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Changes in Brain Levels of Acidic, Basic, and Neutral Amino Acids After Consumption of Single Meals Containing Various Proportions of Protein

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Abstract: Rats fasted overnight were allowed to consume single meals containing 0, 18, or 40% protein or continued to fast; after 2 h, brains and sera were taken and assayed for various amino acids. In general, serum levels of most amino acids were reduced by the 0% protein meal and elevated by the high-protein meal when compared with those associated with fasting conditions. Exceptions were those not diminished by the 0% protein meal (tryptophan, methionine, proline) and those increased (alanine) or decreased (glycine) by all of the test meals. Amino acids exhibiting the broadest normal ranges (estimated by comparing their serum levels after 40% protein with those after 0% protein) were tyrosine, leucine, valine, isoleucine, and proline; serum lysine and histidine, two basic amino acids, also varied more than threefold. Brain levels of lysine, histidine, and some of the large neutral amino acids (LNAAs) also exhibited clear relationships to the protein content of the test meal: those of valine, leucine, and isoleucine were depressed by the 0% protein but increased (compared with 0% protein) when protein was added to the meal: brain tyrosine was increased by all of the test meals in proportion to their protein contents; tryptophan, phenylalanine, and glutamate were increased after the 0% protein meal but not by protein-containing meals; brain lysine, histidine, and methionine were increased after the high-protein meal, and brain alanine was increased slightly by all of the meals. For each of the LNAAs, significant correlations were observed between its brain level in any animal and the ratio of its serum concentration to the sum of the concentrations of its LNAA competitors (for blood–brain barrier transport). For valine, tyrosine, lysine, and histidine, significant correlations were obtained between their brain and serum levels. **Key Words:** Tyrosine—Tryptophan—Lysine—Histidine—Methionine. **Glaeser B. S. et al.** Changes in brain levels of acidic, basic, and neutral amino acids after consumption of single meals containing various proportions of protein. *J. Neurochem.* **41**, 1016–1021 (1983).

The fluxes of amino acids across the blood–brain barrier are mediated by three known transport systems, probably localized within the capillary endothelia (Pardridge, 1977), which specifically recognize acidic, basic, or large neutral amino acids (LNAA) (Oldendorf and Szabo, 1976; Pardridge and Oldendorf, 1977). These systems do not require energy and cannot maintain concentration gradients; rather, they facilitate the diffusion of their substrates between the plasma and the brain's extracellular fluid.

The system that transports the LNAAs has two kinetic properties that govern the responses of brain amino acid levels to treatments (such as food consumption or amino acid administration) that vary serum LNAAs within their physiologic ranges: it is unsaturated with its ligands, and the individual amino acids compete with each other for attachment to transport sites. As a consequence brain LNAA levels rise when serum LNAAs are elevated (Fernstrom and Wurtman, 1972; Fernstrom and Faller, 1978), and the level of any particular LNAA in brain depends not only on its own serum concentration but also, inversely, on the sum of the serum concentrations of its competitors (Fernstrom and Wurtman, 1972; Perez-Cruet et al., 1974; Fern-

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Abbreviations used: LNAA, Large neutral amino acid.

strom et al., 1975; Fernstrom and Faller, 1978). Thus, consumption of a high-carbohydrate, protein-free meal can increase brain tryptophan levels (Fernstrom and Wurtman, 1971), without dramatically elevating serum tryptophan, because the insulin secretion elicited by the food depresses serum levels of competing LNAAs such as leucine, isoleucine, and valine (Fernstrom et al., 1973, 1979; Fernstrom and Faller, 1978). In contrast, consumption of a protein-rich meal—which does elevate serum tryptophan (Fernstrom et al., 1979)—depresses brain tryptophan (Fernstrom and Wurtman, 1972), because the meal causes much greater elevations in serum concentrations of tryptophan's LNAA competitors. These food-induced alterations in brain tryptophan level affect the rate at which neurons convert the amino acid to its neurotransmitter product, serotonin, and can thereby affect brain function (Fernstrom and Wurtman, 1972). The increases in brain tyrosine (Gibson and Wurtman, 1978) and methionine (Rubin et al., 1974) that follow consumption of a protein-rich (40%) meal can similarly enhance production of their products, the catecholamines (Gibson and Wurtman, 1978; Wurtman et al., 1980) and *S*-adenosylmethionine (Rubin et al., 1974).

The present study extends the characterization of meal-induced changes in brain and serum amino acid levels to include some of the basic, acidic, and small neutral compounds.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150–200 g; Charles River Breeding Labs, Wilmington, MA) were housed for 7 days in our temperature/humidity-controlled animal facility, and exposed to light (Vita-lite, Duro-Test, N. Bergen, NJ; 300 $\mu W/cm^2$) between 7 a.m. and 7 p.m. daily. All rats had free access to rat chow (Charles River Rat, Mouse and Hamster Maintenance Formula; 22% protein) and water until the evening preceding the experiment. At 7–8 a.m. the following morning, groups of eight rats received one of four dietary regimens: no food, the control group; a 0% protein diet; an 18% protein diet; or a 40% protein diet. Two hours after access to the test diet, between 9 and 10 a.m., animals were decapitated. Blood was collected from the cervical wound and brains were removed within 30 s.

Blood samples were kept on ice until they could be centrifuged for sera, which were stored at −20°C. Brain samples were kept on Dry Ice and then stored at −70°C. Serum samples were deproteinized by addition of sulfosalicylic acid (50 mg/kg). Brain samples were deproteinized with 10% trichloroacetic acid. Amino acids were analyzed on a Beckman Model 119C amino acid analyzer with lithium citrate buffers; brain tryptophan was assayed fluorimetrically (Denkla and Dewey, 1967; Bloxam and Warren, 1974).

Diets prepared as previously described (Fernstrom and Faller, 1978) were composed of Mazola oil, Rogers-Harpers salt mixture, agar, sucrose, dextrose, dextrin, and casein. Data (means ± SD) are given for all of the circulating amino acids incorporated into protein, except for cystine, which does not react with ninhydrin reliably (Friedman et al., 1979); glutamine, which is unstable and not reliably measured by automatic amino acid analyzers (Bermer et al., 1981); and aspartate, which may coelute with reduced glutathione when a lithium citrate buffer system is used (Bermer et al., 1981). Particular attention was paid to glutamate levels because of the known *in vitro* conversion of glutamine to glutamate. Our estimates of serum and brain glutamate levels fell within published ranges (Banos et al., 1975; Koyuncuoglu et al., 1975; Tyfield and Holton, 1976; Eriksson et al., 1980; Kamata et al., 1980; Toth and Lajtha, 1981).

All data were analyzed by one-way analysis of variance. Rank order analysis of the data was achieved by the use of the Newman-Keuls test (Zivin and Bartko, 1976). The statistical significance of correlations was tested by the Student's *t* test against the null hypothesis. Serum amino acid ratios were calculated by dividing the serum concentration of each amino acid by the sum of the concentrations of other amino acids (as indicated) in its postulated transport group (Oldendorf and Szabo, 1976).

RESULTS

Effects of meal consumption on serum amino acid concentrations

In general, serum levels of most amino acids were lowest after consumption of the 0% protein meal, unchanged (compared with those of fasting animals) after the 18% protein meal, and elevated by the 40% protein meal (Tables 1 and 2). Exceptions were: (a) those unchanged by 0% protein but increased significantly by protein-containing meals, in proportion to the protein contents of the meals (tryptophan, methionine, proline); (b) those increased by 18% as well as by 40% protein, compared with fasting values (tyrosine); and (c) those increased (alanine) or decreased (glycine) by all of the test meals. The normal range of each amino acid's serum levels was estimated by comparing its mean levels after a 40% protein meal with those following a 0% protein meal. Broadest ranges (fivefold or greater) were observed for tyrosine, leucine, valine, isoleucine, and proline; narrowest ranges (twofold or less) were observed for glycine, arginine, and glutamate; others were intermediate. Among the amino acids examined that are not present in protein (ornithine and citrulline), serum levels also were lowest after consumption of the 0% protein meal (data not shown).

Effects of meal consumption on brain amino acid levels

In general, only brain levels of lysine, histidine, and some of the LNAAs exhibited clear relationships to the protein content of the last meal (Tables 3 and 4). As described earlier (Fernstrom and Faller, 1978), brain valine, isoleucine, and leucine levels were depressed after the 0% protein meal, and elevated (compared with levels after 0% protein) in rats consuming the 40% protein meal. Tyrosine levels were increased by the test meals, in proportion to

TABLE 1. *Effect of dietary protein content on serum concentrations of neutral amino acids*

Amino acid	Serum concentration (nmol/ml)				
	Fasting	0% protein	18% protein	40% protein	40%/0% ratio
Valine	241 ± 31[a]	81 ± 18	243 ± 51[a]	513 ± 72[a]	6.33
Isoleucine	143 ± 19[a]	37 ± 7	100 ± 22[a]	192 ± 16[a]	5.19
Leucine	200 ± 24[a]	53 ± 10	173 ± 37[a]	357 ± 151[a]	6.74
Tyrosine	78 ± 9[a]	47 ± 7	173 ± 37[a]	417 ± 74[a]	8.87
Phenylalanine	77 ± 8[a]	45 ± 5	93 ± 10[a]	134 ± 8[a]	2.98
Tryptophan	61 ± 15	70 ± 14	123 ± 16[a]	173 ± 16[a]	2.47
Methionine	56 ± 6	48 ± 17	98 ± 18[a]	204 ± 47[a]	4.25
Threonine	373 ± 65[a]	202 ± 37	395 ± 119[a]	613 ± 52[a]	3.03
Serine	356 ± 40[a]	222 ± 12	385 ± 56[a]	485 ± 48[a]	2.18
Proline	185 ± 21	139 ± 12	540 ± 83[a]	703 ± 62[a]	5.06
Glycine	516 ± 61[a]	342 ± 12	345 ± 64	277 ± 43[a]	0.81
Alanine	427 ± 72[a]	604 ± 27	1104 ± 86[a]	1295 ± 157[a]	2.14
Asparagine	81 ± 10[a]	48 ± 5	124 ± 24[a]	189 ± 27[a]	3.94

Groups of seven or eight rats, starved overnight, were given access to the diet indicated at 7–8 a.m. (except those continuing to fast), and all were sacrificed 2 h later. Serum and brain samples were assayed for the amino acids indicated. Data are given as means ± SD. The 40%/0% protein column describes the ratio of serum concentrations of that amino acid after animals consumed the 40% protein meal to concentrations after the 0% protein meal.

[a] $p < 0.05$ significantly differs from the 0% protein group, by rank order correlations using the Newman-Keuls test (Zivin and Bartko, 1976).

their protein content. Phenylalanine and glutamate levels tended to be increased after the 0% protein meal, but not by protein-containing meals; tryptophan also was depressed by dietary protein. Brain lysine, histidine, and methionine were increased after the high-protein (40%) meal, and brain alanine rose slightly after all of the meals.

We attempted to correlate brain levels of each amino acid, in each animal, with its serum levels, or with—for LNAA—the ratio of its serum concentration to the sum of its major transport competitors [leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine (Pardridge, 1977)]. Significant correlations ($p < 0.05$) were observed between brain and serum concentrations for valine, methionine, and tyrosine (Table 3), as well as for isoleucine ($r = 0.68$), leucine ($r = 0.75$), alanine ($r = 0.44$), lysine ($r = 0.77$), and histidine ($r = 0.50$); significant correlations were also observed between brain levels of each LNAA and the ratio of its serum concentration to the sum of its transport competitors (Table 3).

For leucine the best correlation ($r = 0.89$) was obtained when brain levels were associated with the ratio of its serum concentration to the sum of those of valine, isoleucine, and phenylalanine. The same was observed for phenylalanine (0.73; brain phenylalanine versus serum phenylalanine/serum valine + leucine + isoleucine). The best correlations ($r = 0.69$) for brain methionine and brain isoleucine ($r = 0.86$) were with the ratios of serum methionine or serum isoleucine to the sums of all of the neutral amino acids listed in Table 1 (minus asparagine). The superiority of these correlations may be related to the high affinities of the branched-chain amino acids phenylalanine and methionine for the L trans-

TABLE 2. *Effect of dietary protein contents on serum concentrations of acidic and basic amino acids*

Amino acid	Serum concentration (nmol/ml)				
	Fasting	0% protein	18% protein	40% protein	40%/0% protein
Glutamate	162 ± 33[a]	119 ± 19	174 ± 47[a]	214 ± 30[a]	1.80
Lysine	526 ± 124[a]	275 ± 51	524 ± 96[a]	832 ± 120[a]	3.03
Arginine	165 ± 30[a]	114 ± 14	145 ± 37[a]	200 ± 18[a]	1.75
Histidine	86 ± 11[a]	52 ± 6	97 ± 12[a]	159 ± 9[a]	3.06

Animals were prepared, tissues were examined, and data are described as in the footnote to Table 1.

[a] $p < 0.05$ significantly differs from the 0% protein group, by rank order correlations using the Newman-Keuls test (Zivin and Bartko, 1976).

TABLE 3. *Effect of dietary protein content on brain levels of neutral amino acids*

Amino acid	Brain concentration (nmol/g)					
	Fasting	0% protein	18% protein	40% protein	40%/0% protein	Best serum correlation
Valine	106 ± 12[a]	82 ± 14	110 ± 21[a]	118 ± 19[a]	1.44	0.65 Serum valine 0.59 valine/LNAA
Isoleucine	43 ± 5[a]	25 ± 5	26 ± 5	36 ± 4[a]	1.44	0.70 isoleucine/LNAA
Leucine	72 ± 12[a]	39 ± 10	54 ± 8[a]	84 ± 10[a]	2.15	0.73 leucine/LNAA
Tyrosine	47 ± 5	65 ± 8	91 ± 29[a]	155 ± 32[a]	2.38	0.92 Serum tyrosine 0.90 tyrosine/LNAA
Phenylalanine	43 ± 7[a]	64 ± 10	48 ± 7[a]	43 ± 3[a]	0.67	0.58 phenylalanine/LNAA
Tryptophan	42 ± 8	43 ± 8	32 ± 5[a]	27 ± 1[a]	0.60	
Methionine	39 ± 17	47 ± 19	50 ± 5	64 ± 11[a]	1.36	0.60 Serum methionine
Threonine	605 ± 106	572 ± 91	447 ± 63[a]	603 ± 65	1.05	
Serine	823 ± 116	872 ± 126	790 ± 174	800 ± 138	0.92	
Proline	62 ± 19[a]	101 ± 18	98 ± 26	96 ± 22	0.95	
Glycine	819 ± 80[a]	944 ± 161	905 ± 171	860 ± 222[a]	0.91	
Alanine	348 ± 67[a]	481 ± 59	472 ± 39	445 ± 40	0.93	
Asparagine	75 ± 18	110 ± 44	104 ± 26	70 ± 30	0.64	

Animals were prepared, tissues were examined, and data are described as in the footnote to Table 1. The correlation coefficients indicated were statistically significant in all cases ($p < 0.05$). For each amino acid, correlations were examined between its brain and serum levels and between its brain level and serum ratio (i.e., the ratio of its serum concentration to the sum of the other amino acids in its blood–brain barrier transport group) (LNAA = valine, leucine, isoleucine, tyrosine, tryptophan, phenylalanine, methionine).

[a] $p < 0.05$ significantly differs from the 0% protein group, by rank order correlations using the Newman-Keuls test (Zivin and Bartko, 1976).

port system of Christensen (Christensen, 1973; Pardridge, 1977) and the fact that methionine is also a preferred substrate for the A transport system.

DISCUSSION

These studies confirm and extend previous observations concerning the changes in serum (Fernstrom et al., 1973, 1979) and brain (Fernstrom and Wurtman, 1972; Fernstrom and Faller, 1978) amino acid concentrations that follow consumption of meals containing various proportions of protein. As found earlier (Fernstrom and Faller, 1978), a high-protein (40%) meal increased serum levels of all of the branched-chain and aromatic LNAAs, and also increased brain tyrosine levels (Fernstrom and Faller, 1978; Gibson and Wurtman, 1978). Moreover, this meal increased serum methionine, threonine, serine, proline, alanine, asparagine, lysine, and histidine concentrations (Tables 1 and 2) as well as brain methionine, lysine, and histidine (Tables 3 and 4). The 0% protein meal reduced serum levels of the branched-chain amino acids by 65–75%, and also significantly lowered those of tyrosine, phenylalanine, threonine, serine, glycine, asparagine, glutamate, lysine, arginine, and histidine while raising that of alanine (Tables 1 and 2). It reduced brain levels of the branched-chain amino acids but elevated those of the aromatic LNAAs and alanine (Tables 3 and 4). An increase in brain methionine among rats consuming a high-protein meal was previously described by Rubin et al. (1974); food-induced changes in brain lysine and histidine concentrations apparently have not been described previously.

The increases in brain tryptophan after consumption of a 0% protein meal (Colmenares et al., 1975),

TABLE 4. *Effect of dietary protein content on brain levels of acidic and basic amino acids*

Amino acid	Brain concentration (nmol/g)				
	Fasting	0% protein	18% protein	40% protein	40%/0% protein
Glutamate	5550 ± 2209[a]	8434 ± 949	5230 ± 1437[a]	6760 ± 2098	0.80
Lysine	286 ± 47	247 ± 69	274 ± 26	391 ± 84[a]	1.58
Arginine	73 ± 16	70 ± 23	69 ± 19	69 ± 15	0.99
Histidine	97 ± 50	72 ± 15	107 ± 74	181 ± 80[a]	2.51

Animals were prepared, tissues were examined, and data are described as in the footnote to Table 3.

[a] $p < 0.05$ significantly differs from the 0% protein group, by rank order correlations using the Newman-Keuls test (Zivin and Bartko, 1976).

and in brain tyrosine after a protein-containing meal (Fernstrom and Faller, 1978; Gibson and Wurtman, 1978) have previously been shown to be associated with parallel changes in the synthesis or release of their neurotransmitter products, serotonin and the catecholamines, under appropriate experimental conditions [e.g., when dopaminergic (Wurtman et al., 1980) or noradrenergic (Conlay et al., 1981) neurons are physiologically active]. The rise in brain methionine after a high-protein meal has similarly been correlated with increases in brain levels of *S*-adenosylmethionine (Rubin et al., 1974). The physiologic consequences of the elevations in brain histidine or lysine that follow consumption of the 40% protein meal await discovery; however, it is known that histidine administration can increase brain histamine levels (Schwartz et al., 1972). Lysine is used in the brain for formation of pipecolic acid (Chang, 1978) and carnitine; it will be interesting to determine whether the formation of these or other lysine products is influenced by food-induced changes in brain lysine levels.

The acidic amino acid glutamate is a putative neurotransmitter and food constituent (Shank and Graham, 1978; Filer et al., 1979); moreover, it is added to foods as a flavor enhancer in the form of monosodium glutamate. The fact that serum glutamate levels can normally vary in rats by almost 100%, depending on whether the animal has recently eaten and what it has eaten (Table 2), provides some basis for assessing the significance of the changes in its serum concentrations produced when this compound is added to foods. Brain glutamate levels were elevated when fasting animals consumed a protein-free meal; otherwise its brain levels were not influenced by any of the nutritional manipulations tested here. The transport system that mediates the flux of this amino acid across the blood–brain barrier is asymmetrically distributed, such that the rate of glutamate *efflux* from rat brain is calculated to be as much as sevenfold greater than its rate of *influx* (Pardridge, 1978).

The basic amino acids lysine and histidine behaved like the LNAAs with reference to the changes in their brain levels that followed consumption of various meals. Both decreased insignificantly after animals ate the 0% protein meal, but rose significantly as protein was added to the diet (Table 4). We attempted to determine whether, in individual animals, brain levels of these compounds could be better correlated with a serum ratio (e.g., the ratio of the amino acid's serum concentration to the sum of other basic compounds) than with its serum concentration alone, but failed to discern significant correlations with ratios. Although histidine is generally classified as a basic amino acid, less than 10% of it is charged at pH 7.0, and it may thus be transported as a neutral amino acid at physiological pH (Oldendorf and Szabo, 1976).

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