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Dietary Carbohydrate Increases Brain Tryptophan and Decreases Serum-free Tryptophan

THE rate at which the brain synthesizes serotonin varies physiologically as a function of its tryptophan concentration[1,2]. It has been shown that brain tryptophan, in turn, depends on the relative concentrations in plasma or serum of tryptophan and of the other neutral amino acids which compete with it for uptake into the brain[3]. Thus, carbohydrate ingestion, which raises plasma tryptophan while depressing the concentrations of its competitors, increases the amount of tryptophan in the brain and accelerates synthesis of serotonin in young rats[4]; on the other hand, protein consumption causes proportionately greater increases in the other neutral amino acids than in plasma tryptophan, and thus fails to elevate brain tryptophan or serotonin[3].

Tryptophan exists in plasma in two forms, namely in a "bound" form, reversibly associated with albumin, and as the "free" amino acid[5]. It has been suggested that the level of free tryptophan in serum determines the concentration of tryptophan in the brain and, consequently, the rate of synthesis of serotonin[6]. We have recently shown that the administration of glucose to human subjects causes a decrease in the quantity of free tryptophan in serum, without affecting the concentration of that bound to albumin[7]. Evidence is presented here that carbohydrate consumption also reduces the amount of free tryptophan in the serum of rats, and at the same time elevates brain tryptophan. Thus the concentration of free tryptophan in the serum does not predict the changes in brain tryptophan that normally accompany such physiological occurrences as eating.

Male Sprague-Dawley rats kept at 20–22° C in a room lit between 0900 and 2100 h by 'Vita-Lite' (50–75 foot candles; Duro-Test Corp., North Bergen, New Jersey) were deprived of food, but not water, for 15–18 h before the start of each experiment. The animals were decapitated and blood collected from the cervical wound into tubes previously flushed with a mixture of 5% CO_2 and 95% N_2 to ensure maintenance of *p*H. Serum was collected by centrifugation using conditions of minimum haemolysis. The concentration of free tryptophan in serum was estimated by equilibrium dialysis[7,8]. Visking dialysis tubing (flat width 1 cm, lengths 18 cm) was boiled twice in 0.0002 M disodium EDTA and twice in distilled water, and stored in distilled water. The dialysis buffer was Krebs-Ringer improved bicarbonate, *p*H 7.45. In the glucose experiment, the buffer also contained small molecular weight, constituents of plasma, as recommended by McMenamy *et al.*[8]; however, this addition had no significant effect, and was omitted in later studies. The tubing was first knotted at one end and 0.5 ml of buffer added. This was followed by a second knot, approximately 6.5 cm from the first, and the dialysis bag was folded in half and put in a tube containing 2 ml of serum. (The same concentrations of free tryptophan were obtained using serum: buffer ratios of 4 : 1 and 15 : 1.) After flushing with the CO_2-N_2 mixture, the tube was stoppered tightly. Dialysis was at 37° C in a shaking water bath for 3.5 h to achieve equilibrium. In establishing the method, the levels of free tryptophan were found to be 6.72 μg ml^{-1} at 2.5 h, 7.2 μg ml^{-1} at 3.5 h and 7.27 μg ml^{-1} at 4.5 h. Concentrations of total and free serum tryptophan were determined on aliquots of the serum and buffer, respectively, using the fluorescence assay of Denckla and Dewey[9]. Bound tryptophan was calculated from these concentrations with appropriate volume corrections. Serum NEFA was estimated by the method of Dole and Meinertz[10], and serum tyrosine by that of Waalkes and Udenfriend[11]. Brains were removed immediately after decapitation, frozen on dry ice, and stored at −70° C until tryptophan was assayed[9].

In the first experiment, rats were killed 1 or 2 h after being fed a glucose solution (2 g in 4 ml) through a stomach tube. This treatment caused a large increase in the total concentration of tryptophan in serum; this increase was restricted to the albumin-bound fraction while free tryptophan decreased significantly from 5.5 to 4.2 μg ml^{-1} (Table 1). As in human subjects consuming a glucose load[7], NEFA fell by almost 50%. Brain tryptophan rose significantly even though free tryptophan in serum declined.

To confirm that the above glucose effect was mediated by insulin secretion, other rats received a dose of exogenous insulin (2 U kg^{-1}, i.p.) previously shown to increase plasma and

Table 1 Effect of Glucose Ingestion on Serum and Brain Tryptophan

	Control	Glucose (1 h)	Glucose (2 h)
Serum total tryptophan ($\mu g\ ml^{-1}$)	16.2±0.2	19.6±0.6†	19.9±0.4‡
Serum free tryptophan ($\mu g\ ml^{-1}$)	5.5±0.1	4.8±0.3*	4.2±0.2‡
Free (% of total)	34	25	21
Serum bound tryptophan ($\mu g\ ml^{-1}$)	10.7±0.3	14.8±0.6†	15.7±0.5‡
NEFA ($meq\ l^{-1}$)	1.147±0.034	0.648±0.077‡	0.604±0.044‡
Brain tryptophan ($\mu g\ g^{-1}$)	4.16±0.42	6.42±0.56†	5.93±0.72†

D-Glucose (2 g 4 ml^{-1} tap water) was administered to three groups of thirty-five rats (108–146 g) through a stomach tube. Controls received tap water through a stomach tube. Serum was pooled from seven samples; all values are given as mean ± s.e.m.

* $P<0.05$, differs from controls.

† $P<0.01$, differs from controls.

‡ $P<0.001$, differs from controls.

brain tryptophan[12]. This altered the distribution of plasma tryptophan between free and albumin-bound forms in a manner similar to dietary glucose: free tryptophan was 6.1±0.3 and 7.7±0.6 $\mu g\ ml^{-1}$ in hormone-treated and control animals, respectively ($P<0.05$); serum total tryptophan was 22.2±0.1 and 18.0±1.4 ($P<0.02$). Confirming previous findings in rats[4], exogenous insulin increased the total amount of tryptophan in plasma. In humans, total tryptophan does not rise after insulin injection; however, the circulating neutral amino acids which compete with it for entry into the brain fall markedly[13].

In a second dietary experiment, fasted animals were killed after being allowed to consume one of two protein-free synthetic diets for 2 h. The first diet, used in previous studies in this laboratory[3,4], contained both carbohydrate and fat; the second lacked fat. Both diets raised serum total tryptophan, serum albumin-bound tryptophan, and brain tryptophan; both also depressed serum free tryptophan (Table 2) and the concentrations of serum NEFA and of tyrosine, one of the group of neutral amino acids whose omission from the diet has been shown to facilitate the post-prandial increase in brain tryptophan[3]. This correlation supports our previous suggestion that carbohydrates (and insulin) increase the albumin-binding of tryptophan in serum by decreasing the extent of saturation

Table 2 Effects of Carbohydrate or Carbohydrate-Fat Diets on Serum and Brain Tryptophan

	Fasted controls	Diets	
		Carbohydrate + fat	Carbohydrate
Serum total tryptophan ($\mu g\ ml^{-1}$)	16.5±0.3	18.4±0.5†	19.1±0.4‡
Serum free tryptophan ($\mu g\ ml^{-1}$)	6.2±0.1	5.7±0.2*	3.4±0.2‡
Free (% of total)	37	33	18
Serum bound tryptophan ($\mu g\ ml^{-1}$)	10.3±0.4	12.7±0.7*	15.7±0.5‡
Serum tyrosine ($\mu g\ ml^{-1}$)	19.5±0.7	11.7±0.4	14.4±0.5
NEFA ($meq\ l^{-1}$)	0.831±0.021	0.615±0.029‡	0.301±0.024‡
Brain tryptophan ($\mu g\ g^{-1}$)	2.24±0.11	3.07±0.18‡	3.45±0.19‡

Groups of twenty-two rats weighing 170–200 g were deprived of food but not water at 1400 h and presented with one of the experimental diets at 1030 h the following day. After 2 h, the animals were decapitated and serum and brains taken for assay. Controls had free access to water and were killed during the experiment. Each serum value is obtained from two pooled samples. Each diet contained: dextrose 270 g, sucrose 221 g, dextrin 270 g, Harper's salt mix 40 g, vitamin mix[15] 10 g, and choline 2 g, to which was added 35 g of agar in 1 l of water. The fat diet had an additional 150 g of mazola oil. All values are given as mean ± s.e.m.

* $P<0.05$, differs from controls.

† $P<0.01$, differs from controls.

‡ $P<0.001$, differs from controls.

of albumin with free fatty acids, and thus enhancing albumin's affinity for tryptophan[7]. Animals fed diets containing fat showed lesser falls in NEFA and serum free tryptophan than those eating the fat-free diet (Table 2), and in consequence, serum free tryptophan levels differed significantly between groups of rats eating the two diets (5.7 as opposed to 3.4 $\mu g\ ml^{1}$, $P<0.001$). Even so, brain tryptophan levels were not dissimilar (Table 2).

These observations indicate that the concentration of unbound, or free, tryptophan in the serum does not predict the changes in brain tryptophan caused by such physiological inputs as eating. Free tryptophan might be correlated with brain tryptophan after certain treatments such as drug administration[14] or a prolonged fasting period[6].

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B. K. MADRAS
E. L. COHEN
J. D. FERNSTROM
F. LARIN
H. N. MUNRO
R. J. WURTMAN

Laboratories of Neuroendocrine Regulation and Physiological Chemistry, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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