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LETTER TO THE EDITOR

The Validity of the PET/α-[^{11}C]Methyl-L-Tryptophan Method for Measuring Rates of Serotonin Synthesis in the Human Brain

The recent article by Shoaf et al. (1998) is an interesting contribution to the debate on the validity of the α-[^{11}C]methyl-L-trypotophan (αMTrp) method for measuring rates of 5-hydroxytryptamine (serotonin, 5-HT) synthesis in brain. In this article, the authors conclude that the method may not determine 5-HT synthesis rates accurately. The primary concerns were: (1) the lack of a significant correlation between measured rates of 5-HT synthesis and cerebrospinal fluid (CSF) concentrations of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in animals thought to be at steady state; (2) the strong association between plasma concentrations of free tryptophan and the rate of 5-HT synthesis; and (3) the lack of a clear linear portion in the obtained Patlak plots.

First, no evidence is provided demonstrating that steady state was indeed achieved. Moreover, although CSF 5-HIAA is thought to provide a crude estimate of 5-HT synthesis, it, like most methods, has limitations. Small differences in CSF 5-HIAA levels are particularly unlikely to reflect differences in brain 5-HT synthesis. Instead, the amount of 5-HIAA in CSF is influenced not only by the rate of 5-HT synthesis, but also by: (1) the rate of 5-HT release in brain and spinal cord; (2) the extent to which 5-HT is taken back up into neurons after it is released; (3) the rate at which 5-HT is metabolized to 5-HIAA; (4) the activities of the transport systems that move 5-HIAA from brain or spinal cord to blood and CSF; (5) the rate of production and mixing of CSF; and (6) the activity of the transport system that removes 5-HIAA from CSF to blood. Each of these factors may vary between animals or over time. Moreover, because the pool of 5-HIAA in the CSF does not turn over quickly, CSF 5-HIAA will change only slowly in response to changes in the rate of 5-HT synthesis. Thus, when probenecid is used to block the transport of 5-HIAA out of CSF, it takes several hours for CSF 5-HIAA levels to increase appreciably (Kopin 1978). Similarly, when 5-HT synthesis is lowered using the acute tryptophan depletion technique, the decline in CSF 5-HIAA is modest, and occurs only gradually over a period of hours (Carpenter et al. 1998). These results suggest that baseline levels of CSF 5-HIAA are, at very best, a rough indication of the integrated rate of 5-HT synthesis over many hours. Variations of 5-HT synthesis occurring over a period of less than a day would not necessarily be detected by measurements of CSF 5-HIAA; they might, however, be detected by PET. Thus, the lack of a significant correlation between CSF 5-HIAA and rates of 5-HT synthesis measured by the αMTrp method does not necessarily reflect the lack of validity of the αMTrp method. The relevance of Shoaf et al.'s comparison is further questioned since CSF and PET studies were conducted under different conditions; e.g., ketamine anesthesia vs. isoflurane anesthesia preceded by two injections of ketamine plus one injection of sodium pentobarbital.

Shoaf et al. (1998) expressed concern that the calculated rates of 5-HT synthesis are dependent on plasma concentrations of free tryptophan. Serotonin is made from tryptophan, and plasma-free tryptophan is one of the factors that controls the availability of the 5-HT precursor in brain. Because the rate-limiting enzyme in 5-HT synthesis, tryptophan hydroxylase, is not normally saturated with tryptophan, brain 5-HT synthesis would be expected to covary with free plasma tryptophan. Of course, numerous other factors will cause variations in brain 5-HT synthesis, including the level of the other large neutral amino acids in plasma (which inhibit the transport of tryptophan into brain), the activity of the actual transport system, and the amount of

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active tryptophan hydroxylase. Not much is known about factors that induce variations in the activity of the transport system or of tryptophan hydroxylase. Shoaf et al. provide no information on levels of the other large neutral amino acids. In comparison, they do provide evidence that their measurements were not done under conditions in which free plasma tryptophan concentrations were stable. For example, when monkeys were tested on more than one occasion, the average percent difference for free plasma tryptophan levels was 48%, a difference that might well be expected to produce a difference in brain 5-HT synthesis.

Shoaf et al. (1998) agree that tryptophan hydroxylase is not normally saturated with tryptophan, but they state that the variations of free plasma tryptophan seen in their study are too small to result in variations in brain 5-HT synthesis. In support of this contention they cite several studies (Fernstrom and Wurtman 1972; Leathwood 1987; Leathwood and Fernstrom 1990). However, none of these studies involved measurements of brain 5-HT synthesis. What was measured was brain concentrations of 5-HT and 5-HIAA. Like concentrations of 5-HIAA in CSF, levels of these compounds in brain tissue may give a rough index of 5-HT synthesis, but the measures are, at best, crude and insensitive approximations. One difficulty in assessing the validity of the αMTrp method is the lack of a valid standard. The best methods available in the past looked at the accumulation of 5-hydroxytryptophan following inhibition of aromatic amino acid decarboxylase, or the accumulation of 5-HT after the inhibition of monoamine oxidase. However, both of these methods involve upsetting the homeostasis of the 5-HT neuron, and therefore may involve changes in the rate of 5-HT synthesis, the variable one is attempting to measure.

A final concern expressed by Shoaf et al. is that the Patlak plots published in their report did not have an unambiguously linear portion. In comparison, Patlak plots meeting criteria for linearity have been obtained in other laboratories. Four linear Patlak plots were provided in our first αMTrp/PET article in humans (Figure 1 in Nishizawa et al. 1997) and we show additional examples here (Figure 1). Linear Patlak plots have also been obtained by the one other group of researchers reporting αMTrp/PET studies in humans (see Figure 5 in Chugani et al. 1998). If Shoaf et al. did not obtain linear portions in monkeys, the reason for this is unclear, but it might reflect methodological or species differences.

The αMTrp method may well need further refinements before it gives valid measurements. However, the studies reported by Shoaf et al. are not adequate tests of the model, and their data do not provide a convincing argument that the method lacks validity.

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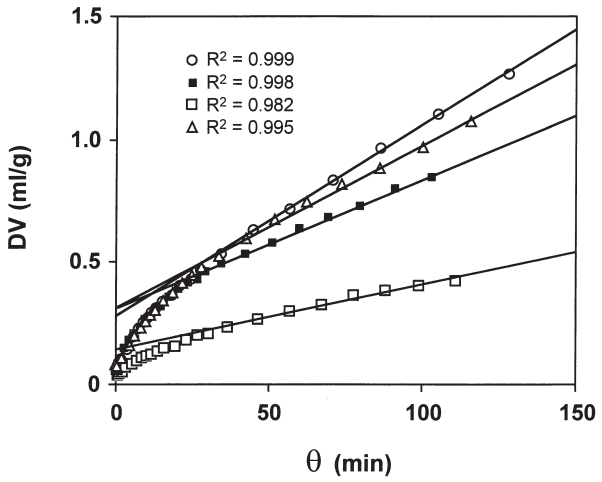

Figure 1. Patlak plots of four of the last five scanned healthy volunteers. Tissue activities of the brain were obtained from a large region of interest drawn in the slice of centrum semiovale. The linear lines are regression lines for the time period of 20 to 60 minutes following tracer injections. Each regression coefficient (r) was higher than 0.99. DV is volume of distribution and ø is the exposure time.

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