Brain tryptophan concentrations and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals\textsuperscript{1–3}

Madelyn H Fernstrom and John D Fernstrom

**ABSTRACT** The response of brain tryptophan concentrations and serotonin synthesis to the ingestion of two sequential meals was examined in rats. Fasted rats ingested a carbohydrate meal followed 2 h later by a protein-containing meal and were examined 2 or 4 h after the first meal. Other rats ingested a protein meal first, followed by a carbohydrate meal. When the carbohydrate meal was fed first, brain tryptophan concentrations and serotonin synthesis increased at 2 h; these changes were reversed at 4 h if the second meal contained protein. When the protein meal was fed first, there were no changes in brain tryptophan or serotonin at 2 h, and a second carbohydrate meal at 2 h did not raise brain tryptophan or serotonin 2 h later. Carbohydrate ingestion 3 h after a protein meal, however, did raise brain tryptophan and serotonin 2 h later. Brain tryptophan concentrations and serotonin synthesis are thus responsive to the sequential ingestion of protein and carbohydrate meals if there is a sufficient interval between meals. *Am J Clin Nutr* 1995;61:312–9

**KEY WORDS** Tryptophan, serotonin, brain, rat, diet, dietary protein, plasma amino acids, large neutral amino acids

**Introduction**

The synthesis and release of serotonin by brain neurons is rapidly influenced by the local tryptophan (Trp) concentration (1, 2). Brain Trp concentrations, in turn, appear to reflect Trp uptake from the circulation; uptake occurs via a transport carrier, located at the blood-brain barrier (the brain capillary endothelial cells). The carrier is shared between Trp and several other large neutral amino acids (LNAA s) and is competitive (3). Changes in the blood concentration of Trp or any of the other LNAA s can thus alter competition at the transport sites and thereby influence Trp uptake into brain and brain Trp concentrations (1).

The ingestion of food is one of the most potent physiological processes to alter the blood concentrations of Trp and its LNAA competitors (principally leucine, isoleucine, valine, tyrosine, and phenylalanine). By this mechanism, food ingestion influences serotonin synthesis in brain. It has been observed that the ingestion of a largely carbohydrate, protein-free meal (the fat content is not important (4)) by fasting rats rapidly raises brain Trp concentrations and stimulates serotonin synthesis, whereas the consumption of a protein-containing meal (typically 18–40% protein by wt) fails to raise brain Trp concentrations or to stimulate serotonin synthesis, despite very large increments in serum Trp concentrations (1). The carbohydrate meal is known to produce its effects on brain Trp and serotonin via insulin secretion (5), which raises serum Trp concentrations and lowers the serum concentrations of the other LNAA s, thereby giving Trp a competitive advantage for brain transport (1). The protein-containing meal fails to raise brain Trp concentrations because its ingestion causes serum Trp concentrations and the concentrations of its transport competitors to rise by proportionally similar amounts, resulting in no net change in competition for uptake. Meal-induced changes in brain Trp concentrations thus appear to be predicted by the alterations produced by the meal in the serum concentration of Trp relative to that of the other LNAA s, a relationship that can be simply expressed as a serum ratio of the Trp concentration to the sum of the concentrations of the other LNAA s (Trp/ΣLNAA); this ratio rises when carbohydrates are ingested and fails to change after the consumption of protein-containing meals (1).

Over the past decade, the recognition that single meals can influence serum LNAA concentrations, and thus ultimately brain serotonin synthesis, has led to the suggestion that the brain may use this metabolic and neurochemical cascade to monitor the recent history of carbohydrate and/or protein consumption. Indeed, in some hypotheses of macronutrient appetite regulation, brain Trp concentrations and serotonin synthesis are viewed as fluctuating from meal to meal as a function of the protein and carbohydrate contents of the meal (6, 7). These food-induced neurochemical changes are then said to be used by the brain to decide the macronutrient selection at the next meal.

Appealing though such hypotheses are, they involve a major assumption regarding food intake and serotonin synthesis, namely that brain Trp uptake and serotonin synthesis can vary.

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\textsuperscript{2} Supported by a grant from the National Institutes of Health (HD24730) and an NIMH Research Scientist Award (MH00254) to JDF.

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Received November 29, 1993.

Accepted for publication September 8, 1994.
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on a meal-to-meal basis. However, the only studies that have examined the relationship of food ingestion to brain serotonin synthesis have involved feeding large, single meals to rats after an overnight fast. No attempts have been made to determine whether the ingestion of a carbohydrate- or protein-containing meal by a nonfasting rat would produce the same effects on brain Trp concentrations and serotonin synthesis as occur after their ingestion by fasting animals.

The present studies therefore examine the issue of whether nonfasting rats that ingest a carbohydrate- or protein-containing meal show changes in the serum LNAA pattern, brain Trp concentration, and serotonin synthesis similar to those observed in fasting rats. In particular, we conducted studies in rats fed two sequential meals after an overnight fast. The meal size (16 kcal, 67 kJ) and intermeal interval (2 h) were high, but in the physiological range for rats (8, 9), and were chosen to ensure that biochemical changes would be detected if they occurred.

Materials and methods

All experimental procedures were approved by the University of Pittsburgh Animal Care and Use Committee.

Male Sprague-Dawley rats weighing 175–200 g (Hilltop Laboratories, Scottdale, PA) were acclimated to our animal quarters for 7 d before experimentation. During this time, water and food (Purina Rodent Laboratory Chow 5001; Purina Mills, St Louis) were provided ad libitum. The animals were exposed to 12 h of light daily (0700–1900) and an ambient temperature of 22 °C. At 1700 on the day before each experiment, rats were deprived of food but not water. At 0900 the next morning, groups of seven rats were given free access to a 4-g meal (dry wt), or remained fasting. All food was consumed within 15 min. In some experiments, rats received only one meal while in others they received a second 4-g meal, presented 2 or 3 h after the first meal. All rats were decapitated 2 h after their last meal. Thirty minutes beforehand, they received an injection of m-hydroxybenzylhydrazine (NSD-1015), an inhibitor of aromatic L-amino acid decarboxylase, to allow estimation of serotonin synthesis rate (10). The brains were rapidly removed and placed on an ice-cold glass plate. The hypothalamus and pieces of cerebral cortex were removed from each brain (11) and rapidly frozen on dry ice. Blood was collected from the cervical wound into glass tubes and allowed to clot in an ice bath. The sera were then harvested after low-speed centrifugation at 1200 × g for 20 min at 4 °C and aliquoted into test tubes. All samples were stored at −70 °C until assayed.

The meals were prepared by using ingredients obtained from Teklad (Madison, WI). The 0% protein (carbohydrate) diet contained (in g/kg dry wt) 250 g sucrose, 200 g dextrose, and 460 g dextrin. The 6% protein diet (6% protein by wt, 5.9% of total energy) contained 60 g casein, 250 g sucrose, 200 g dextrose, and 400 g dextrin. The 12% protein diet (12% by wt, 11.7% of total energy) contained 120 g casein, 250 g sucrose, 200 g dextrose, and 340 g dextrin. The 24% protein diet (24% by wt, 23.5% of total energy) contained 240 g casein, 250 g sucrose, 200 g dextrose, and 220 g dextrin. The 40% protein diet (40% by wt, 39.1% of total energy) contained 400 g casein, 250 g sucrose, 200 g dextrose, and 60 g dextrin. In addition to these ingredients, each diet contained 50 g Mazola Oil (CPC International, Englewood Cliffs, NJ) and 40 g agar. The agar was mixed with 1 L water, heated, and then homogenized with the other ingredients. After cooling, the final food had a firm consistency. The energy density of the diets was ~17.2 kJ/g dry wt (4.1 kcal/g dry wt).

Trp concentrations in serum and brain were quantitated fluorometrically (12–14) with a Hitachi F-2000 (Hitachi Instruments, Danbury, CT) spectrophotofluorometer. The concentrations of other LNAAAs in serum (tyrosine, phenylalanine, leucine, isoleucine, and valine) were quantitated by using a Beckman 6300 amino acid analyzer, equipped for fluorometric detection of o-phthalaldehyde derivatives of the amino acids (15). 5-Hydroxytryptophan (5-HTP) concentrations in brain were measured by using HPLC and electrochemical detection (16).

The data were analyzed statistically by using one-way analysis of variance and the Newman-Keuls test, or Student’s t test (17).
In this study all animals rapidly consumed all of the food at both meals. The ingestion of any of the meal pairs raised serum Trp concentrations significantly over fasting values (Table 1). As in the initial experiments, the ingestion of two sequential carbohydrate meals caused the serum Trp/ILNAA to rise significantly vs fasted groups at 2 h (t test). * P < 0.05, ** P < 0.01 vs fasted groups at 2 h (t test).

Another series of studies examined the ability of a second meal of carbohydrates to increase the serum Trp/ILNAA and brain Trp and 5-HTP concentrations after an initial meal containing protein. As a preliminary step in this study, we evaluated the effects of single meals containing different amounts of protein on the serum Trp/ILNAA, and on cortical and hypothalamic concentrations of Trp and 5-HTP synthesis. As anticipated, a single meal of carbohydrates caused all of these variables to rise significantly 2 h later (Table 2). Smaller but nonetheless significant increases were also evident after inges-

**TABLE 1**

Changes in tryptophan (Trp) concentrations and 5-hydroxytryptophan (5-HTP) synthesis rate in cerebral cortex and hypothalamus in rats ingesting a carbohydrate (CHO) meal followed by a protein-containing meal.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Trp</th>
<th>Serum LNAA</th>
<th>Serum Trp/ΣLNAA</th>
<th>Cortex Trp</th>
<th>Hypothalamus Trp</th>
<th>5-HTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>μmol/L</td>
<td></td>
<td>nmol/g</td>
<td>μmol/g protein</td>
<td>ng/g</td>
</tr>
<tr>
<td>No food</td>
<td>96 ± 6</td>
<td>482 ± 14</td>
<td>0.19 ± 0.01</td>
<td>28 ± 2</td>
<td>0.34 ± 0.01</td>
<td>113 ± 7</td>
</tr>
<tr>
<td>CHO-CHO</td>
<td>140 ± 6</td>
<td>376 ± 21</td>
<td>0.38 ± 0.02</td>
<td>36 ± 2</td>
<td>0.41 ± 0.03</td>
<td>159 ± 11</td>
</tr>
<tr>
<td>CHO-6% protein</td>
<td>150 ± 5</td>
<td>437 ± 28</td>
<td>0.35 ± 0.02</td>
<td>36 ± 2</td>
<td>0.38 ± 0.01</td>
<td>171 ± 12</td>
</tr>
<tr>
<td>CHO-12% protein</td>
<td>129 ± 6</td>
<td>385 ± 27</td>
<td>0.33 ± 0.03</td>
<td>35 ± 1</td>
<td>0.35 ± 0.01</td>
<td>136 ± 8</td>
</tr>
<tr>
<td>CHO-24% protein</td>
<td>158 ± 10</td>
<td>675 ± 43</td>
<td>0.24 ± 0.01</td>
<td>28 ± 1</td>
<td>0.30 ± 0.01</td>
<td>110 ± 5</td>
</tr>
<tr>
<td>CHO-40% protein</td>
<td>159 ± 9</td>
<td>976 ± 47</td>
<td>0.16 ± 0.01</td>
<td>23 ± 1</td>
<td>0.28 ± 0.01</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>F</td>
<td>10.74</td>
<td>45.47</td>
<td>26.67</td>
<td>17.90</td>
<td>22.35</td>
<td>13.22</td>
</tr>
</tbody>
</table>

1 x ± SE. Groups of seven rats, fasted overnight, ingested at 0 h either no food or CHO (4 g dry wt). At 2 h the animals received a second meal: 4 g dry wt of 0%, 6%, 12%, 24%, or 40% protein (except for the fasted group); 90 min thereafter, all rats received NSD-1015 and were killed 30 min later. LNAA, large neutral amino acids.

2 Statistically significant vs no food values (Newman-Keuls test): * P < 0.01, ** P < 0.005.

3 Statistically significant vs no food values, P < 0.01 (ANOVA)
tion of the 6% protein meal (except for cortical Trp, which increased but not significantly so in this experiment). Consumption of the 12%, 24%, or 40% protein meals did not elevate any of the brain variables above fasting control values, though ingestion of 12% protein did cause a small rise in the serum Trp/LNAA. Also, the 40% protein meal caused no increase over fasting values in the serum Trp/LNAA despite the large rise in serum Trp concentrations, because of the substantial increments in the serum concentrations of the other LNAA. Significant reductions in cortical and hypothalamic concentrations of Trp also occurred (5-HTP synthesis also declined, but not significantly so).

Other groups of fasted rats were then given an initial meal of either carbohydrates or 6%, 12%, 24%, or 40% protein. Two hours later a second meal was offered that consisted of carbohydrates only. The rats were killed 2 h after the second meal, and had received NSD-1015 30 min beforehand. As in the other studies, serum Trp concentrations rose after the ingestion of each of the meals (Table 3). The serum Trp/LNAA and cortical and hypothalamic concentrations of Trp and 5-HTP were increased at the 4-h time point in animals consuming two consecutive carbohydrate meals (Table 3). If the rats consumed 6% protein as their first meal, the serum Trp/LNAA and all of the brain variables measured were almost as high after the ingestion of the second carbohydrate meal as they were when both meals had been carbohydrate. When the initial meal contained 12% or 24% protein, the second (carbohydrate) meal raised the serum Trp/LNAA above fasting values, but cortical and hypothalamic Trp concentrations were not significantly increased, and 5-HTP accumulation did not rise significantly over fasting values (for cortex or hypothalamus). At 40% protein, neither the serum Trp/LNAA nor any of the brain variables was significantly increased over fasting values (Table 3). The principal conclusion from these results is that a second meal of carbohydrates, 2 h after an initial meal of 12-40% protein, does not significantly increase brain Trp concentrations or serotonin synthesis.

A final series of studies was conducted to determine whether a longer interval between the first and second meals would allow a second carbohydrate meal to elevate brain Trp and 5-HTP after an initial meal of high protein content (24% protein). An intermeal period of 3 h was selected. As a preliminary step, we measured cortical and hypothalamic Trp concentrations and 5-HTP synthesis 3 h after fasting rats re-

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Trp</th>
<th>Serum LNAA</th>
<th>Serum Trp/ LNAA</th>
<th>Cortex Trp</th>
<th>Hypothalamus</th>
<th>5-HTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>μmol/L</td>
<td>Trp: LNAA</td>
<td>nmol/g</td>
<td>μmol/g protein</td>
<td>ng/g</td>
</tr>
<tr>
<td>No food</td>
<td>85 ± 8</td>
<td>603 ± 16</td>
<td>0.15 ± 0.01</td>
<td>25 ± 1</td>
<td>0.39 ± 0.01</td>
<td>124 ± 5</td>
</tr>
<tr>
<td>CHO</td>
<td>128 ± 7²</td>
<td>454 ± 28</td>
<td>0.30 ± 0.04²</td>
<td>34 ± 2²</td>
<td>0.54 ± 0.01²</td>
<td>184 ± 8²</td>
</tr>
<tr>
<td>6% Protein</td>
<td>130 ± 4²</td>
<td>464 ± 14</td>
<td>0.29 ± 0.01²</td>
<td>29 ± 2</td>
<td>0.48 ± 0.01²</td>
<td>149 ± 6²</td>
</tr>
<tr>
<td>12% Protein</td>
<td>123 ± 9²</td>
<td>578 ± 48</td>
<td>0.21 ± 0.02²</td>
<td>23 ± 2</td>
<td>0.42 ± 0.01</td>
<td>136 ± 10</td>
</tr>
<tr>
<td>24% Protein</td>
<td>137 ± 12²</td>
<td>728 ± 39²</td>
<td>0.17 ± 0.01</td>
<td>27 ± 1</td>
<td>0.38 ± 0.01</td>
<td>133 ± 5</td>
</tr>
<tr>
<td>40% Protein</td>
<td>170 ± 11²</td>
<td>1223 ± 59²</td>
<td>0.14 ± 0.01</td>
<td>20 ± 1²</td>
<td>0.35 ± 0.01³</td>
<td>111 ± 5</td>
</tr>
</tbody>
</table>

² p < 0.01, ³ p < 0.05.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Trp</th>
<th>Serum LNAA</th>
<th>Serum Trp/ LNAA</th>
<th>Cortex Trp</th>
<th>Hypothalamus</th>
<th>5-HTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>μmol/L</td>
<td>Trp: LNAA</td>
<td>nmol/g</td>
<td>μmol/g protein</td>
<td>ng/g</td>
</tr>
<tr>
<td>No food</td>
<td>142 ± 3</td>
<td>731 ± 36</td>
<td>0.20 ± 0.01</td>
<td>26 ± 1</td>
<td>0.35 ± 0.01</td>
<td>125 ± 1</td>
</tr>
<tr>
<td>CHO</td>
<td>162 ± 8²</td>
<td>497 ± 44²</td>
<td>0.33 ± 0.01²</td>
<td>33 ± 1²</td>
<td>0.41 ± 0.01²</td>
<td>181 ± 10²</td>
</tr>
<tr>
<td>6%→CHO</td>
<td>165 ± 7²</td>
<td>533 ± 13²</td>
<td>0.31 ± 0.01²</td>
<td>31 ± 1²</td>
<td>0.40 ± 0.01²</td>
<td>173 ± 10²</td>
</tr>
<tr>
<td>12%→CHO</td>
<td>190 ± 9²</td>
<td>521 ± 27²</td>
<td>0.31 ± 0.02²</td>
<td>27 ± 1</td>
<td>0.34 ± 0.02</td>
<td>152 ± 3</td>
</tr>
<tr>
<td>24%→CHO</td>
<td>184 ± 7²</td>
<td>643 ± 12²</td>
<td>0.28 ± 0.01²</td>
<td>28 ± 1</td>
<td>0.33 ± 0.01</td>
<td>141 ± 6</td>
</tr>
<tr>
<td>40%→CHO</td>
<td>194 ± 6²</td>
<td>814 ± 36²</td>
<td>0.24 ± 0.01</td>
<td>25 ± 1</td>
<td>0.35 ± 0.01</td>
<td>141 ± 12</td>
</tr>
</tbody>
</table>

¹ p < 0.05. Groups of seven male rats, fasted overnight, ingested at 0 h either no food or 4 g dry wt of 0%, 6%, 12%, 24%, or 40% protein. At 2 h the animals received a second meal of CHO (4 g dry wt, except for the fasted group); 90 min thereafter all rats received NSD-1015 and were killed 30 min later. LNAA, large neutral amino acids.

² Statistically significant vs no food values (Newman-Keuls test): ² P < 0.01, ³ P < 0.05.

² Statistically significant vs no food values, P < 0.01 (ANOVA).
received a 4-g meal (dry wt) of carbohydrates or 24% protein (Table 4). The results were very similar to those obtained 2 h after the ingestion of either meal (Table 2). That is, ingestion of the carbohydrate meal raised Trp concentrations and 5-HTP synthesis rates in brain, whereas the consumption of the 24% protein meal did not (Table 4). (Both meals raised serum Trp concentrations, with the protein-containing meal producing a greater effect, as in the 2-h studies; the serum concentrations of the other LNAAs were not measured in this experiment.) Other groups of fasted rats were then studied by using the two-meal paradigm: one group ingested two meals of carbohydrates (0% protein), a second group consumed two meals of 24% protein, and a third group consumed an initial meal of 24% protein followed by a second meal of carbohydrates (Table 5). The second meal was offered 3 h after the initial meal. As before, all rats received NSD-1015 30 min before being killed (at 5 h). The serum Trp/LNAA, cortical and hypothalamic Trp concentrations, and 5-HTP synthesis rates were all significantly higher in rats ingesting two sequential carbohydrate meals than in animals consuming two 24% protein meals. These variables were all significantly elevated as well in rats ingesting the protein meal followed by the carbohydrate meal, when compared with values in animals consuming two 24% protein meals. Thus, an intermeal interval of 3 h appears to be sufficient to allow a meal of carbohydrates subsequent to a meal of 24% protein to raise brain Trp concentrations and 5-HTP synthesis to values comparable with those obtained when two meals of carbohydrates are consumed.

**Discussion**

These results demonstrate that brain Trp concentrations and the rate of serotonin synthesis [as estimated via 5-HTP accumulation rate (10)] respond predictably to the sequential ingestion of two meals differing in protein content, though with some qualifications. An initial meal of carbohydrates (0% protein) raised brain Trp concentrations and serotonin synthesis 2 h after its ingestion. A second meal containing moderate to large amounts of protein, ingested 2 h after the first, generally reversed these effects 2 h later. If the initial meal contained protein (≥12%), brain Trp concentrations and serotonin synthesis were usually unchanged at 2 h (an anticipated result). However, a second meal containing carbohydrates (0% protein) offered at 2 h failed to raise brain Trp concentrations or to stimulate serotonin synthesis 2 h thereafter. However, if the second meal of carbohydrates was offered 3 h after the protein-containing meal, Trp concentrations and serotonin synthesis 2 h later were as high as those seen in animals consuming two meals of carbohydrates. We conclude that brain Trp concentrations and the serotonin synthesis rate, at least in the present context, can respond to the presence or absence of protein in a meal, even in nonfasted animals, but that this continued responsivity requires a significant intermeal interval.

Some of the present findings were not unexpected; namely, that when a protein meal follows a carbohydrate meal, the serum Trp/LNAA would fall and brain Trp concentrations and 5-HTP synthesis would decline. This expectation derived from some [though not all (18)] earlier reports showing that protein ingestion can lower the serum Trp/LNAA and brain Trp (19, 20). The same effect could be predicted in rats ingesting protein after a carbohydrate meal: the influx of competitors to Trp into the blood after the protein meal would lower the already elevated serum Trp/LNAA (Table 1). The unanticipated result was that a carbohydrate meal failed to raise brain Trp concentrations and 5-HTP synthesis when consumed 2 h after a protein meal (12–40% by wt; Table 3). This nonresponsiveness to carbohydrate might have been due to the lingering of elevated serum concentrations of Trp’s LNAA competitors after the first protein meal (Table 2). This would make it difficult for the second carbohydrate meal (and insulin secretion) to reduce LNAA concentrations sufficiently to raise the serum Trp/LNAA (and thus brain Trp concentrations and serotonin synthesis). This possibility is consistent with the results of an experiment in which the intermeal interval was lengthened to 3 h. In this case, the serum Trp/LNAA, brain Trp concentrations, and Trp hydroxylation rate were all increased by a carbohydrate meal that followed a 24% protein meal (Table 5). Perhaps the longer intermeal interval allowed time for serum LNAA concentrations and insulin concentrations to fall sufficiently toward baseline to permit a subsequent carbohydrate meal (and insulin secretion) to produce a marked lowering of serum LNAA concentrations, and thus an increase in the serum Trp/LNAA (Table 5).

Is this temporal difference in the ability of a carbohydrate meal to raise brain Trp concentrations and serotonin synthesis compatible with current hypotheses regarding the role of meal-induced changes in brain serotonin synthesis in carbohydrate intake regulation? The notion that carbohydrate intake is reg-

**TABLE 4**

Changes in tryptophan (Trp) concentrations and 5-hydroxytryptophan (5-HTP) synthesis rate in cerebral cortex and hypothalamus in rats 3 h after ingesting single meals containing different amounts of protein

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Trp</th>
<th>Cortex</th>
<th>Hypothalamus</th>
<th>5-HTP</th>
<th>Cortex</th>
<th>Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/L</td>
<td></td>
<td></td>
<td>ng/g</td>
<td></td>
<td>µg/g protein</td>
</tr>
<tr>
<td>No food</td>
<td>88 ± 6</td>
<td>20 ± 1</td>
<td>0.45 ± 0.02</td>
<td>113 ± 5</td>
<td>4.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>116 ± 6*</td>
<td>26 ± 1*</td>
<td>0.57 ± 0.02*</td>
<td>156 ± 4*</td>
<td>5.3 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>24% Protein</td>
<td>140 ± 7*</td>
<td>19 ± 1</td>
<td>0.47 ± 0.02*</td>
<td>115 ± 5</td>
<td>4.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>16.14*</td>
<td>17.50*</td>
<td>12.27*</td>
<td>23.59</td>
<td>20.57*</td>
<td></td>
</tr>
</tbody>
</table>

1. x ± SE. Groups of seven male rats, fasted overnight, ingested at 0 h either no food or 4 g dry wt of one of the diets indicated in the table (all 4 g was consumed); 150 min thereafter all rats received NSD-1015 and were killed 3 min later. CHO, carbohydrate.
2. Statistically significant vs no food values, P < 0.01 (Newman-Keuls test).
3. Statistically significant vs no food values, P < 0.01 (ANOVA).
TABLE 5
Changes in tryptophan (Trp) concentrations and 5-hydroxytryptophan (5-HTP) synthesis rate in cerebral cortex and hypothalamus in rats ingesting a 24% protein meal or a carbohydrate (CHO) meal followed 3 h later by a second CHO or protein meal

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Trp</th>
<th>Serum LNAA</th>
<th>Serum Trp/(\Sigma)LNAA</th>
<th>Cortex Trp</th>
<th>Hypothalamus Trp</th>
<th>Cortex 5-HTP</th>
<th>Hypothalamus 5-HTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein—protein</td>
<td>155 ± 7</td>
<td>680 ± 18</td>
<td>0.24 ± 0.01</td>
<td>21 ± 7</td>
<td>0.39 ± 0.02</td>
<td>123 ± 5</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Protein—CHO</td>
<td>157 ± 7</td>
<td>475 ± 25</td>
<td>0.34 ± 0.02</td>
<td>27 ± 12</td>
<td>0.49 ± 0.03</td>
<td>169 ± 7</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>CHO—CHO</td>
<td>132 ± 34</td>
<td>408 ± 28</td>
<td>0.34 ± 0.03</td>
<td>29 ± 12</td>
<td>0.52 ± 0.04</td>
<td>186 ± 7</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>Control (F)</td>
<td>5.47</td>
<td>26.16</td>
<td>5.88</td>
<td>8.89</td>
<td>6.10</td>
<td>25.79</td>
<td>22.08</td>
</tr>
</tbody>
</table>

1 \(\pm\) SE. Groups of seven male rats, fasted overnight, ingested at 0 h 4 g dry wt of either 0% (CHO) or 24% protein. At 3 h the animals received a second 4-g meal of protein or CHO; 90 min thereafter all rats received NSD-1015 and were killed 30 min later. Each rat consumed all of the first and second meals (4 g: 68.6 kJ, 16.4 kcal). LNAA, large neutral amino acids.

2 Statistically significant vs protein—protein values (Newman-Keuls test); \(P < 0.01\), \(j P < 0.05\).

3 Statistically significant vs protein—protein values, \(P < 0.01\) (ANOVA).

utilated, and that serotonin neurons participate in this regulation, was assembled primarily from three sets of data: 1) that single carbohydrate meals consumed by fasted rats would rapidly elevate the serum Trp/\(\Sigma\)LNAA, brain Trp concentrations, and serotonin synthesis, whereas ingestion of a protein-containing meal would not (21); 2) that the injection of drugs that selectively enhance transmission across serotonin synapses (ie, serotonin agonists and re-uptake blockers) selectively reduces the ingestion of carbohydrates by rats and humans (22–29); and 3) that rats can change their selection of carbohydrates from meal to meal in both experimental (29, 30) and free-feeding (31, 32) contexts. Together, these data have been synthesized into a negative feedback model for regulating meal-to-meal appetite for carbohydrates (30, 33). When a rat consumes carbohydrates, the serum Trp/\(\Sigma\)LNAA rises, causing brain Trp uptake and serotonin synthesis to increase (21), which presumably leads to an enhancement of neuronal serotonin release (2). The result is a reduction in carbohydrate (and an increase in protein) intake at the next meal. At the next meal, more protein (and less carbohydrate) is ingested, leading to a reduction in the serum Trp/\(\Sigma\)LNAA and a decline in brain Trp concentrations and serotonin synthesis and release. The inhibition of carbohydrate intake is thus relieved, and carbohydrate ingestion increases at the succeeding meal. Thus, this feedback mechanism, as envisioned, has both rats and humans proceeding to each meal, choosing more carbohydrate or protein on the basis of whether brain Trp concentrations and serotonin synthesis have been raised or lowered at the preceding meal. Indeed, certain forms of obesity in humans have been attributed to a breakdown in this feedback loop, with obesity resulting from a deficiency of serotonin in the brain and thus an excessive craving for carbohydrates (23).

The present results probably detract from this hypothesis because of the findings associated with feeding carbohydrate meals after protein-containing meals. For the feedback loop to work, it must be responsive to each meal. However, our data show that a rat needs 3 h after a protein-containing meal to exhibit an increase in brain serotonin synthesis when a subsequent carbohydrate meal is consumed. When the interval is 2 h, carbohydrate ingestion does not stimulate serotonin synthesis (Table 3). In a free-feeding context, rats consume many meals during the night (the normal feeding period), sometimes separated by 2 h but more often by less (8, 9, 32). Given such short time intervals, it is difficult to envision in most cases that a carbohydrate meal would be able to raise the serum Trp/\(\Sigma\)LNAA when consumed after a protein meal. Such nonresponsiveness, if it exists, makes the model untenable. However, this conclusion cannot be drawn definitively until macronutrient intake patterns are followed temporally in a free-feeding context, and accompanied by on-line measurements of serotonin synthesis or release [such as might be possible using in vivo microdialysis (34)].

The issue of the intermeal interval between protein and carbohydrate meals is the same for humans. However, because the rate of metabolism in rats is much greater than that of humans (35), a minimum intermeal interval of 3 h or less might be substantially longer in humans. Normal-weight humans eat on average 5–7 times/d (though some eat 10 times/d) (36, 37). Hence, the intermeal-intersnack intervals in humans may be too short at some times of day to allow a carbohydrate meal or snack to raise the serum Trp/\(\Sigma\)LNAA if it follows a protein-containing meal. In this case, the meal-to-meal feedback hypothesis, as described above, could not reliably work in humans. A similar argument would apply to individuals (eg, so-called “carbohydrate cravers”) who consume above-normal numbers of snacks each day (22). To clarify this issue further, it would be of interest to conduct a study in which the normal food-intake pattern of human subjects was recorded throughout the day, in association with frequent measurements of the serum Trp/\(\Sigma\)LNAA.

A broader question is whether the serum Trp/\(\Sigma\)LNAA is sufficiently responsive to meals and snacks in general to produce notable changes in serotonin synthesis and attendant brain functions. If not, then it is of little interest whether this ratio rises after one carbohydrate meal but not after another. In both cases there would be no effect on the brain (at least in relation to serotonin synthesis and function). Indeed, some investigators suggest as much. Teff et al (38) report that the modest changes in the serum Trp/\(\Sigma\)LNAA that follow the ingestion of a carbohydrate or protein-containing breakfast are not sufficient to influence cerebrospinal fluid concentrations of Trp (an index of brain Trp concentrations in humans). The ratio changes they obtained are of similar magnitude to those observed by others using similar dietary treatments (39, 40). Teff et al (38) conclude from their studies that protein and carbohydrate meals fail to alter human brain Trp concentrations sufficiently to...
influence serotonin synthesis. Delgado et al (41), however, observed a short-term, substantial lowering of mood in remitted depressed patients after the oral administration of an amino acid mixture that reduces brain Trp concentrations in rats (42). Such an effect is consistent with the well-established relationship between affective state and brain serotonin (ie, that enhancing serotonin release elevates mood, whereas reducing it depresses mood) (43). Their amino acid treatment causes a short-term reduction in the serum Trp/SLNAA (44), the magnitude of which is similar to that observed after the ingestion of carbohydrate or protein meals by humans (39, 40, 45). This recognition suggests that changes in the serum Trp/SLNAA of this size might well produce changes in brain function that signal significant alterations in brain Trp uptake and serotonin synthesis. Regardless of the ultimate resolution of this issue, note that this issue remains unresolved.

Three technical points deserve comment. First, we have focused on Trp concentrations and serotonin synthesis in the cerebral cortex and the hypothalamus. The hypothalamus was selected for study because of its role in appetite regulation (46). An examination of the ability of meals to influence biochemically serotonin neurons projecting into this region was thus of great interest, particularly because these serotonin projections are likely to be involved in food-intake regulation (47). Cerebral cortex was examined because it is a major recipient of serotonin axons and nerve terminals (48), but is not known to have a key role in food-intake regulation. Using cortex, we could thus explore whether serotonin synthesis in nerve terminals not linked to appetite control is likely to be influenced by meal-induced changes in Trp concentrations. Clearly, the results support the view that all serotonin projections are likely to be influenced by meals, a finding consistent with an earlier report that carbohydrate ingestion increases Trp concentrations and serotonin synthesis in several brain regions (49). Though it is not presently clear why all serotonin neurons should be susceptible to food-induced changes in transmitter synthesis, the effects on those projecting into the hypothalamus might be to influence local appetite circuits.

Second, the present studies have used meals of large, but normal size (8, 9). The issue of meal size is important because, in previous single meal studies using fasting rats, animals consumed a high proportion of their daily energy intake as a single, large meal. The neurochemical responses, it could thus be argued, might not be predictive of effects obtained after a meal of normal size. The present results suggest that the responses may be the same in the two dietary contexts. And third, the present results indicate that a meal containing ≥6% but <12% protein raises the serum Trp/SLNAA, brain Trp concentrations, and serotonin synthesis (Table 2). This finding differs from that of Yokogoshi and Wurtman (50), who reported that a meal containing 5% casein was associated with no postgingestion increase in the serum Trp/SLNAA (neither brain Trp concentrations nor serotonin synthesis were measured in their study). We have no simple explanation for this difference, except to note that several days before experimentation, they entrained their animals to a feeding schedule that included an extended period of fasting each day (20 h). Metabolic effects may therefore have been present during their test meal that do not occur in animals that are fasted only once, the night before the test meal is presented.

Finally, although the present results focus on serotonin’s role in food-intake sensing and regulation by the brain, serotonin neurons are but one of many neurochemically specific cells in the brain that participate in appetite regulation. Ultimately, information regarding the participation of serotonin neurons in brain circuits that monitor nutrient intake and influence appetite must be integrated with knowledge regarding other neurochemically specific neurons that function in the same brain circuitry before a full understanding of the brain’s role in food-intake regulation can be achieved.

We gratefully acknowledge the expert technical assistance of Alissa Ebaugh, Karl Kovalkovich, and Terre Constantine.

References


