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The first *Science* article showed that small, physiologic variations in brain tryptophan levels could readily influence the synthesis in the brain of the tryptophan-derived neurotransmitter, serotonin. The second *Science* article presented evidence showing that a physiologic event, the ingestion of food, by influencing the plasma levels of tryptophan relative to those of other amino acids competing with it for brain uptake, could predictably alter brain tryptophan levels and serotonin synthesis. The third *Science* paper tested if the ingestion of protein would increase brain tryptophan levels and serotonin production. [The *SCI*® indicates that these papers have been cited in more than 580, 340, and 625 publications, respectively.]

Searching for a Dietary Connection to Neurotransmitters

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The experiments described in these three reports in *Science* constituted a portion of the research associated with my PhD dissertation at the Massachusetts Institute of Technology (MIT), Cambridge. I was then a graduate student in the Department of Nutrition at MIT, training with the neuropharmacologist/neuroendocrinologist Richard J. Wurtman; together, we were exploring for a dietary connection to the synthesis of neurotransmitters in the brain. At that time (late 1960s), this connection was viewed solely in the context of malnutrition. However, my feeling then (as now) was that malnutrition was largely a political/economic—not a basic research—problem, particularly since the solution was known *and* available: adequate nutrition. Consequently, I did not want to examine the diet-neurotransmitter connection in malnourished animals, and instead studied healthy rats.

At the time, neuropharmacologists were focused on the catecholamine transmitters, but we instead selected serotonin for study. This decision was influenced by a view held by Hamish Munro of our department concerning hepatic protein synthesis; viz., tryptophan is the most limiting amino acid in mammalian proteins, and thus its cellular level should limit the rate of protein synthesis. We hoped that such might also be true for serotonin, which is derived from tryptophan. The first *Science* article examined this possibility.

We found that small, physiologic-sized increases in plasma and brain tryptophan levels were sufficient to stimulate serotonin synthesis. This observation then led us to search for physiologic phenomena that would indirectly alter brain tryptophan levels, and, thus, possibly also serotonin synthesis. The resulting experiments appeared in the second article in this *Classic* series. To alter brain tryptophan levels, we turned to an endocrine paradigm, insulin injection, which was known to produce rapid, marked changes in plasma amino acid levels. Using this approach, we found that insulin injection altered plasma tryptophan and led to a parallel change in brain tryptophan (both increased). We then found that brain serotonin production also was increased. We next tested the insulin effect in a more physiologic context: viz., the rat releasing its own insulin by ingesting carbohydrates. This experiment also worked, and we concluded in this article that, in addition to insulin secretion indirectly stimulating brain tryptophan uptake and serotonin production, the ingestion of any insulin-secreting meal/food should produce the same effects.

In the third *Science* article, we examined this latter conclusion by testing if the ingestion of protein would also increase brain tryptophan levels and serotonin production (because it greatly increases plasma tryptophan levels). This experiment produced an unexpected result: despite very large increases in plasma tryptophan produced by the protein meal, brain tryptophan levels

(and serotonin production) did not change from control values. This finding led us to search for an explanation, one eventually offered by another area of nutritional/metabolic investigation—that dealing with amino acid transport.

The view was that tryptophan is transported into the brain via a carrier at the blood-brain barrier, which it shares competitively with several other large, neutral amino acids.[1] From this perspective, we quickly hypothesized that protein ingestion did not raise brain tryptophan levels because the protein contributed so many molecules of tryptophan's transport *competitors* that, despite the large rise in plasma tryptophan, this increase was easily offset at the transport sites by the comparable, proportional increases in the plasma levels of tryptophan's competitors.

We borrowed a well-known nutritional paradigm to test this hypothesis: the use of meals containing amino acid mixtures in lieu of intact proteins.[2] The rats consuming a meal containing all amino acids in a typical protein showed no increase in brain tryptophan; the rats consuming this meal lacking the suspected transport competitors showed an enormous rise in brain tryptophan (and serotonin). These were the key results in the third *Science* article, pointing to the pivotal role of the competitive transport carrier in determining how a meal (or any metabolic event) will alter brain tryptophan uptake.

I learned a number of lessons from this work. First, it helps to have a little luck. In this regard, we accidentally picked the right transmitter to study. Unlike other transmitters, serotonin levels go up and down in parallel with synthesis rate, allowing us to have a simple method for studying synthesis. Had we picked a different transmitter for study, we would have seen no changes and quickly abandoned the project.

Second, we employed an axiom often stated by Julius Axelrod on his visits to MIT: "We followed our noses," aggressively poking into areas of pharmacology, endocrinology, nutrition, and metabolism to examine this issue. Needless to say, the time spent in the literature was not modest.

And third, we benefited greatly by existing in a very unusual intellectual environment—the Department of Nutrition at MIT (which, sadly, was recently disbanded by the MIT administration). By being a repository of creative investigators from a variety of disciplines (nutrition, nutritional biochemistry, metabolism, endocrinology, and neuropharmacology), it fostered an interdisciplinary energy I personally have not seen since. It represents an ideal, at least in the nutritional sciences, that should have been (and should be) encouraged, not destroyed.

Most likely, the interest in these articles (and the basis for their frequent citation) over the years relates in part to their relevance to the control of food intake/appetite by metabolic cues.[3] If food ingestion can influence an important brain neurotransmitter (serotonin), then such effects may inform the brain via chemical changes about what has recently been eaten. Their citation may also derive from the fact that these results provided some of the first evidence that the competitive transport of amino acids into the brain has a demonstrable *physiologic* context. And finally, interest may derive in part from an application of the concepts examined in these articles to pathophysiologic situations in which metabolic disturbances may lead to predictable alterations in brain amino acid uptake, and, consequently, transmitter synthesis (e.g., diabetes, cirrhosis of the liver).[4,5]

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