Effects of aspartame ingestion on the carbohydrate-induced rise in tryptophan hydroxylation rate in rat brain[1-3]

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ABSTRACT Effects of aspartame (aspartyl-phenylalanine-methylester) on increases in brain-tryptophan level and hydroxylation rate following a high-carbohydrate, protein-free meal were tested. After an overnight fast, rats consumed a protein-free meal containing one of several levels of aspartame. Blood and brain amino acid levels and the in vivo rate of tryptophan hydroxylation in brain were estimated at intervals thereafter. Ingestion of the meal alone increased brain-tryptophan level and hydroxylation rate. Aspartame did not modify these effects, except at doses of 530 mg/kg body weight or more. Results suggest a threshold dose of aspartame can be identified for the rat in single-meal studies above which suppression of carbohydrate-induced increases in brain-tryptophan level and serotonin synthesis occurs. This dose, however, is large and, when corrected for species differences in metabolic rate, is unlikely to be ingested by a human subject as a single load. *Am J Clin Nutr* 1986;44:195-205.

KEY WORDS Aspartame, serum amino acids, brain tryptophan, brain serotonin, tryptophan hydroxylation, rat, diet

Introduction

The artificial sweetener aspartame is a phenylalanine-containing dipeptide (aspartyl-phenylalanine-methylester). Because it is about 200 times sweeter than sugar (1), normally individuals are not likely to consume it in sizeable amounts, but some might conceivably ingest large amounts in a relatively short period (2). One potentially significant consequence of such megadosing could be suppression of the rise in brain-tryptophan level and serotonin synthesis that normally accompanies the ingestion of a carbohydrate meal (2).

Such an effect is predicted from the known competition among the large neutral amino acids (phenylalanine, tyrosine, tryptophan, leucine, isoleucine, valine) for transport across the blood-brain barrier. That is, when a carbohydrate meal is consumed by a fasting rat, the secretion of insulin lowers considerably the blood levels of the branched-chain amino acids, those of phenylalanine and tyrosine modestly, but does not modify (and sometimes raises) blood-tryptophan levels (3). As a consequence, tryptophan gains a competitive advantage over the other large neutral amino acids (LNAA) for transport into brain. Thus, brain-tryptophan levels rise soon after carbohydrate ingestion (3, 4). (The competitive standing between tryptophan and the other LNAA in blood following meal ingestion has been expressed in the form of a serum-tryptophan:LNAA ratio [tryptophan:tyrosine + phenylalanine + leucine + isoleucine + valine]; this ratio is increased following carbohydrate ingestion [5].)

Tryptophan hydroxylase, the enzyme that catalyzes the rate-limiting step in serotonin (5HT) synthesis in brain, is normally only

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about half-saturated with substrate (6, 7). Consequently, when carbohydrate ingestion raises brain-tryptophan level, it also increases the substrate saturation of tryptophan hydroxylase (and thus increases tryptophan-hydroxylation rate). As a result, 5HT synthesis rate increases (4, 8).

The expressed concern about aspartame (2) is that if phenylalanine as aspartame were to be consumed with carbohydrate in sufficiently large amounts, blood-phenylalanine (and tyrosine) levels would rise so high as to neutralize the normal enhancement of tryptophan's competitive advantage for brain transport (reflected as a suppression of the carbohydrate-induced rise in the serum-tryptophan:LNAA ratio). As a result, brain-tryptophan level would not rise following carbohydrate ingestion, and serotonin synthesis would not be stimulated (2).

There can be no doubt that the administration of very high doses of phenylalanine can inhibit the uptake of tryptophan into brain (eg, 7). Thus, the issue for aspartame is also one of dose. In previous studies (9), we have observed that the oral intubation of rats with aspartame in doses up to 200 mg/kg body weight produces no effects on the brain level of tryptophan, the rate of tryptophan hydroxylation in vivo, or the brain levels of 5HT and its principal metabolite, 5-hydroxyindoleacetic acid (5HIAA). It thus seemed to us that if the ingestion of aspartame could modify the carbohydrate-induced rise in brain-tryptophan level (and its rate of hydroxylation), high doses of the sweetener would probably be required (higher than 200 mg/kg body weight).

The results presented below explore the relationship between aspartame dose and its hypothesized suppression of the carbohydrate-induced rise in brain 5HT synthesis, using doses up to about 1500 mg/kg body weight. They show that the ingestion of only very large doses of aspartame in combination with a high-carbohydrate, protein-free meal succeed in blunting the carbohydrate-induced rise in the serum-tryptophan:LNAA ratio and in brain-tryptophan level and hydroxylation rate. To achieve such high doses, the sweetener was included in the meal. This vehicle for dipeptide administration was necessitated by our experience in previous studies (9) in which we found it quite difficult to administer aspartame by gavage when doses exceeded 200 mg/kg body weight (owing to its poor solubility in water). We observed in the present studies that rats will consume single meals containing very large amounts of the dipeptide, probably because aspartame is not perceived as sweet by rodents (10, 11).

Methods

Initial studies

In initial studies, we obtained a time-course of the effects of ingesting aspartame (APM) with a high-carbohydrate, protein-free meal on serum and brain levels of tyrosine and phenylalanine to ascertain the optimal time for dose-response studies.

Groups of eight food-deprived adult male Sprague-Dawley rats (COBS, Charles River Breeding Laboratories, Wilmington, MA) weighing 343 ± 10 g each consumed a high-carbohydrate, protein-free meal containing aspartame (64 g/kg diet, dry weight). They were killed at 0, 30, 60, 120, or 240 min thereafter. Animals killed after 30 min each consumed 3.5 g food (dry weight), giving an aspartame dose of 650 mg/kg body weight; after 60 min, 4.3 g, giving an aspartame dose of 800 mg/kg body weight; after 120 min, 5.5 g, giving an aspartame dose of 1025 mg/kg body weight; and after 240 min, 6.5 g, giving an aspartame dose of 1215 mg/kg body weight.

As indicated in **Table 1**, ingestion of this meal containing 64 g/kg APM (dry weight) caused serum-phenylalanine concentrations to rise monotonically to a peak 2 h after food presentation. Brain-phenylalanine levels also rose during the first 2 h and then plateaued. Serum-tyrosine levels increased during the 2-h period after food presentation and then plateaued, while brain-tyrosine levels were higher at 4 h than at 2 h (though the change between 2 h and 4 h is about one-third that during the first 2 h).

Serum-tyrosine:LNAA and serum-phenylalanine:LNAA ratios (predictors of the competitive uptake into brain of tyrosine and phenylalanine, respectively, and analogous to the tryptophan:LNAA ratio [*see* ref 3]), also rose during the first 2 h after food presentation. The tyrosine ratio showed a small, further increment at 4 h, while the phenylalanine ratio showed a small decline during this period. On the basis of these results, principally that the major increments in serum- and brain-tyrosine and phenylalanine occurred during the first 2 h after meal presentation, we selected the 2-h timepoint for subsequent, dose-response experiments.

Dose-response studies

Experimental animals. Adult male Sprague-Dawley rats weighing 200–350 g were housed six per cage and given free access to water and food (Wayne Lab Blox, Continental Grain Co, Chicago, IL). They were exposed to light from 0700 h to 1900 h daily; the ambient temperature was 22°C. All rats were acclimated to our animal quarters for 1 wk prior to experimentation.

The night before an experiment, rats were deprived of food (but not water). The next morning, they were either deprived of food during the 2-h experimental period (which

TABLE 1
Time-course of the effect of ingesting a high-carbohydrate, protein-free meal containing aspartame on phenylalanine and tyrosine levels in serum and brain*

Time after food presentation	Tyrosine			Phenylalanine		
	Serum	Ratio	Brain	Serum	Ratio	Brain
minutes	*nmol/ml*		*nmol/g*	*nmol/ml*		*nmol/g*
Fasting	74 ± 5	0.119 ± 0.005	70 ± 3	59 ± 6	0.092 ± 0.007	87 ± 5
30	113 ± 6	0.242 ± 0.020	94 ± 5	86 ± 4	0.172 ± 0.012	110 ± 9
60	132 ± 8	0.362 ± 0.028	120 ± 2	97 ± 4	0.237 ± 0.016	118 ± 3
120	205 ± 13	0.597 ± 0.036	225 ± 5	104 ± 5	0.235 ± 0.010	140 ± 3
240	214 ± 11	0.663 ± 0.051	275 ± 14	89 ± 4	0.198 ± 0.007	140 ± 6

* Data are means ± SEM. By analysis of variance, a statistically significant effect of time ($p < 0.01$) was noted for each variable.

began at 0900 h) or given free access for this period to one of the diets described below. After 90 min, each rat received an intraperitoneal injection of m-hydroxybenzyl-hydrazine (100 mg/kg in water vehicle). This drug, NSD-1015, (Sigma Chemical Co, St Louis, MO) is an inhibitor of aromatic L-amino acid decarboxylase; it blocks 5-hydroxytryptophan decarboxylation in serotonin-synthesizing neurons. (Following its injection, 5-hydroxytryptophan levels increase linearly for 30–45 min. As a result, quantitation of 5HTP levels during this time period allows an estimation of the in vivo rate of tryptophan hydroxylation [12].)

Thirty minutes after NSD-1015 injection (7, 12), animals were sacrificed by decapitation. Trunk-blood samples were collected, placed in an ice bath, and centrifuged at the end of the experiment to obtain serum. Serum samples were then frozen. The brains were collected, the cerebelli removed, the remainder of the brains bisected midsagitally, and then all half-brain and cerebellum samples were frozen on dry ice within 2 min of sacrifice. Stomach contents were examined to ensure each rat had consumed a substantial amount of food.

Diet. The base diet, to which varying amounts of aspartame were added, contained the following ingredients (in g/kg diet, dry weight): sucrose, 200; dextrin, 548; mazola oil, 150; vitamins (Vitamin Diet Fortification Mixture, ICN/Nutritional Biochemicals, Cleveland, OH [13]), 22; salt mix (Rogers-Harper Salt Mix, Teklab, Madison, WI [14]), 40; and agar (Teklab), 40. The diet was formulated with an equal weight of water and had a cheese-like consistency. When aspartame (GD Searle and Company, Skokie, IL) was added, an equivalent amount of carbohydrate was deleted. The levels of aspartame substituted in the base diet were (in g/kg, dry weight): 0, 4, 8, 16, 32, or 64. The dose of aspartame received by each rat was calculated from its body weight and the amount of diet consumed during the experimental period. These data are provided in the legends to the tables for each experiment.

Laboratory determinations. Tryptophan levels were determined fluorimetrically in cerebelli and serum samples by a modification of the method of Denckla and Dewey (15–17). We have previously shown (8) that cerebellar-tryptophan levels accurately reflect whole-brain tryptophan levels. We assayed 5-hydroxytryptophan levels by high-performance liquid chromatography (HPLC) using the Reinhard et al (18) procedure. We differed from this published procedure only in that we used an HPLC buffer containing no methanol, to allow 5HTP peaks to emerge well removed from the solvent front.

Tyrosine and phenylalanine levels in blood and brain were quantitated fluorimetrically (19, 20) in some experiments (*see* Results, Table 3). Otherwise, the levels of tyrosine, phenylalanine, and the branched-chain amino acids were quantitated in blood and brain using a Beckman Model 6300 amino acid analyzer (Beckman Instruments, Palo Alto, CA) equipped with a fluorimetric detector. Aminoethylcysteine was added to all samples as an internal standard at the initial homogenization or precipitation step.

Serum samples were precipitated using sulfosalicylic acid (50 mg/ml serum) and centrifuged at 5000 × *g*. We added 500 μl of each resulting supernatant to 1 ml 0.1% trifluoroacetic acid (TFA) containing 30% methanol and injected this solution onto a Waters Sep-Pak (Waters Division, Millipore Corp, Milford, MA) that had first been prepared by sequential injections of 10 ml isopropanol and 10 ml water.

Brain samples (half-brains) were homogenized in 4.5 volumes of 6% trichloroacetic acid, and the homogenates were centrifuged. The resulting supernatants were extracted three times with equal volumes of diethylether, and the final aqueous phases were lyophilized on a Speed-Vac concentrator (Savant Instruments, Hicksville, NY) attached to a lyophilizer (Virtis Instruments, Gardiner, NY). The residues were reconstituted in 1 ml of 0.1% TFA: methanol (80:20 by volume), and these solutions were injected onto Waters Sep-Paks first prepared with isopropanol and water, as above.

Following injection onto Sep-Paks, effluents of both serum and brain samples, which contained the amino acids, were collected. Then we washed the Sep-Paks with 0.1% TFA containing 20% methanol (4.0 ml for serum samples; 6.0 ml for brain samples), and combined the resulting eluates with the effluents. (We employed the Sep-Pak procedure principally as a filtration procedure to protect the HPLC column of the amino acid analyzer from particulate matter.) We injected 50 μl of these final solutions onto the amino acid analyzer. Calculations were made using the internal standard method. Recoveries (% ± SEM; n = 5) for each amino acid measured by the analyzer in these studies were as follows: tyrosine, 101.3 ± 5.1%; phenylalanine, 89.2 ± 11.5%; leucine, 96.3 ± 5.3%; isoleucine, 98.1 ± 14.2%; valine, 93.4 ± 6.6%.

Statistical analyses. Data are presented as means ± the

standard errors of the mean. All data were subjected to one-way analysis of variance; post-hoc testing was performed using the Newman-Keuls test. Occasionally, standard deviations were noted to increase linearly with the mean. In such cases, natural log transforms of the data were made prior to statistical testing (21). The studies reported here, which employed different doses of aspartame, are representative of the findings in 10 different experiments.

Results

As previously shown (3–5), ingestion of a high-carbohydrate, protein-free meal (CHO) alone significantly elevated the serum-tryptophan level, serum-tryptophan:LNAA ratio, brain-tryptophan level, and 5HTP accumulation 2 h after food presentation (**Tables 2–4,** CHO + 0 mg/kg group). Also resulting from the ingestion of this meal were insignificant reductions in serum-phenylalanine and serum-tyrosine levels. However, the serum-phenylalanine:LNAA ratio and brain-phenylalanine levels increased significantly, though the serum tyrosine:LNAA ratio and brain-tyrosine level did not (Table 2). In addition, ingestion of the meal produced significant reductions in serum level, serum ratio, and brain level of each of the branched-chain amino acids (Table 2). These findings are almost all compatible with previous findings (3, 22).

The addition of aspartame to the high-carbohydrate, protein-free meal caused increments in serum-phenylalanine and serum-tyrosine levels in the lower range of dipeptide doses (up to 530 mg/kg body weight; *see* **Figure 1** and Tables 2–4). No further increments in the serum level of either amino acid were apparent, above this dose. In general, serum-tyrosine levels usually rose proportionally more than serum-phenylalanine concentrations (Figure 1). The serum levels of none of the other LNAA appeared consistently to be affected by aspartame ingestion (Table 2).

Aspartame ingestion produced anticipated effects on the serum-LNAA ratios. The serum-phenylalanine:LNAA and tyrosine:LNAA ratios generally rose with increasing dose, while the ratios for each of the other LNAA fell (tryptophan, leucine, isoleucine, valine: Table 2).

Brain-phenylalanine levels increased with increasing aspartame load up to a dose of 166 mg/kg body weight, and then plateaued (*see* Figure 1). Brain-tyrosine levels also rose with increasing aspartame dose, but continued to rise up to an aspartame dose of 656 mg/kg. The increments in brain-tyrosine and brain-phenylalanine levels were proportionally similar in the lower aspartame dose range (up to 166 mg/kg body weight; *see* Figure 1); but at the higher doses, brain-tyrosine increased consistently more than brain-phenylalanine levels.

The brain level of each of the branched-chain amino acids appeared unaffected by the presence of aspartame in the diet, at least for doses up to 656 mg/kg (the levels of these amino acids in brain were not quantitated in experiments involving higher doses [Table 2]).

Tables 2–4 also summarize the relationship between dietary aspartame content and brain-tryptophan levels and 5HTP accumulation. The data in the three tables provide a considerable dose-response relationship for aspartame doses below 656 mg/kg: 56, 122, 144, 166, 267, 315, 386, 530, and 656 mg/kg body weight. In addition, effects of two very high doses are presented (881 and 1440 mg/kg body weight).

In Table 2, it is apparent that ingestion of the high-carbohydrate, protein-free meal alone (CHO + 0 mg/kg diet APM) increased brain 5HTP levels significantly above fasting values. However, of the doses achieved in this study, none significantly attenuated this meal-induced increment in 5HTP accumulation. Brain-tryptophan concentrations were also significantly elevated following ingestion of the meal, and no dose of APM tested in this study significantly antagonized this effect (Table 2). In Table 3, brain-tryptophan and 5HTP levels rose significantly above fasting values in the groups ingesting the same CHO containing aspartame in doses up to and including 386 mg/kg body weight. (The absence of a suppressive effect of aspartame on the carbohydrate-induced rise in these brain indoles in the 400 mg/kg body weight dose range was confirmed in another experiment [data not shown], in which the ingestion of CHO with an aspartame dose of 440 mg/kg body weight produced the same increase in brain-tryptophan level and 5HTP accumulation as that obtained following the consumption of this same meal containing no APM.)

Ingestion of 881 mg/kg body weight aspartame along with the test meal, however, clearly

TABLE 2

Effect of aspartame ingestion on the changes in serum and brain levels of large neutral amino acids induced by the ingestion of a high-carbohydrate, protein-free meal by fasting rats*

	Fasting	CHO + 0 mg/kg	CHO + 56 mg/kg	CHO + 144 mg/kg	CHO + 267 mg/kg	CHO + 656 mg/kg
Serum–amino acid concentration (nmol/ml)						
Tryptophan	82 ± 5	118 ± 10†	101 ± 15	104 ± 10	121 ± 8†	132 ± 9†
Tyrosine	81 ± 5	69 ± 3	96 ± 9	118 ± 9	117 ± 6†	233 ± 16†
Phenylalanine	43 ± 3	39 ± 2	48 ± 3	58 ± 3†	67 ± 6†	65 ± 7†
Leucine	123 ± 9	56 ± 6†	55 ± 7†	60 ± 4†	51 ± 6†	56 ± 7†
Isoleucine	74 ± 10	33 ± 4†	36 ± 5†	27 ± 4†	26 ± 3†	33 ± 5†
Valine	143 ± 9	73 ± 9†	77 ± 6†	79 ± 8†	62 ± 6†	67 ± 5†
Serum–amino acid ratio						
Tryptophan:LNAA	0.177 ± 0.013	0.436 ± 0.040†	0.326 ± 0.029†	0.308 ± 0.035†	0.385 ± 0.047†	0.321 ± 0.044†
Tyrosine:LNAA	0.194 ± 0.016	0.219 ± 0.014	0.303 ± 0.025	0.360 ± 0.025†	0.391 ± 0.035†	0.599 ± 0.057†
Phenylalanine:LNAA	0.084 ± 0.004	0.113 ± 0.008†	0.121 ± 0.006†	0.150 ± 0.007†	0.175 ± 0.015†	0.128 ± 0.007†
Leucine:LNAA	0.283 ± 0.013	0.164 ± 0.009†	0.153 ± 0.012†	0.142 ± 0.020†	0.114 ± 0.014†	0.112 ± 0.015†
Isoleucine:LNAA	0.154 ± 0.017	0.106 ± 0.013†	0.095 ± 0.010†	0.078 ± 0.026†	0.070 ± 0.010†	0.065 ± 0.012†
Valine:LNAA	0.353 ± 0.024	0.205 ± 0.010†	0.231 ± 0.015†	0.193 ± 0.009†	0.158 ± 0.013†	0.133 ± 0.005†
Brain–amino acid (nmol/g)						
Tryptophan	38 ± 2	51 ± 2†	47 ± 3†	51 ± 2†	48 ± 1†	49 ± 3†
Tyrosine	104 ± 5	129 ± 14	130 ± 7†	146 ± 8†	168 ± 7†	289 ± 30†
Phenylalanine	63 ± 3	82 ± 8†	84 ± 7	85 ± 5	102 ± 14†	112 ± 16†
Leucine	105 ± 5	78 ± 3†	88 ± 7	74 ± 4†	76 ± 5†	81 ± 9
Isoleucine	58 ± 3	41 ± 4†	43 ± 3†	34 ± 2†	40 ± 4†	43 ± 6†
Valine	112 ± 5	88 ± 4†	93 ± 5†	72 ± 4†	78 ± 6†	88 ± 9†
5-hydroxytryptophan	0.67 ± 0.03	1.04 ± 0.04†	0.91 ± 0.04†	0.92 ± 0.06†	1.02 ± 0.10†	0.95 ± 0.06†

* Groups of six food-deprived rats (195 ± 3 g) were given free access to either the high carbohydrate, protein-free meal (CHO) alone, or the same meal containing aspartame (4, 8, 16, or 32 g/kg diet, dry weight). All rats received NSD-1015 90 min later, and were killed 30 min thereafter. Data are presented as the means ± SEM. The serum tryptophan(TRP):LNAA ratio is the ratio of the serum levels of TRP:tyrosine(TYR) + phenylalanine (PHE) + leucine (LEU) + isoleucine ILE + valine (VAL); the serum-TYR:LNAA ratio is serum TYR:serum TRP + PHE + LEU + ILE + VAL; the serum-PHE:LNAA ratio is serum PHE:serum TRP + TYR + LEU + ILE + VAL; the serum-LEU:LNAA ratio is serum LEU:serum TRP + TYR + PHE + ILE + VAL; the serum-ILE:LNAA ratio is serum ILE:serum TRP + TYR + PHE + LEU + VAL; and the serum-VAL:LNAA ratio is serum VAL:serum TRP + TYR + PHE + LEU + ILE. Rats in each of the groups consumed the amounts of food listed below (in g/rat, dry weight), yielding the indicated dosages (in mg/kg body weight): carbohydrate (CHO) alone, 3.5 g/rat = 0 mg/kg APM; CHO + 4 g/kg APM, 2.75 g/rat = 56 mg/kg APM; CHO + 8 g/kg, 3.5 g/rat = 144 mg/kg APM; CHO + 16 g/kg, 3.25 g/rat = 267 mg/kg; and CHO + 32 g/kg, 4 g/rat = 656 mg/kg APM.

† Group differs from fasting group, $p < 0.05$ (ANOVA, Newman-Keuls).

TABLE 3
Effects of different doses of aspartame on the increments in brain aromatic amino acids produced by the ingestion of a high-carbohydrate, protein-free meal by fasting rats*

Treatment group	Serum PHE	Serum TYR	Serum TRP	Brain PHE	Brain TYR	Brain TRP	Brain 5-HTP
	nmol/ml	*nmol/ml*	*nmol/ml*	*nmol/g*	*nmol/g*	*nmol/g*	*nmol/g*
Fasting	45 ± 4	102 ± 4	79 ± 8	90 ± 4	103 ± 4	29 ± 2	0.47 ± 0.02
CHO + 0 mg/kg APM	44 ± 4	81 ± 2	114 ± 6†	102 ± 2	133 ± 5‡	40 ± 2†	0.74 ± 0.04†
CHO + 122 mg/kg APM	60 ± 3	99 ± 5	98 ± 6‡	123 ± 3†	131 ± 7‡	32 ± 3	0.70 ± 0.04†
CHO + 166 mg/kg APM	68 ± 2	114 ± 5	109 ± 4†	133 ± 9†	171 ± 2†	40 ± 1†	0.68 ± 0.02†
CHO + 386 mg/kg APM	93 ± 13†	179 ± 12†	134 ± 2†	137 ± 6†	195 ± 12†	40 ± 2†	0.63 ± 0.08†
CHO + 881 mg/kg APM	101 ± 9†	245 ± 8†	116 ± 7†	144 ± 7†	259 ± 11†	28 ± 2	0.57 ± 0.05

* Groups of six food-deprived rats (207 ± 3 g) were given free access to either the high-carbohydrate, protein-free meal (CHO) alone, or the same meal containing aspartame (8, 16, 32, or 64 g/kg diet, dry weight). All rats received NSD-1015 90 min later, and were killed 30 min thereafter. Data are presented as the means ± SEM. In this experiment, tyrosine and phenylalanine (as well as tryptophan) were quantitated by fluorimetric assay (19, 20). Rats in each of the groups consumed the amounts of food listed below (in g/rat, dry weight), yielding the indicated dosages (in mg/kg body weight): carbohydrate (CHO) alone, 3.4 g/rat = 0 mg/kg APM; CHO + 8 g/kg APM, 3.15 g/rat = 122 mg/kg APM; CHO + 16 g/kg, 2.15 g/rat = 166 mg/kg APM; CHO + 32 g/kg, 2.5 g/rat = 386 mg/kg; and CHO + 64 g/kg, 2.85 g/rat = 881 mg/kg APM.

† Group differs significantly from fasting values ($p < 0.01$).

‡ Group differs significantly from fasting values ($p < 0.05$).

suppressed the carbohydrate-induced increments in brain-tryptophan levels and 5HTP accumulation. In addition, as shown in Table 4 (experiment II), 530 mg/kg body weight, but not 315 mg/kg body weight aspartame also prevented the elevation of brain-tryptophan and 5HTP levels (and the serum-tryptophan:LNAA ratio). The highest dose of APM tested (1440 mg/kg) also completely blocked the carbohydrate-induced increase in 5HTP accumulation and almost fully prevented any rise in brain-tryptophan level and the serum-tryptophan:LNAA ratio (Table 4, experiment I).

TABLE 4
Antagonism by high doses of aspartame of the increases in brain indoles produced by the ingestion of a high-carbohydrate, protein-free meal by fasting rats*

Treatment group	Serum PHE	Serum TYR	Serum TRP	Serum TRP:LNAA ratio	Brain TRP	Brain 5-HTP
	nmol/ml	*nmol/ml*	*nmol/ml*		*nmol/g*	*nmol/g*
Experiment I:						
Fasting	55 ± 4	128 ± 11	94 ± 6	0.136 ± 0.010	23 ± 2	0.47 ± 0.04
CHO + 0 mg/kg APM	46 ± 2	77 ± 2	119 ± 4†	0.405 ± 0.013†	37 ± 1†	0.71 ± 0.10†
CHO + 1440 mg/kg APM	115 ± 4†	293 ± 45†	123 ± 8†	0.231 ± 0.029†	28 ± 1†	0.36 ± 0.07
Experiment II:						
Fasting	46 ± 2	135 ± 10	87 ± 5	0.155 ± 0.007	22 ± 1	0.75 ± 0.04
CHO + 0 mg/kg APM	51 ± 2	112 ± 5	149 ± 9†	0.332 ± 0.020†	30 ± 1†	0.97 ± 0.05†
CHO + 315 mg/kg APM	68 ± 7‡	189 ± 16	149 ± 7†	0.293 ± 0.033†	30 ± 1†	0.99 ± 0.03†
CHO + 530 mg/kg APM	126 ± 9†	297 ± 37†	129 ± 13†	0.171 ± 0.014	24 ± 1	0.80 ± 0.07

* Groups of six rats were food-deprived overnight, and the next morning given free access to either a high-carbohydrate, protein-free meal (CHO) alone, or the same meal containing aspartame (experiment I: 64 g/kg diet, dry weight; and experiment II: 16 or 32 g/kg diet, dry weight). All rats received NSD-1015 90 min later, and were killed 30 min thereafter. Data are presented as the means ± SEM. In experiment I, rats weighed 244 ± 3 g and ingested 5.5 g of the APM-containing food (dry weight), giving an APM dose of 1440 mg/kg body weight. In experiment II, one group of rats ingested 5.5 g/rat of a 16-g/kg diet (dry weight) (animal weight = 280 ± 9 g; APM dose = 315 mg/kg body weight); another group ingested 5 g/rat of a 32-g/kg APM diet (animal weight = 302 ± 6 g; APM dose = 530 mg/kg body weight).

† Group differs significantly from fasting values ($p < 0.05$).

‡ Group differs significantly from fasting values ($p = 0.05$).

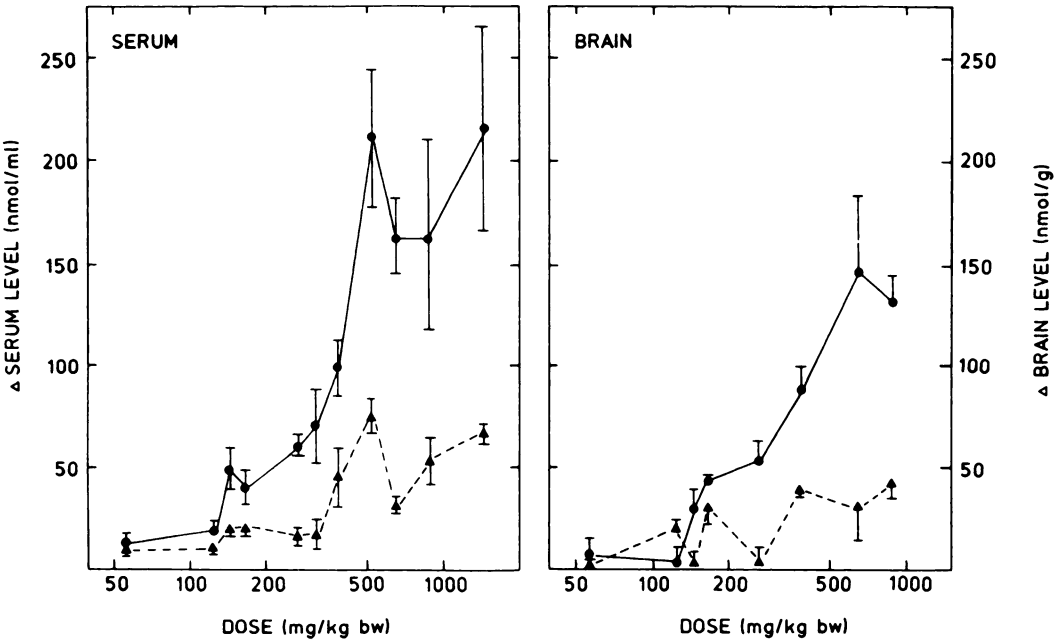

FIG 1. Increments in the serum (left panel) and brain (right panel) levels of tyrosine and phenylalanine produced by ingesting various doses of aspartame in combination with a high-carbohydrate, protein-free meal. The points were derived from data in Tables 2–4. They represent the means ± SEM of the differences between individual values in each dose group and the mean value for the group ingesting no aspartame in the same experiment. Circles are tyrosine values; triangles are phenylalanine values. Vertical bars are standard errors of the mean.

Discussion

These results indicate that a dose-response relationship can be constructed between the amount of aspartame consumed in a single meal and the effect it has on several variables relevant to serotonin synthesis in the brain. In particular, we observed that the increments in brain-tryptophan level and the in vivo rate of tryptophan hydroxylation produced by the ingestion of a high-carbohydrate, protein-free meal were unaffected by the concomitant ingestion of aspartame in doses up to at least 440 mg/kg body weight. At very high doses (881 and 1440 mg/kg body weight), clear suppression of the carbohydrate-induced rise in brain-tryptophan level and hydroxylation rate was evident.

Such was also observed at the 530 mg/kg body weight dose of aspartame (Table 4), though in a different experiment, an effect at 656 mg/kg was not obtained (*see* Table 2 and discussion below). If the results at 530 mg/kg body weight are conservatively taken as definitive, the threshold dose for an effect of aspartame on the carbohydrate-induced rise in brain-tryptophan level (and hydroxylation rate) is between 440 and 530 mg/kg. It is difficult to identify the exact threshold dose in such free-feeding experiments; precision is sacrificed to enable very large doses of the dipeptide to be self-administered.

In these studies, the in vivo rate of tryptophan hydroxylation was used to estimate the rate of serotonin production. Tryptophan hydroxylase catalyzes the rate-limiting reaction in serotonin synthesis (6) and estimates of its in vivo activity are generally believed to provide accurate reflections of the overall rate of serotonin synthesis (eg, 7, 8, 12, 23). Measurements of tryptophan hydroxylation were preferred because they provide direct kinetic information about the fate of tryptophan in serotonin-synthesizing neurons. Though changes in 5HT and 5HIAA levels have been used to infer changes in 5HT synthesis rate (eg, 2, 4, 5), such changes in levels, at least in our hands, have been more variable from experiment to experiment than direct measurements of synthesis. This might be anticipated

inasmuch as changes in the levels of a transmitter and its metabolite reflect alterations, not simply in synthesis rate but also in the rate of catabolism and removal from the brain. The in vivo rate of tryptophan hydroxylation also seems a particularly appropriate index of 5HT synthesis to use in these experiments because it is known from previous studies to be stimulated by the ingestion of carbohydrates (eg, 8).

Two possible mechanisms might explain the ability of high doses of aspartame to block the rise in tryptophan-hydroxylation rate. Either phenylalanine might inhibit tryptophan hydroxylation indirectly, by blocking tryptophan transport into brain, or it might itself directly inhibit tryptophan hydroxylase (6). The latter possibility seems unlikely: Although phenylalanine can inhibit tryptophan hydroxylase directly, the k_i of the enzyme for phenylalanine is reputed to be about 210 nmol/g (6), a level well above that achieved in our experiments. For example, in Table 2, the 656 mg/kg body weight dose raised brain-phenylalanine only to 112 ± 16 nmol/g. In the experiment in Table 3, the 881 mg/kg dose raised brain phenylalanine to only 144 ± 7 nmol/g, while in Table 1, the highest level achieved was 140 ± 3 nmol/g (at 2 h). Hence, the inhibition of tryptophan hydroxylation probably follows indirectly from a reduction of tryptophan transport into brain.

This view is consistent with the findings. That is, whenever a particular dose of aspartame suppressed the rise in 5HTP accumulation that accompanied carbohydrate ingestion, the increase in brain-tryptophan level (Tables 3 and 4, at doses of 530, 881, and 1400 mg/kg body weight) and the serum-tryptophan ratio (Table 4, at doses of 530 and 1440 mg/kg body weight) were also suppressed.

If the minimum effective dose of aspartame is in the range of 530 mg/kg, this result would fit with the findings of Carlsson and Lindqvist (7). These investigators studied brain-tryptophan levels soon after rats received an injection of any of several LNAA, including phenylalanine and tyrosine. A dose of 100 mg/kg of phenylalanine produced no effect on brain-tryptophan level, while 300 mg/kg elicited a 33% fall in brain tryptophan. A higher dose, 1000 mg/kg, reduced brain tryptophan to 50% of control levels. Tyrosine was of similar potency. The 530 mg/kg dose of aspartame represents a dose of approximately 300 mg/kg phenylalanine. Hence, it would be consistent with the findings of Carlsson and Lindqvist that the 530 mg/kg dose of aspartame would be sufficient to inhibit tryptophan transport into brain.

The 530 mg/kg body weight dose of APM blocked the carbohydrate-induced rise in brain-tryptophan level and hydroxylation rate (Table 4), but the 656 mg/kg dose did not (Table 2). The 656 mg/kg dose also did not appreciably suppress the rise in the serum-tryptophan ratio, while the 530 mg/kg dose did. Hence, the explanation for the impotence of the 656 mg/kg dose may lie in the serum–amino acid responses in this study.

The increments in serum tyrosine and phenylalanine (and tryptophan) in this experiment were almost as large as in the experiment containing the 530 mg/kg dose. However, the carbohydrate-induced reductions in the serum levels of the branched-chain amino acids were not as great in the animals ingesting 656 mg/kg aspartame (Table 2) as in those consuming the 530 mg/kg dose. For example, the reduction in serum leucine in the experiment in Table 2 was from 123 ± 9 to 56 ± 6 nmol/ml (54% reduction); in the experiment in Table 4, it was from 142 ± 13 to 106 ± 5 nmol/ml (25% reduction). Similar differences between the two studies were noted for isoleucine and valine (data not shown). Hence, perhaps variability in the response of serum branched-chain amino acid levels to insulin secretion, or in the degree of insulin secretion in response to carbohydrate ingestion, produced this difference in the ability of aspartame to suppress the carbohydrate-induced rise in brain-tryptophan levels at these doses. (Differences in the amounts of carbohydrate consumed in the two experiments, and thus possibly in the stimulus for insulin secretion, cannot account for the difference. The animals showing the smaller branched-chain amino acid reductions actually ate more food than those showing the larger reductions [*see* Tables 2 and 4].)

Interestingly, when the aspartame dose exceeded approximately 500 mg/kg body weight, no further increments occurred in the levels of tyrosine and phenylalanine in serum (*see* Figure 1 and Tables 1–4). This phenomenon explains why, for example, doses of 1440 and

530 mg/kg body weight (Table 4) are of approximately equal effectiveness in blunting the carbohydrate-induced increments in the serum-tryptophan:LNAA ratio (and in brain-tryptophan level). That is, the aspartame-induced increments in serum-tyrosine and serum-phenylalanine levels were about equal in the two studies.

Given that the changes in the serum levels of tryptophan and the branched-chain amino acids (data not shown) in response to carbohydrate ingestion were also about equal in the two experiments, the suppression by either dose of aspartame of the carbohydrate-induced rise in the serum trytophan:LNAA ratio would be expected to be about the same. The question remains, however, as to why the absolute serum levels of tyrosine and phenylalanine should plateau in the higher dose range of aspartame.

Few data have been published using a paradigm similar to ours to further illuminate this issue. One possibility is that the hepatic enzymes that catabolize tyrosine and phenylalanine become more active as local substrate levels increase. If so, then as the levels of these amino acids rise, their rates of catabolism might also increase. At some point, a new steady-state level in blood for each amino acid would presumably be achieved. Some fragmentary data support the view that these enzymes might become activated in the presence of high substrate levels. For example, tyrosine administration has been reported to increase the activity of hepatic tyrosine aminotransferase (24). Of course, this is not thought to be an important factor governing transaminase activity physiologically (*see* 25), but perhaps it is important in this pharmacologic context. In addition, in vitro studies using purified phenylalanine hydroxylase show that increasing the concentration of substrate increases hydroxylase phosphorylation rate (ie, activation of the enzyme) (26). Perhaps this result also holds in vivo.

A second possible explanation for the plateau in serum-tyrosine and serum-phenylalanine levels at high aspartame doses might be that the renal thresholds for tyrosine and phenylalanine have been exceeded. In this case, any amount of either amino acid above a certain blood level would presumably be lost directly into the urine (filtered, but not reabsorbed by the nephrons). Appealing though this hypothesis might be, few data are available to evaluate it (*see* 27). In one study in dogs, the blood level of phenylalanine was increased tenfold via intravenous infusion. A small increase was observed in the amount of phenylalanine excreted, but 85% of the filtered amino acid was nonetheless reabsorbed (28). The authors speculated that the maximal reabsorptive capacity might have been achieved at a little higher blood-phenylalanine level, though they did not test this possibility. Despite the species differences, the fact that in the present studies blood-phenylalanine levels at most doubled suggests that the renal threshold for phenylalanine reabsorbtion was not exceeded. Presumably, an analogous argument could also be made for tyrosine.

One other report considers the possibility that aspartame ingestion in large amounts antagonizes the carbohydrate-induced increments in brain-tryptophan level and serotonin synthesis (2). In these studies, a single dose of aspartame (200 mg/kg) was tested and the investigators reported this dose to suppress the increments in the serum-tryptophan:LNAA ratio, brain-tryptophan level, and brain 5-HT and 5HIAA levels produced by glucose intubation. This dose is less than half that required in our studies to produce this same inhibition. What might the basis for this difference in dose-response be? Perhaps it is related to differences in the actual peak increments in serum-phenylalanine and serum-tyrosine levels produced by the two methods of APM administration. For example, if a 200 mg/kg body weight dose given by gavage produced larger increments in serum-phenylalanine and serum-tyrosine levels than those resulting from ingestion of the same dose as a part of a meal, perhaps only the higher levels produced by gavage sufficed to block the carbohydrate-induced rise in tryptophan uptake and serotonin synthesis. Certainly, in the present studies, doses in the 200 mg/kg body weight range did not produce the same elevations of serum-phenylalanine and serum-tyrosine as seen in the intubation study of Yokogoshi et al (2).

But if this is the explanation, then the converse should also have been true. That is, the APM doses in our study that produced elevations in serum-phenylalanine and serum-tyrosine levels that did match (approximately)

those produced in the Yokogoshi et al (2) study should also have blocked the carbohydrate-induced rise in brain indoles. However, this was not the case. In Table 3, the 386 mg/kg body weight dose produced a combined increment in serum-phenylalanine and serum-tyrosine levels that matched that produced by Yokogoshi et al (2), and yet the rise in brain-tryptophan level and 5HTP accumulation was not suppressed. A similar result is apparent for the 656 mg/kg body weight dose in Table 2. For the doses that did suppress the carbohydrate-induced elevations of brain-indole levels, the combined increases in serum-phenylalanine and serum-tyrosine levels were much greater than that produced by Yokogoshi et al (2) (eg, the 881 mg/kg dose in Table 3, and the 530 mg/kg dose in Table 4).

Of course, the objection can be offered that Yokogoshi et al (2) may have achieved higher serum levels of tyrosine and phenylalanine before 60 min post-APM gavage that matched those in the present studies. However, this argument probably cannot account for the discrepancy. For though Yokogoshi et al (2) did not provide time-course data following APM gavage, we have done so in an earlier study (9). The increases produced in serum-phenylalanine and serum-tyrosine levels by an oral gavage of 200 mg/kg body weight APM to rats are practically identical 30 and 60 min postintubation. Therefore, their serum values for phenylalanine and tyrosine at 60 min postgavage were probably maximal. Hence, in sum, it is not presently possible to reconcile the dose differences in the results obtained in our studies with those obtained by Yokogoshi et al (2).

Nonetheless, we feel confident of the results obtained using our method of APM administration because most previous studies describing the effect of carbohydrate ingestion on the serum-tryptophan:LNAA ratio, brain-tryptophan levels, and brain-serotonin synthesis have employed the same paradigm (eg, 3, 4, 5, 22). Thus, determining effects of aspartame by using this paradigm places our studies in an experimental context that has been well defined.

In conclusion, the present results show that the rat requires an aspartame dose on the order of 500 mg/kg body weight to experience suppression of the normal carbohydrate-induced rise in brain-tryptophan level and 5HT synthesis rate. In humans, if a metabolic rate adjustment of five is used (29), the theoretical-dose level needed to produce the same effects would be 100 mg/kg or more. This dose would have to be ingested in a single load.

Is such a practice likely to occur? If a human child weighing 30 kg consumed this amount, the total load would be 3000 mg or more (it would be considerably more for a 50–70+ kg adult). Given that aspartame is about 200 times sweeter than sucrose (1), this dose represents such a substantial level of sweetness (the equivalent of 600 g or 1.3 pounds of sucrose) that it is unlikely it would ever be consumed in a single sitting. Hence, it is improbable that a human subject would normally consume the very large amount of aspartame that might be predicted to block the increases in brain-tryptophan uptake and 5HT formation that may accompany carbohydrate consumption in man. It thus seems unlikely that any effects on brain function would result (2).

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