



Meal ingestion, amino acids and brain neurotransmitters: Effects of dietary protein source on serotonin and catecholamine synthesis rates

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ABSTRACT

Carbohydrate ingestion raises tryptophan uptake and serotonin synthesis in rat brain. The addition of protein is generally believed only to block such increases. However, some recent evidence suggests dietary protein may not be limited to this action. In the present studies, we fed rats single meals containing one of 5 proteins (zein, wheat gluten, soy protein isolate, casein, lactalbumin, 17% by weight) or no protein, and killed them 2.5 h later, 30 min after the injection of *m*-hydroxybenzylhydrazine, to allow serotonin and catecholamine synthesis rates to be measured in brain. Blood and cerebral cortex samples were analyzed for tryptophan and other large, neutral amino acids; 5-hydroxytryptophan and dihydroxyphenylalanine were measured in hypothalamus, hippocampus and cerebral cortex as indices of serotonin and catecholamine synthesis, respectively. An 8-fold variation occurred in cortex tryptophan: a marked decline followed zein ingestion, and modest reductions after casein or gluten. A large rise in cortex tryptophan occurred after lactalbumin consumption, and smaller increases after soy protein or carbohydrate (no protein). In the brain regions examined, a 4–8-fold range in serotonin synthesis occurred which closely followed the tryptophan alterations. No effects were observed in regional catecholamine synthesis rates. Cortical concentrations of leucine showed small changes; leucine has been linked to mTOR (mammalian target of rapamycin) signaling in brain circuits regulating food intake. The data suggest that tryptophan concentrations and serotonin synthesis in brain neurons are remarkably sensitive to which protein is present in a meal. Conceivably, this relationship might inform the brain about the nutritional quality of the protein ingested.

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1. Introduction

The rate of serotonin (5HT) synthesis in brain is tied to local concentrations of its amino acid precursor, L-tryptophan (TRP) [1]. Brain TRP concentrations, in turn, reflect uptake from the circulation, which occurs via a blood–brain barrier transport carrier shared among several large, neutral amino acids (LNAA; including TRP, tyrosine [TYR], phenylalanine [PHE], leucine [LEU], isoleucine [ILE] and valine [VAL]) [2]. Transport is saturable and competitive. Hence, TRP uptake can be modified by altering the blood concentrations of either TRP or one or more of its LNAA competitors. Physiologically, in rats, the impact of

competitive transport on brain TRP and 5HT has been most readily demonstrated by feeding them meals containing either carbohydrates, or carbohydrates in combination with proteins. The ingestion of carbohydrates was found rapidly to raise brain TRP and 5HT synthesis, while the consumption of a meal of carbohydrates and even modest amounts of protein produced no rise in TRP or 5HT [1,3]. This dichotomy occurs because carbohydrate consumption lowers the blood concentrations of TRP's competitors, but not of TRP itself, while the ingestion of protein with carbohydrates offsets the decline in blood levels of TRP's competitors. These effects of the meal on blood LNAA concentrations, in relation to TRP transport at the BBB, are more easily seen with an expression of competition, the TRP/ΣLNAA ratio ($([TRP]/[TYR + PHE + LEU + ILE + VAL])$). Following carbohydrate ingestion, the TRP/ΣLNAA ratio rises; after a protein meal, it does not [4].

In animal studies, casein has typically been used as the dietary protein. Other proteins have rarely been evaluated, to ascertain if the effect seen for casein is true for them as well. Indeed, some evidence suggests it is not: Markus et al. reported in humans that the ingestion of a meal containing lactalbumin produced an increase in the serum TRP/ΣLNAA

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ratio compared to a similar meal containing casein, and predictably altered brain functions linked to 5HT neurons (mood, prolactin and cortisol secretion [5]). This observation was subsequently examined in rats. The ingestion of a casein-based vs. a lactalbumin-based diet (~18% protein) for several days was associated with higher serum TRP/ Σ LNAAs ratios in rats ingesting lactalbumin, compared to those ingesting casein [6]. And, 5HT release from brain neurons, measured using *in vivo* microdialysis, was found to be significantly higher in rats consuming lactalbumin than in those ingesting casein [7]. Although not measured, 5HT synthesis most likely paralleled 5HT release, since 5HT release is known to reflect 5HT synthesis, in paradigms involving LNAAs administration to raise or lower brain TRP uptake and levels [8–10]. Such findings suggest that dietary proteins are not all alike, and may not simply suppress carbohydrate-induced increases in brain 5HT synthesis and release.

We therefore undertook studies to explore this possibility further. At the onset of the daily dark period, we gave rats that had been deprived of food during the day free access to meals containing one of five common dietary proteins, and measured the effects a few hours later on the serum TRP/ Σ LNAAs ratio, brain TRP concentrations and the rate of 5HT synthesis in several brain regions. We also measured the responses of two other amino acids, TYR and LEU. TYR is a precursor for catecholamine synthesis, and diet-induced changes in brain TYR levels have been tied to the rate of catecholamine synthesis [11–13]. LEU levels in brain also change in response to food ingestion [14], and LEU has recently been linked to hypothalamic neurons that influence appetite [15]. Our intent was to examine how each of these chemical signals was influenced by the protein the animals were ingesting.

2. Materials and methods

Male Sprague Dawley rats (initial body weight 125–150 g; Hilltop Lab Animals, Scottsdale, PA) were acclimated to our animal quarters for 10 d before experimentation. During this time, water and food (a 17% casein diet; see below) were provided *ad libitum*. The animals were exposed to 12 h of light daily (2200 h–1000 h; i.e., a reversed lighting cycle) and an ambient temperature of 22 °C. All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. At 2100 h the day before an experiment, rats were deprived of food but not water. At 1000 h the next morning, groups of six rats were given free access to a food containing one of the following proteins (17% by weight): casein, lactalbumin, soy protein isolate, zein (a corn protein) and wheat gluten (diet compositions given below). Other groups of six rats were given a food containing no protein, or remained fasting. Water was freely available. Two h after food presentation, all rats received an injection of *m*-hydroxybenzylhydrazine (catalog number AC22903; Fisher Scientific, Pittsburgh PA), an inhibitor of aromatic L-amino acid decarboxylase, to allow estimation of serotonin and catecholamine synthesis rates [16], and were killed 30 min later by guillotine. The brains were rapidly removed and placed on an ice-cold glass plate. The hypothalamus, hippocampi and two pieces of cerebral cortex were removed from each brain and rapidly frozen on dry ice. The hippocampi were revealed by peeling back the cerebral cortex and bisecting the corpus callosum. A spatula was used to lift and separate the underlying hippocampi [17]; care was taken not to bisect the entire brain, in order that the hypothalamus remained intact. The ventral surface of the hypothalamus was then dissected along the circle of Willis. The anterior cut at the optic chiasm revealed the fornix, which defined the dorsal extent of the hypothalamus and the dissection point from subcortical structures [18]. Blood was collected into glass tubes and allowed to clot in an ice bath. The sera were then harvested after low-speed centrifugation at 1200 \times g for 20 min at 4 °C and aliquoted into microcentrifuge tubes. All samples were stored at –80 °C until assayed.

2.1. Meal/diet composition

Meals and diets were prepared using ingredients obtained from Dyets, Inc. (Bethlehem PA), except where noted. The carbohydrate meal contained (in g/kg dry wt) 250 g sucrose, 153 g dextrose, and 415 g dextrin. The protein-containing meals contained 170 g protein source, 250 g sucrose, 153 g dextrose, and 245 g dextrin. In addition to these ingredients, each diet contained 50 g Corn Oil, 40 g Rogers-Harper Salt Mix (Catalog 200800, Dyets), 22 g Vitamin Mix (Catalog 300000, Dyets) and 70 g agar. Casein, lactalbumin and wheat gluten were obtained from Dyets; soy protein isolate was obtained from Bio-Serv (Frenchtown NJ), and zein from Freeman Industries (food grade, Catalog F4000; Tuckahoe NY). The casein diet also contained 2.55 g L-cystine/1000 g diet, dry weight [19]. The agar was mixed with 1 L water, heated, and then homogenized with the other ingredients, except for the vitamins, which were added and blended when the diet was still liquid, but just warm to the touch. When completely cooled, the final diets had a firm, cheese-like consistency. The energy density of the diets was 15.58 kJ/g dry wt (3.72 kcal/g dry wt).

2.2. Chemical measurements

The large neutral amino acids, including tryptophan, were assayed in serum and brain samples using reversed phase HPLC and electrochemical detection [20]. Briefly, *serum samples* were sonicated in absolute ethanol and centrifuged at 13,600 \times g for 10 min. The supernatants were then spun through a 0.45 μ m filter (Corning Costar 8170 Spin-X Centrifuge Tube Filter; Fisher Scientific, Pittsburgh PA) at 2000 \times g for 1 min, and aliquots of the filtrate reacted with o-phthalaldehyde (OPA) prior to injection onto the HPLC column. Samples of *cerebral cortex* were sonicated in 5 volumes of 0.1 N perchloric acid, and centrifuged at 13,600 \times g for 10 min. Aliquots of the supernatant were then passed over minicolumns of AG 50W-X4 resin (sodium form; Catalog 142–1351, Bio-Rad Laboratories, Hercules CA), prior to reaction with OPA and injection onto the HPLC. (This step removed interfering peaks, and also increased greatly the signal to background ratio for all amino acid peaks.) The minicolumns were prepared by packing a small amount of glass wool into a 200 μ l micropipette tip, and then adding 50 μ l of a thick slurry of the resin. The columns were then subjected to sequential washes with 200 μ l 0.5 M sodium phosphate (pH 6) and 200 μ l 0.1 M sodium citrate (pH 2.0). Following application, each solution was forced through the minicolumn by centrifugation at very low speed (~10 \times g; Sorvall RC5-B centrifuge, RC-3000A head, 200 rpm; Thermo Fisher Scientific, Waltham MA). Samples (200 μ l of a mixture of 100 μ l sample supernatant + 125 μ l 0.1 M sodium citrate, pH 2.0) were then pipeted onto the columns and spun, followed by a 200 μ l wash with deionized water. The amino acids were then eluted from the column with 500 μ l 0.25 M sodium phosphate (pH 6.0). An aliquot of the final eluant was then reacted with OPA (see [20]), and injected onto the HPLC. The HPLC column was an Alltech Allsphere ODS-2, 3 μ m, 100 mm \times 4.6 mm (P/N 778487; Grace, Deerfield IL). The mobile phase was 100 mmol/L Na₂HPO₄, 0.13 mmol/L Na₂EDTA, and 25% methanol, adjusted to pH 6.8 with phosphoric acid, run at 0.5 ml/min. The column temperature was maintained at 30 °C with a column heater. The effluent from the column was directed through a Coulochem III detector (ESA, Inc., Chelmsford MA); the detector settings were: guard cell +700 mV; E₁, +350 mV, R₁, 1 μ A; E₂, +650 mV, R₂, 1 μ A. Under these conditions, with a new column, representative retention times were: tyrosine, 6.5 min; valine, 8.2 min; isoleucine, 17.4 min; leucine, 20.9 min; phenylalanine, 22.2 min; tryptophan, 26.0 min.

Concentrations of individual amino acids were calculated using the external standard method. Brain values are corrected for recovery, which was determined from the recovery of known amounts of standard amino acids added to sample aliquots prior to minicolumn chromatography (72% for leucine, 56% for tyrosine, and 52% for tryptophan). The serum ratio for a given amino acid is the concentration of that amino acid

divided by the sum of the concentrations of its transport competitors (e.g., serum TRP/ Σ LNAAs = TRP/(TYR + PHE + LEU + ILE + VAL)) [14]. Using this method, serum concentrations, serum ratios and cortical concentrations of each amino acid studied were very similar to those reported previously, using other methods [3,14,21,22].

5-hydroxytryptophan and dihydroxyphenylalanine were also measured in brain samples by HPLC/electrochemical detection. Samples were sonicated in 0.1 N perchloric acid containing 200 ng/ml alpha-methyl-dopa (internal standard) and spun at 13,600 \times g for 10 min. Aliquots of the supernatant (100 μ l) were combined with 125 μ l 0.1 M sodium citrate, pH 2.0, and 200 μ l was pipetted onto AG 50W-X4 minicolumns, prepared exactly as above for amino acids. The supernatant was spun through the resin, as above, and was followed by a wash with deionized water (200 μ l). The column was then eluted with 300 μ l 0.25 M sodium phosphate, pH 6.0 [23]. Aliquots of the eluate were then injected directly onto the HPLC. The HPLC column was a Waters Symmetry C18, 5 μ , 150 \times 4.6 mm Column (P/N WAT045905; Waters Corp., Milford MA). The mobile phase was 100 mM KH₂PO₄, pH 2.75 (pH adjusted with phosphoric acid), containing 1.0 mmol/L sodium octyl sulfate, 0.1 mmol/L disodium EDTA, and 18% methanol, run at 1.2 ml/min at room temperature. The effluent from the column was directed through a Coulochem II detector (ESA, Inc., Chelmsford MA); the detector settings were: guard cell + 500 mV; E₁, -350 mV, R₁, 1 μ A; E₂, +250 mV, R₂, 1 μ A. Under these conditions, with a new column, representative retention times were: dihydroxyphenylalanine, 4.5 min; methyl-dopa, 11.9 min; 5-hydroxytryptophan, 13.6 min. Calculations were made using the internal standard method.

This study was performed twice, on two separate occasions, with very similar results. The data are presented for one study, and were analyzed statistically using one-way analysis of variance and the Newman-Keuls test (SigmaPlot 11, Systat Software, San Jose CA).

3. Results

At the time of the experiment, rats weighed 201 \pm 2 g. During the food intake period (2.5 h), rats ingested similar amounts of each diet (11–13 g wet weight, 20–24 kcal energy; ANOVA, $F = 0.676$, $P = 0.645$).

Ingestion of the meals caused marked differences in TRP concentrations in brain (cortex; cortex TRP was measured, as tissue is plentiful, and TRP concentrations are known to respond similarly to meals in all brain regions thus far examined [24]). TRP concentrations dropped (relative to fasting) to very low levels (~3 nmol/g) in rats ingesting the zein-containing diet, and rose to high levels (~24 nmol/g) in rats ingesting the lactalbumin-containing diet. Ingestion of the other diets produced values that were intermediate; the range of variation in cortical TRP values was thus about 8-fold (Table 1). The serum TRP/ Σ LNAAs ratio, an index of the competitive transport of the LNAAs across the BBB, showed the same, marked response to meal ingestion as seen

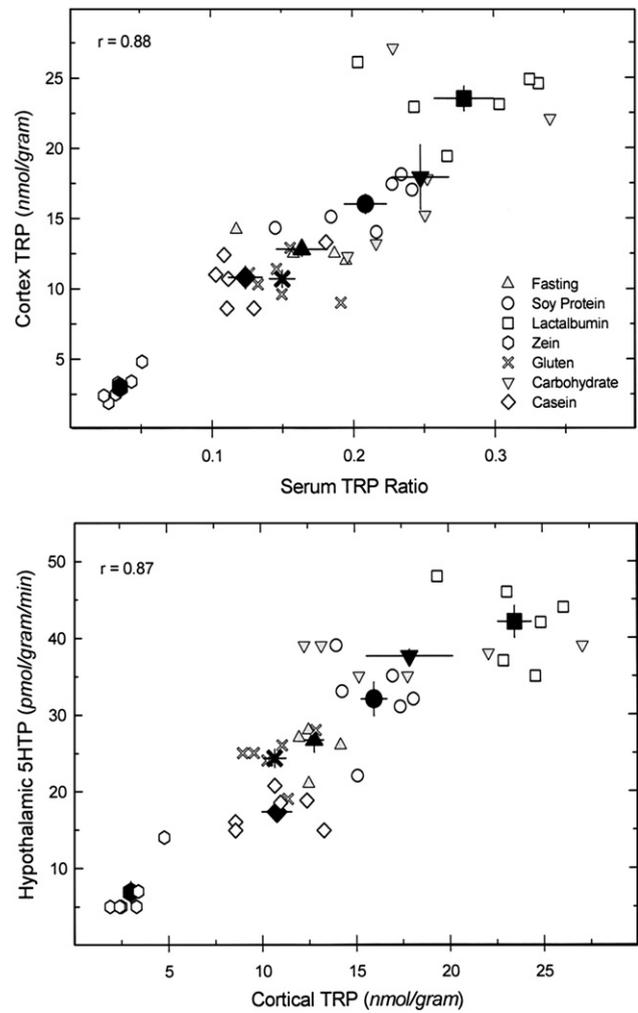


Fig. 1. Correlation between cortical TRP concentration and the serum TRP/ Σ LNAAs ratio (top panel), and hypothalamic 5HTP synthesis rate and cortical TRP concentration (bottom panel) in rats ingesting meals containing different proteins. White (open) symbols represent data from individual rats in each treatment group (symbols defined in top panel); large, black symbols are mean values for each group, and bars are standard errors of the mean. The value of the linear correlation coefficient (r) is indicated in each panel. Experimental design is summarized in legend to Table 1.

for brain TRP (Table 1), and the changes in brain TRP closely followed those in the serum TRP ratio (Fig. 1, top panel).

Meal ingestion also produced considerable changes in 5HTP (i.e., serotonin) synthesis rate, depending on the protein ingested, which

Table 1

Serum TRP ratio, cortical TRP concentrations and regional brain 5HTP synthesis rates in rats ingesting single meals differing in protein source.

Variable	Serum TRP ratio	Cortical TRP (nmol/g)	Hypothalamic 5HTP (pmol/g/min)	Hippocampal 5HTP (pmol/g/min)	Cortical 5HTP (pmol/g/min)
Fasting	0.164 \pm 0.018 ^{a,d}	12.8 \pm 0.5 ^{a,d}	26.7 \pm 1.6 ^a	13.7 \pm 0.8 ^{c,f}	13.4 \pm 1.7 ^{a,d}
Zein	0.035 \pm 0.004 ^b	3.0 \pm 0.4 ^b	6.9 \pm 1.4 ^b	4.0 \pm 0.7 ^b	2.8 \pm 0.7 ^b
Lactalbumin	0.279 \pm 0.021 ^c	23.5 \pm 0.9 ^c	42.1 \pm 2.1 ^c	18.0 \pm 1.3 ^{a,e}	14.7 \pm 0.6 ^a
Soy protein	0.209 \pm 0.015 ^{d,e}	16.0 \pm 0.7 ^{d,e}	32.0 \pm 2.2 ^d	15.3 \pm 1.0 ^{d,e,f}	11.2 \pm 0.7 ^{a,c}
Gluten	0.150 \pm 0.009 ^a	10.7 \pm 0.6 ^a	24.3 \pm 1.2 ^a	12.3 \pm 0.7 ^{c,d}	10.5 \pm 0.6 ^{c,d,e}
Casein	0.124 \pm 0.012 ^a	10.8 \pm 0.8 ^a	17.3 \pm 1.0 ^e	11.1 \pm 1.4 ^c	8.7 \pm 0.6 ^c
Carbohydrates	0.248 \pm 0.020 ^{c,e}	17.9 \pm 2.3 ^e	37.6 \pm 0.9 ^f	19.0 \pm 1.2 ^a	13.5 \pm 1.3 ^{a,e}
F value	30.803	34.226	59.982	22.988	16.642
P value	0.001	0.001	0.001	0.001	0.001

Groups of 6 male rats were fed for 10 days a diet containing 17% casein. On the final day, they were fasted during the light period, and at dark onset, received a free access to a meal containing one of the above proteins (17% by weight), or a meal lacking protein ("carbohydrates"), or nothing ("fasting"). Two h into the dark period, all rats received an injection of m-hydroxybenzylhydrazine (100 mg/kg ip), and were then killed 30 min later. The serum TRP ratio is serum TRP/(TYR + PHE + LEU + ILE + VAL). Data are means \pm SEM. Data were analyzed by ANOVA; different letters within a column indicate value comparison is significantly different, $P < 0.05$ (Newman-Keuls test).

Table 2

Serum TYR ratio, cortical TYR concentrations and regional brain DOPA synthesis rates in rats ingesting single meals differing in protein source.

Variable	Serum TYR ratio	Cortical TYR (nmol/g)	Hypothalamic DOPA (pmol/g/min)	Hippocampal DOPA (pmol/g/min)	Cortical DOPA (pmol/g/min)
Fasting	0.290 ± 0.020 ^a	106.9 ± 8.0 ^a	85.0 ± 4.1	27.1 ± 1.4	88.3 ± 15.3
Zein	0.467 ± 0.009 ^b	118.8 ± 5.7 ^a	94.8 ± 5.9	26.3 ± 2.0	65.5 ± 8.2
Lactalbumin	0.237 ± 0.007 ^c	102.9 ± 5.0 ^a	86.0 ± 5.1	29.1 ± 2.1	75.8 ± 6.9
Soy protein	0.361 ± 0.017 ^d	121.2 ± 3.8 ^a	96.6 ± 5.7	29.2 ± 1.9	80.2 ± 11.5
Gluten	0.426 ± 0.027 ^b	123.1 ± 6.5 ^a	95.1 ± 4.5	28.8 ± 2.9	103.0 ± 14.6
Casein	0.451 ± 0.023 ^b	127.1 ± 2.0 ^a	90.4 ± 1.5	35.7 ± 2.2	82.6 ± 5.5
Carbohydrates	0.227 ± 0.011 ^c	106.3 ± 6.9 ^a	90.7 ± 6.5	31.0 ± 2.3	85.3 ± 15.2
F value	32.972	2.818	0.824	2.047	0.883
P value	0.001	0.024	0.559	0.085	0.518

Experimental design described in Table 1. The serum TYR ratio is serum (TYR)/(TRP + PHE + LEU + ILE + VAL). Data are means ± SEM. Data were analyzed by ANOVA; different letters within a column indicate value comparison is significantly different, $P < 0.05$ (Newman–Keuls test).

paralleled the modifications in brain TRP (and the serum TRP ratio). Hence, for example, hypothalamic 5HTP synthesis rate declined precipitously (relative to fasting values) after ingestion of the zein-based diet (to ~7 pmol/g/min), and increased markedly following ingestion of the lactalbumin-based diet (to ~42 pmol/g/min). 5HTP synthesis values were intermediate for the other protein-containing meals, and followed closely the meal-related changes in brain TRP concentration (Fig. 1, bottom panel). The ingestion of the non-protein

meal (“carbohydrates” in Table 1 and Fig. 1) caused the expected increases in the serum TRP ratio, cortex TRP concentration, and 5HTP synthesis rates in hypothalamus and hippocampus, though in this particular experiment, not in cortex [3,24,25]. The high correlation between cortical TRP and 5HTP synthesis values seen in Fig. 1 (bottom panel) for hypothalamus was also seen in like analyses comparing cortical TRP values with 5HTP synthesis values in hippocampus and cortex (not shown; hippocampus, $r = 0.862$, $F = 107.279$, $P < 0.001$; cortex, $r = 0.864$, $F = 109.424$, $P < 0.001$).

Meal ingestion also caused changes in cortical TYR concentrations, but the magnitude was a fraction of the responses seen for TRP. Hence, the highest mean TYR values were seen following ingestion of the casein-containing meal (~127 nmol/gram), while the lowest occurred after ingesting the lactalbumin-containing meal (~103 nmol/gram) (Table 2). Nevertheless, the meal-induced changes in cortical TYR paralleled the changes in the serum TYR/ΣLNAA ratio (an indicator of its competitive BBB transport [14]) fairly well, though not as tightly as was the case for TRP (Fig. 2, top panel). However, unlike 5HTP synthesis rates, DOPA synthesis rates in hypothalamus, hippocampus and cortex showed no significant variation with meal composition (Table 2; Fig. 2, bottom panel).

Finally, cortical LEU concentrations were also examined, since LEU administration into the third ventricle has been reported to modify food intake [15]. Leucine concentrations showed significant variation with meal composition, but like TYR, the range of variation was relatively small (Table 3). Hence, following meal ingestion, the lowest cortical LEU concentrations were observed in rats ingesting the soy protein-containing meal (~47 nmol/gram), and highest in animals consuming the zein-containing meal (~85 nmol/gram), slightly less than a two-fold variation (Table 3). Serum LEU concentrations showed a much larger excursion, ranging from a low of 74 nmol/ml following carbohydrate ingestion to a high of 274 nmol/ml following zein consumption, almost a 4-fold difference (Table 3).

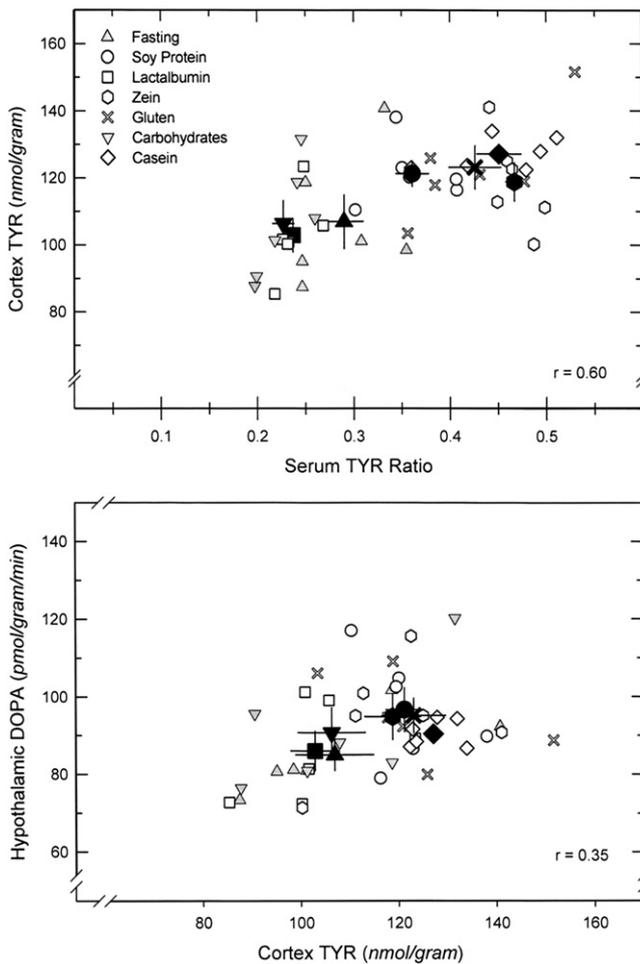


Fig. 2. Correlation between cortical TYR concentration and the serum TYR/ΣLNAA ratio (top panel), and hypothalamic DOPA synthesis rate and cortical TYR concentration (bottom panel) in rats ingesting meals containing different proteins. White (open) symbols represent data from individual rats in each treatment group (symbols defined in top panel); large, black symbols are mean values for each group, and bars are standard errors of the mean. The value of the linear correlation coefficient (r) is indicated in each panel. Experimental design is summarized in legend to Table 1.

Table 3

Serum and cortical LEU concentrations in rats ingesting single meals differing in protein source.

Variable	Serum LEU (nmol/ml)	Serum LEU ratio	Cortical LEU (nmol/g)
Fasting	89 ± 7 ^a	0.249 ± 0.013 ^{a,d,e}	53.6 ± 5.3 ^a
Zein	274 ± 12 ^b	0.632 ± 0.010 ^b	84.7 ± 7.8 ^b
Lactalbumin	253 ± 21 ^b	0.422 ± 0.018 ^c	75.4 ± 3.6 ^b
Soy protein	121 ± 13 ^{a,e}	0.210 ± 0.006 ^a	47.1 ± 0.7 ^a
Gluten	119 ± 21 ^{a,d}	0.259 ± 0.013 ^{d,e}	53.1 ± 4.4 ^a
Casein	147 ± 9 ^{c,d,e}	0.217 ± 0.008 ^{a,d}	56.8 ± 4.9 ^a
Carbohydrates	74 ± 5 ^a	0.275 ± 0.014 ^e	50.0 ± 2.8 ^a
F value	31.866	152.184	8.537
P value	0.001	0.001	0.001

Experimental design described in Table 1. The serum LEU ratio is serum (LEU)/(TRP + TYR + PHE + ILE + VAL). Data are means ± SEM. Data were analyzed by ANOVA; different letters within a column indicate value comparison is significantly different, $P < 0.05$ (Newman–Keuls test).

4. Discussion

These results demonstrate that marked variations occur in brain TRP concentrations and 5HT synthesis rates when rats ingest single meals that differ only in their source of protein. Compared to fasting values, when rats ingest lactalbumin- or soy protein isolate-containing meals, TRP and 5HT synthesis increase; when they ingest meals containing zein, they decrease. And when they ingest casein- or gluten-containing meals, TRP and 5HT synthesis do not change appreciably. The magnitude of the changes is surprisingly large, with cortical TRP values varying over a 8-fold range, and regional 5HT synthesis rates over an 4–8-fold range (Fig. 1). In contrast, in the same animals, the variations in TYR and catecholamine (DOPA) synthesis rates are modest: cortical TYR concentrations range over no more than a two-fold range, while regional catecholamine synthesis rates show no significant variation at all (Fig. 2). Similarly, cortical LEU concentrations demonstrate a relatively modest variation, about 2-fold (Table 3). Together, the results suggest that at the level of dietary protein examined, serotonin neurons, as reflected in their rate of transmitter synthesis, generate a strong signal regarding the protein ingested, while catecholamine neurons do not.

The synthesis of 5HT in brain neurons is known to vary with the supply of its precursor, TRP [1]. Because of this relationship, physiological factors that modify TRP concentrations in 5HT neurons have the potential to influence neurotransmission at 5HT synapses, since TRP-related changes in 5HT synthesis are linked to synaptic 5HT release [8–10,26,27]. Food ingestion is one such physiologic factor demonstrating this relationship [7]. Food ingestion modifies brain TRP levels indirectly, and the effect depends on how the meal alters the serum concentrations of TRP and several other LNAA that compete with it for a shared, competitive transport carrier located at the blood–brain barrier [1]. In this context, the most described effects have been the ability of a carbohydrate meal to increase TRP concentrations in serum relative to those of the other LNAA (i.e., to increase the serum TRP/ Σ LNAA ratio), causing brain TRP uptake and levels to increase, and leading to a stimulation of 5HT synthesis and release [3,28]. In contrast, the ingestion of a protein-containing meal is usually reported to lower serum TRP concentrations relative to those of its competitors (i.e., to lower the serum TRP/ Σ LNAA ratio, though absolute serum TRP levels may rise), reduce brain TRP uptake and levels, and diminish 5HT synthesis and release [3,28]. One consequence of this set of observations, that dietary carbohydrates raise, while dietary proteins lower, 5HT synthesis and release, has been to inspire numerous investigations into the impact of macronutrients on brain functions such as appetite and food cravings, and cognitive functions (e.g., [29,30]).

However, some years ago Markus et al. [5] observed that not all proteins are alike in their effect on the TRP/ Σ LNAA ratio, and reported that the ingestion of lactalbumin by humans caused the serum TRP/ Σ LNAA ratio to rise, compared to the ratio change associated with casein ingestion. This effect in humans was later pursued in rats: neuronal 5HT release was found to rise with lactalbumin ingestion, and fall with casein ingestion [7]. Clearly, not all proteins can be assumed to reduce TRP uptake and 5HT synthesis. The possibility that the ingestion of different proteins could produce more than simply reductions in brain TRP and 5HT synthesis is readily inferred from even earlier studies, such as that of Yokogoshi and Wurtman [31]. These authors fed rats 10% protein meals containing either casein, lactalbumin, egg white, peanut meal or gelatin, and measured changes in the serum TRP/ Σ LNAA ratio a few hours later. Compared to fasting, carbohydrate ingestion caused the largest rise in the ratio, with lactalbumin consumption causing an increase that was smaller by comparison, but still large. Egg white and peanut meal caused smaller increases over fasting (not significant); only casein and gelatin caused reductions. Neither brain TRP levels nor 5HT measures were included in this study. The authors' perspective was primarily oriented toward the dichotomy between carbohydrate and protein effects, namely that carbohydrates should raise brain TRP and 5HT, while proteins should

not. In this context, the clearly contrary effect of lactalbumin was not emphasized [32].

The present study examines this issue further, and includes a measure of 5HT synthesis rate, as well as comparisons with other amino acids (TYR, LEU) and transmitters (catecholamines) influenced by the ingestion of carbohydrates and proteins. The somewhat surprising finding was not how little, but indeed, how much brain TRP levels and 5HT synthesis varied with the dietary protein ingested. TRP values and 5HT synthesis in brain varied over an 8-fold range within a few hours of food ingestion (Fig. 1). These variations generally followed the proportions of TRP and the other LNAA present in the dietary proteins (the TRP/ Σ LNAA ratios in the dietary protein sources examined are: lactalbumin, 0.048; soy protein isolate, 0.033; wheat gluten, 0.029; casein, 0.026, and zein, 0.015; calculated from [33–35]). Given the size of these changes, it is remarkable they have not been reported previously.

The TYR effects are also of interest. TYR levels in brain followed the serum TYR/ Σ LNAA ratio (Fig. 2), as would be expected, since TYR is an LNAA and thus transported at the BBB by the competitive LNAA carrier [14]. However, TYR levels varied considerably less than TRP concentrations following meal ingestion, and DOPA synthesis rate (an index of catecholamine synthesis rate [16]) did not vary significantly (Fig. 2; Table 2). While catecholamine synthesis rate is responsive to rapid, meal-induced changes in TYR levels in neurons that are active, the TYR changes in the present study were not large by comparison [12,13]. However, in a chronic dietary paradigm, in which dietary protein content (not protein source) was varied, two-fold differences in brain TYR concentrations were associated with significant changes in hypothalamic DOPA synthesis [11]. Further work is thus necessary to evaluate the TYR–catecholamine relationship more fully.

Oral leucine stimulates muscle protein synthesis via the mTOR (mammalian target of rapamycin) pathway [36,37]. This relationship was recently extended to hypothalamic neurons with the report that LEU injection into the third ventricle reduced food intake in rats [15]. The effect was blocked by rapamycin, suggesting that a local mTOR signaling pathway was involved. No LEU measurements were made. This observation was recently extended by others [38], though again, no hypothalamic LEU measurements were made. In the present study using different dietary proteins, cortical LEU concentrations varied over about a two-fold range (Table 3). A similar variation has been observed when the meal's protein concentration is varied [14]. Such changes would also be expected in hypothalamus, since the blood–brain barrier amino acid transporters operate similarly in both regions (e.g., [39]). It would thus be of interest to know if 2-fold LEU variations in hypothalamus are sufficient to produce mTOR and behavioral effects like those seen following intraventricular LEU injection [15,38].

These findings may have application to human paradigms that use the effects of food and amino acids on the serum TRP/ Σ LNAA ratio to examine brain functions that involve 5HT neurons. In one paradigm, a large, oral mixture of amino acids lacking TRP reduces brain 5HT [40,41]. The clearest effect of this treatment is to lower mood [42,43]. The change in the serum TRP/ Σ LNAA ratio elicited by this treatment is smaller than that achieved in the present studies. Hence, the use of natural proteins in human studies may produce changes in the serum TRP/ Σ LNAA ratio much greater than those produced by an amino acid drink, and not have the unpleasant taste and smell associated with the ingestion of a large dose of free amino acids [44]. Conceivably, larger ratio differences might also produce greater behavioral effects. In another human paradigm, a chocolate drink containing lactalbumin or casein has been used to produce differences in the serum TRP/ Σ LNAA ratio and in behavior [45]. The present findings suggest that larger effects are likely to be seen, if the comparison is between a beverage containing zein and one containing lactalbumin.

The present findings, together with other reports [7], clearly indicate that the earlier view that dietary carbohydrates raise brain TRP and 5HT, while proteins do not, is incomplete at best. Earlier dietary studies routinely used casein as the dietary protein (e.g., [14]),

and indeed, its inclusion in a meal is associated with no increases in brain TRP levels or 5HT synthesis (Table 1; Fig. 1). However, the relationship is much more robust, as indicated in Fig. 1, in relation to what protein is ingested; carbohydrate and casein effects appear modest, and no longer unique. Indeed, not just carbohydrates, but also proteins like lactalbumin and soy protein are seen to raise brain TRP and 5HT. Such findings produce difficulties for hypotheses built on the earlier perceived relationships. For example, the concept of “carbohydrate craving” is based on the notion that only carbohydrates raise brain TRP and 5HT, and such cravers seek carbohydrate to rectify a 5HT deficiency in brain (e.g., see [30]). Based on the present findings, such is unlikely to be true. Or, if it is, there must be others who seek food to raise brain 5HT, but are drawn to foods that contain lactalbumin, soy protein, or combinations of these proteins with carbohydrates instead of pure carbohydrate foods.

Finally, we only examined one level of protein in the meals (17%). This level was selected, since it is a little above the daily requirement for protein in rats [33], and thus can be considered a normal dietary proportion. By way of comparison, humans select a level of protein in their diet that is modestly above requirement [46]. Conceivably, meals containing more or less protein may produce different effects on TRP and 5HT; further work seems warranted. In addition, one interesting implication from the present findings is that combining proteins should produce predictable, moderating effects. For example, a combination of zein and milk proteins should moderate the severe suppressing action of zein on TRP and 5HT (Fig. 1). An examination of the effects of dietary protein combinations would thus also be of interest. Given the relationship described by Fig. 1, it is possible that the effect of the protein in a meal on brain TRP and 5HT may provide the brain with some reflection of dietary protein quality, which it may use to direct behavior toward dietary protein combinations optimal for nitrogen balance and growth. One problem with such a hypothesis, however, is that carbohydrates alone raise brain TRP and 5HT, providing the brain with faulty information regarding protein ingestion (i.e., carbohydrates would appear like soy or lactalbumin). However, the brain may use more than a single signal to evaluate dietary protein quality and quantity. For example, recent data indicate that enteroendocrine cells of the gut express receptors for glutamate. Moreover, the stimulation of such receptors by luminal glutamate activates the afferent vagus [47]. Glutamate is the most prevalent amino acid in almost all dietary proteins. Perhaps, therefore, dietary glutamate sensing in the gut conveys information to brain, via the vagus, indicating the ingestion of protein, and how much. In this case, together with a TRP–5HT signal reflecting some aspect of protein quality, the carbohydrate effect would cease to be ambiguous. In such a case, it would only signal carbohydrate ingestion. Such speculations hopefully suggest interesting avenues for future studies.

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