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RESPONSE

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

The comments made by Benkelfat et al. raise some interesting points. Their comments were that (1) "no evidence . . . that steady state was indeed achieved," and (2) ". . . evidence that their measurements were not done under conditions in which free plasma tryptophan concentrations were stabilized."

During the PET scans, plasma tryptophan (Trp) concentrations were in fact at steady state. As stated in our article, animals were fasted from 7 p.m. the previous night until the time of the scan. During the 18 months of the study, all the monkeys consumed the same diet with identical feeding schedules. Not included in our article was the fact that plasma samples were taken before and after the PET scans and analyzed for Trp. The average difference for total Trp was less than 5 nmol/ml and for free Trp was less than 0.5 nmol/ml.

Benkelfat et al. also commented that (1) "CSF 5-HIAA will change only slowly in response to changes in the rate of 5-HT synthesis," and (2) "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."

The turnover of the cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) is more rapid than they state. Plasma tryptophan loading experiments in dog (Eccelston et al. 1968) and human (Eccelston et al. 1970), and a human plasma tryptophan depletion study in our laboratory (Williams et al. 1999) show that CSF 5-HIAA concentrations are different than baseline within 2 hours of significant changes in CSF TRP. The slow change in CSF 5-HIAA concentrations seen following oral probenecid administration is because absorption of probenecid is slow [t_{max} of approximately 4 hours, Emmanuelson et al. (1987a)]. The acid transporters are probably not completely blockaded until total plasma concentrations of probenecid reach more than 200 μg/ml (Emmanuelson et al. 1987b). The rate of rise of CSF 5-HIAA will be dependent on the degree of saturation of the transporters.

Benkelfat et al. further commented that (1) "Small differences in CSF 5-HIAA levels are particularly unlikely to reflect differences in brain 5-HT synthesis." and (2) "One difficulty in assessing the validity of the αMTP method is lack of a valid standard."

Our monkeys had CSF 5-HIAA concentrations that ranged from 157–345 pmol/ml, hardly a small difference. Note that the data point for 5-HIAA of 345 pmol/ml was inadvertently cropped from Figure 4A in Shoaf et al.; the line and correlation are correct as indicated. For many years, CSF 5-HIAA concentrations have been considered to be the marker of serotonin turnover for correlation with disease state and behavior (Bjorntorp 1995; Linnoila et al. 1994; Meltzer 1990; Owens and Nemeroff 1994; Tuinier et al. 1995).

Benkelfat et al. also commented that mention of the possibility that different anesthesias might be the reason we see no correlation between serotonin synthesis rate and CSF 5-HIAA.

CSF samples are withdrawn within 15 minutes of ketamine administration. It is unlikely that isoflurane anesthesia greatly suppresses 5-HT synthesis rates but no published data are available. If it is true, then the results from the dog PET study (Diksic et al. 1991) may also have to be reinterpreted. It has been our experience that the length of isoflurane anesthesia does not affect αMTP uptake. In four paired αMTP studies separated by 3 hours, the mean K-values changed by an average (SD) of −0.2 (9.8)% (unpublished observation).

Benkelfat et al. commented that "Patlak plots meeting criteria for linearity have been obtained in other laboratories." We have redrawn our Figure 1A (Figure 1) with regression lines for data collected between 30–60 minutes and 60–90 minutes. In both cases, we get high correlations. When each line is viewed independently, one might conclude that an irreversible uptake phase of the Patlak plot had been reached. However, when viewing the whole curve, it becomes obvious that the assumption of linearity in any part of the curve is a result of the slow kinetics of αMTP. In our discussion (p. 351) we stated "One interpretation is that equilibrium of the αMTP precursor pool has not yet occurred by the 30 to 60 min period used for calculation of K. If so, we have underestimated the true K values." This statement should have read "overestimated". We find that the K-values estimated from the data between 60-90 minutes are significantly lower than those from 30–60 min-

Published 1999 by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010
0893-133X/99/$–see front matter
PII S0893-133X(99)00002-0

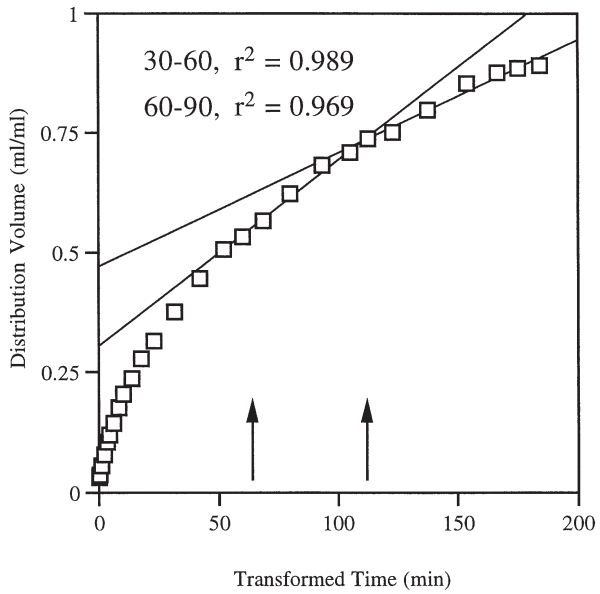

Figure 1. The Patlak plot for a cortical region of rhesus monkey brain now shows the regression lines drawn through data obtained from 30–60 minutes and 60–90 minutes of actual time. The transformed times for 30 and 60 minutes, 60 and 112 minutes are indicated by arrows. Although the r^2-values are greater than 0.96, there is an under, over, under pattern to the y-residuals. (Redrawn from Fig. 1A of Shoaf et al.)

utes data; for a cortical region from 12 monkeys, K-values were 3.62 ± 0.78 versus 2.39 ± 0.74, $p < .00001$. This was a 34 ± 14% reduction. Presumably, if the human data acquisition in other laboratories had continued longer than 60 minutes similar results would have been found.

Susan E. Shoaf, Ph.D.[b]
Daniel Hommer, M.D.[b]
Wendol Williams, M.D.[b]
J. Dee Higley, Ph.D.[b]
Richard Carson, Ph.D.[a]
Peter Herscovitch, M.D.[a]
William Eckelman, Ph.D.[a]

[a]PET Department
National Institutes of Health
Clinical Center
Bethesda, Maryland

[b]Laboratory of Clinical Studies, DICBR
National Institute on Alcohol Abuse & Alcoholism
Bethesda, MD

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