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REVIEW ARTICLE

Large neutral amino acids: dietary effects on brain neurochemistry and function

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Abstract The ingestion of large neutral amino acids (LNAA), notably tryptophan, tyrosine and the branched-chain amino acids (BCAA), modifies tryptophan and tyrosine uptake into brain and their conversion to serotonin and catecholamines, respectively. The particular effect reflects the competitive nature of the transporter for LNAA at the blood–brain barrier. For example, raising blood tryptophan or tyrosine levels raises their uptake into brain, while raising blood BCAA levels lowers tryptophan and tyrosine uptake; serotonin and catecholamine synthesis in brain parallel the tryptophan and tyrosine changes. By changing blood LNAA levels, the ingestion of particular proteins causes surprisingly large variations in brain tryptophan uptake and serotonin synthesis, with minimal effects on tyrosine uptake and catecholamine synthesis. Such variations elicit predictable effects on mood, cognition and hormone secretion (prolactin, cortisol). The ingestion of mixtures of LNAA, particularly BCAA, lowers brain tryptophan uptake and serotonin synthesis. Though argued to improve physical performance by reducing serotonin function, such effects are generally considered modest at best. However, BCAA ingestion also lowers tyrosine uptake, and dopamine synthesis in brain. Increasing dopamine function in brain improves performance, suggesting that BCAA may fail to increase performance because dopamine is reduced. Conceivably, BCAA administered with tyrosine could prevent the decline in dopamine, while still eliciting a drop in serotonin. Such an LNAA mixture might thus prove an effective enhancer of physical performance. The thoughtful development and application of dietary proteins and LNAA mixtures may thus produce treatments with predictable and useful functional effects.

Keywords Tryptophan · Serotonin · Brain · Diet · Exercise

Abbreviations

AN	Anorexia nervosa
ATD	Acute tryptophan depletion
BBB	Blood–brain barrier
BCAA	Branched-chain amino acids
LNAA	Large neutral amino acids
NEFA	Non-esterified fatty acids

Introduction

Tryptophan and tyrosine are unique (so far) among amino acids in being precursors to brain neurotransmitters, the synthesis and release of which are sensitive to relatively small, physiologic changes in precursor concentrations (Fernstrom 1983). Hence, variations in brain tryptophan concentrations modify the synthesis and release of its transmitter product, serotonin (5-hydroxytryptamine), while variations in brain tyrosine concentration produce such effects on its products, the catecholamine neurotransmitters (dopamine, norepinephrine and epinephrine). The brain concentrations of both amino acids are readily modified by the ingestion of either amino acid, as well as other amino acids that share a competitive transporter for uptake into brain from the circulation. By modifying serotonin and catecholamine synthesis and release, tryptophan, tyrosine

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and its transport competitors can influence central nervous system function (Fernstrom 1983). Such effects can be produced either by ingesting free amino acids, or a variety of dietary proteins. This review describes these relationships, and how they have been and are being used to apply amino acid supplements and dietary proteins to the modification of mood, cognition, and physical performance.

Tryptophan, tyrosine and the factors governing the synthesis of their neurotransmitter products

Tryptophan–serotonin

Serotonin is synthesized from tryptophan via a two-step reaction. The initial step is hydroxylation, and is rate limiting for the pathway (Fig. 1). The product, 5-hydroxytryptophan, is decarboxylated to serotonin. Serotonin is released from nerve terminals when the neuron depolarizes, and interacts with serotonin receptors on adjacent neurons. Transmission is terminated by the rapid removal of transmitter from the immediate extracellular space (the synaptic cleft), mediated by a serotonin transporter. Once taken back up into the terminal, serotonin can either be metabolized by monoamine oxidase to 5-hydroxyindoleacetic acid, or stored for future release. At normal brain tryptophan concentrations, the enzyme catalyzing the initial, rate-limiting reaction (tryptophan hydroxylase) is only partly saturated with substrate. As a result, the rate of serotonin synthesis can be increased or decreased rapidly by raising or lowering brain tryptophan concentrations (Fernstrom 1983). For example, serotonin synthesis rises quickly following an injection of tryptophan into rats that raises brain tryptophan concentrations (Carlsson and Lindqvist 1978; Fernstrom and Wurtman 1971b; Moir and Eccleston 1968). The rate of serotonin synthesis can be lowered by injecting amino acids that reduce brain tryptophan concentrations. This latter effect occurs because of the mechanism by which tryptophan is taken up into the brain. Tryptophan enters brain via a transporter located on capillary endothelial cells [the locus of the "blood–brain barrier" (BBB)] (Pardridge and Oldendorf 1975), which it shares with several other amino acids [the large neutral amino acids (LNAA)]. The LNAA include tryptophan, tyrosine, phenylalanine, and the branched-chain amino acids (BCAA; leucine, isoleucine and valine). The transporter is saturable and competitive, so that raising the blood (or plasma or serum) concentration of one LNAA raises the brain uptake of that LNAA, and reduces those of the others. For example, tryptophan uptake into brain and brain tryptophan concentrations fall within 60 min of an injection of leucine, isoleucine or valine (Carlsson and Lindqvist 1978). The influence of tryptophan supply on serotonin synthesis in brain neurons is thought to be functionally important, since increases or decreases in serotonin synthesis produced by injections of tryptophan or other LNAA cause rapid (within 60–90 min) and like changes in neuronal serotonin release (Gartside et al. 1992; Sharp et al. 1992). The rate of serotonin synthesis is also influenced by neuronal activity. In rats, e.g., tryptophan hydroxylase activity rises in response to the depolarization of serotonin neurons by electrical stimulation, and falls

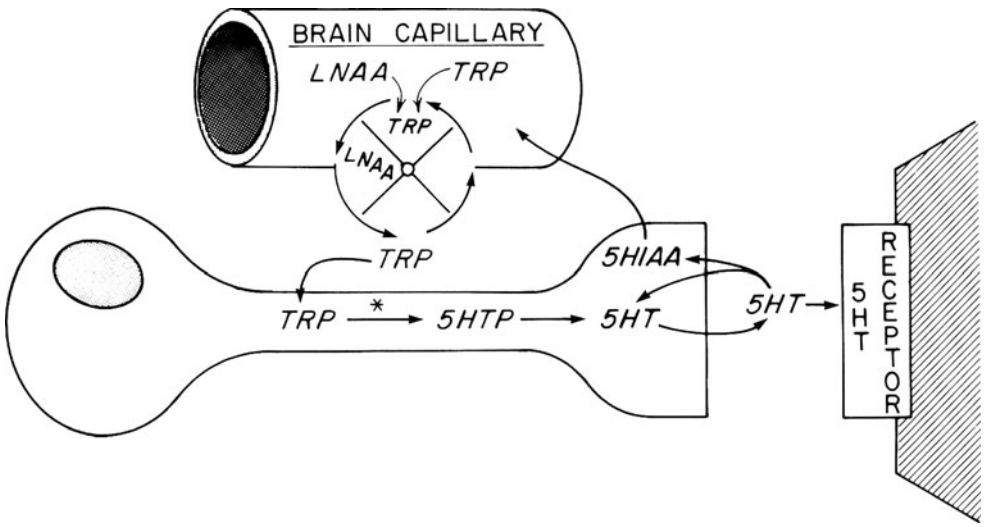

Fig. 1 Brain tryptophan uptake and serotonin (5HT) synthesis in neurons. Tryptophan (TRP) in blood is transported into brain via a carrier it shares (competitively) with other large, neutral amino acids (LNAA). In brain neurons, tryptophan is hydroxylated to 5-hydroxytryptophan (5HTP), catalyzed by tryptophan hydroxylase; 5-hydroxytryptophan is decarboxylated to serotonin, catalyzed by aromatic L-amino acid decarboxylase. Monoamine oxidase initiates the catabolism of serotonin to 5-hydroxyindoleacetic acid (5HIAA), the principal serotonin metabolite in brain. *Asterisk* indicates rate-limiting step in serotonin formation, tryptophan hydroxylation

when depolarization declines, such as following an injection of 8-hydroxy-2-(di-*n*-propylamino)tetralin, an agonist of inhibitory serotonin autoreceptors (Boadle-Biber 1993). This relationship can influence the ability of changes in brain tryptophan concentration to modify serotonin synthesis: e.g., when serotonin neurons are inactivated by 8-hydroxy-2-(di-*n*-propylamino)tetralin, an increase in brain tryptophan is less effective in stimulating serotonin synthesis than when neurons are active (Fernstrom et al. 1990).

Tyrosine–catecholamines

The catecholamine transmitters are synthesized from tyrosine. Figure 2 shows the pathway in the dopamine neuron. The initial step involves hydroxylation to dihydroxyphenylalanine, catalyzed by the enzyme tyrosine hydroxylase. Dihydroxyphenylalanine is rapidly decarboxylated to dopamine by aromatic L-amino acid decarboxylase, the same enzyme that converts 5-hydroxytryptophan to serotonin. In dopamine neurons, no additional enzymatic modification occurs. Neurons using norepinephrine as a transmitter contain one additional enzyme, dopamine-β-hydroxylase, to convert dopamine to norepinephrine. Epinephrine neurons contain one further enzyme, phenylethanolamine-*N*-methyl transferase, that converts norepinephrine to epinephrine (not shown in Fig. 2). Tyrosine hydroxylation is rate limiting in the pathway, and thus controls the overall rate of synthesis (Kaufman and Kaufman 1985). The rate of hydroxylation is governed by a number of factors, including end-product inhibition and neuronal activity (Iuvone et al. 1982; Spector et al. 1967). However, substrate availability is also a factor. Raising brain tyrosine concentrations by injecting the amino acid stimulates hydroxylation rate, while lowering brain tyrosine by injecting other LNAA (competitive LNAA transport effects at the BBB occur for tyrosine as well as for tryptophan) reduces tyrosine hydroxylation (Carlsson and Lindqvist 1978; Wurtman et al. 1974). The influence of tyrosine concentration on hydroxylation rate is sensitive to neuronal activity, and has been demonstrated most clearly using retinal dopamine neurons (the retina is part of the central nervous system). These neurons become very active when exposed to light, and are relatively inactive in darkness (Iuvone et al. 1978). In rats, injections of tyrosine (or meals) that raise retinal tyrosine concentrations stimulate tyrosine hydroxylation rate in retinal dopamine neurons, but only in light-exposed animals (Fernstrom et al. 1986; Fernstrom and Fernstrom 1987). Tyrosine injection also stimulates tyrosine hydroxylation in dopamine neurons in the brain that terminate in the corpus striatum, but only in the presence of a dopamine receptor blocker (haloperidol, 2 mg/kg bw, i.p.), which is thought indirectly to increase the activity of these neurons (Carlsson and Lindqvist 1978; Scally et al. 1977).

Tryptophan, serotonin synthesis and food ingestion

Since changes in the blood concentrations of tryptophan and the other LNAA produced by injecting amino acids rapidly modify brain tryptophan uptake and serotonin synthesis, other phenomenon that modify blood LNAA

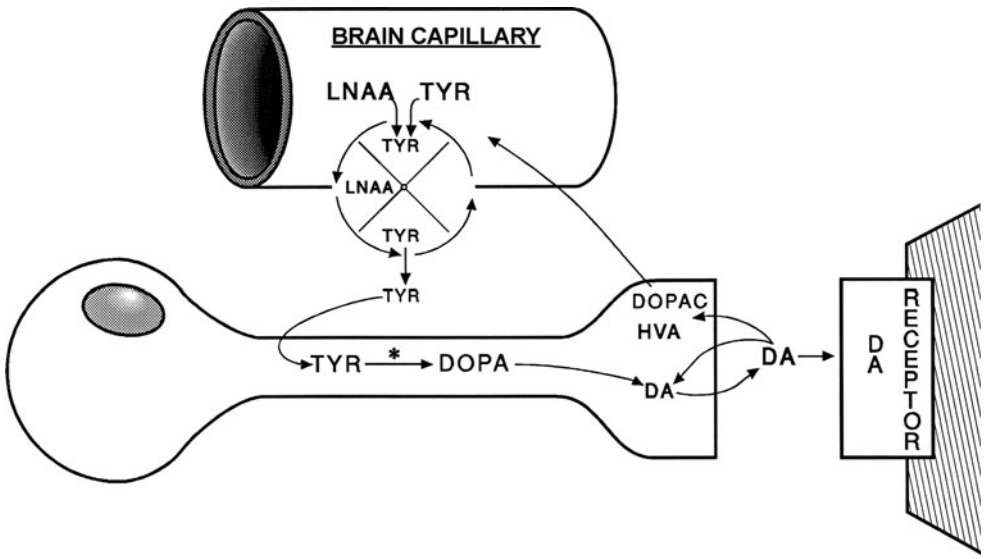

Fig. 2 Brain tyrosine uptake and dopamine (DA) synthesis in neurons. Tyrosine (TYR) in blood is transported into brain via a carrier it shares (competitively) with other large, neutral amino acids (LNAA). Tyrosine is converted to dihydroxyphenylalanine (DOPA) in neurons containing tyrosine hydroxylase (*asterisk*), the rate-limiting enzyme in catecholamine synthesis. Dihydroxyphenylalanine is decarboxylated to dopamine, catalyzed by aromatic L-amino acid decarboxylase. Dopamine is metabolized to dihydroxyphenylacetic acid (DOPAC) in a reaction initiated by monoamine oxidase, and can be further catabolized to homovanillic acid (HVA) by catechol-*O*-methyltransferase

concentrations might also be anticipated to have similar effects. One physiological example is food consumption, since the ingestion of protein supplies exogenous amino acids to the circulation, causing their concentrations to rise (for some), while the ingestion of any macronutrient induces the secretion of insulin, which directly promotes the uptake of most amino acids into peripheral tissues (such as muscle, though not brain), causing blood concentrations to fall (Crofford et al. 1964; Fukagawa et al. 1986). Consequently, the impact of a meal might be expected indirectly to alter brain tryptophan uptake (and serotonin synthesis), depending on how it modified the blood concentration of tryptophan relative to those of its LNAA transport competitors. Early studies focused on the ingestion of carbohydrates and protein. When fasting rats ingested a carbohydrate meal, brain tryptophan concentrations and serotonin synthesis increased, an effect linked to insulin action (Crandall and Fernstrom 1980; Fernstrom and Wurtman 1971a): insulin increased plasma concentrations of tryptophan [in rats, though not in humans (Fernstrom and Wurtman 1972b; Filip et al. 1974; Lipsett et al. 1973)], and decreased those of the other LNAA, thus favoring the competitive transport of tryptophan into brain. When rats ingested a protein-containing meal, brain tryptophan and serotonin did not change, a response also linked to the effect of the meal on the plasma concentrations of tryptophan and other LNAA (Fernstrom and Wurtman 1972a): the meal raised plasma tryptophan, but also the concentrations of other LNAA by a proportionally similar amount (i.e., competition for the transporter did not change). The changes in the plasma LNAA concentration linked to brain tryptophan uptake and serotonin synthesis were summarized as a plasma (or serum) ratio of tryptophan to the sum of the other LNAA [tryptophan ratio: (tryptophan)/(tyrosine + phenylalanine + leucine + isoleucine + valine)]. The ratio rises when carbohydrates are ingested, and does not rise when protein is included in a meal, in parallel with the changes in brain tryptophan and serotonin (Fernstrom and Wurtman 1972a).

These studies with protein involved a single protein, casein (a typical, high-quality animal protein), but led to a generalization: the ingestion of any protein would produce like effects (no changes in brain tryptophan concentration or serotonin synthesis) (e.g., Wurtman et al. 1980). Curiously, this view persisted for about 25 years, even though results with only a single dietary protein formed the basis for the hypothesis. It changed when Markus and associates presented evidence in humans that the ingestion of different proteins can produce different effects on the plasma tryptophan ratio and serotonin-linked behaviors (Markus et al. 2000). The proteins studied were α-lactalbumin and casein, both milk proteins. α-Lactalbumin has a higher content of tryptophan and a lower content of other LNAA than casein. Hence, when humans consumed a meal containing α-lactalbumin, the plasma tryptophan ratio rose to a higher value than that observed when a meal containing casein was ingested. Predictable alterations in brain functions linked to serotonin neurons also occurred (mood, prolactin and cortisol secretion) (Markus et al. 2000). Subsequently, Feurte et al. (2001) reported that plasma tryptophan ratios similar to those observed in humans occurred in rats ingesting these proteins. Orosco et al. (2004) observed that neuronal serotonin release in brain was greater in rats ingesting an α-lactalbumin-containing meal than in those consuming casein, in parallel with the changes in the plasma tryptophan ratio (Feurte et al. 2001). While not measured, the rate of serotonin synthesis most likely paralleled that of serotonin release, inasmuch as serotonin release reflects serotonin synthesis in paradigms involving LNAA administration to raise or lower brain tryptophan uptake and concentrations (Gartside et al. 1992; Sharp et al. 1992). These findings suggested that dietary proteins are not all alike in their effects on brain tryptophan concentrations, and on serotonin synthesis and release.

If a predictor of the effect of ingesting protein on the plasma tryptophan ratio is the tryptophan ratio of the protein itself, there are much greater variations in the tryptophan ratio of proteins than that occurring between α-lactalbumin and casein. We therefore explored whether the ingestion of a variety of dietary proteins by rats might produce larger differences in the serum tryptophan ratio (and brain tryptophan concentrations and serotonin synthesis) than that seen between casein and α-lactalbumin. To this end, we fed groups of rats a meal containing one of several proteins, and measured the effects on the serum tryptophan ratio, brain tryptophan concentration, and serotonin synthesis rate (Choi et al. 2009). The test meals were prepared using a standard rat diet formulation, which all rats consumed as their stock diet for the week before the experiment. This diet contained approximately 17 % protein (% dry weight), typical for rat diets, and all other essential nutrients (Reeves et al. 1993). Casein was employed as the protein in the stock diet because it is a standard protein used in formulated diets (Reeves et al. 1993). On the day of an experiment, rats were deprived of food during the light period (when they normally eat little), and then given access to a diet at dark onset [when they normally eat (Rosenwasser et al. 1981)]. The food presented was the same formulation as their stock diet, differing only in the protein. One group received the stock casein diet; other groups received this same formulation, but containing in lieu of casein α-lactalbumin, soy protein, wheat gluten, or corn zein (17 % dry weight). Another group consumed a meal of similar formulation, but containing no protein (a carbohydrate meal), and yet another group remained fasting. Two hours later, all rats received a

drug (*m*-hydroxybenzylhydrazine, 100 mg/kg bw, i.p.) that allowed measurement of serotonin synthesis rate, and were killed 30 min later. [The method estimates serotonin synthesis rate by measuring the accumulation for 30 min of the product of the first and rate-limiting step in the pathway, 5-hydroxytryptophan, after blockade of the second, decarboxylase step by the drug (see Fig. 1) (Carlsson and Lindqvist 1978).]

The results are presented graphically in Fig. 3. First, rats ingesting α-lactalbumin (squares in top panel) had a higher serum tryptophan ratio and cerebral cortical tryptophan concentration than those consuming casein [diamonds in figure; cortex tryptophan reflects tryptophan concentrations in other brain regions (Colmenares et al. 1975)]. In addition, serotonin synthesis rate in hypothalamus, a prominent locus of serotonin nerve terminals (bottom panel of figure, reported as 5-hydroxytryptophan synthesis), was greater in rats ingesting α-lactalbumin than in those consuming casein (Choi et al. 2009). Other brain regions showed similar effects (cerebral cortex, hippocampus). These results are consistent with the findings of Feurte et al. (2001) and Orosco et al. (2004) in rats, and those of Markus et al. (2000) in humans. Second, much larger differences are evident for other dietary proteins. For example, the difference in effect between ingesting zein (a tryptophan-poor plant protein; hexagons in Fig. 3) and α-lactalbumin (a tryptophan-rich animal protein) is much greater for each variable measured (serum tryptophan ratio, cortex tryptophan, hypothalamic 5-hydroxytryptophan synthesis) than that seen between α-lactalbumin and casein. Values for rats ingesting soy protein (circles in figure) are higher than those for casein, while wheat gluten ("multi" symbols in figure) values are similar to those for casein. Third, the figures also show a remarkably close correlation between the serum tryptophan ratio and cortical tryptophan concentrations (top panel), and cortical tryptophan and 5-hydroxytryptophan synthesis rate (bottom panel), supporting the notions that (a) competition among LNAA is a key feature of the transport of tryptophan into brain, and (b) the tryptophan concentration is an important determinant of the rate of serotonin synthesis. Fourth, the ingestion of the carbohydrate meal (no protein), as expected (Fernstrom and Wurtman 1971a; Fernstrom and Fernstrom 1995a), elevated the serum tryptophan ratio, cortical tryptophan and serotonin synthesis rate (inverted triangles in figure) over fasting values (triangles in figure). The carbohydrate treatment was included as a positive control. It was also included to evaluate how large the changes were for individual proteins, in comparison to those for carbohydrates alone. In an earlier hypothesis, in which carbohydrates, but not proteins, were postulated to increase brain tryptophan and serotonin, this effect of carbohydrates was hypothesized to be a unique signal to brain during a meal to

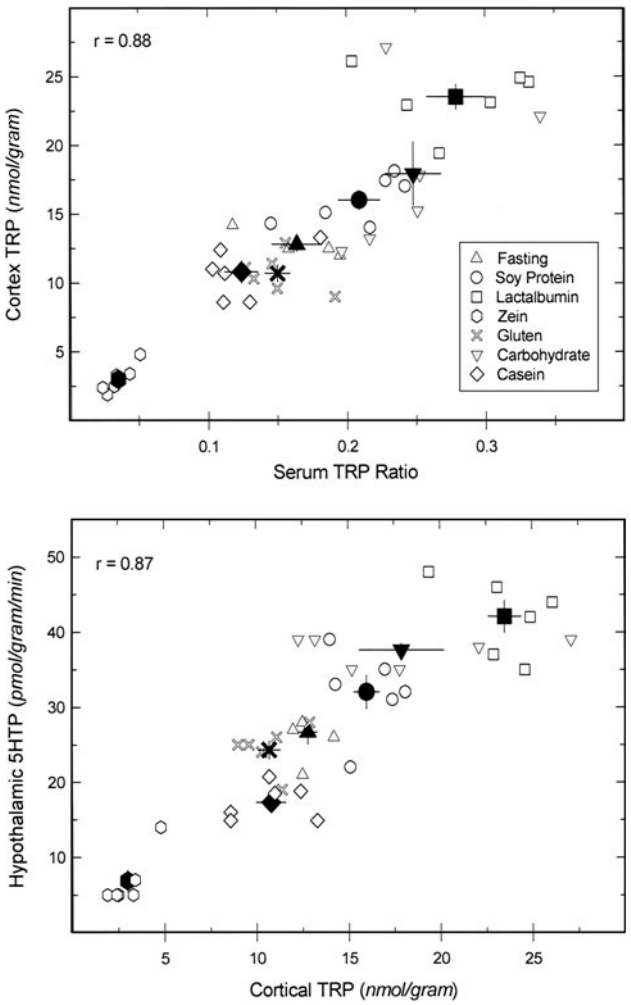

Fig. 3 Brain tryptophan concentrations and serotonin synthesis in rats ingesting a single meal containing one of several dietary proteins. Rats were fasted during the daily light period, and supplied with food at the onset of the daily dark period. Blood and brain samples were obtained 2.5 h into the dark period, after they had consumed a meal and then received a drug (*m*-hydroxybenzylhydrazine, 100 mg/kg bw, i.p.) to allow serotonin synthesis to be estimated (30 min before sacrifice). Tryptophan was measured in cerebral cortex (representative of all brain regions); 5-hydroxytryptophan (an index of serotonin synthesis rate) was measured in hypothalamus. *Large, black symbols* represent group means; *lines* are standard error bars. *Small white symbols* are data from individual animals. The dietary protein represented by each *symbol* is indicated in the figure. Adapted from Choi et al. (2009)

reduce the desire to ingest carbohydrates (Wurtman and Wurtman 1979). A corollary notion was that individuals with below normal serotonin transmission in brain might unknowingly seek out carbohydrate-containing foods to elevate serotonin synthesis and release (dubbed "carbohydrate cravers") to ameliorate disorders linked to low serotonin, such as depression (Wurtman et al. 1987; Wurtman 2011). Clearly, these notions cannot be correct, given that carbohydrate ingestion not only produces

stimulatory effects on TRP and 5HT that are smaller than those observed following α-lactalbumin ingestion, but more generally, such effects are quite modest compared to those elicited by the range of proteins tested, at least in rats. Hence, if carbohydrate craving exists, it is probably not related to the effects of carbohydrate ingestion on brain tryptophan uptake and serotonin synthesis/release, as originally proposed: individuals unknowingly attempting to elevate low serotonin levels would presumably identify protein foods (e.g., those containing soy protein) that produce larger increases in tryptophan and serotonin than those produced by carbohydrates. Incidentally, it is also no small point that in humans, carbohydrate ingestion does not appear to modify the tryptophan ratio or indices of serotonin synthesis and release (Ashley et al. 1982, 1985; Schweiger et al. 1986; Teff et al. 1989a, b), further to the detriment of the carbohydrate craver hypothesis as it relates to tryptophan and serotonin.

In this study, we also examined the effect of ingesting these proteins on the synthesis of catecholamines in brain. As noted above, tyrosine hydroxylation rate is influenced by local concentrations of tyrosine. Moreover, the ingestion of protein by rats was known to elevate brain tyrosine concentrations, and stimulate hydroxylation rate (Fernstrom and Fernstrom 1995b, 1987). In the earlier protein studies examining tryptophan and serotonin, the protein employed in tyrosine studies was casein. We therefore wondered if varying the dietary protein produced effects on tyrosine and tyrosine hydroxylation rate as remarkable as those observed on tryptophan and serotonin synthesis. The answer was no. Table 1 shows data for cortical tyrosine concentration and hypothalamic tyrosine hydroxylation (dihydroxyphenylalanine synthesis) rate from the same study described in Fig. 3. The hydroxylation rates of both tryptophan and tyrosine can be measured in the same animals, since the method involves inhibition of one enzyme, aromatic L-amino acid decarboxylase, that is responsible for catalyzing the conversion of tryptophan to 5-hydroxytryptophan and tyrosine to dihydroxyphenylalanine (the same enzyme participates in both synthetic pathways, though in different neurons) (Carlsson and Lindqvist 1978). Surprisingly, ingestion of the different proteins produced very small differences in cortical tyrosine concentrations, and statistically insignificant differences in hypothalamic tyrosine hydroxylation rate (dihydroxyphenylalanine synthesis). Hence, the dietary protein effect is specific, in the sense that it occurs in serotonin but not catecholamine neurons.

Recently, we have begun to examine this dietary protein effect in human subjects. In a pilot study, we have given normal males a beverage containing 40 g of α-lactalbumin, wheat gluten, corn zein or starch (carbohydrate) in the morning, after overnight fasting, and followed the plasma tryptophan ratio for 4 h. [A protein load of 40 g is similar to the protein content of a large fast-food hamburger/cheeseburger in the United States (US Department of Agriculture and Agricultural Research Service 2011)]. The findings indicated that changes in the plasma tryptophan ratio following each protein (though not carbohydrate) are essentially the same as those seen in rats (Fernstrom et al. 2011). Of particular note, α-lactalbumin caused a marked increase in the plasma tryptophan ratio, as reported previously (Markus et al. 2000), while zein lowered the ratio.

The focus of Markus and associates has been to identify dietary proteins that raise the plasma tryptophan ratio, presumably because of beneficial effects that are associated with increasing serotonin function in brain. Recently, another protein has been identified having a higher proportion of tryptophan to other LNAA than α-lactalbumin: lysozyme from egg white. The ingestion of this protein (in the form of a partial hydrolysate, perhaps to eliminate enzymatic activity) causes a greater rise in the plasma tryptophan ratio in humans than that produced by α-lactalbumin consumption (Markus et al. 2008; Mitchell et al. 2011). Consumption of the hydrolysate also produced a significant elevation of mood (Markus et al. 2008), consistent with some earlier findings that tryptophan administration can improve mood (Jensen et al. 1975; Rao and Broadhurst 1976). Such dietary protein products are viewed as possibly a better route to raising serotonin in brain via precursor-induced stimulation of synthesis than tryptophan itself, given past concerns regarding the purity of manufactured tryptophan (Kaufman and Philen 1993; Markus et al. 2008).

In contrast, we are presently beginning studies on the effects of the protein that lowers the plasma tryptophan ratio: zein. This direction is based on earlier findings using the acute tryptophan depletion (ATD) paradigm in patients with anorexia nervosa (AN). ATD is a biochemical phenomenon produced when animals and humans ingest an amino acid mixture (in beverage form) that reduces tryptophan concentrations in brain and slows serotonin synthesis and turnover (Gessa et al. 1974; Le Floc'h et al. 2011; Williams et al. 1999). Typically, human subjects ingest 100 g of amino acids, in a mixture that mimics the amino acid composition of a protein-containing food [e.g., steak (Young et al. 1985)]. This formulation is the "control" mixture, containing almost all amino acids including tryptophan and the other LNAA. The "experimental" mixture (ATD mixture) contains the same complement of amino acids, but no tryptophan. In early studies, only plasma tryptophan was measured, and its concentrations fell rapidly when the ATD mixture was ingested, an effect attributed to increased uptake into muscle for protein synthesis (Gessa et al. 1974). Ingestion of the control mixture resulted in no decline in plasma tryptophan concentration (Young et al. 1985). When normal subjects

Table 1 Serum tyrosine ratios, cortical tyrosine concentrations and hypothalamic dihydroxyphenylalanine synthesis rates in rats ingesting single meals differing in protein source

Variable	Serum tyrosine ratio	Cortical tyrosine (nmol/g)	Hypothalamic dihydroxyphenylalanine (pmol/g/min)
Fasting	0.290 ± 0.020^{a}	106.9 ± 8.0^{a}	85.0 ± 4.1
Zein	0.467 ± 0.009^{b}	118.8 ± 5.7^{a}	94.8 ± 5.9
Lactalbumin	0.237 ± 0.007^{c}	102.9 ± 5.0^{a}	86.0 ± 5.1
Soy protein	0.361 ± 0.017^{d}	121.2 ± 3.8^{a}	96.6 ± 5.7
Gluten	0.426 ± 0.027^{b}	123.1 ± 6.5^{a}	95.1 ± 4.5
Casein	0.451 ± 0.023^{b}	127.1 ± 2.0^{a}	90.4 ± 1.5
Carbohydrates	0.227 ± 0.011^{c}	106.3 ± 6.9^{a}	90.7 ± 6.5
F value	32.972	2.818	0.824
P value	0.001	0.024	0.559

Groups of six 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 free access to a meal containing one of the proteins listed in the table (17 % by weight), or a meal lacking protein ("carbohydrates"), or nothing ("fasting"). Two hour into the dark period, all rats received an injection of *m*-hydroxybenzylhydrazine (100 mg/kg bw, i.p.), and were killed 30 min thereafter. The serum tyrosine ratio is serum (tyrosine)/(tryptophan + phenylalanine + leucine + isoleucine + valine)

Data are mean ± SEM. Data were analyzed by ANOVA; different letters within a column indicate value comparison is significantly different, $P < 0.05$ (Newman–Keuls test). Adapted from Choi et al. (2009)

ingested the ATD mixture, mood was significantly lowered, a finding consistent with earlier findings with drugs showing that reducing serotonin lowered mood, while raising serotonin elevated mood (van Praag 1982). The control mixture did not reduce mood. A subsequent study in depressed subjects in remission elicited the same results (Delgado et al. 1990).

As noted, we adopted the ATD paradigm for studies in AN patients. AN individuals restrict food intake, resulting in serious weight loss (Gwirtsman et al. 1989; Klein and Walsh 2003). The etiology of the behavior is hypothesized to be linked to abnormally high serotonin neuronal activity in some brain circuits, which precipitates some of the behavioral features seen in AN subjects, including inhibited, anxious behavior (Kaye et al. 2005). A current hypothesis suggests that AN patients restrict food intake to reduce serotonin transmission and thereby relieve anxiety and other serotonin-linked unpleasant feelings (Kaye et al. 2005). If so, it is possible that ATD would ameliorate anxiety in AN patients by lowering serotonin synthesis (and release). We therefore administered to AN women the ATD and control amino acid solutions (on different days), and monitored anxiety. ATD significantly reduced anxiety in both active and recovered AN subjects (but had no effect on mood), while the control solution did not. The effect was not observed in normal women, as expected (Kaye et al. 2003).

Since the ingestion of zein lowers the plasma tryptophan ratio, like the ATD treatment, it is possible that its ingestion would reduce anxiety in AN women (whereas α-lactalbumin would not), an issue that we are presently evaluating. Parenthetically, zein is not the only protein that may elicit sizeable reductions in brain tryptophan and serotonin. An hydrolysate of collagen has also been reported to reduce brain tryptophan and serotonin in rats, and to lower the serum tryptophan ratio in both rats and humans, and is employed as an alternate to the free amino acid mixture used in the ATD paradigm (Evers et al. 2005; Lieben et al. 2004; Sambeth et al. 2009).

BCAA and central fatigue during exercise

The effects of ingested protein, and tryptophan-depleting amino acid mixtures, on serotonin function center on the LNAA they contain, principally tryptophan, tyrosine, phenylalanine, leucine, isoleucine and valine. As noted above, the effects of such treatments on tryptophan uptake into brain depend on how they modify the plasma tryptophan ratio, which reflects the contribution of exogenous amino acids to the circulation (from the gut), and the removal of amino acids into peripheral tissues (principally muscle, an effect of insulin secretion and action). The above studies show that protein ingestion is selective in modifying the plasma tryptophan ratio, and not the tyrosine ratio. The administration of LNAA, particularly BCAA, has also been examined in the context of another metabolic issue, physical exercise and fatigue, and provides useful insights into their use.

Prolonged exercise produces physical fatigue. Fatigue is typically thought of in relation to muscle function, and considered to be the result of chemical alterations in the muscle itself. For example, such alterations include the depletion of energy substrates and build up of metabolites.

Fatigue due to changes to the muscle itself is termed "peripheral fatigue" (Davis and Bailey 1997). Peripheral fatigue, however, is probably not the only cause of fatigue. A component termed "central fatigue" has been described, which cannot be explained by changes in the muscle itself; it appears to be associated with changes in central nervous system function. One example of central fatigue is the gradual reduction in the ability of an individual to lift a weight repeatedly, despite no evidence that the maximal contractile force of the muscle has diminished (as measured by the electrically stimulated force that the muscle continues to be able to generate) (Gandevia et al. 1996). One notion is that central fatigue can result from changes in corticospinal neuronal outflow to lower motor neurons (Taylor and Gandevia 2008), perhaps mediated by neuronal inputs to upper motor neurons, including those provided by serotonin and dopamine neurons (Davis and Bailey 1997).

One hypothesis regarding the etiology of central fatigue is that exercise-induced changes in energy and amino acid metabolism within muscle indirectly cause increases in brain tryptophan uptake and serotonin synthesis and release. Increased serotonin release by brain neurons is argued to produce the features of central fatigue (Blomstrand 2001). Briefly, physical exercise increases the metabolism of BCAA (Rennie and Tipton 2000; Tarnopolsky 2004), causing a reduction in their plasma concentrations (Blomstrand 2001). In addition, adipocyte fatty acid mobilization during exercise results in increases in the plasma concentrations of non-esterified fatty acids (NEFA, which are used by muscle as an energy substrate during exercise). The rise in plasma NEFA concentrations causes circulating tryptophan to be displaced from serum albumin [most tryptophan in blood circulates loosely associated with albumin (McMenamy and Oncley 1958)], causing the proportion of unbound tryptophan in blood to increase. This free pool of tryptophan in blood has been argued to be the fraction available for transport across the BBB (Chaouloff et al. 1986), though this is unlikely to be the case (Fernstrom and Fernstrom 2006; Le Floc'h et al. 2011). The consequence of these exercise-associated amino acid changes in blood (rise in free tryptophan and fall in BCAA concentrations) is reputed to cause an increase in the competitive tryptophan transport into brain, raising brain tryptophan concentrations and accelerating neuronal serotonin synthesis and release. The increase in serotonin release promotes drowsiness (serotonin neurons are linked to sleep), precipitating the perception of fatigue (Blomstrand 2001). Exercise certainly does increase tryptophan concentrations and serotonin synthesis and release in brain (Chaouloff et al. 1985; Davis and Bailey 1997; Gomez-Merino et al. 2001a; Meeusen et al. 1996; Smriga et al. 2002), and this argument led to the proposal that the ingestion of BCAA, by raising their plasma concentrations, could prevent exercise-induced increases in brain tryptophan concentrations and serotonin function, and thereby improve performance (Blomstrand et al. 1991). Subsequent studies, notably in animals, have indeed shown that the ingestion of BCAA can attenuate the exercise-induced rise in neuronal serotonin synthesis and release in brain (Gomez-Merino et al. 2001b; Smriga et al. 2002). However, the BCAA effect on central fatigue and physical performance in exercising humans has been questioned (Bishop 2010; Davis et al. 2000; Davis and Bailey 1997; Meeusen et al. 2006).

One possible reason that BCAA ingestion shows marginal or no effects on performance, despite blocking the exercise-induced rise in brain serotonin, may involve an overlooked consequence of BCAA use. The administration of BCAA also reduces tyrosine uptake into brain, and the synthesis and release of catecholamines, most notably dopamine. Dopamine-stimulating drugs, such as amphetamine and selective dopamine-reuptake blockers, enhance aspects of physical performance (Bouchard et al. 2002; Davis and Bailey 1997; Laties and Weiss 1981; Meeusen et al. 2006; Roelands et al. 2008). Hence, if BCAA treatment slows both serotonin and dopamine synthesis, secondary to reductions in brain precursor levels, then the positive effect on "fatigue" due to the serotonin reduction might be offset by the negative effects of a dopamine decline. BCAA administration can lower both serotonin and dopamine synthesis in brain, as illustrated by the observation that the injection of one or more BCAA rapidly reduces tryptophan and tyrosine concentrations and the rates of both tryptophan and tyrosine hydroxylation in rat brain (Carlsson and Lindqvist 1978). In humans, BCAA ingestion has been noted to reduce dopamine-dependent functions in both normal subjects and manic patients (Gijsman et al. 2002; Scarna et al. 2003). We have begun to evaluate this possibility further by administering oral doses of BCAA to sedentary and exercising rats, to assess if both tryptophan and tyrosine hydroxylation rates are depressed. We looked for a dose of BCAA used previously in rat exercise studies that produced a significant serotonin effect, and adapted the oral dose regimen used by Smriga et al. (2002). Preliminary studies showed that an oral BCAA dose of 268 mg/kg bw worked well in producing reductions in tryptophan hydroxylation rate in brain, using a protocol in which hydroxylation rate was measured 1 h after BCAA ingestion. Representative results are presented in Table 2. In this study, sedentary rats received the oral BCAA load or the vehicle (water) 2 h into the daily dark period. *m*-Hydroxybenzylhydrazine was injected 30 min later, and the animals were sacrificed after an additional 30 min. As noted, the drug blocks the decarboxylase enzyme in both serotonin and catecholamine neurons, preventing the conversion of 5-hydroxytryptophan and

Table 2 Branched-chain amino acid administration reduces both serotonin and catecholamine synthesis rates in rat brain

Variable	Vehicle	BCAA
Serum tryptophan (nmol/ml)	49.1 ± 2.4	33.4 ± 1.1*
Serum tryptophan ratio	0.124 ± 0.007	0.047 ± 0.003*
Cortex tryptophan (nmol/g)	19.5 ± 1.5	6.6 ± 0.5*
Hypothalamic 5-hydroxytryptophan (pmol/g/min)	29 ± 1	13 ± 1*
Cortex 5-hydroxytryptophan (pmol/g/min)	14 ± 1	5 ± 1*
Serum tyrosine (nmol/ml)	80.3 ± 3.4	40.2 ± 2.1*
Serum tyrosine ratio	0.219 ± 0.005	0.056 ± 0.005*
Cortex tyrosine (nmol/g)	181.8 ± 7.3	64.8 ± 5.4*
Hypothalamic dihydroxylphenylalanine (pmol/g/min)	53 ± 1	39 ± 2*
Cortex dihydroxylphenylalanine (pmol/g/min)	59 ± 6	37 ± 4*

Groups of ten sedentary male rats were fasted during the daily light period, and intubated with BCAA or vehicle (water) at dark onset. The BCAA solution (administered by feeding tube at 10 ml/kg bw) provided the following dose of each amino acid (in mg/kg bw): leucine, 116; isoleucine, 76; valine, 76; glutamine, 142; and arginine, 140. Thirty min later, they received an injection of *m*-hydroxybenzylhydrazine (100 mg/kg bw, i.p.) and were killed 30 min thereafter. The serum tryptophan ratio is serum (tryptophan)/(tyrosine + phenylalanine + leucine + isoleucine + valine); the serum tyrosine ratio is serum (tyrosine)/(tryptophan + phenylalanine + leucine + isoleucine + valine)

Data are mean ± SEM. (Choi S, DiSilvio B, Fernstrom MH, Fernstrom JD, unpublished observations)

* $P < 0.01$ (two-tailed t test)

dihydroxyphenylalanine to serotonin and dopamine, respectively. 5-Hydroxytryptophan and dihydroxyphenylalanine accumulate linearly for 30 min, at which point their concentrations are determined as indices of the rates of tryptophan and tyrosine hydroxylation (Carlsson and Lindqvist 1978). The BCAA load significantly reduced the serum tryptophan ratio, brain (cerebral cortical) tryptophan concentrations, and tryptophan hydroxylation rate in the two brain regions examined, cerebral cortex and hypothalamus. These results are thus consistent with the findings of Smriga et al. (2002). In addition, it can be seen in Table 2 that the BCAA treatment reduced the serum tyrosine ratio, cortical tyrosine concentrations, and tyrosine hydroxylation rate in both cortex and hypothalamus (Fernstrom et al. 2008). Similar results have also been obtained in rats exercising ad libitum on running wheels in their home cages for a week prior to experimentation. All studies are initiated 2 h into the daily dark period, when the animals are quite active (in running wheel studies, rats run about 1 km during the 2-h dark period preceding the study).

Hence, it seems possible that BCAA ingestion reduces serotonin and catecholamine synthesis rates in human brain. If this is the case, it is possible that the marginal effects of BCAA ingestion on physical performance are related to the reduction in tyrosine uptake into brain and dopamine synthesis/release caused by their use. It is of interest that this effect could most likely be eliminated by adding an appropriate amount of tyrosine to the BCAA mixture. It would be interesting to evaluate the effect of such a mixture on physical performance. We are currently conducting studies to evaluate this possibility neurochemically in rats.

Conclusions

This review has focused primarily on the susceptibility of serotonin synthesis and release in brain neurons to variations in the supply of the serotonin precursor, tryptophan. Since tryptophan concentrations in brain are readily influenced by changes in circulating concentrations of tryptophan and its LNAA transport competitors, and LNAA concentrations in blood are modified by their ingestion (as free amino acids or constituents of proteins), serotonin synthesis, release, and ultimately serotonin-linked brain functions can be influenced by what is eaten. As noted, this has led to some interesting attempts to modify serotonin function through the use of dietary proteins and LNAA. Such attempts illustrate that LNAA do not always produce effects specific to serotonin: the administration of BCAA, e.g., reduces not only tryptophan and serotonin in brain, but also tyrosine and dopamine. The dual effect may have unwanted functional consequences. Such problems may be easily solved, e.g., by adding tyrosine to a BCAA mixture to prevent a decline in tyrosine and dopamine in brain. But the example points up a broader issue. The development of proteins and LNAA mixtures for study generally seems to have been somewhat haphazard. Hence, there are wide differences among studies in the amount of a protein or amino acid mixture administered, and little consideration given to the formulation of LNAA mixtures. It would be useful to know how much protein or BCAA is needed to elicit a particular effect. How often should the treatment be given to maintain the observed effect? In addition, does the effect persist if the treatment is provided chronically, or does tolerance (tachyphylaxis) develop? Tolerance might develop, e.g., if continuous stimulation of serotonin synthesis and release occurs with repeated administration of tryptophan or α-lactalbumin, through a reflex reduction in serotonin neuronal activity or the development of reduced sensitivity of post-synaptic receptors to serotonin. Surprisingly, such simple pharmacologic questions have rarely, if ever, been examined.

A broader, physiologic question, not addressed here, is why serotonin neurons (and catecholamine neurons to a lesser extent) are at all susceptible to food, most notably protein intake. So far, no other transmitter has demonstrated such sensitivity to dietary precursor supply. One possibility, in relation to protein ingestion, is that the brain may receive information regarding dietary protein quality via serotonin neurons, though the correlation between dietary protein quality (usually measured as its effectiveness in promoting nitrogen retention or net protein synthesis in the body) (Raghunath and Rao 1984) and the influence of protein ingestion on brain tryptophan and serotonin (Fig. 3) is not perfect. But it may be premature to dismiss this possibility, since the relevant databases are quite small. If there is a functionally important link between tryptophan or LNAA intake as constituents of dietary protein, brain serotonin and the perception of protein quality, it might have been most useful when humans were hunter-gatherers, often subsisting on near or below requirement intakes of protein, with intermittent access to protein. To date, our examination of this issue has never involved experimental models that take such nutritional milieus into account. It might prove interesting to do so.

Conflict of interest The author declares that he has no conflict of interest.

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