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The lumped constant of α -methyl-L-tryptophan is not influenced by drugs acting through serotonergic system

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Abstract

Lumped constant (LC) is a constant used to convert brain trapping constant of α -methyl-L-tryptophan (using α -¹⁴C-methyl-L-tryptophan) into the constant for conversion of tryptophan into serotonin (5-hydroxytryptamine, 5-HT), which can be then used with certain assumptions in the calculation of the brain regional 5-HT synthesis rate. The aim of the present study was to investigate the acute effects of two drugs on the regional stability of the LC and possible effect on its value. Drugs used were a selective 5-HT reuptake inhibitor, fluoxetine, and a drug that releases 5-HT and inhibits 5-HT uptake, D,L-fenfluramine. The values of the LC from those experiments were compared with the value of LC obtained in the saline treated rats. KT is the constant for tryptophan conversion into 5-HT, which was measured by an autoradiographic method in more than twenty brain regions, using labelled tryptophan (¹⁴C-Trp) as tracer, after the fraction incorporated into proteins was removed. The trapping constant K_a for α -methyl-L-tryptophan was also measured autoradiographically in a separate group of rats. All measurements were done in drug and saline (control) treated rats. The regional LC constants were calculated as the ratios between KT and K_a. Statistical evaluation showed that the regional values in each of these three sets were normally distributed, and that the three sets of LC values calculated as the mean of logarithmic differences (saline 0.450±0.055; fluoxetine 0.429±0.091; D,L-fenfluramine 0.48±0.09) did not differ significantly. The overall weighted mean value of the LC from all three sets of measurements was 0.452±0.041, and this value was not significantly different from the LV value of 0.42±0.07, i.e. value used in our previous studies. These results showed the unaltered LC value in discrete rat brain regions after treatment with fluoxetine or D,L-fenfluramine, and confirmed that the numerical value of the LC used in our previous studies was not altered by treatment with drugs affecting 5-HT transmission and 5-HT synthesis rate.

Keywords

lumped constant; serotonin synthesis; fluoxetine; D,L-fenfluramine; saline; rat brain; 14 C-tryptophan; autoradiography

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Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important and widely distributed neurotransmitter. The alterations in central 5-HT functions are involved in a variety of physiological abnormalities, altered behaviors (anxiety, extreme violence, hostility, aggression, suicide, migraine, addictive behaviors, depressed mood, impulsivity), and some psychiatric disorders, such as depression, autism, eating disorders, schizophrenia, and obsessive/compulsive disorder (Lucki, 1998; Stahl, 1998; Yulug, 2009; Andrade et al., 2010). Antidepressant treatments have a wide effect on the brain neurochemistry; e.g. elevation in creatine kinase activity (Santos et al. 2009), modulation of the regional 5-HT synthesis (Berney et al. 2008), changes in 5-HT receptors (Sato et al. 2008 and 2010; Sargent et al. 2010; Bhagwagar et al. 2006). These divergent roles of central 5-HT stimulated research aimed to develop methods for studying alterations in central 5-HT neurotransmission, especially 5-HT synthesis. Studies in laboratory animals are of a special importance to obtain information on the influence of different drugs on the brain serotonergic system and relationship between those changes and behaviour.

5-HT is synthesized from its precursor, L-tryptophan (Trp), an essential amino acid. The transformation of Trp into 5-hydroxy-L-tryptophan (5-HTP) is catalysed by the tryptophan hydroxylase (TPH) the enzyme exclusive present only in serotonergic neurons (Kuhar et al. 1972), in the presence of tetrahydrobioptine and molecular oxygen (Fuller et al., 1980; Kema et al., 2000). After that 5-HTP undergoes a decarboxylation with aromatic acid decarboxylase (AAAD) into 5-HT (Kema et al., 2000). Because it is not practical to use labelled tryptophan as a tracer to measure this biochemical conversion, an autoradiographic method was developed using labelled α -methyl-L-tryptophan (α -MTrp) as an analog of tryptophan (Diksic et al., 1990; Nagahiro et al., 1999; Diksic and Young, 2001). The method is based on the measurements of the trapping constant of α -MTrp ($K\alpha$; ml/g/min) and its conversion to the constant representing Trp metabolism (KT; ml/g/min) through 5-HT pathway (Diksic et al. 2000) by a division with a conversion factor (lumped constant, LC). Subsequently the synthesis can be calculated as a product of KT and the plasma free tryptophan concentration C_p (nmol/ml). This conversion constant, LC, has been so far measured in normal rats without any treatment and a group of rats treated with probenecid (Vanier et al. 1995). Probenecid inhibits an efflux of 5-HT metabolite, 5-HIAA, from the brain but at the same time elevates the plasma non-protein bound tryptophan (van Wijk M et al. 1979), as well as the plasma concentration of amino acids competing with tryptophan for the blood brain barrier transporter (Vanier et al. 1995), which might represent rather complex biochemical condition and might complicate interpretation of results. Those experiments showed that probenecid does not influence the value of the LC or its stability across different brain regions, while fluoxetine is a non-tricyclic compound, with predominantly 5-HT reuptake inhibiting properties (Fuller et al., 1991). Fenfluramine is a drug that releases 5-HT (Fuller et al., 1991; Bonanno et al., 1994) and inhibits 5-HT uptake into synaptosomes (Bonanno et al., 1994). Fluoxetine has been widely used as antidepressant (Casack et al. 1994; Sanchez and Hyttel, 1999) while D,L-fenfluramine has been used as anorectic (Samanin and Garattini 1990; Goldstein and Potvin, 1994). In our previous studies effects of different drugs on the brain 5-HT synthesis rate using autoradiography were determined (Diksic and Young, 2001; Tohyama et al., 2007; Hasegawa et al. 2005; Watanabe et al. 2006; Nishi et al. 2006 and 2009; Skelin et al. 2008 and 2009; Kanemaru et al. 2008 and 2009) using LC value measured in normal rats (Vanier et al. 1995) and an assumption that serotonergic drugs do not have influence on the value of LC.

To obtain a confirmation of the LC value as well as its uniformity throughout the brain further evaluation with drugs known to act more directly on the brain serotonergic system

was termed necessary. Here we report LC determination in experiments in which two somewhat different drugs were used; fluoxetine, serotonin selective reuptake inhibitor (Fuller et al., 1991), which elevates extracellular concentration of 5-HT and influences 5-HT synthesis in a region specific manner (Mück-Seler 1996). In addition measurements done with D,L-fenfluramine, a drug which releases 5-HT from the vesicular storage (Mennini et al. 1985; Garattini, Meunini et al. 1986) and influences 5-HT synthesis in a region specific manner (Mück-Seler and Diksic, 1996).

An hypothesis of having LC normally distributed throughout rat brain and without change by different drugs was also tested. The test of the last hypothesis will be able to answer the question if serotonergic drugs influence the value of LC.

Methods

Drugs

Rats were injected intraperitoneally (i.p) with fluoxetine (10 mg/kg), received as a gift from the Eli Lilly, Indianapolis, IN, USA or saline (controls), or D,L-fenfluramine (10 mg/kg) purchased from RBI Chemicals, USA or saline (controls), immediately after the surgical procedure, and about 2 h before infusion of the tracer. The doses of fluoxetine and D,L-fenfluramine were those used in previously reported experiments (Mück-Seler et al., 1996; Mück-Seler and Diksic, 1996) to permit a direct comparison of results.

Tracers

In experiments reported here two tracers were used: 1) L- [3-¹⁴C] Trp (specific radioactivity 50 mCi/mmol) obtained from E.I. Du Pont de Nemours and Co (NEN, Boston, MA, USA), and 2) α-[¹⁴C]MTrp (specific activity of about 50 mCi/mmol) synthesized using the procedure described previously (Mzengeza et al., 1993). Both tracers had radiochemical purity above 96% and the chemical purity above 99%.

Experimental animals

Sprague-Dawley male rats (Charles River) weighing between 200–220 g were used in the study. Rats were housed in the animal facility (room temperature 22°C and 12 h day-night cycle; dark 19:00 to 7:00) for at least 3 days before the beginning of an experiment. The night before the experiment the animals were fasted overnight to stabilize the plasma concentration of amino acids, especially tryptophan, but with water supply *ad libitum*. All rats were sacrificed between 2 p.m.–4 p.m. The physiological parameters and the plasma tryptophan concentrations of rats used in the experiments described here were within the range of previously published in saline treated rats (e.g. Nagahiro et al., 1990; Pivac et al 2003). All of the animal use procedures were in the strict accordance with the Canadian Council on Animal Care guidelines and were approved by the Animal Care Committee of McGill University.

Surgical procedure and tracer injections

On the day of the experiment the rats were anaesthetized with 1.5% of halothane during the surgical implantation of the plastic catheters into femoral artery (for blood sampling) and vein (for tracer injection). The lower body of the rat was placed in a loose-fitting plaster cast and the animal was allowed to awake. Two hours after the i.p. administration of fluoxetine, D,L-fenfluramine, or saline, 27 µCi of [3-¹⁴C]Trp was injected over 2 min by an injection pump, except for the 5 min experiments (see schedule below), where a continuous infusion was carried out. Another group of rats was injected 30 µCi of α-[¹⁴C]MTrp in 1 ml of saline through a catheter into the femoral vein over 2 min as above, and 2 h after drug injection. Plasma samples were taken at progressively increased time intervals for the input function as

detailed before (Nagahiro et al. 1990). The blood samples were centrifuged for 3 min at 9300xg. Twenty μL of plasma was taken for the radioactivity determination by a liquid scintillation counting to measure plasma [^{14}C]radioactivity, needed for the input function. Blood gasses (pO_2 , pCO_2) and hematocrit and pH were determined with a blood gas analyzer (Model 178, CIBA-CORNING, Canada). In the experiments with α -[^{14}C] MTrp half of rats were decapitated 60 min and another half 150 min after the beginning of the tracer injection.

Tracer experiments

Number of rats to which L-[3- ^{14}C]Trp was injected in the protocol with fluoxetine (10 mg/kg) and saline, and in the protocol with D,L-fenfluramine (10 mg/kg) and saline are identified in Table 1. Five rats per group were sacrificed (with a guillotine) at 5, 10, 20, 30, 40, 50, 60, and 70 min after the beginning of tracer injection. At each time point, 2 rats received saline and 3–4 rats were injected with fluoxetine (10 mg/kg) dissolved in saline. A similar protocol was used for D,L-fenfluramine: 2 rats were injected with saline (note these are the same rats used in the fluoxetine protocol) and 3 rats were injected with D,L-fenfluramine (10 mg/kg) for each time point. With the beginning of the tracer (α -[^{14}C] MTrp) or L-[3- ^{14}C]Trp injection, arterial blood samples (12 – 15) were taken at progressively increased time intervals up to a sacrifice. Experiments with saline treated rats from fluoxetine and D,L-fenfluramine protocols were combined into a single group, because there was no significant difference in the tissue tracer concentrations.

Brain Slices Processing

The brains of the rats were immediately extracted and frozen in a cold (-20°C) 2-methylbutane, and stored at -80°C until cut for autoradiography. Brains were cut into 30 μm thickness in a cryostat at -24°C . The brain slices were mounted on the glass slides, dried on a hot plate kept at about 60°C , and contacted with X-ray films along with ^{14}C -polymer standards for 3–5 weeks in order to obtain autoradiograms. In experiments with labelled Trp the brain slices were cut in duplicate, and one set was used to determine regional total tissue concentrations of tracer (representing sum of labelled Trp incorporated into proteins, present in labelled 5-HT and 5-HIAA, and free labelled Trp), while another sets of adjacent brain slices from the same animal were washed three times with 20% trichloroacetic acid (TCA) (Vanier et al. 1995) to remove all non-protein bound radioactivity to obtain autoradiograms representing labelled Trp incorporated into tissue proteins. The washed slices were covered with Mylar (to prevent the effect of trichloroacetic acid on film) and along with ^{14}C -standards contacted to X-ray film. After films were developed, radioactivity concentrations in different structures were identified with reference to a rat brain atlas (Paxinos and Watson, 1995) using the Microcomputer Imaging System (MCID/M4- Image Analysis System; Imaging research, St. Catharines, Ontario, Canada). Optical densities were converted into tissue tracer concentration, utilizing a calibration curve made by plotting the optical density of ^{14}C standards as a function of their tissue equivalent concentration.

Quantification of the regional tracer concentration

The concentrations of the tissue radioactivity in different brain structures, were converted into volume of distribution (DV_α ; ml/g) by dividing the tissue concentrations at time T (min) [$\text{Ct}^*(T)$; nCi/g] by the plasma tracer concentration at time T [$\text{Cp}^*(T)$; nCi/ml] at the end of the experiment (Nagahiro et al. 1990). Similarly images from experiments in which labelled Trp were converted to tissue radioactivity concentration and the differences between two sets of images at the same time gave labelled Trp concentrations in the tissue soluble fraction (sum of labelled 5-HT, 5-HIAA, and labelled Trp) (Vanier et al. 1995). A tree compartment model describing metabolic pathway of tryptophan through 5-HT pathway

and irreversible trapping of labelled α -MTrp is shown in Fig. 1. Equation 1 was derived after solution of differential equations representing tracer movement between different tissue compartments (Nagahiro et al. 1990; Vanier et al. 1995). The method for measurement of 5-HT synthesis using labelled α -MTrp is based on irreversible trapping of α -MTrp and/or its metabolite (Diksic and Young, 2001; Diksic, 2001). The movement of the tracers either labelled α -MTrp or labelled tryptophan could be represented with differential equations (Diksic et al. 1990; Vanier et al. 1995). After equations are solved with initial condition of having concentration zero at time zero in all tissue compartments and rearrangements yields equation for the tracer DV in the brain soluble pool as presented by Eq. 1 (Vanier et al. 1995). A fitting of Eq.1 to the tissue soluble pool gives K_T [ml/g/min], the constant for tryptophan metabolism through 5-HT metabolic pathway, for different brain regions.

$$DV = \frac{V_1}{C_p(T)} \int_0^T \exp[-k_{el}(T-t)] \times C_p(t) dt + \frac{V_2}{C_p(T)} \int_0^T \exp[-V_3(T-t)] \times C_p(t) dt$$

$$V_1 = K_1 k_3 / (V_3 - k_{el}); V_2 = K_1 (k_2 + k_{el}) / (V_3 - k_{el}); V_3 = k_2 + k_3$$

Here K_1 , k_2 and k_3 are the first order rate constants describing transfer of tracer between different compartments (see Fig. 1). The k_{el} is the constant governing removal of labelled 5-HIAA, 5-HT metabolite, from the brain tissue. This rate constant was used as a constant and had a value of $k_{el} = 0.017 \text{ min}^{-1}$ (Burns et al. 1976).

Calculation of K_a

The regional K_a for fluoxetine for D,L-fenfluramine experiments were calculated as described before using linear fitting to values of DV_a as function of Θ (Nagahiro et al. 1990). The $DV_a(T)$ was equal to the ratio between tissue and plasma concentrations at the end of experiment (T). The Θ (min) was calculated as the ratio of the plasma integral of the tracer concentration and the plasma tracer concentration at the end of experiment. The values are given in Tables 3 and 4. The regional K_a values for the saline experiments were calculated as the mean for rats injected with saline in these two sets of experiments and are given in Table 2.

Determination of plasma tryptophan (Trp) concentrations

At the beginning, at midpoint, and at the end of each experiment, a further 50 μl of plasma was deproteinized with 25 μl of 20% trichloroacetic acid and supernatant was used for total Trp determination. A plasma ultrafiltrate (Ultrafree-MC filter with 10,000 MW cut off; spun at 12,000 rpm for 10 min) was used for determination of non-albumin-bound (free) Trp in the plasma. Trp concentrations were measured by the HPLC using a fluorescence detector. The measurements of the total (2 samples) and non-protein bound (free) Trp were done in five samples taken at different times after the beginning of tracer injection. The HPLC was done using a reverse phase column and an isocratic elution with a mixture of methanol (18%) and phosphate buffer (Missala and Sourkes, 1988). A Shimatsu fluorescence detector with 285 nm excitation and 340 nm emission filters was used for quantification of tryptophan using external tryptophan standard (Missala and Sourkes, 1988).

LC Calculation

The rate of 5-HT synthesis (R_{Trp} ; pmol/g/min) by definition of the model is equal to the product K_{Trp} and C_{Trp} , while in experiments with labelled α -[^{14}C]MTrp, $R_a = K_a / \text{LC} \cdot C_{\text{Trp}}$ [pmol/g/min]. With an assumption that the regional values of R_{Trp} and R_a should be same in rats used in two sets of experiments in which labelled Trp and α -[^{14}C]MTrp were used as

tracers (the same strain and age/weight of rats used and all were done under same experimental conditions) the $LC = K_{\alpha}/K_{Trp}$. The regional LC values were calculated for each brain region as K_{α}/K_{Trp} (note that the plasma concentrations of the free tryptophan were not significantly different between different experiments) (Table 1). Since plasma fraction of Trp is in dynamic equilibrium with brain Trp (Bloxam and Curzon, 1978, Salter et al., 1989; Tagliamonte et al., 1973; Takada et al., 1993) the plasma free Trp was used in this LC calculation. The regional values were calculated separately for experiments in which rats were injected saline and different drugs. This way three sets of the regional LC values were obtained permitting evaluation of drug effect on the regional distribution of LC as well as influence of drugs on the mean brain values (Tables 2, 3, and 4).

Statistical analyses

All data are expressed as mean $\pm$ SD. The differences between groups (free or total Trp) were assessed by one-way ANOVA, followed by a Tukey's multiple comparison test. χ^2 statistics on logarithmically transformed data was used to assess if the LC value belong to the same population (Vanier et al. 1995). Because the LC was calculated as the ratio between two uptake constants (K_{α} and K_{Trp}), it was easier to carry out an error analysis on the log scale, where those ratios become differences, and errors are additive. The errors were calculated as square root of sum of squares. The LC values were calculated separately for experiments done with saline and those done with fluoxetine and D,L-fenfluramine, by using respective uptake constants for α -MTrp and Trp as tracers. The simplicity of the error analysis comes from the fact that errors on the log scale are equal to the proportional errors, or coefficients of variation, in the original variables. The proportional error in log (LC) is the root sum of squares of the proportional errors in log (K_{α}), log (K_{Trp}), and plasma tryptophan for each of experiments. The consistency of the regional LC values (e.g. all of them coming from the same population meaning uniform value throughout the brain) was assessed by comparing the SD of the regional LC values to the root mean squared error in each regional value. This ratio r should be close to 1 if the regional values are consistent and > 1 if the regional values are different for each region. Assuming normality and independence of then regional LC values (on the log scale), the appropriate test statistic is $\chi^2 = (n - 1)r^2$, which can be compared to a χ^2 distribution with $n - 1$ degrees of freedom. Because this test in the present experiments was not significant, our results confirmed that regional LC values are equal, and we can test the equality of the saline, fluoxetine and D,L-fenfluramine mean LC values by a simple paired difference t-test on $(n - 1)$ degrees of freedom. The criterion for significance was $p < 0.05$.

Results

There was no significant difference (ANOVA; $F(5,113)=2.0$; $p > 0.05$) between different groups of rats in any of the physiological variables (e.g. blood gases, body weight) (actual data not given). The plasma free and total tryptophan concentrations and the sample sizes in each set of the data are given in Table 1. Plasma free (ANOVA; $F(5,113)=1.04$; $p > 0.05$) or total (ANOVA; $F(5,113)=2.08$; $p > 0.05$) tryptophan concentration did not differ significantly between rats treated with saline, fluoxetine or D,L-fenfluramine, respectively, and in addition, values of plasma free and total tryptophan were similar among rats used in different protocols (labelled α -MTrp vs. labelled Trp) (see Table 1).

The representative autoradiograms of rat brain slices after injections of L- [3 - ^{14}C], showing different rat brain regions, from control rats treated with saline (Fig. 2), exemplifying the effect of the TCA washing. Washed slices contain only radioactive Trp incorporated into proteins (Fig. 2B), since washing procedure with 20% TCA removes all non-protein bound radioactivity. The visual examination of the autoradiograms detects structures which contain the high concentration of 5-HT producing cells, such as pineal body, dorsal and median

raphe nucleus. The autoradiograms shown in the figures were obtained from the rats treated with saline. The differences between optical densities (tracer concentrations) in the images in Figs. 2A and 2B relate to the 5-HT synthesis rates.

The regional LC in more than twenty brain regions was calculated and presented in the 4th column in Tables 2–4, for saline, fluoxetine, and D,L-fenfluramine experiments, respectively. Tables show evident particular regional variability in the LC values between different brain regions, requiring a formal test to assess regional variability in relation to the standard deviation of individual values. χ^2 tests of the regional LC-s and their standard deviations in each of three sets suggested that the regional variability occurred by chance alone, as they were within the statistical fluctuations expected in these sets. The values of χ^2 were: $\chi^2=9.89$ (N=24); $p>0.2$ for the saline treatment group; $\chi^2=9.06$ (N=27); $p>0.2$ for the fluoxetine treatment group, and $\chi^2=2.1$ (N=22), $p>0.2$ for the D,L-fenfluramine treatment group, respectively. The Q-Q plots of these three sets clearly show a normal distribution of the regional values for each of these sets (Fig. 3). The mean values of the LC calculated from the log data were 0.450 ± 0.055 for the saline experiment, 0.429 ± 0.091 for fluoxetine experiment, and 0.48 ± 0.09 for D,L-fenfluramine experiment. These values were not significantly different (ANOVA, $F(2,70)=0.94$; $p>0.3$). The weighted mean of these values was $LC=0.45\pm0.04$.

The results of the present study showed that the value of LC did not differ significantly throughout different rat brain regions, as χ^2 test revealed (see above) that the regional variability, which can be seen in the Tables, occurred by chance and was not significant.

Discussion

The determination of brain 5-HT synthesis rate with the autoradiographic α -MTrp method is based on the assumption that labeled α -MTrp is trapped in an irreversible pool from which Trp is used for 5-HT synthesis (Diksic, 2001; Diksic and Young, 2001). The theoretical basis of the method and assumptions on which the method is based were reported and discussed in previous publications (Nagahiro et al. 1990; Vanier et al. 1995; Diksic et al. 2000; Diksic, 2001; Diksic and Young, 2001). The need of LC for the conversion of measurements done with α -MTrp into 5-HT synthesis was also discussed in those publications.

The main findings reported here are: 1) the value of the mean LC for conversion of the α -MTrp trapping into 5-HT synthesis rate is not influenced by fluoxetine or fenfluramine treatments; 2) the LC is, as before reported for saline and probenecid treated rats (Vanier et al. 1995), the same across the brain (observed variability between different brain regions could be explained by chance alone); and 3) the mean value of the LC in experiments reported here is not significantly different from the value reported before for naive rats (Vanier et al. 1995).

The mean value of LC was not significantly different in rats injected with saline or those injected fluoxetine or D,L-fenfluramine. This finding is especially interesting because acute treatment with the same dose of fluoxetine (10 mg/kg), as the one used in the present study, significantly affected the 5-HT synthesis rate: acute fluoxetine increased the 5-HT synthesis rate in regions containing nerve terminals like cortex (visual, auditory and parietal cortices), hypothalamus, ventral thalamus, dorsal hippocampus and substantia nigra (Mück-Šeler et al., 1996), but reduced the synthesis in DR (cell body region). In short the results suggest that fluoxetine treatment does not influence the value of the LC. Similarly acute treatment with the same dose of D,L-fenfluramine (10 mg/kg), as used in the present study, increased the rate of 5-HT synthesis in auditory and parietal-sensory motor cortices, striatum (globus

palidus and median part of caudatus putamen), superior olive, dorsal hippocampus and ventral thalamus, and decreased it in 5-HT cell bodies (dorsal and medial raphe nuclei), (Mück-Šeler and Diksic, 1996). The increase in the 5-HT synthesis rate was observed also after treatment with a dose of D-fenfluramine (5 mg/kg; it is equivalent to the dose of racemic mixture used here), which increased the 5-HT synthesis rate in the parietal, visual and sensory motor cortices, medial and lateral caudate nucleus, while 5-HT synthesis rate was decreased in dorsal raphe nucleus (Yamane et al., 1999). Again this result suggests that D,L-fenfluramine treatment does not have influence on the brain LC. Similar results to those reported before (Mück-Šeler and Diksic, 1996; Mück-Šeler et al. 1996) were obtained in the present study when published results are compared to those given in columns 3 of Tables 2–4.

Central 5-HT is a main controller of the formation and integration of neural networks, synaptic function, neurite outgrowth, synaptogenesis and cell survival, and it modulates many physiological and pathological functions and behaviors (Lucki, 1998; Stahl, 1998; Gil et al. 2011). The impaired 5-HT neurotransmission elicits a variety of behavioral and physiological abnormalities and psychiatric disorders, including depression (Mattson et al., 2004). One of the most important presynaptic steps in the 5-HT neurotransmission is 5-HT synthesis (Nelson 1993). As shown in depressed subjects antidepressant treatment have influence on the regional 5-HT synthesis (Berney et al. 2008). It has been also shown that striatal 5-HT levels have a positive association with abnormal involuntary movements in L-DOPA chronically treated hemiparkinsonian rats (Gil et al. 2011).

The LC could also be seen as conglomerate of constants related to the transport and trapping of tracer which are somewhat related to the final step of Trp conversion to 5-HT (Diksic et al. 2000). There is a possibility that these constants are effected by drugs in a region specific manner, but the constancy of the LC throughout the brain and different drug treatments suggest a presence of a compensatory mechanism(s) which insures a constancy of the LC. Because Trp entering the brain pool used for 5-HT synthesis and α -MTrp share common transport (Diksic et al. 2000) and enzymes (Roberge et al. 1972) it is likely that changes produced by drugs effect parameters for both Trp and α -MTrp which in the LC appear as ratios and as such do not influence the value of LC. The measurements done with labelled Trp could be directly related to the regional 5-HT synthesis; they do not need any LC for a conversion. When these comparisons of results given here (Table 2–4) and those reported before (Mück-Šeler et al. 1996; Mück-Šeler and Diksic, 1995) were done one finds very good agreement with 5-HT synthesis between these reports. Note that the measurements done with labelled tryptophan when part incorporated into proteins is separated give directly 5-HT synthesis rates (columns 3 in Tables 2–4). This autoradiographic methods offer the insight in the central 5-HT system by studying 5-HT synthesis rate in different brain regions in control rats and rats treated with different drugs affecting 5-HT transmission. It could be concluded that LC for α -MTrp method is not influenced by drugs which elevate tissue 5-HT (probenecid; Vanier et al. 1995), release 5-HT from neuronal storage (D,L-fenfluramine; Mück-Šeler and Diksic, 1996), and those which elevate extracellular levels of 5-HT (fluoxetine). This stability of LC is important for a better understanding of 5-HT synthesis measurements with pharmacological manipulations. The experiments presented here do not reveal if the LC is same in brain with a pathology as it is in normal brain. Because the LC can be seen as a conglomerate of different rate constants for tryptophan and α -MTrp (Diksic et al. 1999) the stability of it could be expected in a pathology; the changes in the constants are correlated and the ratio stays the same.

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α -MTrp Model

Figure 1.

A schematic representation of a biological model identifying the apparent brain compartments for and Trp whenever either of them is used as a tracer. The first-order rate constants shown in the scheme are constants for the movement of tracers between plasma and brain [K_1 ($\text{ml g}^{-1} \text{min}^{-1}$) and K_1^* ($\text{ml g}^{-1} \text{min}^{-1}$), are the plasma clearance constant obtained by multiplying the first-order rate constant k_1 (min^{-1}) or k_1^* and the plasma volume in 1 g of brain)]; the rate constants are the first-order rate constants for the transfer of the tracer from the precursor compartment into the plasma (k_2 (min^{-1}) and k_2^* (min^{-1})) and irreversible compartment (k_3 (min^{-1}) and k_3^* (min^{-1})), respectively. For the modelling purpose k_3^* (min^{-1}) and k_3 (min^{-1}), could be directly related to enzyme activity or could be a constant for transfer into an irreversible compartment (tracer does not return from it) that serves as an immediate precursor pool for the enzyme (Diksic et al. 2000). The rate constant k_{el} is a first-order rate constant representing brain clearance of 5-HIAA. The k_{el} represents the loss of the radioactive metabolite of the L-Trp tracer from the brain tissue. The rate constants without an asterisk refer to the transport of Trp. It should be emphasized that for this purpose, compartments do not need to have a biological significance; they are mathematical approximations of a biological (physical) reality (Norwich, 1977). TPH stands for tryptophan hydroxylase; 5-HTP stand for 5-hydroxy tryptophan; AAAD stands for aromatic acid decarboxylase; 5-HIAA stand for 5-hydroxyindolacetic acid; Alb stands for albumin (broadly represents plasma proteins) to which both tryptophan (Trp) and α -MTrp are bound; and 5-HT stands for serotonin.

Figure 2.

A set of autoradiograms exemplifying the effect of washing with TCA on the brain tissue radioactivity and with this experimental procedure used to obtain tissue soluble pool. Images in the column A are those obtained on 30 μ m thick brain slices before washing, and those in column B are adjacent slices after washing with TCA.

Figure 3.

A set of three Q-Q plots exemplifying the normal distribution of calculated values of the LC. In the insert A are values obtained in experiments with fluoxetine; insert B are the values from saline experiments, and in insert C are values from fenfluramine experiments.

Table 1

The plasma concentrations of free and total tryptophan, and the number of rats in each of the experimental groups

Experiment (sample size)	Tracer used	Plasma free Trp [nmol/mL]	Plasma total Trp [nmol/mL]
Saline (N=20)	α -MTrp	10.6 $\pm$ 2.0	108 $\pm$ 17
Saline (N=20)	Trp	11.6 $\pm$ 1.8	101 $\pm$ 11
Fluoxetine (N=13)	α -MTrp	10.4 $\pm$ 2.2	96 $\pm$ 12
Fluoxetine (n=30)	Trp	10.7 $\pm$ 2.1	105 $\pm$ 20
D,L-fenfluramine (N=12)	α -MTrp	11.1 $\pm$ 5.2	116 $\pm$ 21
D,L-fenfluramine (N=24)	Trp	9.9 $\pm$ 2.4	103 $\pm$ 18

Table 2

The brain trapping constants of tryptophan (Trp) and α -MTrp multiplied by the plasma free tryptophan concentrations and the regional lumped constant in saline treated rats¹

Brain Region	K _{CP} (α -MTrp)[pmol/g/min] ²	K _{CP} (Trp) [pmol/g/min]	LC
Raphe dorsal	108.±12.	337.±41.	0.32±0.05
Raphe median	70.4±10.2	169.±16.	0.42±0.07
Raphe magnus	18.8±3.9	38.7±10.1	0.49±0.16
Visual cortex	18.5±2.3	28.4±3.6	0.65±0.11
Auditory cortex	18.7±3.0	59.1±8.2	0.32±0.07
Parietal cortex	16.3±3.0	39.9±2.4	0.41±0.08
Sensory motor cortex	16.9±2.9	42.1±8.9	0.40±0.11
Frontal cortex	20.1±0.4	38.5±5.5	0.52±0.08
Caudate-putamen lateral	20.7±2.8	46.0±4.8	0.45±0.08
Caudate-putamen medial	28.3±3.6	68.1±6.3	0.42±0.07
Ventral tegmental area	20.7±3.5	38.9±11.6	0.53±0.18
MFB	19.0±2.8	40.0±13.1	0.47±0.17
Inferior colliculus	12.0±2.9	27.4±7.3	0.44±0.16
Superior colliculus	19.9±3.6	39.5±8.3	0.50±0.14
Thalamus ventral	17.9±3.2	29.1±3.2	0.62±0.13
Thalamus dorsal	19.5±3.4	46.9±9.4	0.42±0.11
Hypothalamus	20.4±3.1	34.3±5.2	0.60±0.13
Hippocampus ventral	25.2±3.3	48.0±7.1	0.52±0.10
Hippocampus dorsal	22.3±3.1	53.1±9.5	0.42±0.09
Nucleus accumbens	32.3±4.4	65.1±10.4	0.50±0.10
Superior olive	7.4±2.1	38.3±2.5	0.19±0.06
Medial geniculate	18.9±3.3	34.8±4.3	0.54±0.12
Lateral geniculate	20.4±3.1	43.2±6.1	0.47±0.10
Substantia nigra	15.9±2.9	31.8±4.9	0.50±0.12

¹The values are given as the mean ± standard deviation (SD). The SD was calculated from the log transformed data.

²The total number of rats for α -MTrp as tracer experiments was 20 while in experiments in which labelled Trp was used as the tracer the total number was 32.

Table 3

The brain trapping constants of tryptophan (Trp) and α -MTrp multiplied by the plasma free tryptophan concentrations and the regional lumped constant in D,L-fenfluramine treated rats¹

Brain Region	K _{CP} (α -MTrp)[pmol/g/min] ²	K _{CP} (Trp) [pmol/g/min]	LC
Raphe dorsal	78.5±8.0	272.±28.	0.29±0.04
Raphe median	61.7±5.0	131.±15.	0.47±0.07
Raphe magnus	26.0±3.4	55.8±7.5	0.47±0.09
Visual cortex	26.5±2.5	50.1±21.7	0.53±0.23
Auditory cortex	26.5±2.9	62.3±21.8	0.43±0.16
Parietal cortex	28.6±2.5	73.6±18.6	0.39±0.10
Sensory-motor cortex	26.5±2.5	51.8±17.3	0.51±0.18
Frontal cortex	28.1±2.1	44.5±10.8	0.63±0.16
Caudate-putamen lateral	26.5±2.9	54.3±14.8	0.49±0.14
Caudate-putamen medial	34.9±3.8	84.7±15.5	0.41±0.09
Inferior colliculus	17.6±2.5	40.4±12.8	0.44±0.15
Superior colliculus	24.8±3.4	44.5±10.0	0.56±0.15
Thalamus ventral	25.2±2.9	54.8±13.3	0.46±0.12
Thalamus dorsal	26.0±2.5	43.8±12.8	0.59±0.18
Hypothalamus	27.7±3.4	50.9±20.2	0.54±0.23
Hippocampus ventral	31.1±3.8	60.5±11.6	0.51±0.12
Hippocampus dorsal	32.8±3.4	60.4±13.4	0.54±0.13
Nucleus accumbens	42.0±3.8	88.7±15.9	0.47±0.10
Superior olive	24.4±3.4	49.5±11.6	0.49±0.13
Medial geniculate	23.5±2.9	45.5±17.5	0.52±0.21
Lateral geniculate	25.6±3.4	57.7±16.5	0.44±0.14
Substantia nigra	16.0±2.1	32.0±22.1	0.50±0.35

¹The values are given as the mean ± standard deviation (SD). The SD was calculated from the log transformed data.

²These values were calculated from those reported in Mück-Šeler et al. (1996) using the lumped constant value of 0.42.

Table 4

The brain trapping constants of tryptophan (Trp) and α -MTrp multiplied by the plasma free tryptophan concentrations and the regional lumped constant in fluoxetine treated rats¹

Brain Region	$K_{Cp}(\alpha\text{-Trp})[\text{pmol/g/min}]^2$	$K_{Cp}(\text{Trp}) [\text{pmol/g/min}]$	LC
Raphe Dorsal	86.9±11.2	205.±50.	0.42±0.15
Raphe Median	63.3±7.9	122.±19.	0.52±0.13
Raphe Magnus	24.1±4.8	39.9±3.0	0.61±0.13
Visual cortex	23.0±4.5	78.8±11.9	0.29±0.08
Auditory cortex	24.3±4.7	47.9±11.5	0.51±0.19
Parietal cortex	21.4±4.3	49.2±6.3	0.44±0.11
Sensory-motor cortex	20.3±4.3	37.0±6.4	0.55±0.17
Frontal cortex	22.7±4.7	57.6±10.1	0.39±0.12
Globus pallidus	21.7±4.0	67.8±7.1	0.32±0.07
Caudatus-putamen lateral	21.5±4.4	69.0±17.0	0.31±0.12
Caudatus-putamen medial	28.0±5.5	66.4±7.2	0.42±0.10
Ventral tegmental area	22.7±4.5	115.±19.	0.20±0.06
MFB	20.7±3.8	29.7±2.3	0.70±0.15
Inferior colliculus	12.7±5.0	38.0±6.3	0.33±0.15
Superior colliculus	19.5±4.7	42.8±3.9	0.45±0.12
Thalamus ventral	21.0±4.1	44.3±4.7	0.47±0.11
Thalamus dorsal	21.9±4.4	36.3±2.8	0.60±0.14
Hypothalamus	22.6±3.9	85.1±18.7	0.27±0.09
Hippocampus ventral	26.7±4.8	61.9±9.2	0.43±0.11
Hippocampus-dorsal	25.0±4.0	65.1±11.8	0.38±0.11
Nucleus accumbens	32.7±5.8	75.0±15.3	0.44±0.14
Anterior olfactory nucleus	29.2±5.8	39.8±4.5	0.73±0.18
Superior olive	19.2±5.4	41.1±9.4	0.47±0.19
Medial geniculate	20.4±3.9	50.9±12.6	0.40±0.15
Lateral geniculate	20.8±4.1	45.1±9.2	0.46±0.15
Substantia nigra reticulata	15.8±2.6	35.7±6.7	0.44±0.13
Substantia nigra compacta	23.8±4.3	50.9±5.5	0.47±0.11

¹The values are given as the mean ± standard deviation (SD). The SD was calculated from the log transformed data.

²These values were calculated with the lumped constant value of 0.42.