Adaptation of Rats to Diets Containing Different Levels of Protein: Effects on Food Intake, Plasma and Brain Amino Acid Concentrations and Brain Neurotransmitter Metabolism

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ABSTRACT Food intake, plasma and brain amino acid concentrations, liver amino acid catabolic enzyme activities, and whole-brain neurotransmitter and metabolite concentrations were measured in young rats adapted for 11 d to diets containing from 5 to 75% (in increments of 5%) casein. Food intake was depressed initially in rats fed diets containing 5, 10% or greater than 35% casein. For the duration of the experiment, food intakes of the groups fed the higher protein diets improved on successive days; the length and severity of the depression were proportional to the protein content of the diet fed. Rats fed low levels of protein grew poorly, and their food intake remained depressed. The gradual improvement in growth and food intake of rats fed diets containing more than 35% casein was accompanied by dramatic increases in the activities of serine-threonine dehydratase (SDH, EC 4.2.1.16) and glutamate-pyruvate aminotransferase (GPT, EC 2.6.1.1) in liver. The increase in amino acid catabolic activity was accompanied by decreases in the concentrations of most amino acids in plasma and brain. However, concentrations of branched-chain amino acids, in both plasma and brain, increased in direct proportion to the protein concentration of the diet fed. As a result of these reciprocal responses, the total concentration of dispensable amino acids in brain (IAA) was maintained within a narrow range of values, despite a sixfold range of protein intakes. Whole-brain concentrations of norepinephrine, dopamine and serotonin were not correlated with dietary protein concentration, total food intake or protein intake. Brain concentrations of homovanillic acid and 5-hydroxyindoleacetic acid were correlated inversely with protein intake and that of 3,4-dihydroxyphenylacetic acid was correlated directly with food intake. Protein intake appeared to be related to the animal's ability to maintain brain total IAA content within some upper and lower limits. Our results indicate that this was accomplished initially through downward adjustment of protein intake and subsequently through an increase in catabolic capacity for the amino acids.


INDEXING KEY WORDS food intake • protein intake • plasma amino acids • brain amino acids • neurotransmitters • serine dehydratase • alanine aminotransferase

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protein diet is associated with elevated concentrations of plasma (1, 4, 5) and brain (4, 6, 7) total indispensable amino acids (IAA). This, in turn, is associated with a limited capacity of the animal's amino acid-catabolic enzymes to degrade surplus amino acids. After rats have been fed a high protein diet for several days, their capacity to degrade amino acids increases; the increases in catabolic enzyme activities are accompanied by increased growth and food intake, and a decline in plasma amino acid concentrations to control levels or less (1, 5, 8). The depressed food intake of rats fed a protein-free or low protein diet is associated with decreased plasma (5, 9) and brain (5) concentrations of most IAA.

Also, if young rats are given the opportunity to select between high and low protein diets, they will usually choose an amount of protein that meets or moderately exceeds their amino acid requirements for growth (2, 10–13). The proportion of total calories selected as protein under these conditions depends on both the protein content and the amino acid composition of the diets offered and the amino acid-catabolic state of the animal (6, 9, 10, 13). Interestingly, rats adapted to a high protein diet that are allowed to select between a high and a low protein diet will, within a few days, reduce their protein intake to obtain only a moderate proportion of calories from protein (13).

In diet selection studies, Anderson and associates (14, 15) observed an inverse relationship between long-term (2–4 wk) protein intake and the ratio of plasma tryptophan concentration to the sum of the concentrations of other large neutral amino acids (NAA), which compete with tryptophan for uptake into brain (tryptophan/NAA; NAA = leucine + isoleucine + valine + phenylalanine + tyrosine). Since this ratio is often a good index of brain tryptophan concentration (16, 17), and hence brain serotonin (5-HT) synthesis (18), they proposed that diet-induced changes in brain 5-HT content are involved in the regulation of protein intake (18, 20). Wurtman and Wurtman (21, 22), in contrast, from results of studies in which the activity of central serotonergic neurons was altered with drugs, have concluded that diet-mediated changes in brain 5-HT synthesis may be involved in the control of carbohydrate intake and selection.

In previous studies with rats either allowed to self-select for protein (12) or fed a single meal containing 0–55% protein (6), we did not detect relationships between protein intake and whole-brain 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA). In our studies, protein intake appeared to be controlled at a level that would maintain brain concentrations of total IAA between tolerable minimum and maximum values.

Little information is available on the concentrations of plasma and brain amino acids and brain neurotransmitters in rats adapted to diets containing various levels of protein. Such information might be expected to provide clues about control of protein intake by rats offered single diets or about control of protein preference by rats offered a choice between two diets. We have therefore measured free amino acid concentrations in plasma and brain, and whole-brain contents of NE, DA, 5-HT, 5-HIAA, DOPAC and HVA in young rats after allowing them to adapt to diets containing from 5 to 75% casein. In addition, to identify some of the metabolic variables that may influence brain amino acid and neurotransmitter concentrations, we have measured the adaptive responses of two liver enzymes involved in amino acid degradation; serine-threonine dehydratase (SDH, EC 4.2.1.16) and glutamate-pyruvate transaminase (GPT, EC 2.6.1.1).

MATERIALS AND METHODS

Seventy-five male King rats (King Animal Labs, Oregon, WI) initially weighing 50–60 g were housed individually in suspended metal cages with wire-mesh bottoms in a room lighted for 12 h daily and maintained at approximately 23°C. On receipt, the animals were fasted overnight and then divided into 15 groups of five rats each. For the next 11 d each group was fed one of the 15 diets differing in casein content. Food was provided daily during the last 8 h of the dark period, and water was provided ad libitum.
The diets contained the following ingredients (expressed as a percentage of the total dry matter): mineral mix, 5% (Teklad Test Diets, Madison, WI) (23); vitamin mix, 0.5% (23); corn oil, 5% (Mazola corn oil, Best Foods International, Englewood Cliffs, NJ); choline chloride, 0.2%; vitamin-free casein (Teklad Test Diets), 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 or 75%; glucose monohydrate (CPC International, Inc., Englewood Cliffs, NJ) and cornstarch (A. E. Staley Manufacturing Co., Oak Brook, IL), added in equal amounts to make 100%. The diets were prepared in agar-gel form (30 g agar/kg diet; BactoAgar, Difco Labs, Detroit, MI) and were lightly colored with food dye for identification.

Growth and food intake were measured daily, and protein intake was calculated based on the casein content of each diet. Immediately after the onset of the light cycle on d 12, rats were killed by decapitation, and blood was collected from the trunk into chilled heparinized tubes and stored frozen until analyzed. Brains (without cerebella) were quickly removed, frozen and pulverized; the powder was thoroughly mixed and stored in liquid nitrogen until analyzed. Livers were removed and placed in ice-cold 0.9% saline until they were prepared for enzyme assays later that same day. Pooled samples of plasma or brain were prepared by mixing equal amounts of brain powder or plasma from animals of each dietary treatment group. Protein-free sulfosalicylic acid extracts of either plasma or brain were prepared as described previously (24), and were analyzed for free amino acid content (Beckman Model 119CL, Beckman Instruments, Palo Alto, CA). Plasma and brain tryptophan concentrations were measured separately by the fluorometric assay of Denckla and Dewey (25) as modified by Bloxam and Warren (26).

Measurements of whole-brain NE, DA, 5-HT, DOPAC, HVA and 5-HIAA were carried out on portions (400-500 mg) of the pooled brain powders from rats in each diet group by a method described previously (27) that involved solvent extraction of the neurotransmitters and metabolites followed by high performance liquid chromatography with electrochemical detection.

Pooled samples of liver were prepared by combining 1 g of tissue (taken from the median lobe of each liver) from five rats of each diet group. The activities of the enzymes GPT and SDH were measured essentially as described by Freedland et al. (28) and Freedland and Avery (29), respectively. A 20% homogenate of each pooled liver sample was made in ice-cold distilled-deionized water by using a Potter-Elvehjem tube fitted with a motor-drive Teflon pestle. The homogenates were centrifuged at 30,000 × g at 4°C for 45 min. The resulting undiluted supernatant solution was assayed for SDH; 1 ml of each supernate was diluted with 11.5 volumes of cold distilled-deionized water and the resultant solution was assayed for GPT activity.

For the GPT assay each cuvette contained: 0.2 M Tris buffer, pH 7.4, 0.38 mM NADH, 16.7 mM L-alanine, 10 U beef heart lactic acid dehydrogenase (Sigma #L2625, Sigma Chemical Co., St. Louis, MO) and 0.1 ml of the diluted liver homogenate supernatant solution to make a final volume of 3 ml. The reaction was initiated by the addition of 0.025 ml of α-ketoglutaric acid (1.92 mM, pH 7.4).

For the SDH assay each cuvette contained: 0.1 M Tris buffer, pH 8.5, 0.38 mM NADH, 3.58 mM pyridoxal-5-phosphate, 30 U lactic acid dehydrogenase and 0.15 ml of the undiluted liver homogenate supernate to make a final volume of 2.9 ml. The reaction was initiated by the addition of 0.1 ml of 1 M L-serine.

The rate of each reaction was measured by monitoring the rate of disappearance of NADH at 340 μM at room temperature in a dual-beam spectrophotometer (GCA/McPherson Instruments, Acton, MA) against a blank solution in which water was added in place of either α-ketoglutaric acid or serine. Enzyme activities are reported as micromoles NADH oxidized per minute per gram liver (wet weight).

The concentration of protein in each liver homogenate sample was determined by the method of Lowry et al. (30).

The statistical significance of differences between the means of individual treatments was determined by using one-way ANOVA followed by the Newman-Kuels multiple-range test. Differences were declared significant if the calculated P-value was 0.05 or
less (31).

The entire study was repeated, with the exception of measurement of brain neurotransmitter concentrations, and the results were essentially the same as those reported here.

RESULTS

Growth and food intake. The 11-d cumulative weight gains of rats consuming diets containing less than 20% casein or greater than 35% casein were significantly less \((P < 0.05)\) than those of animals fed the diets containing intermediate levels of casein (table 1). Rats fed the 5% casein diet gained significantly less \((P < 0.05)\) weight than any other diet group.

On the first 2 d of the experiment, growth rates of rats fed diets containing greater than 45% or less than 15% casein were significantly lower \((P < 0.05)\) than those of rats fed the diets containing from 15 to 45% casein (data not shown but derivable from table 1). Growth rates of almost all groups increased between d 3 and 6; those of rats fed diets containing greater than 45% casein increased up to three- to fourfold. During the last 5 d of the experiment, growth rates of animals fed diets containing from 15 to 55% casein were not significantly different. In contrast, growth rates of animals fed the diets containing only 5 or 10% casein did not improve significantly with time.

The change over time in daily food intake in relation to dietary protein level followed a pattern similar to that observed for growth (table 1). Since growth rates of different groups differed widely, comparisons between daily food intakes of groups were performed only after the data were adjusted to account for differences in metabolic body size \((\text{body weight})^{0.75}\) (32).

On the first day of the experiment, rats fed the 20% casein diet consumed significantly more food \((P < 0.05)\) than any other diet group. Food intakes of rats fed diets containing from 5 to 15% or from 25 to 45% casein were similar, while rats fed from 50 to 75% casein diets exhibited depressed food intake \((P < 0.05)\) in relation to animals fed low to intermediate levels of dietary casein. On d 3, rats fed 5, 10 or 40–75% casein diets ate significantly less \((P < 0.05)\) than animals fed 20% casein, while food intakes of rats fed diets containing from 15 to 35% casein were not different. On d 6, rats in groups fed diets containing from 10 to 55% casein consumed similar quantities of food, while animals fed 5%

<table>
<thead>
<tr>
<th>Dietary protein conc (%)</th>
<th>d 1</th>
<th>d 3</th>
<th>d 6</th>
<th>d 11</th>
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<tbody>
<tr>
<td></td>
<td>Body wt</td>
<td>Intake</td>
<td>Body wt</td>
<td>Intake</td>
</tr>
<tr>
<td>5</td>
<td>55 ± 1</td>
<td>3.7 ± 0.2</td>
<td>57 ± 2</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>54 ± 1</td>
<td>3.7 ± 0.2</td>
<td>58 ± 1</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>15</td>
<td>56 ± 1</td>
<td>3.9 ± 0.3</td>
<td>61 ± 1</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>20</td>
<td>54 ± 1</td>
<td>5.0 ± 0.3</td>
<td>63 ± 2</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>25</td>
<td>55 ± 1</td>
<td>4.3 ± 0.1</td>
<td>64 ± 2</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
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<td>55 ± 1</td>
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<td>62 ± 1</td>
<td>6.8 ± 0.2</td>
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<tr>
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<td>65 ± 1</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
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<td>3.4 ± 0.2</td>
<td>59 ± 1</td>
<td>5.5 ± 0.2</td>
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<tr>
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<td>3.5 ± 0.2</td>
<td>59 ± 1</td>
<td>5.9 ± 0.4</td>
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<td>54 ± 1</td>
<td>2.8 ± 0.2</td>
<td>57 ± 1</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
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<td>55 ± 1</td>
<td>2.3 ± 0.1</td>
<td>56 ± 2</td>
<td>3.7 ± 0.5</td>
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<tr>
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<td>57 ± 1</td>
<td>2.6 ± 0.2</td>
<td>58 ± 1</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>70</td>
<td>54 ± 1</td>
<td>2.7 ± 0.1</td>
<td>55 ± 2</td>
<td>3.8 ± 0.6</td>
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<tr>
<td>75</td>
<td>57 ± 1</td>
<td>2.4 ± 0.2</td>
<td>59 ± 1</td>
<td>3.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SEM for five rats/group.
casein or those fed diets containing from 60 to 75% casein ate significantly less \( P < 0.05 \) than rats fed intermediate levels of protein. On the final day of the experiment, food intakes of rats fed diets over the range of 5 to 55% casein were not significantly different, although animals fed diets having 60% casein or more still exhibited significantly depressed food consumption.

The ability of rats to adapt to the high protein diets and increase their total food and protein intakes (relative to metabolic body size) over time is best exemplified by comparing the responses of animals fed diets containing from 35 to 75% casein with those fed 20% casein (this level of casein is near the requirement for rapid growth and is a level of protein similar to that found in most commercial nonpurified diets). Food intake (or protein intake), when expressed relative to metabolic body size \([\text{weight}]^{0.75}\), of rats fed 20% casein was constant from d 3 through d 11. In contrast, between d 3 and 11 food intakes, relative to metabolic body size, of animals fed 35, 45, 55, 65 or 75% casein diets increased by 10, 11, 21, 42 and 52%, respectively. This graded adaptive response in animals fed high protein diets allowed them to consumed nearly the same amount of total energy per unit metabolic body size by the end of the study as rats fed lower protein diets, despite a continued protein intake in excess of needs for growth.

Liver enzyme activities. Dietary protein content of less than 20% casein had no effect on either of the enzyme activities measured; however, for groups consuming diets with more than 20% casein the activities of both GPT and SDH were elevated in almost direct proportion to the protein content of the diet. SDH activity per gram of liver increased 198-fold over the range of dietary protein concentration from 5 to 75% (fig. 1). The activity of GPT increased by ninefold over the same range of dietary protein levels, and the pattern of its increase (not shown) was similar to that seen for SDH. The relative increases in GPT and SDH activities were approximately one-half as great when expressed per gram of total liver protein (not shown).

Plasma and brain amino acid concentrations. Among the dispensable amino acids (DAA) in plasma, alanine, serine and glycine concentrations decreased by 47, 56 and 73%, respectively, from the maximum values observed in rats fed the 5% casein diet (table 2). Glutamic and aspartic acid concentrations were not affected by increased dietary protein content. The concentration of tyrosine in plasma increased as the dietary casein level was increased from 5 to 20%; it declined by 34% as the casein content of the diet was increased further from 25 to 75%.

Overall, the sum of DAA concentrations in plasma was inversely proportional to dietary protein content (fig. 2B; \( r = -0.967, P < 0.01, 13 \) degrees of freedom), with the high correlation determined primarily by changes in alanine, serine, glycine and tyrosine. Plasma total DAA concentration was also highly correlated (inversely) with both liver GPT \( (r = -0.878, P < 0.001, 13 \) degrees of freedom) and SDH \( (r = -0.849, P < 0.001, 13 \) degrees of freedom) activities.

The plasma concentration of threonine increased more than fivefold when the dietary casein content was increased from 5 to 20%; above this level, threonine concentration was inversely proportional to dietary protein content and declined by more than 60% when the casein content of the diet was increased from 25 to 75%. Plasma histidine concentration was greatest in rats fed 15%
### Table 2

**Plasma amino acid concentrations of rats adapted to different levels of dietary protein**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Plasma amino acid concen for dietary casein level, %</th>
<th>μmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
</tr>
</tbody>
</table>

**Amino acid**
- Asp
- Thr
- Ser
- Glu
- Gly
- Ala
- Val
- Ile
- Leu
- Met
- Tyr
- Phe
- Trp
- Lys
- His
- Arg

**Data**
- Data were obtained from analyses of pooled samples from five rats per group as described in the materials and methods section.
- Neutral amino acids except tryptophan: NAA = Leu + Ile + Val + Phe + Tyr.
- Neutral amino acids except tyrosine: NAA = Leu + Ile + Val + Phe + Trp.
casein and declined in proportion to further increases in dietary casein content. Lysine concentration in plasma was twice as great in rats fed the 15% casein diet as in those fed the 5% casein diet, but above 15% casein it did not change in a consistent pattern with respect to increased dietary protein content. Arginine concentration increased gradually in animals fed diets containing from 5 to 60% casein and declined from its peak value in rats fed diets containing greater than 60% casein. The plasma concentration of phenylalanine increased by 75% as dietary casein content was increased from 5 to 35%, but remained stable as the casein content of the diet was increased from 35 to 75%. The concentration of tryptophan in plasma changed in a pattern similar to that seen for phenylalanine; tryptophan concentration increased twofold when the casein content of the diet was increased from 5 to 35% and remained constant or declined slightly with further increases in dietary protein content. The concentration of methionine in plasma increased nearly 13-fold in rats when the dietary casein content was increased from 5 to 55% and declined only slightly from its peak value when the dietary protein content was increased above 55%.

In contrast to the behavior of most other IAA in plasma (fig. 2A; $r = 0.202$, not significant, 13 degrees of freedom), the branched-chain amino acid (BCAA) concentrations were elevated in direct proportion to the protein content of the diet (fig. 2C; $r = 0.982$, $P < 0.01$, 13 degrees of freedom). The sum of BCAA concentrations was nearly eightfold higher in rats fed the 75% casein diet than in those fed the 5% casein diet.

In brain, glutamic and aspartic acid concentrations were unaffected by increases in the dietary protein content (table 3). Glycine and serine concentrations in brain were greatest in rats fed the 5% casein diet and declined by 28 and 45%, respectively, when the casein content of the diet was increased from 5 to 75%. Brain alanine content was at its peak value in rats fed the 10% casein diet and did not change consistently as dietary protein content increased.

The concentration of tyrosine in brain was greatest in animals fed either 10 or 15%...
### Table 3
Brain amino acid concentrations of rats adapted to different levels of dietary protein

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
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<th>50</th>
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<th>60</th>
<th>65</th>
<th>70</th>
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<tbody>
<tr>
<td></td>
<td>μmol/g</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>4.001</td>
<td>5.150</td>
<td>4.644</td>
<td>4.790</td>
<td>4.751</td>
<td>5.242</td>
<td>5.536</td>
<td>5.853</td>
<td>5.415</td>
<td>4.927</td>
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<td>5.322</td>
<td>5.201</td>
<td>5.241</td>
<td>4.735</td>
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<tr>
<td>Thr</td>
<td>0.432</td>
<td>0.827</td>
<td>1.282</td>
<td>1.375</td>
<td>1.164</td>
<td>1.089</td>
<td>1.050</td>
<td>1.077</td>
<td>0.822</td>
<td>0.675</td>
<td>0.711</td>
<td>0.666</td>
<td>0.579</td>
<td>0.563</td>
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<td>Ser</td>
<td>1.881</td>
<td>1.658</td>
<td>1.431</td>
<td>1.295</td>
<td>1.199</td>
<td>1.343</td>
<td>1.242</td>
<td>1.365</td>
<td>1.234</td>
<td>1.045</td>
<td>1.113</td>
<td>1.103</td>
<td>1.044</td>
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<td>1.025</td>
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<td>Gly</td>
<td>0.991</td>
<td>0.825</td>
<td>0.875</td>
<td>0.811</td>
<td>0.735</td>
<td>0.830</td>
<td>0.793</td>
<td>0.820</td>
<td>0.779</td>
<td>0.724</td>
<td>0.776</td>
<td>0.820</td>
<td>0.786</td>
<td>0.757</td>
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<td>0.702</td>
<td>0.606</td>
<td>0.612</td>
<td>0.586</td>
<td>0.628</td>
<td>0.603</td>
<td>0.687</td>
<td>0.624</td>
<td>0.517</td>
<td>0.599</td>
<td>0.601</td>
<td>0.537</td>
<td>0.634</td>
<td>0.553</td>
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<tr>
<td>Val</td>
<td>0.101</td>
<td>0.101</td>
<td>0.085</td>
<td>0.095</td>
<td>0.110</td>
<td>0.140</td>
<td>0.152</td>
<td>0.177</td>
<td>0.168</td>
<td>0.184</td>
<td>0.231</td>
<td>0.223</td>
<td>0.245</td>
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<tr>
<td>Ile</td>
<td>0.054</td>
<td>0.051</td>
<td>0.025</td>
<td>0.030</td>
<td>0.035</td>
<td>0.046</td>
<td>0.041</td>
<td>0.050</td>
<td>0.055</td>
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1Data were obtained from analyses of pooled samples from five rats per group as described in the materials and methods section.
casein, and its concentration in rats fed diets containing from 15 to 75% casein was inversely proportional to the dietary protein content. Over the entire range of dietary-protein levels examined, the ratio of tyrosine concentration in plasma to the sum of the concentrations of other large NAA (expressed tyrosine/NAA; NAA = valine + leucine + isoleucine + phenylalanine + tryptophan) was a good index of brain tyrosine content ($r = 0.984, P < 0.001, 13$ degrees of freedom).

Changes in threonine content of brain with increasing level of dietary protein were similar to those observed in plasma; brain threonine concentration increased in proportion to dietary protein in animals fed from 5 to 20% casein, but was inversely proportional to dietary protein in rats fed from 25 to 75% casein.

Lysine and arginine concentrations in brain did not change consistently with increased dietary protein. Brain histidine content was greatest in animals fed the 5% casein diet and declined steadily in rats consuming diets containing from 10 to 70% casein. In animals fed diets containing from 5 to 20% casein, phenylalanine concentrations in brain declined in proportion to dietary protein increases. Brain phenylalanine content remained stable over the range of casein levels from 20 to 40%, and above this level of casein it was inversely proportional to the protein content of the diet. The concentration of tryptophan in brain was greatest in rats fed either 10 or 15% casein; with diets containing greater than 40% casein, brain tryptophan content was inversely proportional to the protein content of the diet and decreased by more than 35% in rats fed the 75% casein diet compared with values for animals fed from 20 to 40% casein. Brain tryptophan concentration was highly correlated ($r = 0.851, P < 0.001, 13$ degrees of freedom) with the ratio of tryptophan concentration to the sum of the concentrations of other large NAA (tryptophan/NAA; NAA = valine + leucine + isoleucine + phenylalanine + tyrosine) in the plasma.

Methionine concentrations in brain were highly variable and did not change consistently with increases in dietary protein level. BCAA concentrations, whether considered individually or as a sum, declined slightly with increases in dietary protein content in groups of rats fed diets containing from 5 to 15% casein. In rats fed from 15 to 75% casein, BCAA concentrations increased linearly with dietary casein additions such that in rats fed the 75% casein diet, total BCAA concentration of the brain was nearly threefold higher than in rats fed the 15% casein diet.

Despite a 15-fold range in dietary protein content and a 13-fold range in actual protein intake during the 8 h prior to the time samples were taken, the total content of IAA in brain was maintained within a relatively narrow range of concentrations from 1.5 to 2.3 μmol/g (fig. 3).

*Brain neurotransmitter and metabolite concentrations.* No relationship was observed between dietary protein content or the actual amount of casein ingested and whole-brain NE concentration (fig. 4A). Likewise, no significant relationship was found between brain DA concentration and either dietary protein content or protein intake (fig. 4B). Brain tyrosine concentration, which has in some studies been found to correlate positively with the rate of catecholamine synthesis (33–35), was not significantly correlated with either NE or DA concentration.

Brain content of HVA was positively and significantly correlated with brain tyrosine concentration ($r = 0.673, P < 0.01, 13$ degrees of freedom). Also, an inverse correlation was found between brain HVA concentration and long-term protein intake ($r = -0.521, 13$ degrees of freedom).

Brain DOPAC concentration in our studies was not correlated with protein intake, but was significantly correlated with both short-term (last 24 h) ($r = 0.687, P < 0.01, 13$ degrees of freedom) and long-term (cumulative 11-d) food intake ($r = 0.740, P < 0.002, 13$ degrees of freedom). Although brain tyrosine and HVA concentrations were highly correlated in these studies, brain tyrosine concentration was poorly correlated with brain DOPAC concentration.

Whole-brain 5-HT concentration was not affected consistently by changes in protein content of the diet or protein intake (fig. 4C)
Fig. 3  Brain total indispensable amino acid content in relation to protein intake (per 100 g body weight) by rats adapted for 11 d to diets containing from 5 to 75% casein. Rats were killed immediately after feeding period on the final day. Points represent results from analyses of pooled brain samples from five rats per group as described in the materials and methods section. Rats fed diets containing 5 or 10% casein are shown by open circles.

Also, the concentration of tryptophan in brain was poorly correlated with brain 5-HT content. The concentration of 5-HIAA in brain, however, was positively and significantly correlated with brain tryptophan content ($r = 0.827, P < 0.001$, 13 degrees of freedom). Since brain tryptophan content was negatively correlated with protein intake in these studies, brain 5-HIAA concentration was also inversely correlated with both short-term (last 24 h) ($r = -0.721, P < 0.01$, 13 degrees of freedom) and long-term (cumulative 11-d) ($r = -0.645, P < 0.01$, 13 degrees of freedom) protein intake (fig. 4C). In addition, as a result of changes in brain 5-HIAA content in response to dietary treatment, the sum of brain 5-HT and 5-HIAA concentrations (not shown), often used as an index of the rate of serotonin turnover, was highly inversely correlated ($r = -0.707, P < 0.01$, 13 degrees of freedom) with the protein concentration of the diet.

DISCUSSION

Dietary protein content, blood and brain amino acids and food intake. These results are consistent with the concept that food intake of animals consuming diets graded in protein content is related to their capacity to degrade the amino acids ingested in excess of needs for tissue protein synthesis. When first exposed to a diet containing excess amino acids, food intake is depressed in direct proportion to the degree of amino acid surplus (1, 2, 5, 13, 36). The depression of food intake is associated with elevated concentrations of total IAA in plasma (1, 4) and brain (4). As the rat adapts to a high protein diet, the capacity of the liver to degrade most amino acids increases, and the concentrations of amino acids other than the BCAA in the peripheral blood do not exceed those of animals consuming much less protein (1, 5, 8).

In our studies, the improved growth and food intake of animals after they had been consuming a high protein diet for 3 d or longer is consistent with the time course of the increase in catabolic enzyme activities for amino acids in the liver, as observed previously by others with similar dietary conditions (1, 5, 36–39). The sensitivity of the adaptive mechanism to increasing protein intake is exemplified by the incremental
increases observed in liver GPT and SDH activities, which were almost directly proportional to the degree of dietary amino acid excess (fig. 1). As a result of metabolic adaptation, the plasma concentrations of most IAA (except BCAA) of animals fed the 75% casein diet were as low as, or lower than, those of animals fed only 35 to 40% casein.

Unlike most other amino acid catabolic enzyme systems, the aminotransferase responsible for the initial step in the degradation of the BCAA is not concentrated in liver (40) and does not increase in activity when the dietary protein content exceeds the requirement (41). Also, in contrast to most other IAA, BCAA tend to accumulate in plasma and brain directly in proportion to their concentrations in the diet (12, 13, 42). Neither the physiological nor the behavioral significance of this strong correlation between protein intake and the concentration of BCAA in plasma and brain (1, 4, 12, 13, 42, 43) has been established. Uptake of amino acids into the brain is dependent on the activity of at least three separate blood-brain barrier transport systems, which demonstrate affinities for acidic, basic or neutral amino acids, and the competition for transport among amino acids that share the same carrier mechanism is a prominent feature of brain amino acid uptake (44-47). The accumulation of BCAA in blood plasma and brain extracellular fluid when animals ingest a high protein diet may protect the brain and certain neurons, in particular, from large influxes of amino acids that are precursors of neuroactive compounds.

In our studies, competition for transport among large NAAs can explain, in part, the changes observed in their concentrations and patterns in brain relative to changes in protein intake. BCAA concentrations in brain were directly proportional to their plasma concentrations, which in turn increased linearly with additions of protein to the diet. As the plasma concentrations of most other NAAs were either unaltered or reduced with increases in dietary protein, the accumulation of BCAA would be expected to reduce the influx of other large NAAs (i.e., tyrosine, phenylalanine, tryptophan, methionine) into brain. Such an effect was especially notable for tryptophan and
tyrosine, whose concentrations in brain were inversely proportional to the sum of BCAA concentrations in the plasma (which accounted for the greatest proportion of the total large NAA content of plasma).

The decline in the concentrations of threonine, histidine and the aromatic amino acids in the brain of animals fed high protein diets was offset by increases in the concentrations of BCAA in brain, such that the total concentration of NAA (or of IAA) was maintained between 1.5 and 2.3 μmol/g over the entire range of dietary protein levels tested. In other studies (6), in which rats were given a single meal of a diet containing from 0 to 55% casein, the sum of the concentrations of IAA was maintained within the same narrow range of values by downward adjustments in food intake of animals consuming high protein diets. In the present study, in contrast to those findings in short-term studies, when animals were given time to adapt to diets having different concentrations of protein and were consuming protein in proportion to the protein content of the diet, a relatively constant level of total IAA was maintained in the brain through adjustments of the animal's capacity to degrade amino acids. Thus, despite a sixfold range of protein intakes (per unit body weight), total IAA content of the brain was maintained between 1.53 and 2.33 nmol/g. These observations, together with those on the depression of intake of high protein diets during the early adaptive period, suggest that animals control protein intake at a level that maintains total concentration of IAA in the brain between some desirable minimum and tolerable maximum limits.

Previous studies (48, 49) have shown that feeding a protein-free or a low protein diet causes a rapid reduction in brain IAA concentrations. In the present study and in other studies (50–52), rats fed a low protein diet ate the same amount or more food per unit of body weight than rats fed a diet providing an adequate amount of protein. Animals fed low protein diets may consume excess calories in an effort to meet requirements and to maintain brain IAA content above the desirable minimum limit. Nonetheless, rats fed the low protein diets in the present study were unable to consume enough protein to support rapid growth, presumably because protein intake was limited by their inability to dispose of dietary sources of energy in excess of their needs for growth (53–55).

Protein intake and brain neurotransmitter concentrations. Wurtman, Fernstrom and associates (16, 17, 33) have demonstrated that ratios of tryptophan to NAA and tyrosine to NAA in plasma are influenced by the carbohydrate and protein contents of the diet consumed and that there is a direct relationship between the magnitude of these ratios and concentrations of tryptophan and tyrosine, respectively, in the brain. Furthermore, under certain conditions, the rates of synthesis of 5-HT and the catecholamines have been shown to depend on the concentrations of their amino acid precursors, tryptophan and tyrosine, respectively, in the brain (18).

In studies of the rats allowed to select from two diets differing in protein content, Ashley and Anderson (14, 15) noted that there was an inverse relationship between protein intake and the ratio of tryptophan to NAA in the plasma measured on the last day of 2- to 4-wk long experiments. Although 5-HT concentrations in the brain were not measured in their studies, Anderson and associates (19, 20, 56) proposed, on the basis of the relationships observed previously between tryptophan/NAA of the plasma and 5-HT concentrations in the brain (18), that protein intake is regulated by diet-induced changes in 5-HT synthesis in the brain. On the other hand, results of studies in which pharmacologic agents were used to modify neurotransmitter content of the brain have indicated that the serotonergic system is involved in the regulation of carbohydrate intake (21, 22), and that changes in the activity of catecholaminergic systems of the brain may influence protein intake specifically (57).

McArthur and Blundell (58) have demonstrated that self-selection of diet by the animals can be modified by various physiological and environmental factors, and McArthur (59) has concluded that protein and carbohydrate selection may not be under fine physiological control. Leathwood and Ashley (60), in studies of rats allowed to
select sequentially from different pairs of diets did not observe a significant relationship between 5-HT concentration in the brain and protein intake. They also concluded that protein intake was not tightly controlled. We have not observed a consistent relationship between 5-HT content of the brain and protein intake (12) and did not observe changes in either protein (or carbohydrate) intake or selection by rats after they had been injected with an amount of tryptophan (100 mg/kg), which caused substantial elevations in concentrations of both 5-HT and 5-HIAA in the brain (27). It is thus evident that the observed relationships between neurotransmitter concentrations in the brain and protein or carbohydrate intake have not been consistent.

In the present study, with rats allowed to adapt for 11 d to diets differing greatly in protein content, as in the short-term studies of Fernstrom and Wurtman (18, 61), a high and significantly positive correlation was observed between the ratios of tryptophan to NAA and tyrosine to NAA in the plasma and tryptophan and tyrosine concentrations in the brain, respectively. Also, both the plasma concentration ratios and brain tryptophan and tyrosine concentrations were inversely related (tyrosine more closely than tryptophan) to dietary level of protein. However, since the concentrations of tryptophan and tyrosine in the brain were not closely correlated with 5-HT and DA, NE or DOPAC, respectively, there was no significant correlation between protein intake (or protein concentration of the diet) and the concentrations of any of these neurotransmitters or metabolites in the brain. Whole-brain 5-HIAA and HVA concentrations were, however, correlated negatively with protein intake and positively (and significantly) with tryptophan and tyrosine concentrations, respectively, in the brain. The poor correlation between protein intake and 5-HT concentration in the brain is not surprising as tryptophan-NAA ratios are generally below 0.2 with diets containing protein (12, 59, 62). The high correlation observed between tryptophan-NAA ratios and brain 5-HT in earlier studies (61) depended on modification of the diets with amino acid supplements to produce unusually high tryptophan-NAA ratios ranging between 0.4 and 0.8; no clear relationship was observed when values were below 0.2. Likewise, the high correlation previously observed (61) between tryptophan and 5-HT concentrations in the brain was primarily dependent on values from animals that received treatments that elevated tryptophan in the brain by two- to fourfold, whereas the relationship was not strong among rats displaying tryptophan values for brain in the normal range. Thus, in the present study the decline in tryptophan concentration from 0.017 mol/g to 0.010 mol/g in the brains of rats fed increasing amounts of protein may not have been large enough to produce corresponding changes in whole-brain 5-HT content.

The highly significant negative correlation observed in the present study between protein intake and whole-brain 5-HIAA content suggests that the turnover of 5-HT was progressively diminished in rats consuming graded amounts of protein. However, since the concentration of 5-HT in brain was not depressed similarly with increasing protein content of the diet, this seems unlikely. Graham-Smith (63, 64) has suggested that serotonergic neurons may synthesize 5-HT in excess of functional needs and that excess newly synthesized 5-HT may be degraded within the cell without being released. It is possible that the decrease in 5-HIAA content that we observed, as a function of increases in dietary protein level and decreasing tryptophan supply to the brain, may merely reflect a decrease in the amount of 5-HT that was synthesized in excess of functional needs. This interpretation is supported by the finding that fluoxetine, a 5-HT reuptake blocker, failed to abolish the increase in the 5-HIAA content of brain after the injection of incremental doses of tryptophan (Peters, J. C. & Harper, A. E., unpublished observations). Thus, a proportion of the 5-HIAA produced in normal animals may be derived from 5-HT that has not been released from the neuron upon depolarization.

The HVA content of brain in adapted rats was directly proportional to whole-brain tyrosine concentration and was inversely proportional to dietary protein. This observation is somewhat different from that of Gibson and Wurtman (34), who found that
the synthesis and turnover of brain NE was directly proportional to the tyrosine content of brain, which, in other studies, was directly proportional to the protein content of the diet (33). The relationship between tyrosine concentration in the brain and dietary protein evidently changes during the course of adaptation to a high protein intake in the rat.

The highly significant correlation between the DOPAC content of brain and either short-term or long-term food intake may not necessarily be the result of diet-induced changes in DA metabolism of the brain. Other workers have found that the act of feeding is itself associated with increased dopaminergic activity and increased DOPAC content of the brain (65). It was concluded that feeding-induced increases in DA metabolism were not dependent on the nutrient content of the diet. Similarly, Biggio et al. (66) observed elevated concentrations of both DOPAC and HVA in fed compared with fasted rats, and these changes were independent of changes in the tyrosine concentration in the brain.

CONCLUSIONS

The most striking and consistent relationships between protein intake and the variables measured in these studies of rats allowed to adapt to a wide range of dietary levels of protein were those between protein intake and the concentrations of certain amino acids in plasma and brain. Notable among these were: 1) the direct relationship between BCAA concentrations in plasma and protein intake over the entire range of dietary protein levels studied; 2) the direct relationship between BCAA concentrations in the brain and protein intake in the range of dietary protein levels above 15%; 3) the failure of IAA (other than the BCAA) concentrations in plasma to increase with increasing protein intake in the range of dietary protein levels above about 20% and 4) the inverse relationship between concentrations of threonine, phenylalanine, tyrosine, tryptophan and histidine in the brain and protein intake in the range of dietary protein levels above about 15%. The pattern of change in IAA concentrations of brain as the result of the combination of metabolic adaptations that increase amino acid degrading capacity and the elevations in plasma BCAA that compete with other NAA for uptake into brain, were such as to maintain the total IAA concentration of brain below 2.5 mM. We have obtained similar results in short-term studies (6, 48) in which rats, previously adapted to a 20% casein diet, were fed only a single meal of a diet containing up to 55% protein. In those studies, animals fed diets containing 45% casein or more exhibited depressed food intake that was associated with a total IAA content of brain that approached 2.5 mM. Although a consistent association has been found between elevated or depressed IAA content of the brain and depressed food intake, the data do not provide proof that changes in IAA content of the brain actually cause alterations in feeding behavior. However, these observations indicate that protein consumption is controlled, even at the expense of adequate caloric intake, at a level that will preclude an inordinate increase (or decrease) in the concentrations of IAA in the brain.

Despite a remarkable ability to adapt to a high protein diet, rats so adapted will shift to a much lower protein intake if they are allowed to self-select between diets differing in protein content (13). What serves as the signal to bring about this shift has not been established. The changes observed in brain neurotransmitter concentrations would appear to be neither sufficiently consistent nor large enough to serve as a reliable signal (12, 58–60). In the present study, the two responses to a high protein intake that persisted after the period of adaptation were elevated concentrations of BCAA in the brain and depressed concentrations of several other IAA in the brain. We considered previously (6, 13) that the elevated BCAA concentrations in the brain might serve as a signal of excessive protein intake, but results of self-selection studies (Anderson, S. A. & Harper, A. E., unpublished observations) in which BCAA content of the diet was manipulated independently of the protein content have not supported this assumption. The depressions in the concentrations of several IAA in the brain of rats adapted to a high protein intake are striking. Some of the values for threonine, phenylalanine, tyrosine and histidine were
only about one-third of those for rats consuming diets with protein contents from 15 to 25%. Both depressed food intake and preference for a diet having a balanced pattern of amino acids are associated with depressed concentrations of specific IAA in the brain of rats consuming diets with imbalanced amino acid patterns (6). The possibility that consumption of a large amount of protein results in a metabolically induced amino acid imbalance deserves serious consideration.

LITERATURE CITED


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