA Synthesis by Cereal Tyrosinases in Human Foods

Foods	μg of DOPA Synthesized
Cookies containing cereals	
Wheat Thins (Nabisco)	23.10
Oatmeal (Nabisco)	3.00
Triscuit	0
Crackers containing corn	
Cheeze Doodles	0
Tortilla Chips	0
Breakfast foods containing cereal	
Kellogg's Concentrate	10.50
Product 19 (Kellogg's)	3.75
Special K (Kellogg's)	4.05
Cheerios	0.90
Raisin Bran	0
Breakfast foods containing corn	
Corn Flakes	0.35
Sugar Pops	0.29
Miscellaneous	
Rice Krispies	0
Cocoa Krispies	0
Wheat germ (Kretchmer's)	32.00
Whole wheat bread	1.04
White bread	0
White flour	0

Two-tenths grams of food was suspended in 5 ml of 0.1% ethanol, and 2 mg of tyrosine was added. Incubation was performed for 3 hr at 37°C.

Table 3. Design of Clinical Study 2: Controlled Cereal Intake vs. Cereal-like Formula Diet

	Phase 1	Phase 2
	Monday–Tuesday	Wednesday–Thursday (Urine Collection Period)
Diet 1 High cereal	General diet and cereals	Cereals only
Diet 2 No cereal	General diet no cereal	Egg formula diet

Bananas, cheese, avocados, oranges, vanilla, and tomatoes were excluded from the diet throughout the experiment.[3] Coffee was excluded from the diet during phase 2.

of each dietary treatment. While on the cereal diet, each subject consumed 75 g of wheat germ, 150 g of wheat thins, 227 g of oatmeal cookies, and 19 g of Special K per 24 hr. The nutrient content of the two diets was quite similar (Table 4). The formula diet contained dried eggs, and its protein content was lower than that of the cereal diet. Since the biological value of egg protein is considerably greater than that of cereal protein, there was not a large difference in the protein nutrition of the subjects ingesting the two diets. We could not precisely control the intake of ascorbic acid, pyridoxine, and tocopherol since we did not have information on the amounts of these vitamins in some of the cereal products.

Assay of Catecholamines and Metabolites

Free and conjugated DA and NE were separated from the urine by column chromatography, and then assayed fluorimetrically by a technique previously described.[4] The additional of EDTA to the alumina filtrate prior to hydrolysis was omitted to lessen quenching in the assay of conjugated NE. Free DOPAC was measured by the same method described for rat urine.[2] Conjugated DOPAC was converted to free DOPAC by hydrolysis of the alumina filtrate.[5] The hydrolysate was then recycled through alumina, as described for the catecholamines.[4,6] The DOPAC excreted as a conjugate (present in the hydrolyzed alumina filtrate as free DOPAC) was identified in some of the samples on paper chromatograms. The DOPAC was extracted from the 1N sulfuric-acid eluate of the alumina columns into ether. After dihydroxymandelic acid was removed by washing the ether phase with a pH 3.3 phosphate buffer,[7] the ether was flash evaporated. The DOPAC was then taken up into several drops of 50% ethanol/0.01 *N* HCL and spotted on paper chromatograms (Whatman No. 1), which were developed in butanol/acetic acid/water 4:1:1. The extracted DOPAC had the same R_f (0.81) as the authentic compound.

HVA was extracted from 7.5 ml of urine and isolated on paper chromatograms according to the technique of Armstrong.[8] The section of the chromatogram on which the HVA was located was cut out and placed in a 13-ml glass-stoppered centrifuge tube. The HVA was eluted by shaking the paper with 5 ml of 5 *N* NH_4OH for 10 min. The tubes were centrifuged at 2300 g for 10 min, and 1-ml aliquots of the resulting clear supernate were used for the fluorimetric assay of HVA.[9]

RESULTS

None of the foods contained measurable quantities of free DOPA but many cereal products had the capacity to synthesize DOPA when suspensions were incubated with tyrosine (Table 2). The largest amount of DOPA was synthesized by Kretchmer's

Table 4. Nutrient Composition of the Cereal and the Egg Formula Diets

	Calories	Protein (g)	Fat (g)	Carbohydrate	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Iron (mg)
Diet 1 Cereal	2116	45.3	76.0	318	2.7	1.7	14.3	17.2
Diet 2 Egg formula	2047	19.7	76.3	318	2.7	1.7	14.3	13.9

Table 5. Study 1: Ad-lib Cereals vs. All-milk Diet

		Cereals		Milk		Significance Level
		Day 3	Day 4	Day 3	Day 4	
Free	NE	1.83 ± 0.49	2.94 ± 1.16	1.56 ± 0.45	1.47 ± 0.67	$p < 0.05$
Conjugated	NE	2.52 ± 1.24	2.48 ± 0.93	0.61 ± 0.15	1.65 ± 0.44	$p < 0.10$
Total	NE	4.35 ± 1.76	5.42 ± 1.67	2.16 ± 0.39	3.12 ± 0.95	$p < 0.05$
Free	DA	4.07 ± 1.30	4.14 ± 1.60	4.61 ± 1.50	3.60 ± 2.30	NS
Conjugated	DA	77.20 ± 26.00	56.70 ± 12.30	8.56 ± 4.30	9.42 ± 2.10	$p < 0.001$
Total	DA	81.30 ± 26.10	60.80 ± 12.50	13.20 ± 4.10	13.00 ± 3.80	$p < 0.005$
Free	DOPAC	47.00 ± 11.90	47.90 ± 3.10	37.60 ± 5.00	25.00 ± 2.80	$p < 0.05$
Conjugated	DOPAC	123.80 ± 43.00	84.60 ± 11.10	87.50 ± 36.80	30.60 ± 5.10	$p < 0.10$
Total	DOPAC	170.80 ± 54.30	132.50 ± 13.00	125.10 ± 41.60	55.60 ± 7.90	$p < 0.10$
HVA		248.00 ± 91.00	123.00 ± 29.00	194.00 ± 57.00	172.00 ± 54.00	
Creatinine		68.60 ± 4.60	70.30 ± 3.50	73.00 ± 2.70	70.10 ± 1.80	NS
Urine volume		45.30 ± 6.60	47.30 ± 2.50	49.40 ± 5.60	36.50 ± 4.20	NS

Data for catecholamines and metabolites expressed as μg excreted/hr. Creatinine expressed as mg excreted/hr. Urine volume expressed as ml/hr.

Table 6. Study 2: Controlled Cereal Intake vs. Cereal-like Formula Diet

		Cereals		Formula		Significance Level
		Day 3	Day 4	Day 3	Day 4	
Free	NE	1.63 ± 0.48	2.22 ± 0.92	1.03 ± 0.31	0.95 ± 0.16	$p < 0.10$
Conjugated	NE	3.16 ± 0.76	2.41 ± 1.00	1.92 ± 1.10	1.94 ± 0.53	NS
Total	NE	4.80 ± 1.10	4.63 ± 1.10	2.16 ± 1.10	2.88 ± 0.43	$p < 0.10$
Free	DA	19.90 ± 7.90	10.70 ± 3.70	13.00 ± 4.20	11.90 ± 3.90	NS
Conjugated	DA	27.20 ± 3.30	33.20 ± 6.30	13.90 ± 1.70	14.80 ± 2.60	$p < 0.005$
Total	DA	47.10 ± 9.70	43.90 ± 6.70	26.90 ± 3.70	26.70 ± 5.10	$p < 0.01$
Free	DOPAC	35.20 ± 7.20	25.70 ± 5.20	33.50 ± 10.80	24.80 ± 8.20	NS
Conjugated	DOPAC	159.10 ± 43.70	85.50 ± 35.00	86.60 ± 23.00	35.50 ± 28.00	$p < 0.02$
Total	DOPAC	194.30 ± 49.10	111.20 ± 40.20	120.10 ± 32.60	60.30 ± 35.10	$p < 0.05$
HVA		163.00 ± 57.00	120.00 ± 70.00	108.00 ± 57.00	145.00 ± 47.00	NS
Creatinine		72.90 ± 1.40	67.00 ± 1.80	68.00 ± 6.90	65.70 ± 4.00	NS
Urine volume		38.50 ± 3.60	28.50 ± 1.80	33.80 ± 13.30	37.50 ± 12.00	NS

Data for catecholamines and metabolites expressed as μg excreted/hr. Creatinine expressed as mg excreted/hr. Urine volume expressed as ml/hr.

Table 7. Combined Data for Clinical Studies 1 and 2

		Cereals		Noncereals		Significance Level
		Day 3	Day 4	Day 3	Day 4	
Free	NE	1.73 ± 0.32	2.58 ± 0.70	1.29 ± 0.27	1.21 ± 0.33	$p < 0.05$
Conjugated	NE	2.84 ± 0.69	2.44 ± 0.64	1.26 ± 0.55	1.79 ± 0.32	$p < 0.10$
Total	NE	4.57 ± 0.98	5.02 ± 0.94	2.55 ± 0.56	3.00 ± 0.49	$p < 0.02$
Free	DA	12.00 ± 4.75	7.42 ± 2.20	8.81 ± 2.60	7.75 ± 2.60	NS
Conjugated	DA	52.20 ± 15.40	44.90 ± 7.80	11.20 ± 2.40	12.10 ± 1.80	$p < 0.001$
Total	DA	64.20 ± 14.40	52.30 ± 7.30	20.00 ± 3.60	19.90 ± 3.90	$p < 0.005$
Free	DOPAC	41.10 ± 6.80	36.80 ± 5.00	35.50 ± 5.60	24.90 ± 4.00	NS
Conjugated	DOPAC	141.50 ± 29.00	85.00 ± 17.20	87.00 ± 20.10	33.00 ± 13.60	$p < 0.02$
Total	DOPAC	182.70 ± 34.40	121.90 ± 19.80	122.50 ± 24.00	57.90 ± 17.00	$p < 0.02$
HVA		205.00 ± 52.00	121.00 ± 35.00	151.00 ± 40.00	158.00 ± 34.00	
Urine volume		41.60 ± 3.70	37.00 ± 3.80	41.60 ± 7.30	37.00 ± 5.90	NS

Data for catecholamines and metabolites expressed as μg excreted/hr. Urine volume expressed as ml/hr.

Wheat Germ and Nabisco Wheat Thins. Negligible tyrosine-hydroxylating activity was detected in the corn and rice products.

The clinical data were analyzed by a two-way analysis of variance involving partitioning of the between subjects variance. The between conditions variance for DA and NE excretion was analyzed by orthagonal comparison in which days 3 and 4 of the cereal diet were rated +1, +1, and days 3 and 4 of the noncereal diet were rated -1, -1.[10] For the DOPAC data, the fourth day of the noncereal diet was rated -3, and each of the other days was rated +1.

The results of clinical study 1 (Table 5) and clinical study 2 (Table 6) were quite similar. Cereal ingestion was associated with an eightfold increase in conjugated DA excretion in study 1 ($p < 0.001$) and a twofold increase in study 2 ($p < 0.005$). Cereal ingestion led to nearly a twofold increase in free NE ($p < 0.05$) and total NE ($p < 0.02$) (Table 7). DOPAC excretion followed a slightly different pattern, and was only altered on the fourth day of the noncereal diet ($p < 0.02$). HVA excretion was not significantly altered in either study. Total DA, NE, DOPAC, and HVA excretion for the pooled data can be seen in Fig. 1.

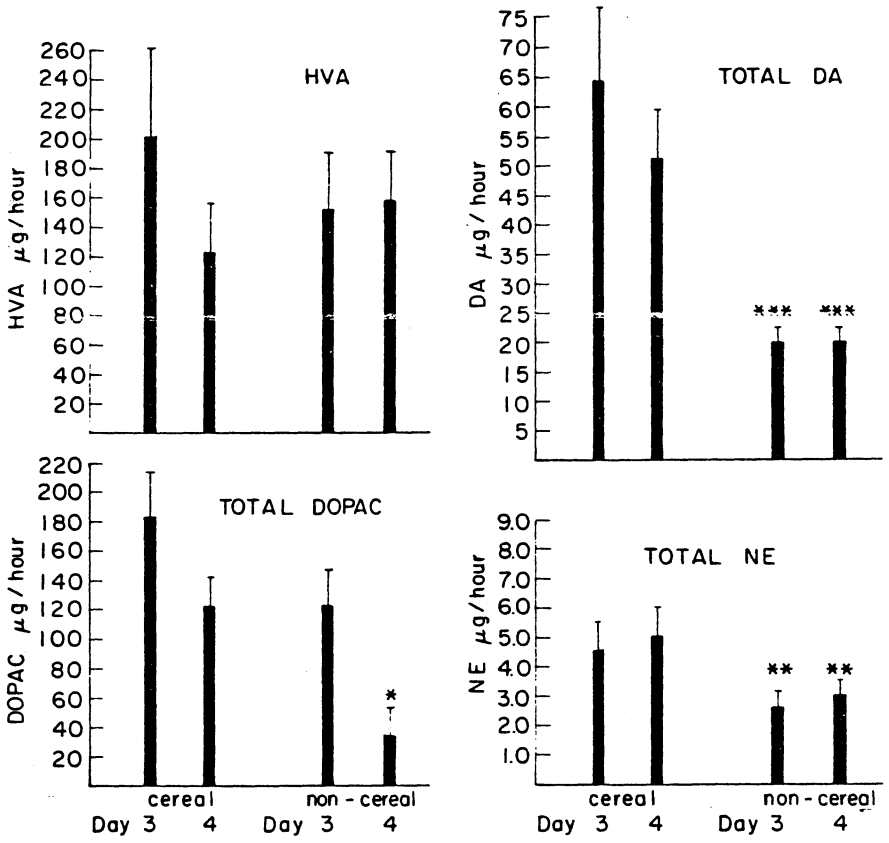

Fig. 1. Combined data from Studies 1 and 2 are represented. Asterisk, $p < 0.02$. Day 4 of the noncereal diet was significantly lower when compared with the other three days (see text for explanation of statistics). Double asterisks, $p < 0.02$. Cereal diet was significantly higher than the noncereal. Triple asterisks, $p < 0.005$. Cereal diet was significantly higher than the noncereal.

DISCUSSION

These observations suggest that cereals in human foods contain tyrosine-hydroxylating activity, presumably related to an enzyme, that catalyzes DOPA synthesis during the passage of the food through the stomach. The metabolism and excretion of dietary DOPA apparently differs slightly from that following administration of large doses of the drug. Exogenous DOPA is taken up into many tissues and decarboxylated to form DA,[11] which is then further metabolized in the liver and kidney by monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) to form the two major end products of DOPA metabolism, DOPAC and HVA.[12] Thus when patients with Parkinson's disease are given large doses of oral DOPA, there occurs a dramatic increase in the excretion of free and conjugated DA,[13,14] free DOPAC, and HVA.[15,16] The ingestion of the cereal diet did not lead to changes in the excretions of free DA, DOPAC, or HVA, but did increase the urinary concentrations of conjugated DA and conjugated DOPAC. Orally administered catecholamines are metabolized in the stomach and/or liver to conjugates (presumably ethereal sulfates) that can be released by acid hydrolysis of the plasma[17] or urine.[18] The ingestion of pure NE caused a marked increase in conjugated NE, but only moderately affected normetanephrine excretion.[19] When epinephrine was injected into the portal vein the major metabolite fractions were O-methylated.[20] These findings suggest that the conjugation pathway predominates in the gastrointestinal tract, and the O-methylation pathway predominates in the liver. Thus it seems possible that DOPA derived from the diet is converted to DA and DOPAC in the gastrointestinal tract, and then conjugated prior to reaching the liver. This would explain the lack of change in the excretion of HVA, the O-methylated product. When large doses of DOPA are administered by mouth, the capacity of the conjugation pathway is exceeded, and some of the DOPA, DA, and DOPAC reach the liver, brain, and other tissues where O-methylation occurs.[21]

The effect of cereal ingestion on NE excretion is not readily explained. It is unlikely that total synthesis of NE from tyrosine occurs in the gut following cereal ingestion; cereal suspensions convert tyrosine-H^3 to DOPA-^3H, but further conversion to catecholamines-H^3 does not occur.[2] It also seems unlikely that sufficient levels of DOPA are derived from the diet to alter NE excretion. Routh et al. observed an increase in NE excretion after therapeutic doses of DOPA.[22] However, other observers have not seen any alteration.[13,15] Perhaps the small amount of DOPA derived from the diet gains more ready access to the β-hydroxyating enzyme (presumably within sympathetic neurons) than do pharmacologic doses of the pure compound.

ACKNOWLEDGMENT

Gratitude is expressed to Miss Edwina Murray for help in preparing the diets, and to Miss Jacqueline Allen for performing the creatinine determinations.

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