Effects of aspartame and glucose administration on brain and plasma levels of large neutral amino acids and brain 5-hydroxyindoles

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ABSTRACT Administration of the artificial sweetener aspartame (L-aspartyl-L-phenylalanyl-methyl ester; 200 mg/kg) by gavage to rats caused large increments in brain and plasma levels of phenylalanine and its product tyrosine. Glucose administration (3 g/kg, by gavage, a dose sufficient to cause insulin-mediated reductions in plasma levels of the large neutral amino acids leucine, isoleucine, and valine) also elevated brain phenylalanine and tyrosine, and enhanced the increments caused by the aspartame, nearly doubling the rise in brain phenylalanine. Each animal's brain phenylalanine or tyrosine levels were highly correlated (r = 0.97 and 0.99, respectively) with its plasma phenylalanine or tyrosine ratios, affirming that aspartame's effects on the brain amino acids result from the changes it produces in plasma composition. As described previously, glucose consumption increased brain tryptophan levels, and consequently, brain levels of the 5-hydroxyindoles serotonin and 5-hydroxyindoleacetic acid. Aspartame alone had no effect on these compounds but completely blocked the changes in 5-hydroxyindoles caused by glucose. Each animal's brain level of tryptophan (r = 0.89) and 5-hydroxyindoles (r = 0.74) was also significantly correlated with its plasma tryptophan ratio, affirming that the effects of glucose or aspartame on these brain constituents also result from the changes they produce in plasma composition. The aspartame-glucose combination also reduced brain levels of leucine, isoleucine, and valine to a significantly greater extent than aspartame or glucose alone. These observations indicate that high aspartame doses can generate major neurochemical changes in rats, especially when consumed along with carbohydrate-containing foods. However, they should not in any way be interpreted as demonstrating that aspartame significantly affects the human brain.

KEY WORDS Aspartame, glucose, amino acids, tryptophan, tyrosine, phenylalanine, serotonin, 5-hydroxyindoleacetic acid, sweeteners

Introduction

Aspartame (L-aspartyl-L-phenylalanyl methyl ester), a synthetic dipeptide ester, provides the blood stream with phenylalanine but not with the other large neutral amino acids (LNAA), which are present in all proteins and which normally compete with phenylalanine for transport across the blood-brain barrier (1-4). Hence consumption of sufficient doses of the sweetener can

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raise brain phenylalanine levels beyond their normal range (5, 6). Since a major fraction of dietary phenylalanine is converted to tyrosine in the liver, the synthetic sweetener also elevates plasma and brain (5, 6) tyrosine levels. Moreover, it might be anticipated that the combined increase in plasma phenylalanine and tyrosine that follows aspartame's consumption would suppress the uptake into brain of other LNAA such as tryptophan and the branched-chain amino acids (3).

The levels of any of the LNAA in a rat's brain are highly correlated with that LNAA's "plasma ratio" [ie, the ratio of its plasma concentration to the sum of the concentrations of the other LNAA (4, 7)] a relationship that makes it possible to estimate these brain levels indirectly after an animal or human has been exposed to a treatment that changes its plasma LNAA concentrations. To determine whether consumption of aspartame, in the high doses that some people might occasionally take via cold drinks (8), increases human brain phenylalanine or tyrosine levels beyond their normal ranges, we performed a preliminary experiment in which we measured the plasma phenylalanine and tyrosine ratios among six normal subjects given an aspartame dose (15 mg/kg, po) well within current consumption ranges (8), along with a snack that provided 200 g of carbohydrate. [We had previously observed (9) that dietary carbohydrates, by eliciting insulin secretion, can selectively lower plasma levels of the branched-chain LNAA to the point of raising the phenylalanine and tyrosine ratios; thus we anticipated that giving the aspartame along with a carbohydrate (as might occur when one eats cookies along with an aspartame-sweetened beverage) would amplify the sweetener's effects on the plasma phenylalanine and tyrosine ratios and thus on brain phenylalanine and tyrosine.] Peak plasma phenylalanine and tyrosine ratios (0.170 and 0.128, respectively) were found to be beyond the normal ranges noted previously (10, 11), for any hour of day or night, among subjects consuming aspartame-free meals providing up to 150 g of protein daily; a finding that suggested that brain phenylalanine and tyrosine levels might also have been elevated unphysiologically. To examine this hypothesis we have carried out the studies described below on brain LNAA levels in rats given aspartame or a carbohydrate, alone or conjointly.

Materials and methods

Adult male Sprague-Dawley rats weighing about 170 g (Charles River Breeding Laboratories, Wilmington, MA) were housed in individual cages in our animal facility for 7 days, to adjust to the new environment. Food (Charles River Rat, Mouse and Hamster Maintenance Formula) and tap water were provided ad libitum. The room temperature was maintained at 22°C, and animals were exposed to 12-h cycles of light (Vita-Lite; Duro-Test Corp, North Bergen, NJ) and darkness. The National Research Council's guidelines for the care and use of laboratory animals was followed in this study.

In a typical experiment, 32 animals were divided into four groups and deprived of food the night before the experiment. At 9 AM the next morning they received aspartame (Ajinomoto Co, Inc, New York NY) or glucose (Baker Chemical Co, Phillipsburg, NJ) by gavage, as slurries in distilled water. Rats in groups 1 and 4 first received water (1 ml/150 g body weight by gavage) and those in groups 2 and 3 initially received glucose (3 g/kg; 1 ml/150 g body weight). One hour after the initial intubation, rats in groups 1 and 2 received water, again by gavage, while those in groups 3 and 4 were intubated with aspartame (200 mg/kg; 1 ml/150 g body weight). One hour after the second intubation, rats were decapitated, and their brains were immediately removed, dissected, frozen on dry ice, and stored at −70°C until assayed. Blood was collected from the cervical wound, and plasma samples were stored at −20°C until assayed. Amino acid concentrations in plasmas and brains were assayed by high-performance liquid chromatography (Waters Associates, Milford, MA) and by amino acid auto analyzer (Beckman, model 119C, Palo Alto, CA), respectively. Tryptophan (12), serotonin (13), and 5-hydroxyindoleacetic acid (5-HIAA) (13) were assayed fluorimetrically.

The statistical significances of differences between values were determined by analysis of variance and Duncan's multiple range test (14). Data relating each animal's brain amino acid or 5-hydroxyindole concentrations to its plasma amino acid ratios were correlated by regression analysis using the least-squares method.

Results

Phenylalanine and tyrosine

Aspartame administration increased plasma phenylalanine levels by 62% and those of tyrosine by 142% (Table 1). Glucose alone failed to modify these levels and it also did not affect the magnitude of the increases produced by aspartame. However, the reductions that the glucose caused in plasma
branched-chain amino acid levels (Table 1) were sufficient to increase the plasma phenylalanine and tyrosine ratios and to potentiate the increases in these ratios produced by aspartame. Hence the increment in the plasma phenylalanine ratio caused by giving aspartame with glucose (from 0.118 to 0.250, or +0.132) was about double that seen after aspartame alone (+0.065) (Table 1), and the corresponding rise in the plasma tyrosine ratio was about 50% greater.

Brain phenylalanine and tyrosine levels of rats within each of the four treatment groups tended to parallel their plasma ratios (Table 2), such that the correlation coefficients relating each animal’s brain phenylalanine or tyrosine level to its plasma phenylalanine or tyrosine ratio were 0.97 and 0.99 (Figure 1). The increase in brain phenylalanine seen in rats given aspartame plus glucose was about double that seen after aspartame alone, and that in brain tyrosine about 50% greater (Table 2).

**Branched-chain amino acids**

Aspartame administration caused small but significant decreases in plasma leucine, isoleucine, and valine levels (Table 1). Much larger decreases (36 to 65%) were caused by giving glucose. The reduction in plasma leucine observed among animals given aspartame plus glucose was significantly greater than the decreases seen after either sweetener alone (Table 1) (p < 0.05). Aspartame given alone caused larger decreases, proportionately, in each branched-chain amino acid’s plasma ratio than in its absolute plasma concentration. The decreases in these ratios caused by giving glucose were about as great as those caused by aspartame, while the decreases caused by giving both compounds (about 50%) were significantly greater than those observed after either treatment alone (Table 1).

As with phenylalanine and tyrosine, the brain level of each branched-chain amino acid tended, in each animal, to parallel its corresponding plasma ratio. Both aspartame and glucose, given alone, significantly re-

### TABLE 1

| Effects of aspartame and glucose on plasma amino acid concentrations and ratios in rats* |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Water and water                              | Glucose and water | Glucose and aspartame | Water and aspartame |
| Plasma Amino Acid Concentrations (nmol/ml)    |                  |                  |                  |
| Tyrosine                                      | 64.9 ± 2.8b      | 58.9 ± 2.6b      | 152.9 ± 4.9b     | 157.2 ± 11.3b   |
| Valine                                        | 148.7 ± 4.5b     | 83.7 ± 3.6c      | 76.2 ± 2.4b      | 126.0 ± 3.1b    |
| Isoleucine                                    | 90.4 ± 2.8b      | 47.4 ± 2.4c      | 41.4 ± 1.5b      | 74.6 ± 1.9b     |
| Tryptophan                                    | 71.1 ± 4.7c      | 81.1 ± 4.5*      | 67.8 ± 2.6b      | 69.3 ± 2.7b     |
| Leucine                                       | 132.6 ± 4.4*     | 78.7 ± 3.7c      | 65.6 ± 2.7a      | 104.4 ± 3.4b    |
| Phenylalanine                                 | 59.9 ± 1.7c      | 53.5 ± 1.3b      | 50.3 ± 1.6b      | 97.3 ± 3.9b     |

Plasma amino acid ratios

| Tyrosine/LNAA                                  | 0.130 ± 0.007d   | 0.172 ± 0.007c   | 0.436 ± 0.015a   | 0.336 ± 0.029b  |
| Valine/LNAA                                    | 0.355 ± 0.005a   | 0.262 ± 0.007b   | 0.178 ± 0.005c   | 0.252 ± 0.010b  |
| Isoleucine/LNAA                                | 0.190 ± 0.003a   | 0.133 ± 0.003b   | 0.090 ± 0.003a   | 0.135 ± 0.004b  |
| Tryptophan/LNAA                                | 0.143 ± 0.007a   | 0.252 ± 0.012b   | 0.156 ± 0.005a   | 0.124 ± 0.006c  |
| Leucine/LNAA                                   | 0.305 ± 0.005a   | 0.242 ± 0.006b   | 0.149 ± 0.005a   | 0.201 ± 0.010b  |
| Phenylalanine/LNAA                             | 0.118 ± 0.003c   | 0.154 ± 0.003a   | 0.250 ± 0.016a   | 0.183 ± 0.006b  |

* Groups of eight rats were killed 1 h after receiving water or aspartame (200 mg/kg), and 2 h after receiving water or glucose (3 g/kg). Data are given as mean ± SEM. In each row, means with different superscripts differ, Duncan’s test (p < 0.01).

### TABLE 2

| Effects of aspartame and glucose on brain amino acid levels in rats* |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Water and water                              | Glucose and water | Glucose and aspartame | Water and aspartame |
| Tyrosine                                      | 54.4 ± 2.7a     | 73.1 ± 3.3a      | 187.1 ± 9.5a     | 139.7 ± 8.4b    |
| Valine                                        | 79.1 ± 1.1*     | 59.8 ± 1.7*      | 51.6 ± 1.3*      | 65.8 ± 1.5b     |
| Isoleucine                                    | 33.3 ± 0.8*     | 23.1 ± 0.8*      | 18.2 ± 0.5*      | 26.5 ± 0.6b     |
| Tryptophan                                    | 20.8 ± 0.4c     | 30.2 ± 0.5*      | 25.5 ± 0.7*      | 20.3 ± 0.6c     |
| Leucine                                       | 62.0 ± 0.7a     | 49.9 ± 1.0b      | 41.9 ± 1.0a      | 51.2 ± 1.3b     |
| Phenylalanine                                 | 39.3 ± 0.9a     | 56.4 ± 1.4a      | 108.3 ± 2.4*     | 75.5 ± 2.3b     |

* Animals were treated and data are presented as described in Table 1.
duced brain leucine, isoleucine, and valine, and the reductions seen in animals receiving both compounds were significantly greater than those observed in rats receiving only one of the treatments (Table 2).

Tryptophan, serotonin, and 5-HIAA

Aspartame given alone failed to affect plasma tryptophan levels and decreased the plasma tryptophan ratio only slightly (by causing large increases in plasma phenylalanine and tyrosine; Table 1). Glucose consumption increased plasma tryptophan slightly (14%) but not significantly compared with values observed in control rats receiving only water; however, as noted previously (1, 15), the carbohydrate caused a 76% increase in the plasma tryptophan ratio (p < 0.001) an
effect that was completely blocked if aspartame was administered concurrently (Table 1). The brain tryptophan level in each animal tended to parallel its plasma tryptophan ratio (Fig 1; $r = 0.89$), glucose alone increasing the ratio by about 45%, and concurrent aspartame reducing this to +23% (Table 2).

As described previously (1, 4), consumption of glucose caused major increases in brain levels of the neurotransmitter serotonin and of serotonin's metabolite 5-HIAA (Table 3). Although aspartame alone had no effect on these hydroxyindoles, concurrent administration of the synthetic sweetener completely blocked the increases caused by the dietary carbohydrate. Each animal's total brain 5-hydroxyindole level (serotonin plus 5-HIAA) tended to be highly correlated ($r = 0.74$, $p < 0.001$; Fig 1) with its plasma tryptophan ratio, suggesting that aspartame affects the brain neurotransmitter indirectly, by modifying the plasma amino acid pattern.

**Discussion**

These data show that administration of the phenylalanine-containing dipeptide aspartame to adult rats can cause major increases in brain phenylalanine and tyrosine, and can reduce brain levels of the branched-chain amino acids leucine, isoleucine, and valine (Table 2). Moreover, if the aspartame is consumed along with a dietary carbohydrate (glucose; 3 g/kg), it blocks the physiological increase that carbohydrates normally produce (1) in brain tryptophan, serotonin, and 5-HIAA (Tables 2 and 3), and amplifies the increase or decrease in brain levels of each of the other LNAA (Table 2). These brain changes tend, both in experimental groups (Table 1) and in individual animals (Fig 1), to parallel the changes occurring concurrently in plasma amino acid ratios, suggesting that the major and possibly sole locus through which the sweetener affects the brain is peripheral, ie, by contributing phenylalanine and tyrosine molecules to the blood stream. These molecules then occupy sites on the transport macromolecules (3) that carry LNAA across the blood-brain barrier, accelerating their own uptakes into the brain and suppressing those of the branched-chain amino acids. Phenylalanine and tyrosine have only a small effect on the plasma tryptophan ratio (and, probably, on brain tryptophan uptake) basally; however, they block the increase in brain tryptophan that would otherwise occur after carbohydrate consumption (1, 2) and thereby block the increased saturation of the enzyme tryptophan hydroxylase (16) and the resulting acceleration in serotonin's synthesis and release (1, 15).

The dose of aspartame given to our rats (200 mg/kg) was chosen in the belief that its brain effects would be similar to those occurring if a 30-kg child obtained 20 mg/kg of the sweetener by drinking a quart of some presently available diet beverages (approximately 500 mg), and consumed an additional 100 mg of aspartame from other dietary sources, concurrently eating carbohydrate-rich foods but no protein. The carbohydrate presumably would double the effect of that dose on brain phenylalanine (cf Table 2), and the 5-fold or greater species difference in the rates at which rats and humans metabolize essential amino acids such as phenylalanine (17) would raise it to an equivalent dose of 200 mg/kg for rats. Of course, if humans metabolize phenylalanine to tyrosine less rapidly than rats do, our data on rats would overestimate the changes occurring in human brain tyrosine, and underestimate those in brain phenylalanine. (No data are available concerning the extent to which the blood-brain barrier LNAA transport system in humans is or is not kinetically similar to the system operating in rats.) It now becomes important to test the effects on plasma LNAA ratios of real aspartame-containing foods, given to children and adults as components of typical snacks and meals, to establish whether the dosage and experimental design used here are likely to be predictive of the sweetener's actual effects when used by consumers. Available data (18,

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Effects of aspartame and glucose on brain 5-hydroxyindole levels in rats*</th>
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<tbody>
<tr>
<td>Water and water</td>
<td>Glucose and water</td>
</tr>
<tr>
<td>Serotonin (ng/g tissue)</td>
<td>485 ± 16*</td>
</tr>
<tr>
<td>5-HIAA (ng/g tissue)</td>
<td>361 ± 35*</td>
</tr>
</tbody>
</table>

* Animals were treated and data are presented as described in Table 1.
19, this report) suggest that the plasma phenylalanine ratio in humans is a linear function of aspartame dose at all dosage levels.

The carbohydrate dose used in this study (3 g/kg) was also chosen to be metabolically equivalent to the amount needed to lower plasma branched-chain amino acid levels in normal human subjects. We previously observed that, while some subjects responded to as little as 6 g of glucose (or 90 mg/kg), it requires a dose of 50 g (or 0.8 g/kg) to cause enough of a fall in all subjects to raise the plasma tryptophan ratio (9) as well as other measures of amino acid metabolism (9). Our present 3 g/kg dose for rats is thus about four times the requisite human dose, and parallels the differences in the species' metabolic rates. In other studies in which we gave rats aspartame (200 mg/kg) plus an inadequate glucose dose (200 mg/kg), we observed a slight potentiation of aspartame's effects on the plasma tyrosine ratio (+180% as opposed to +127% after aspartame alone) and on brain tyrosine (+198% versus +136%), but no other effects.

The functional consequences, if any, of aspartame-induced changes in brain amino acid concentrations are unknown, but may turn out to be related to the known functions of the neurotransmitters that are formed from these amino acids. The increase in brain serotonin that follows consumption of a carbohydrate-rich meal (1) seems to be an essential component of the mechanism that “informs” the brain about the nutrient composition of the food then being digested, the release of the serotonin acting to affect subsequent appetites for carbohydrates and proteins (20–22). Serotonin release is also involved in sleep onset (23) and in modulating sensitivity to certain kinds of pain (24). The synthesis of norepinephrine and dopamine are increased within physiologically active neurons (25), when more tyrosine is made available to the brain; hence aspartame administration may, by elevating brain tyrosine levels (Table 2), amplify catecholamine release from such neurons, and thus influence the physiological and behavioral mechanisms that they mediate, eg, the ability to lower blood pressure in certain types of hypertension (26, 27). The effects of high aspartame doses on catecholamine release in humans would probably depend on their effects on tissue phenylalanine, as well as tyrosine levels, since at high concentrations phenylalanine may inhibit tyrosine hydroxylase activity (Kaufman S, personal communication) and catecholamine release.

In summary, the present study demonstrates that aspartame intake significantly increases brain phenylalanine and tyrosine in rats, while decreasing brain levels of leucine, isoleucine, and valine. Moreover, simultaneous ingestion of aspartame with carbohydrates potentiates all of these effects, while blocking the increase in brain serotonin normally seen after a carbohydrate meal. (Dietary protein would be expected to diminish these effects) Each brain amino acid level after each treatment was highly correlated with the corresponding plasma ratio, suggesting that aspartame’s brain effects in the rat result solely from peripheral actions on amino acid metabolism.

References

10. Fernstrom JD, Wurtman RJ, Hammarstrom-Wik-