

# Brain Serotonin Synthesis Rates in Rhesus Monkeys Determined by [<sup>11</sup>C]α-Methyl-L-Tryptophan and Positron Emission Tomography Compared to CSF 5-Hydroxyindole-3-Acetic Acid Concentrations

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Twelve male, fasted, anesthetized rhesus monkeys were studied with positron emission tomography (PET) and [<sup>11</sup>C]α-methyl-L-tryptophan (αMTP) to determine serotonin synthesis rates as described by Diksic et al. (1991). It was expected that the serotonin synthesis rates determined for the whole brain would be correlated with CSF 5-hydroxyindole-3-acetic acid concentrations, a measure of central serotonin turnover, because both measures were obtained at steady state. However, no significant correlation was found. During data analysis, it was noticed that the calculated serotonin synthesis rates were significantly correlated to free plasma tryptophan (TP) concentrations ( $r = 0.88$ ,  $p < .001$ ). From repeat scans conducted in six monkeys, it was determined that day-to-day variability in free plasma TP and the percentage of

protein binding (average percent difference was 48 and 37%, respectively) produced most of the variability in the calculated serotonin synthesis rates (50%); repeat K images, obtained from the PET data alone, differed by only 11%. Calculated serotonin synthesis rates reported for [<sup>11</sup>C]αMTP PET studies of humans (Nishizawa et al. 1997) and dogs (Diksic et al. 1991) were also highly correlated to reported differences in plasma free TP concentrations. It seems that the [<sup>11</sup>C]αMTP model for the computation of serotonin synthesis rates is very dependent on plasma free TP concentration and that it may not accurately determine actual serotonin synthesis rates.

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Early attempts to determine serotonin synthesis rates in small mammals usually involved pharmacological manipulation of the serotonin system followed by the sampling of brain tissue for the determination of tryptophan, tryptophan metabolites, serotonin, and/or serotonin metabolites. These studies were limited by the ability of the investigator to isolate specific brain regions, and the sensitivity of the assays usually required the pooling of tissues from multiple animals (Colmenares et al. 1975; Curzon and Marsden 1975; Fernstrom and Wurtman 1971; Fernstrom and Wurtman

1972; Hamon and Glowinski 1974; Leathwood 1987; Leathwood and Fernstrom 1990; Moir and Eccleston 1968; Ternaux et al. 1976). Recently, a method for measuring serotonin synthesis rates in the brain using  $\alpha$ -methyl-L-tryptophan ( $\alpha$ MTP) was developed in rats (Diksic et al. 1990; Nagahiro et al. 1990) and then tested in dogs using [ $^{11}$ C] $\alpha$ MTP and positron emission tomography (PET) (Diksic et al. 1991).  $\alpha$ MTP is a tracer of tryptophan uptake into the brain and its conversion into serotonin.  $\alpha$ MTP crosses the blood-brain barrier via the same transport mechanism as tryptophan and is converted to  $\alpha$ -methylserotonin ( $\alpha$ M5HT) in serotonin neurons. Because  $\alpha$ M5HT is not a substrate for monoamine oxidase and does not cross the blood-brain barrier, it accumulates in the brain. It is also assumed that there is no loss of radioactivity from the brain during the period of the study; that is, that the uptake of  $\alpha$ MTP is irreversible (Diksic et al. 1990). This method employs the same mathematical model as that developed by Sokoloff et al. (1977) for the use of deoxyglucose to determine glucose metabolism. The use of PET makes it theoretically feasible to investigate serotonin synthesis rates in vivo in such mammals as rhesus monkeys and humans.

For many years, CSF concentrations of 5-hydroxyindole-3-acetic acid (5-HIAA) have been used as a measure of central serotonin turnover rate. Lowered CSF 5-HIAA has been linked to impaired impulse control and aggressive behavior in humans (Brown et al. 1979; Linnoila et al. 1989; Linnoila et al. 1983) and in rhesus monkeys (Higley et al. 1996b, c, d, e). If serotonin pools in the brain are in steady state, we hypothesized that there should be a close correspondence between serotonin synthesis rate and CSF 5-HIAA concentrations. The brains of 12 male rhesus monkeys that have had CSF (obtained from the cisterna magna) 5-HIAA concentrations determined repeatedly throughout their life span were studied with PET and [ $^{11}$ C] $\alpha$ MTP to determine their serotonin synthesis rates.

## METHODS

### Animals

Twelve male rhesus monkeys were housed in mixed-sex groups according to the NIH Animal Care and Use Guidelines. All procedures were approved by the NIAAA Animal Care and Use Committee (protocol LCS-73). Animals weighed between 5 to 9.6 kg and were 4 to 5.5 years old. The day before the PET scan, two animals were removed from the colony and transported to a holding room where they were housed together. The animals were fasted with only water available from 7 P.M. until the scan was complete and they were fully recovered from anesthesia. On the day of the scan, animals were transported to a surgical suite, anes-

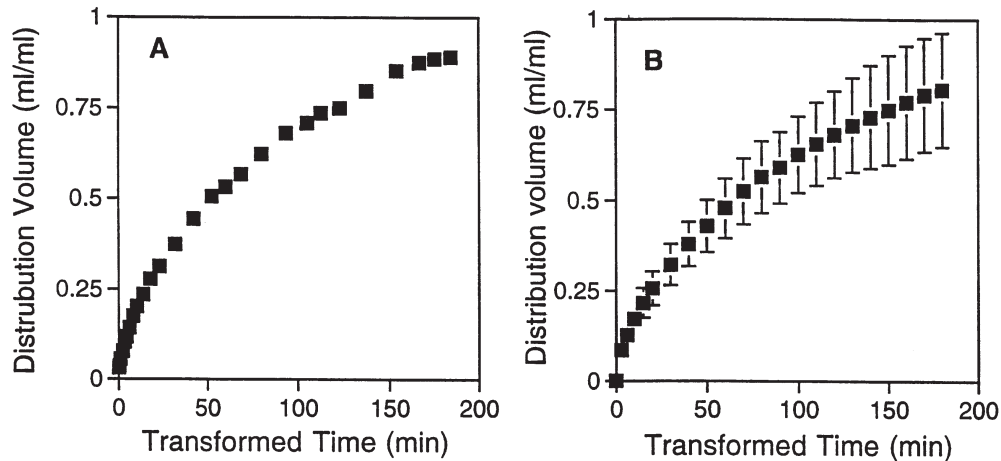
thetized with ketamine (IM, 10 mg/kg) and given IM injections of penicillin G procaine/benzathine (0.15 ml/kg) and glycopyrrolate (0.055 mg/kg). An intravenous catheter was inserted in the saphenous vein and a drip of sterile physiological saline was started. Sodium pentobarbital (9–13 mg/kg, IV) was administered before each animal was intubated. The animals were then placed under inhalation anesthesia (isoflurane) for the insertion of a femoral artery catheter, which was sutured and taped in place after being flushed with 1 ml of 10 U/ml heparin. For transport to the PET suite, the animals were given another IM dose of ketamine. Once in the PET suite, inhalation anesthesia with isoflurane was resumed. Heating pads were used to maintain body temperature. Electrocardiogram, blood pressure, respiratory rate, and end-tidal pCO<sub>2</sub> monitoring were used to regulate the depth of anesthesia.  $\alpha$ MTP was administered at least 2.5 h after the initial induction of anesthesia with ketamine so that any effects of ketamine or pentobarbital should be minimal and any medication effects would be primarily attributable to isoflurane.

### Imaging

A Scanditronix PC2048-15B scanner (Uppsala, Sweden) was used to acquire 15 coronal brain slices. During the PET procedure, the animals were positioned prone in a stereotaxic frame designed for use in the scanner. A transmission scan was obtained before injection of the tracer for attenuation correction. Two injections of 15 mCi of [ $^{15}$ O]water were performed and 60 s scans were acquired, averaged, and used for image registration and ROI placement. A 3 ml sample of arterial blood was taken for the determination of free and total plasma tryptophan (TP). The radiopharmaceutical [ $^{11}$ C] $\alpha$ MTP was prepared according to the method of Schmall et al. (1995). The dose was injected intravenously through the saphenous catheter over a 1 min period using an infusion pump. Injected doses ranged from 1.42 to 16.4 mCi. Twenty-six arterial blood samples were drawn for the determination of  $\alpha$ MTP whole blood and plasma concentration curves; each blood sample had a volume of 0.5 ml. Because no metabolites of  $\alpha$ MTP are present in blood/plasma samples, samples were directly counted on a Packard Cobra Auto-gamma counter. Six of the monkeys were scanned twice, once with low activity of  $\alpha$ MTP ( $3.0 \pm 1.7$  mCi, mean  $\pm$  SD, range 1.42–6.1 mCi, specific activity, Ci/mmol,  $128 \pm 90$ ) and once with high activity ( $11.4 \pm 4.1$  mCi, range 4.2–16.4 mCi, specific activity, Ci/mmol,  $163 \pm 55$ ).

### Data Analysis

Based on the model of Diksic et al. (1990, 1991), the serotonin synthesis rate is calculated as follows:  $R = K \cdot C/LC$  where  $K$  is the net influx constant of  $\alpha$ MTP ( $\mu$ l/



**Figure 1.** (A) A plot of the tissue distribution volume (tissue concentration/plasma concentration; ml/ml brain) as a function of the exposure time (min, see Patlak et al. 1983) for a cortical region of a rhesus monkey brain. (B) A plot of the average ( $\pm$  SD) tissue distribution volume as a function of the exposure time for a cortical region of 12 rhesus monkey brains. Time activity curves and plasma input functions were transformed to produce Patlak plots, and values were interpolated to a standard set of times for purpose of calculation of mean and SD, as shown in the figure. Note that there is no time period where a clearly discernible irreversible uptake (linear portion) is evident.  $K$  values were obtained between 30 and 60 min of actual time, corresponding to  $61 \pm 7$  to  $128 \pm 15$  min of transformed time.

min/ml),  $C$  is the free plasma TP concentration (nmol/ml), and  $LC$  is the lumped constant with a value of 0.18 for rhesus monkeys (Shoaf and Schmall 1996).  $K$  was calculated using a variant of the Patlak analysis (see Figure 1) (Patlak et al. 1983). No portion of the Patlak plots was truly linear, so we chose 30 to 60 min postinjection of  $\alpha$ MTP for our analysis to correspond with analyses that had been performed for humans (Muzik et al. 1997; Nishizawa et al. 1997). The tissue concentration data from each pixel over that time range ( $C_i$ , nCi/ml tissue) are fit to the following linear equation:  $C_i = K \int C_p dt + V_e \cdot C_b$  where  $C_p(t)$  is the measured plasma concentration of  $\alpha$ MTP,  $C_b$  is the whole blood concentration, and  $V_e$  is the exchangeable tissue volume (ml blood/ml tissue). The fit was performed pixel-by-pixel, and images of  $K$  were obtained.

Brains were outlined on the water scans and coregistered to the brain of one monkey by the gray scale matching technique (Thévanaz et al. 1995); cubic spline interpolation was used to determine pixel values on the registered scans. Average  $K$  and water images were produced for the 12 animals (Figure 2). Regions of interest ( $4 \times 4$  pixels; 2 mm/pixel) were placed on the average waterscan, and the  $K$  values for each ROI were determined using the registered scans.

### Chromatographic Assays

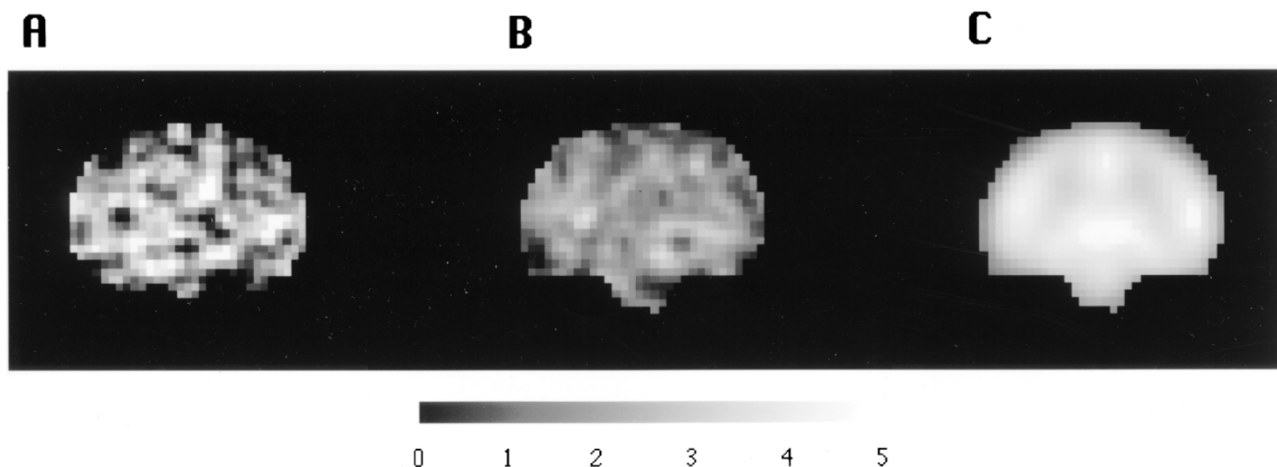
Free and total plasma TP concentrations were determined by high-performance liquid chromatography (HPLC) according to Shoaf and Schmall (1996), except

that fluorometric detection was used. Fluorometric detection (Waters 460 Fluorimeter) used excitation and emission wavelengths of 290 and 340 nm, respectively. Free plasma TP was determined from 250  $\mu$ l aliquots of plasma, incubated for 3 min at 37° C, which were centrifuged through 30,000 mw cutoff filters (Amicon, Beverly, MA) placed in a microfuge rotor that had been brought to 37° C by incubation in a water bath. Samples were spun at 6,000 rpm (approximately,  $3,000 \times g$ ) for 5 min to produce 35 to 40  $\mu$ l of eluant.

CSF 5-HIAA concentrations were determined in samples taken via the cisterna magna under ketamine anesthesia and frozen at  $-80^\circ\text{C}$  until assayed. The assay used gas chromatography with mass spectrophotometric detection following derivitization according to the method of Polinsky et al. (1988). Samples obtained from each individual within 4 days to 1 month of the scan data were used for the correlation with calculated serotonin synthesis rates.

## RESULTS

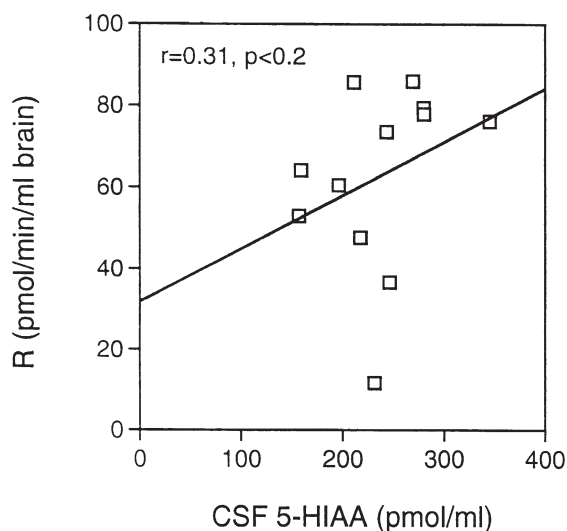
Figure 2A shows a typical  $K$  image, from a single male rhesus monkey, from a coronal slice through the brainstem. The noise of the individual image is great because of low brain uptake (peak % of injected dose/cc cortex was  $0.0096 \pm 0.0018\%$ ), and no anatomical detail is discernible. The averaged  $K$  image (Figure 2B) is less noisy but, again, it seems that no regional variations in  $\alpha$ MTP uptake are present. Brain structure can be more clearly visualized on the average water image (Figure 2C).



**Figure 2.**  $\alpha$ -Methyl-L-tryptophan uptake images ( $K$  values, see Methods) and an [ $^{15}\text{O}$ ]water image from a coronal slice through the brainstem. (A) shows the  $K$  image from a single male rhesus monkey. (B) is the averaged  $K$  image for 12 monkeys. (C) is the averaged water image for that slice, upon which the ROIs were placed. The gray scale bar indicates the range of  $K$  values  $\mu\text{l}/\text{min}/\text{ml}$  brain).

### Whole Brain Analysis

For the whole brain, the average ( $\pm$ SD)  $K$  value ( $\alpha$ MTP uptake) for the 12 monkeys was  $3.33 \pm 0.56$  ( $\mu\text{l}/\text{min}/\text{ml}$ ), the average free plasma TP ( $C$ ) was  $3.36 \pm 1.13$  (nmol/ml), and the average serotonin synthesis rate ( $R$ ) was  $62.7 \pm 22.3$  (pmol/min/ml). The  $K$  and  $R$  values are similar to those reported for human brain (Muzik et al. 1997; Nishizawa et al. 1997). We determined whole brain  $K$  and  $R$  to compare with CSF 5-HIAA concentrations, because CSF 5-HIAA is considered an indicator of whole brain serotonin turnover rate. When we correlated  $R$  with CSF 5-HIAA, pmol/ml (Figure 3), we



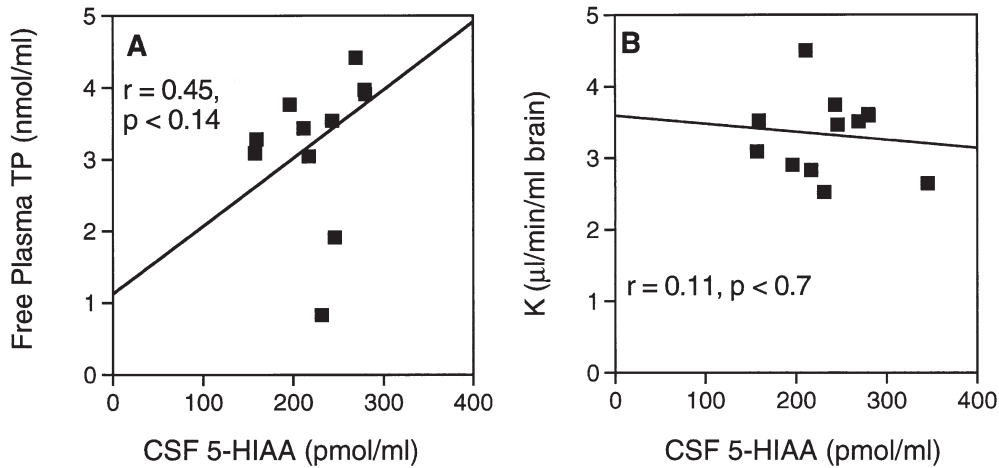
**Figure 3.** Correlation of calculated whole brain serotonin synthesis rates ( $R$ , see Methods) with cerebrospinal fluid 5-hydroxyindole-3-acetic acid concentrations (CSF 5-HIAA) for 12 male rhesus monkeys.

found that the correlation coefficient  $r = 0.31$  was not significant, and the variability in the  $R$  values was high; animals with similar CSF concentrations had  $R$  values that were 2 to 4-fold different.

$R$  is calculated from the equation  $R = C \cdot K/LC$ ; to examine the contributions of  $C$  and  $K$  to the variance of  $R$ , we performed a log transformation of the data where  $\text{var}(\ln R) = \text{var}(\ln C) + \text{var}(\ln K) + 2 \text{cov}(\ln C, \ln K)$ . Approximately 70% of the variance was attributable to  $C$ , 10% was attributable to  $K$ , and 20% was attributable to a mixture of the two. Therefore, free TP is the major source of variance in the  $R$  values. When we compared free TP (Figure 4) with CSF 5-HIAA (Figure 4A) and  $K$  values (Figure 4B), we found no significant correlations. However, the correlation coefficient for free TP with CSF 5-HIAA was 0.45 and would help support the correlation of  $R$  with CSF 5-HIAA.

Because of the large amount of variability in  $R$ , we examined the reproducibility of our measurements of free TP and  $K$ . In the six animals that were scanned twice, we compared the  $K$ ,  $R$ , free plasma TP, and % free TP values obtained from the first scan to those obtained in the second scan (Figure 5). Of all the measurements,  $K$  (Figure 5A) varies the least with an average percent difference of 11%, percent calculated as equal to  $|K_1 - K_2|/(K_1 + K_2)/2 \cdot 100$ . The average percent difference for free TP determinations (Figure 5C) was 48% and this variation produces most of the variation seen in  $R$ , 50% (Figure 5B). Not only does the free TP concentration vary between days, but the percent of free TP also varies (Figure 5D), the average percent difference was 37%.

Since the  $\alpha$ MTP method for the calculation of serotonin synthesis rate seems to be extremely sensitive to the determination of free TP and some investigators (Par-



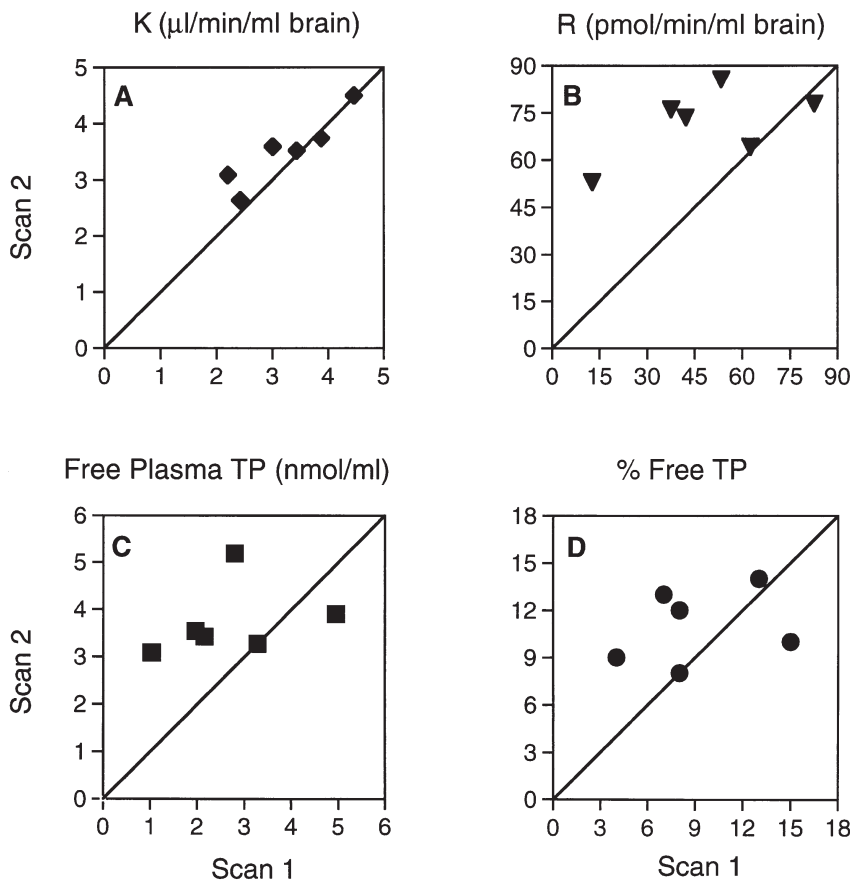
**Figure 4.** Correlations of cerebrospinal fluid 5-hydroxyindole-3-acetic acid concentrations (CSF 5-HIAA) with (A) free plasma tryptophan (TP) concentrations and (B) whole brain  $\alpha$ MTP uptake rate (K) for 12 male rhesus monkeys.

dridge and Fierer 1990; Yuwiler et al. 1977) have suggested that the uptake of TP is not limited by the percent of free TP but by total TP concentration, we investigated the correlation of R with CSF 5-HIAA when total TP concentrations were used to calculate R; there was no correlation,  $r = 0.09$ . Also, total TP concentrations were not correlated with CSF 5-HIAA,  $r = 0.03$ . Total TP concentrations ranged from 19–39 nmol/ml,

average ( $\pm$  SD)  $29.2 \pm 6.9$  nmol/ml. In the six animals scanned twice, the average percent difference of total TP concentrations across days was 20%.

**Region of Interest Analysis**

K and R values (Table 1) were determined for nineteen regions of interest (ROI). Due to the small size of the



**Figure 5.** Correlations between (A) whole brain  $\alpha$ MTP uptake rates (K, see Methods); (B) whole brain serotonin synthesis rates (R); (C) free plasma tryptophan (TP) concentrations; and (D) the percentage of free plasma TP concentrations that were determined on 2 separate days for six male rhesus monkeys. The solid lines indicate lines of identity.

monkey brain, the ROI's on any given slice were limited to  $4 \times 4$  pixels (2 mm/pixel). Analysis of the mean  $K$  and  $R$  values by 1-way repeated measures ANOVA indicated that the means were different but follow-up pairwise comparisons of the means found only a few significant differences,  $p < .0005$ , when Bonferroni corrections were applied. Differences in partial volume effects between regions may be the main contributing factor to any inter-region variability. The range of the average  $K$  values for the different ROI's is only 2.87–4.47 ( $\mu\text{l}/\text{min}/\text{ml}$  brain), indicating that  $\alpha\text{MTP}$  uptake is fairly uniform across the brain. The right insular cortex and the orbital lobe were found to be significantly different than some other regions (Table 1). A similar finding was made in PET scans of human brains (Muzik et al. 1997; Nishizawa et al. 1997).

## DISCUSSION

In steady state, the synthesis rate of serotonin, the conversion rate of serotonin to 5-HIAA, and the elimination rate of 5-HIAA should be equal. The primary route of elimination of 5-HIAA from the brain is via its release into the CSF followed by transfer into blood by acid transporters across the blood–brain barrier. The concentration of 5-HIAA in CSF should therefore, be reflective of the elimination rate of 5-HIAA. (Post and Goodwin 1975). We found no significant correlation be-

tween whole brain serotonin synthesis rates  $R$  as calculated by the [ $^{14}\text{C}$ ] $\alpha\text{MTP}$  technique and CSF 5-HIAA concentrations.

We believe that our CSF 5-HIAA concentrations and serotonin synthesis rates were obtained at steady state. The CSF concentrations determined in these animals were obtained 5 to 7 h following the morning feeding. Ashley et al. (1984) have stated that in free feeding rats, the carbohydrate or protein meals eaten after a 3-h fast had no effect on brain TP or serotonin metabolism. In human diet studies, it was reported that diet or recent meal type (high carbohydrate vs. high protein) had little effect on total plasma TP/LNAA ratios (Fernstrom 1987) or human CSF TP and 5-HIAA concentrations (Teff et al. 1989). 5-HIAA concentrations have been shown to be relatively consistent across repeated samples in adult female rhesus macaques over a 24-week period (Higley et al. 1996a). In the present study, the  $R$  values were determined following at least a 12-h fast. Tryptophan is transported across the blood–brain barrier by an amino acid transporter that also carries phenylalanine, valine, isoleucine, leucine, and tyrosine. Fasting makes the concentrations and proportions of these large neutral amino acids (LNAA) relatively constant in plasma. In cynomolgous monkeys, the TP/LNAA ratio was 0.18 to 0.22 for four different groups of 6 monkeys on 4 different days, and the total concentrations of all six LNAA had a coefficient of variation of approximately 10% (Leathwood and Fernstrom 1990). Thus, it is unlikely that variation in TP/LNAA ratio was a meaningful source of the marked variability observed in the repeat scans in the present study.

We found that whole brain serotonin synthesis rates ( $R$ , range 10–85 pmol/ml/min) were highly correlated to the free plasma TP concentrations ( $r = 0.88$ ,  $p < .001$ ). This finding is unexpected based on the literature. Although in vitro enzyme and brain slice studies have shown that small changes in TP concentration can rapidly change serotonin synthesis rates (Hamon and Glowinski 1974), in vivo studies in rats and monkeys do not show marked changes in  $R$  over small ranges of plasma TP (Fernstrom and Wurtman 1972; Leathwood 1987; Leathwood and Fernstrom 1990); these ranges were similar to the range of plasma TP found in this study,  $3.36 \pm 1.13$  (nmol/ml). In these in vivo studies, the increase of serotonin synthesis rate (as determined by the combined concentrations of 5HT and 5-HIAA) was better correlated to large increases in the TP/LNAA ratio; 2 to 10-fold changes in the ratio were well correlated ( $r > 0.8$ ) with increasing 5HT+5-HIAA concentrations. Leathwood (1987) states that a 4-fold increase in the TP/LNAA ratio produces about an 80% increase in brain TP, which produces a 20% increase in 5HT+5-HIAA concentrations. However, within small ranges of the TP/LNAA ratio, there is no correlation of TP/LNAA to 5HT+5-HIAA concentrations (Fernstrom

**Table 1.** Uptake Slopes ( $K$ ,  $\mu\text{l}/\text{min}/\text{ml}$  brain) and Calculated Serotonin Synthesis Rates ( $R$ , pmol/min/ml brain) for 12 Anesthetized, Male Rhesus Monkeys

Brain Area	$K \pm \text{SD}$	$R \pm \text{SD}$
Occipital cortex	$3.10 \pm 0.71^a$	$57 \pm 20^a$
Cerebellum	$2.98 \pm 0.86^a$	$56 \pm 24^a$
L. parietal	$4.01 \pm 0.79$	$76 \pm 28^b$
R. parietal	$3.69 \pm 1.08$	$70 \pm 29$
L. hippocampus	$3.39 \pm 0.81$	$62 \pm 23$
R. hippocampus	$3.53 \pm 1.26$	$66 \pm 32$
L. white matter	$3.67 \pm 0.94$	$68 \pm 27$
R. white matter	$3.10 \pm 0.91^a$	$58 \pm 25^a$
Thalamus	$3.56 \pm 0.80$	$67 \pm 28$
Cingulate gyrus	$3.15 \pm 1.00^a$	$61 \pm 29^a$
Brainstem	$2.99 \pm 0.84^a$	$56 \pm 23^a$
Basal ganglia	$3.26 \pm 0.68^a$	$61 \pm 23^a$
L. temporal lobe	$4.03 \pm 1.05$	$75 \pm 28$
R. temporal lobe	$3.82 \pm 1.02$	$73 \pm 32$
L. insular cortex	$4.08 \pm 0.98$	$77 \pm 31^b$
R. insular cortex	$4.47 \pm 1.23$	$84 \pm 33^b$
L. prefrontal cortex	$4.05 \pm 0.91$	$78 \pm 30^b$
R. prefrontal cortex	$3.87 \pm 1.08$	$75 \pm 31$
Orbital lobe	$2.87 \pm 0.78$	$52 \pm 20^a$
Whole brain	$3.33 \pm 0.56$	$63 \pm 22$

<sup>a</sup>Significantly different from the R. insular cortex,  $p < .0005$ .

<sup>b</sup>Significantly different from the orbital lobe,  $p < .0005$ .

and Wurtman 1972; Leathwood 1987). We should have expected a 2-fold range of serotonin synthesis rates to parallel our 2-fold variation in cisternal steady state CSF 5-HIAA concentrations (200–360 pmol/ml).

In a recent PET study using [ $^{11}\text{C}$ ] $\alpha\text{MTP}$  to determine serotonin synthesis rates in men and women (Nishizawa et al. 1997), the authors state that “. . . there was no significant correlation between plasma free or total tryptophan levels and rates of serotonin synthesis in either group [men vs. women] of subjects.” But in a review of the data from this study and a dog [ $^{11}\text{C}$ ] $\alpha\text{MTP}$  PET study (Diksic et al. 1991), we find that reported differences in serotonin synthesis rates can be mostly attributed to differences in plasma TP concentrations. In the human study, men were reported to have higher serotonin synthesis rates than women, both at baseline and following plasma TP depletion. When we calculated the average  $K$  values at baseline from the reported  $R$  values ( $K = R * 0.42/C_p$ ) for each region and the average  $C_p$  value, we found that the ratio of the  $R$  values (men/women, range 1.5–1.7) was most similar to the ratio of the average plasma TP concentrations (1.97) and that the ratios of the  $K$  values ranged from 0.75 to 0.86. A similar result was found for the data obtained following TP depletion. Therefore, the sex difference in  $R$  is mostly attributable to the difference in plasma free TP between the sexes. Before TP depletion, the plasma TP concentrations reported for women were approximately half that of the men, and following TP depletion they were 5-fold lower. Differences of these magnitudes between the sexes have never before been reported by other investigators (Caballero et al. 1991; Delgado et al. 1989; Lucini et al. 1996; Maes et al. 1995; Møller et al. 1996; Neumeister et al. 1997) or in previous studies conducted by the authors themselves (Benkelfat et al. 1994; Ellenbogen et al. 1996).

In the dog PET scans (Diksic et al. 1991), the authors reported that the whole brain serotonin synthesis rate ( $R$ ) was  $620 \pm 112$  pmol/g/min for a free plasma TP value of 381 nmol/ml and  $320 \pm 95$  for a free plasma TP value of 191.5 nmol/ml. Calculation of the  $K$  values indicates that there is no difference (0.75 vs. 0.77) and that the increase in  $R$  is attributable totally to the doubling of the free plasma TP concentration. However, rat studies have shown that serotonin synthesis rates seem to peak when the brain and free plasma TP concentrations are in the 90 nmol/ml range (Leathwood 1987) with further increases in plasma TP prolonging the increased rate of serotonin synthesis but not increasing the absolute rate (Eccleston et al. 1965; Moir and Eccleston 1968). Therefore, we would have expected that the  $K$  value would be reduced in the highest TP-loaded dog, because the serotonin synthesis rate would be the same, although plasma TP concentrations were higher. In vitro enzyme studies have shown that TP hydroxylase activity is not solely limited by TP availability but also

by brain oxygen tension and the availability of tetrahydrobiopterin (Hamon and Glowinski 1974). It seems that  $\alpha\text{MTP}$  uptake is not as tightly coupled to serotonin synthesis as, for example, fluorodeoxyglucose uptake is to glucose metabolism (the model upon which the  $\alpha\text{MTP}$  method is based).

The consistency of  $K$  across all brain regions becomes an expected result when we consider that TP uptake into the brain is a fast phenomenon that occurs brain-wide (Colmenares et al. 1975; Curzon and Marsden 1975; Eccleston et al. 1968) so  $\alpha\text{MTP}$  should be taken up into all brain regions. The conversion of  $\alpha\text{MTP}$  to  $\alpha\text{M5HT}$  is slow, even within serotonin neurons. Diksic et al. (1990) found that 31% of the radioactivity in the dorsal raphe nucleus was present as  $\alpha\text{M5HT}$  at 60 min following injection of [ $^{14}\text{C}$ ]  $\alpha\text{MTP}$ , and this brain region had a calculated serotonin synthesis rate 3 to 5-fold higher than all other brain regions studied, except the pineal gland, which is outside the blood–brain barrier (Nagahiro et al. 1990). So for the short duration of the PET scan, the amount of  $\alpha\text{MTP}$  actually “trapped” as  $\alpha\text{M5HT}$  in most brain regions is small. In their analysis of human PET scans, Muzik et al. (1997) noted that the  $K$  value is dominated by the  $k_2$  term and that 2-fold changes in  $k_3$  (the formation rate of  $\alpha\text{M5HT}$ ) produce only a 47% increase in  $K$  “causing a transport dependent blunting of differences in the  $K$  complex among different brain regions.”

We were able to collect images for 90 min post- $\alpha\text{MTP}$  injection, because our highest doses in monkeys (0.6–2.5 mCi/kg) were 3 to 5 times larger than the doses, approximately 0.12 to 0.8 mCi/kg, used in dogs (Diksic et al. 1991) and humans (Muzik et al. 1997; Nishizawa et al. 1997). Based on the model of Diksic et al. (1990) we expected to be able to verify that uptake of the tracer was irreversible, as evidenced by a straight line in the Patlak plot. As seen in Figure 1, this was not the case, and there was no time at which true irreversible uptake was seen. One interpretation is that equilibrium of the  $\alpha\text{MTP}$  precursor pool has not yet occurred by the 30 to 60 min period used for calculation of  $K$  (Diksic et al. 1991; Diksic et al. 1990; Muzik et al. 1997). If so, we have underestimated the true  $K$  values, and our estimates are overly influenced by the rate of transport of  $\alpha\text{MTP}$  instead of the steady-state rate of  $\alpha$ -methyl-serotonin synthesis. If this is the case, then studies with larger doses of  $\alpha\text{MTP}$ , longer data acquisitions, and the use of 3-dimensional PET may permit the accurate determination of an eventual irreversible slope. Note, however, that if the irreversible uptake phase begins much later than 30 to 60 min, the Patlak plots (Figure 1) would have a large  $y$ -intercept (a measure of unmetabolized  $\alpha\text{MTP}$ ) and a small slope (a measure of  $\alpha$ -methyl-serotonin synthesis). This suggests that the  $\alpha\text{MTP}$  method has a low sensitivity to the serotonin synthesis rate because of the high background of unmetabolized  $\alpha\text{MTP}$ .



We conclude that the [ $^{11}\text{C}$ ]αMTP method of determining serotonin synthesis rate has several methodological and theoretical shortcomings. This method is too dependent on the determination of free plasma TP concentrations, a parameter that is not well correlated to the regulation of serotonin synthesis rate under steady state. The rapid uptake of αMTP into all brain regions creates a large background effect and the rapid loss of signal, caused by the short half-life of  $^{11}\text{C}$ , prevents the observation of any regional α-methyl-serotonin accumulation that may be occurring.

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