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Short Communication

The ingestion of different dietary proteins by humans induces large changes in the plasma tryptophan ratio, a predictor of brain tryptophan uptake and serotonin synthesis

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SUMMARY

Background & aims: The ingestion by rats of different proteins causes large differences in the plasma ratio of tryptophan to other large neutral amino acids, which predicts brain tryptophan uptake and serotonin synthesis. We evaluated in humans whether ingesting these proteins also produces large excursions in the tryptophan ratio.

Methods: Fasting males ($n = 6$) ingested V-8 Juice containing 40 g of α-lactalbumin, gluten, zein or starch. Blood was drawn before and at 30 min intervals after ingestion for 4 h; tryptophan and other large neutral amino acids were quantitated.

Results: Pre-meal plasma tryptophan was ~50 nmol/ml; the tryptophan ratio was ~0.010. α-Lactalbumin increased plasma tryptophan (3-fold) and the tryptophan ratio (50%); starch did not change either tryptophan variable, while gluten caused a modest (25%) and zein a large reduction (50%) in plasma tryptophan. Gluten and zein reduced the tryptophan ratio. The maximal difference in the tryptophan ratio occurred between α-lactalbumin and zein and was large (~3-fold).

Conclusion: Since the plasma tryptophan ratio predicts brain tryptophan uptake and serotonin synthesis in rats, the differences in the ratio produced in humans by these proteins may modify serotonin synthesis, and perhaps elicit serotonin-linked changes in behavior.

1. Introduction

The brain neurotransmitter serotonin (5-hydroxytryptamine; 5HT) is synthesized from the essential amino acid tryptophan. The rate of 5HT synthesis is sensitive to tryptophan concentrations in brain.[1] Brain tryptophan concentrations are influenced by tryptophan uptake from the circulation, mediated by a competitive transporter located at the blood–brain barrier (BBB) and shared among several large neutral amino acids (LNAA), including tryptophan, tyrosine, phenylalanine, leucine, isoleucine and valine.[1] Brain tryptophan uptake and 5HT synthesis are therefore influenced by changes in the plasma concentrations of tryptophan and the other LNAA. Because foods containing carbohydrates and proteins modify plasma concentrations of the LNAA, their ingestion by rats alters brain tryptophan uptake and 5HT synthesis. Inasmuch as changes in 5HT synthesis modify neuronal 5HT release, a potential link is made between diet and brain functions involving 5HT neurons.[1–3]

Initial studies in rats connecting food intake and brain tryptophan defined the difference between ingesting carbohydrates alone or carbohydrates with protein. Carbohydrate ingestion increased tryptophan uptake into brain (and 5HT synthesis) by lowering plasma concentrations of the LNAA and raising plasma tryptophan. The combination of protein with carbohydrates blocked the rise in brain tryptophan because the rise in plasma tryptophan was counterbalanced by increases in the plasma concentrations of other LNAA (hence, no net change in competition for transport).[1] The influence of meals on plasma LNAA and the BBB uptake of tryptophan was summarized as a plasma ratio of tryptophan to the sum of its LNAA competitors. When carbohydrates are ingested, plasma tryptophan rises while plasma concentrations of the other LNAA

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decline, resulting in an increase in the plasma tryptophan ratio. When protein is ingested with carbohydrates, both plasma tryptophan and the other LNAA rise, by proportionally-similar amounts, and the ratio does not change.[1]

This basic set of observations, that carbohydrate ingestion raised the plasma tryptophan ratio, while added protein (*any* protein) blocked the effect, began to change when Markus and associates reported that the ingestion of either of two mammalian proteins (casein, α-lactalbumin) produced *different* effects on the plasma tryptophan ratio and on 5HT-mediated behaviors in stress-vulnerable human subjects.[4] Subsequent rat studies confirmed the finding, showing that ingesting α-lactalbumin raised the plasma tryptophan ratio, brain tryptophan and neuronal 5HT release, compared to casein consumption.[5,6] Such effects were then found to be much larger, when a range of animal and plant proteins was examined.[7] Notably, the differences observed between rats ingesting a meal containing α-lactalbumin vs. those ingesting zein were remarkable. Soon after ingesting α-lactalbumin- or zein-containing meals, the plasma tryptophan ratio, brain tryptophan concentrations and 5HT synthesis rate differed by several-fold, effects substantially greater than those seen between casein- and α-lactalbumin-containing meals.[7]

These findings suggest in humans that ingesting different proteins should produce much greater changes in the plasma tryptophan ratio, and ultimately 5HT-linked behaviors, than those previously observed.[4] In the present study, we have begun to explore this possibility by examining the effects of a meal containing each of three dietary proteins or carbohydrates on the plasma tryptophan ratio in normal humans. The proteins selected elicited the largest increase (α-lactalbumin), no change (wheat gluten), or the largest decrease (zein) in the plasma tryptophan ratio in rats.[7]

2. Methods and materials

2.1. Subjects

Male subjects were recruited by advertisements at local universities in San Diego. Respondents were screened briefly over the phone, and following completion of a signed consent form, were interviewed in person and examined by a study physician. Subjects were included who were 18–30 years of age and had a body mass index (mass(kg)/height(m)^2) of 23–27. Subjects were excluded who had (a) an allergy to gluten and/or celiac disease, (b) any metabolic disease (e.g., diabetes (verified by fasting plasma glucose measurement), liver or kidney disease) or digestive disease, (c) a history of an Axis I disorder, including major depression, evidence of current depression, and/or (d) been taking a psychoactive medication.

2.2. Study design

The final study group consisted of six male subjects, 27 ± 2 years old with a body mass index of 24 ± 1 (mean ± SEM). They reported to the lab at 7–8 AM on four occasions, separated by 3–5 days, having consumed no food or beverages (except water) since 10 PM the night before. On admission, an indwelling venous catheter was inserted, and a blood sample taken just prior to beverage ingestion (0 min). The beverage was then ingested in less than 15 min, and blood samples were obtained at 30 min intervals for 4 h. During this time, subjects could read, watch television, or play video games. Water was available for consumption. At the end of the study, the catheter was removed, subjects were offered lunch, and then released. This protocol was conducted by the Eating Disorders Program at the Center for Clinical Research, University of California, San Diego, and was approved by the Human Research Protections Program of the University of California, San Diego.

2.3. Test beverage composition

The base was spicy hot V-8 juice (16 oz; Campbell's Soup Co., Camden NJ; 102 kcal; 4.0 g protein, 20.0 g carbohydrates, 0 g fat), to which was added 40 g protein (α-lactalbumin, Davisco Foods International, Le Sueur MN; wheat gluten, MGP Ingredients Inc., Atchison KS; zein, Freeman Industries, Tuckahoe, NY) or cornstarch (Argo, ACH Food Companies, Oakbrook IL). The total caloric content of each beverage was 262 kcal.

2.4. Biochemical analyses

Blood samples were collected in heparin, and centrifuged to obtain plasma. Plasma samples were assayed for large neutral amino acids by HPLC and electrochemical detection.[7] The plasma tryptophan ratio was calculated as (molar ratio) (tryptophan)/(tyrosine + phenylalanine + leucine + isoleucine + valine), and the plasma tyrosine ratio as (tyrosine)/(tryptophan + phenylalanine + leucine + isoleucine + valine).[1] Plasma insulin concentrations were

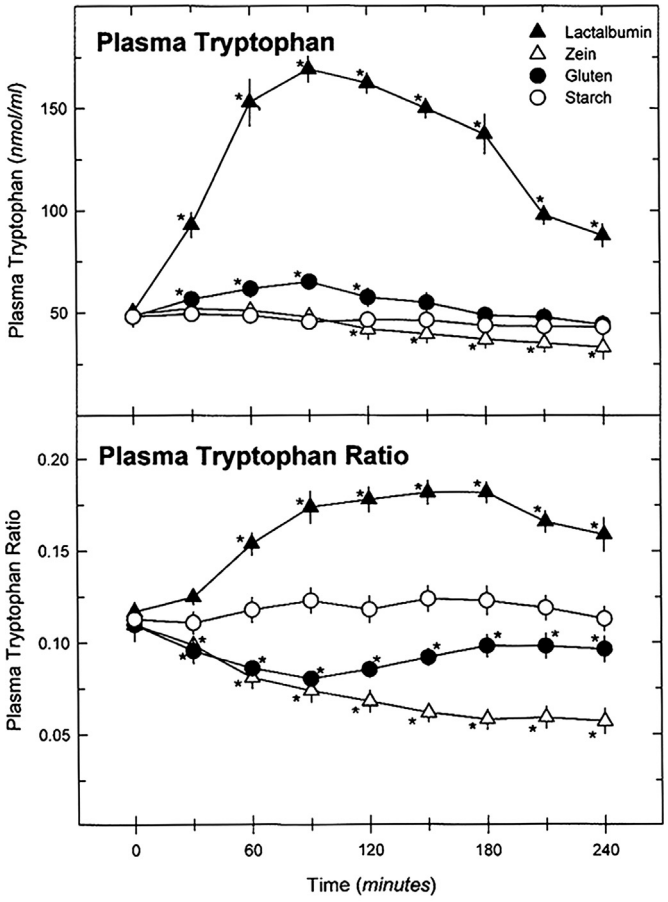

Fig. 1. Effect of protein ingestion by human subjects on plasma tryptophan concentrations and the plasma tryptophan ratio. Fasting male subjects ($n = 6$) ingested a V-8-based drink (16 oz) containing 40 g of the indicated protein on four separate occasions. Blood samples were collected at 0 min and at 30 min intervals thereafter for 240 min. The plasma tryptophan ratio is the molar ratio of the tryptophan concentration divided by the sum of the concentrations of tyrosine, phenylalanine, leucine, isoleucine and valine. Data are presented as the means ± sem. Data were analyzed by 2-way, repeated measures ANOVA. For plasma tryptophan, the effect of protein ($F = 80.36$, df = 3) and of time ($F = 30.49$, df = 8) were significant ($P < 0.001$); the interaction ($F = 26.21$, df = 24) was also significant ($P < 0.001$). For the plasma tryptophan ratio, the effect of protein ($F = 81.95$, df = 3; $P < 0.001$) and of time ($F = 2.48$, df = 8, $P < 0.05$) were significant; the interaction ($F = 46.17$, df = 24) was also significant ($P < 0.01$). *$P < 0.05$ vs. 0-time value (Newman–Keuls test).