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REVIEW

Study of the brain serotonergic system with labeled α-methyl-L-tryptophan

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Abstract

α-Methyl-L-tryptophan (α-MTrp) is an artificial amino acid and an analog of tryptophan (Trp), the precursor of the neurotransmitter serotonin (5-HT). In this article we have summarized available data, which suggest that the measurement of the unidirectional uptake of α-MTrp and its conversion to 5-HT synthesis rates is a valid approach for the determination of brain 5-HT synthesis rates. The main feature on which the model is based is the trapping of labeled α-MTrp in brain tissue. An overview of opposing opinions, which suggest that there is a need for a metabolic conversion of tracer, is also presented and discussed critically. As with all biological modeling there is likely to be room for improvements of the proposed biological model. In addition, there are a limited number of clearly defined circumstances in which the method is confounded by the metabolism of labeled α-MTrp via the kynurenine pathway. Nonetheless, a significant body of evidence suggests that labeled α-MTrp is a useful tracer to study brain 5-HT synthesis in most circumstances. Calculation of 5-HT synthesis rates depends on the plasma-free tryptophan concentration, which, according to the balance of arguments in the literature, is a more appropriate parameter than the total-plasma tryptophan. The method, as proposed by us, can be used in conjunction with autoradiographic measurements in laboratory animals, and with positron emission tomography in large animals and humans. We review studies in animals looking at the normal control of 5-HT synthesis and the way in which it is altered by drugs, as well as initial studies investigating healthy humans and patients with neuropsychiatric disorders.

Keywords: autoradiography, brain synthesis, α-methyl-L-tryptophan, positron emission tomography, serotonin.

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Serotonin: anatomy and metabolism

Serotonin (5-hydroxytryptamine; 5-HT) is one of the less abundant neurotransmitters/neuromodulators in brain. However, serotonin neurons, the cell bodies of which are located in the raphe nuclei of the brainstem, project diffusely to all areas of the central nervous system (Azmitia and Segal 1978). In keeping with its diffuse anatomical distribution, serotonin seems to modulate many different aspects of brain function, and it has been implicated in a variety of disorders of the brain (Heninger 1995). This has stimulated much interest in developing methods for studying different aspects of serotonin function in humans, including serotonin synthesis.

5-Hydroxytryptamine is a biogenic amine with a pK of 9.8. Thus, it is charged at physiological pH and will not cross the blood–brain barrier, or diffuse into cells from the extra cellular space. Because of this, it must be synthesized inside 5-HT neurons; indeed it is the presence of the relevant enzymes that characterizes 5-HT neurons. 5-Hydroxytryptamine is synthesized from tryptophan (Trp), an essential amino acid, in a two-step biochemical reaction. The first rate-limiting step, catalyzed by Trp hydroxylase (TPH; EC 1.14.16.4, hydroxylation of Trp in position 5), forms 5-hydroxytryptophan, which is then converted to 5-HT by aromatic amino acid decarboxylase (AAAD; EC 1.1.4.28). Trp hydroxylase has three substrates, the other two being tetrahydrobiopterin and oxygen. Because tetrahydrobiopterin is converted to dihydrobiopterin, which is then reduced back

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Abbreviations used: AAAD, aromatic amino acid decarboxylase; BBB, blood–brain barrier; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; LC, lumped constant; α-M5-HT, α-methylserotonin; MAO, monoamine oxidase; MDMA, (±)3,4-methylendioxymethamphetamine; α-MTrp, α-methyl-L-tryptophan; PET, positron emission tomography; PS, permeability surface area; SPM, statistical parametric mapping; SSRI, selective 5-HT re-uptake inhibitors; TPH, Trp hydroxylase; Trp, tryptophan.

to tetrahydrobiopterin and reused, it is sometimes referred to as a cofactor. The AADC uses pyridoxal phosphate as a cofactor. Immediately after synthesis, cytosolic 5-HT is protected from monoamine oxidase (MAO; EC 1.4.3.4) by a 5-HT binding protein (Tamir and Gershon 1979), before it is stored in the vesicles. Brain, serotonergic neurons contain MAO-B, in spite of the fact that 5-HT is a preferred substrate for MAO-A but not for MAO-B. However, because MAO is present in a large excess and the affinity of the MAO-B for 5-HT is substantial (K_m = 1150 μM, Fowler and Tipton 1982), 5-HT can be deaminated by MAO-B in serotonergic neurons (Fagervall and Ross 1986).

The TPH is the rate-limiting step in the synthesis of 5-HT, and TPH is not saturated with any of its substrates (Trp, oxygen or tetrahydrobiopterin). Thus, increases in the level of any of the substrates can increase the rate of 5-HT synthesis. This has been demonstrated for tetrahydrobiopterin in the rat (Miwa *et al*. 1985), for oxygen in the rat (Davis *et al*. 1973; Faiman and Mehl 1973) and dog (Diksic *et al*. 1991), and for Trp in several species. In both rats (Fernstrom and Wurtman 1971) and humans (Young and Gauthier 1981) the normal level of Trp in the brain is around the K_m of TPH for Trp. Thus, increases in brain Trp can double the rate of 5-HT synthesis. However an increase in the substrate concentration cannot increase 5-HT synthesis by more than twofold, assuming that a baseline Trp concentration is at about K_m value. The latter might not always be the case (Diksic *et al*. 1991; Diksic 2001). This conclusion assumes that there is a uniformed distribution of Trp throughout brain tissue, and is consistent with experimental data for rats and humans (Fernstrom and Wurtman 1971; Young and Gauthier 1981).

Measurement of various parameters related to 5-HT neurotransmission

The 5-HT function is regulated by numerous factors. The first factor is the synthesis of 5-HT. Thus, increasing 5-HT synthesis by giving Trp can influence a variety of aspects of brain function thought to be mediated by 5-HT (Young 1986). This implies that increasing 5-HT synthesis is increasing the amount of 5-HT released each time a neuron fires. Firing rates of 5-HT neurons are obviously important in regulating 5-HT function, as is the sensitivity of 5-HT receptors and the activity of the re-uptake system that removes 5-HT from the synaptic cleft. Although there is no method for looking at the firing rates of 5-HT neurons in humans, methods have been developed for studying other factors that contribute to 5-HT function in clinical populations.

Various methods have been proposed to image different receptor types in the brain with positron emission tomography (PET), using radioactively labeled radiopharmaceuticals specific for the following receptors: 5-HT_{1A} with [^{11}C]WAY-100635 (Farde *et al*. 1998; Gunn *et al*. 1998); 5-HT_{2A} with [^{18}F]setoperone (Cho *et al*. 1999) or [^{11}C]MDL-100,907 (Ito *et al*. 1998). The 5-HT transporter has been imaged using a variety of tracers including [^{132}I]β-CIT (Semple *et al*. 1999) and [^{11}C]McN-5652 (McCann *et al*. 1998). In addition, PET imaging methods have been proposed for the vesicular uptake sites (Chan *et al*. 1999). The imaging of receptors in the living brain is generally hindered by the fact that current methods cannot obtain information on both the density of the sites and their affinity for a particular radiopharmaceutical. This is because it is not possible to give multiple injections of the radiopharmaceuticals with different specific activities, which would permit an estimation of both parameters.

In the long run the picture that will emerge of 5-HT in clinical populations will be derived from a variety of different techniques. Among these is the measurement of 5-HT synthesis. The purpose of this review is to describe some of the limitations of the older methods for studying the rate of 5-HT synthesis, and give an overview and rational for the use of the α-methyl-L-tryptophan method (α-MTrp).

Methods for measuring 5-HT synthesis

Older methods used in experimental animals

Development of methods for measuring turnover of biogenic amines, including 5-HT, were developed mainly in the 1960s. A review by Neff in 1972 discussed most of the methods that were used over several decades. The majority of these methods employed drugs that inhibited various steps in the synthesis or metabolism of 5-HT. Thus, the rates of decline of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in brain were measured after the inhibition of TPH with *p*-chlorophenylalanine. The assumptions behind this method were that the enzyme was fully inhibited and that the rate of metabolism of 5-HT was unchanged when its synthesis was inhibited, so that the rate of disappearance of 5-HT would be equal to its normal rate of synthesis. Other methods looked at the accumulation of 5-HT or the disappearance of 5-HIAA after inhibition of monoamine oxidase. Finally, the accumulation of 5-HIAA in brain was measured after its efflux from brain was inhibited with probenecid. The major problems with all these methods are that the available drugs do not always result in complete inhibition of the relevant process, and that upsetting the homeostasis of 5-HT neurons by inhibiting, for example, 5-HT catabolism, is unlikely to leave 5-HT synthesis rates unchanged. As discussed by Neff (1972), methods were available that employed either pulse injections or prolonged intravenous infusions of radioactive Trp tracers, and these probably gave more reliable results than the methods employing drugs. Nonetheless, the most commonly used method for studying 5-HT synthesis over the past few

decades looked at the accumulation of 5-hydroxytryptophan after the inhibition of AAAD (Carlsson and Lindqvist 1973). For this method there is evidence that inhibition of AAAD increases the rate of 5-HT synthesis (Mück-Šeler and Diksic 1995). Thus, many studies on the rate of 5-HT synthesis have probably reported erroneous data.

Measurements in cerebrospinal fluid

The methods used to study 5-HT synthesis in rat brain are not in general applicable to humans. In the past the most commonly used method in humans was to measure levels of 5-HIAA in lumbar cerebrospinal fluid (CSF). 5-HT itself is present in human CSF in such low quantities that trace contamination of CSF with blood would distort the levels measured (Anderson *et al*. 1990a). Although there is good evidence that CSF 5-HIAA is a rough index of CNS 5-HT metabolism (Garelis *et al*. 1974; Wood 1980; Bertilsson 1987), this approach has a number of drawbacks.

(i) It is invasive and subjects often do not like the idea of having a lumbar puncture.

(ii) The amount of 5-HIAA in CSF will reflect, in part, the transport of 5-HIAA from brain into CSF, and the activity of the system that transports 5-HIAA out of CSF, as well as the rate of 5-HT synthesis.

(iii) Because of the relatively large pool size of 5-HIAA in CSF, 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). Alternatively, when 5-HT synthesis is lowered, using the acute Trp depletion technique, the decline in CSF 5-HIAA is modest and occurs gradually over a period of hours (Carpenter *et al*. 1998).

(iv) Lumbar CSF 5-HIAA reflects, in part, 5-HT metabolism in the spinal cord, because 5-HT metabolism is more active in the brain than in the spinal cord. Most of the disorders involving serotonin implicate serotonin in the brain rather than in the spinal cord.

(v) Because 5-HT metabolism is more active in the brain than the spinal cord, there is a gradient of CSF 5-HIAA between the cisterna magna and the spinal subarachnoid space. As a result, levels of CSF will vary according to the physical activity of the subject (physical activity can result in CSF mixing), the height of the subject (greater height will increase the distance from the cisterna magna to the spinal subarachnoid space), and the volume of CSF taken (Jakupcevic *et al*. 1977; Bertilsson 1987; Bulat 1996).

Variations in methodology available include using probenecid to inhibit the transport of 5-HIAA out of the CSF, and looking at the accumulation of ^{18}O-labeled 5-HIAA after subjects breathe an atmosphere containing ^{18}O (Kopin 1978). Both these methods make it possible to measure rates of serotonin synthesis. However, these methods also have additional disadvantages. The probenecid method involves ingesting a drug, and then having two lumbar punctures, in order to look at the rate of accumulation of CSF 5-HIAA when its transport out of the CSF is inhibited. As a result, it has not been used extensively. The ^{18}O method involves having a source of ^{18}O, taking several CSF samples, and measuring the [5-^{18}O]HIAA with mass spectrometry. Thus, it is not a practical method for human studies.

In summary, CSF 5-HIAA measurements have been found useful, but are limited because (i) levels reflect other processes in addition to 5-HT metabolism, (ii) levels change only slowly in response to changes in 5-HT synthesis, and (iii) the measurements may reflect spinal cord metabolism more than brain metabolism. There is an important need for a better method for studying 5-HT synthesis in human brain, and in particular one that will give information on regional rates of 5-HT synthesis.

The α-methyltryptophan method

Overview of the method

For a number of years we have been concentrating on a method that permits the imaging of the brain trapping of labeled α-methyl-L-tryptophan (α-MTrp), an analog of Trp, and the calculation of brain 5-HT synthesis from the α-MTrp trapping (unidirectional uptake) constant. We assume that this gives a reasonable estimation of the *in vivo* activity of TPH. Autoradiography can be used to visualize and quantitate 5-HT synthesis in the brains of small laboratory animals (Nagahiro *et al*. 1990b; Diksic *et al*. 1995), whereas positron emission tomography (PET) is used for the study of larger animals (e.g. dogs and monkeys) (Diksic *et al*. 1991; Shoaf *et al*. 1998, 2000; Nishisawa *et al*. 1999), and humans (Nishizawa *et al*. 1997, 1998; Muzik *et al*. 1997; Chugani *et al*. 1998a,b,c, 1999a,b; Okazawa and Diksic 1998).

The α-MTrp method for imaging brain 5-HT synthesis (used here interchangeably with *in vivo* activity of TPH) has been developed during the past 15 years (Diksic *et al*. 1990a,b; Nagahiro *et al*. 1990b; Diksic and Grdiša 1995). The work started with rats (Diksic *et al*. 1990b; Nagahiro *et al*. 1990b), and was then extended to dogs (Diksic *et al*. 1991; Nishisawa *et al*. 1999) and eventually to humans (Nishizawa *et al*. 1997). The method is based on the essential work on the metabolism of α-MTrp in rats performed in the laboratory of T. L. Sourkes (Sourkes 1971), which showed that α-MTrp is a substrate for TPH and that the metabolite produced, which has been hypothesized to be α-methylserotonin (α-M5-HT) (Roberge *et al*. 1972; Diksic *et al*. 1990a), accumulates in brain. Using HPLC analysis on a chiral column, the metabolite has the HPLC characteristics (elution volume) of *S*-α-M5-HT (Diksic *et al*., unpublished). When pharmacological doses

Table 1 The Pearson product moment correlations between the different uptake constants

Parameter[1]	PS^T	K^α	K^{pr}	K^T	K^{pr}_{pb}	K^α_{pb}	K^T_{pb}
PS^α ($n = 33$)	0.71*	− 0.18	− 0.15	0.17	− 0.17	− 0.3	− 0.24
	(5×10^{-6})	(0.4)	(0.48)	(0.44)	(0.42)	(0.18)	(0.27)
PS^T ($n = 24$)		− 0.31	− 0.28	0.12	− 0.29	− 0.34	− 0.22
		(0.14)	(0.19)	(0.57)	(0.18)	(0.13)	(0.31)
K^α ($n = 28$)			0.28	0.6*	0.3	0.90*	0.87*
			(0.14)	(0.0007)	(0.12)	(10^{-9})	(10^{-9})
K^{pr} ($n = 28$)				0.23	0.98*	0.08	0.13
				(0.23)	(10^{-20})	(0.71)	(0.53)
K^T ($n = 28$)					0.29	0.56*	0.63*
					(0.13)	(0.003)	0.0003
K^{pr}_{pb} ($n = 28$)						0.13	0.32
						(0.53)	(0.09)
K^α_{pb} ($n = 25$)							0.86*
							(4×10^{-8})

Permeability surface area products for Trp (PS^T mL/g/min) and α-MTrp (PS^α mL/g/min), the brain uptake and trapping of α-MTrp (K^α mL/g/min, no probenecid; K^α_{pb}mL/g/min with probenecid), the uptake of Trp via the 5-HT metabolic pathway (K^T mL/g/min, no probenecid; K^T_{pb} mL/g/min, with probenecid), and the incorporation of Trp into proteins (K^{pr} mL/g/min, no probenecid; K^{pr}_{pb} mL/g/min with probenecid). In each cell the upper numbers represent the correlation coefficients, whereas the numbers in parentheses represent *p*-values. *Cells with a significant correlation. (See details in Diksic *et al.* 2000b.) [1]Here, *n* represents the number of brain structures used in the calculation of the correlations.

of α-MTrp are given to rats the α-M5-HT formed can replace 5-HT in the neuronal storage pool (Missala and Sourkes 1988). The metabolite of α-MTrp is stored in the brain tissue in the K^+ releasable pool, and it is released on depolarization to approximately the same extent as 5-HT (Cohen *et al.* 1995).

The use of α-MTrp for the measurement of 5-HT synthesis depends in part on the fact that, unlike Trp, it is not incorporated into protein (Madras and Sourkes 1965; Diksic *et al.* 1990b) and that its metabolite α-M5-HT is not metabolized by monoamine oxidase, and thus accumulates in brain. When α-MTrp is used as a tracer, the distribution of radioactivity in brain is similar to the distribution of brain serotonergic cell bodies in the raphe nuclei and their neuronal projections (Diksic *et al.* 1990b; Takada *et al.* 1993; Tsuiki *et al.* 1995a). At the electron microscopic level there is an excellent correlation between the radioactive tracer, TPH and 5-HT (Cohen *et al.* 1995). In addition, we have shown that the brain tissue uptake of this tracer can be differentially affected in different brain structures, and that the effects are different when the animals are treated acutely or chronically with different drugs (e.g. lithium, several selective 5-HT uptake inhibitors, reserpine, 5-HT agonists and 5-HT antagonists) known to affect brain serotonergic transmission and 5-HT synthesis (Nagahiro *et al.* 1990a; Mück-Seler and Diksic 1995; Tsuiki *et al.* 1995c; Mück-Šeler *et al.* 1996, 1998; Nishizawa *et al.* 1998; Okazawa *et al.* 1999; Yamane *et al.* 1999). Taken together these studies suggest that radioactively labeled α-MTrp should be a suitable tracer for the study of the brain serotonergic system, including brain 5-HT synthesis or TPH *in vivo* activity.

Biological model for labeled α-MTrp

The applicability and validity of the α-MTrp method depends in part on the fact that α-MTrp is substrate for TPH and is transported into the brain irreversible compartment proportionally to the rate of 5-HT synthesis. The brain irreversible compartment is the brain compartment from which the tracer does not exchange directly with tracer in the plasma pool/compartment. However, the proportionality between α-MTrp trapping and the rate of 5-HT synthesis may not be true in all clinical conditions. Normally the brain does not metabolize tryptophan by the kynurenine pathway (Saito *et al.* 1993). However, in conditions where there is inflammation of brain tissue (including bacterial, viral, fungal and parasitic infections, meningitis, autoimmune disease and septicemia), induction of indoleamine-2,3-dioxygenase in macrophage infiltrates results in appreciable metabolism of tryptophan via the kynurenine pathway in the brain (Heyes *et al.* 1992, 1993). The substrate specificity of indoleamine-2,3-dioxygenase is low (Shimizu *et al.* 1978), and it will catabolize α-MTrp. Thus, the increased signal in the epileptic tubers in children with tuberous sclerosis complex after administration of α-MTrp tracer (Chugani *et al.* 1998a), is probably caused by metabolism of the α-MTrp via the kynurenine pathway (Chugani and Muzik 2000). However, in conditions in which there is no inflammation in the brain, the use of α-MTrp can be used to obtain information about brain 5-HT synthesis.

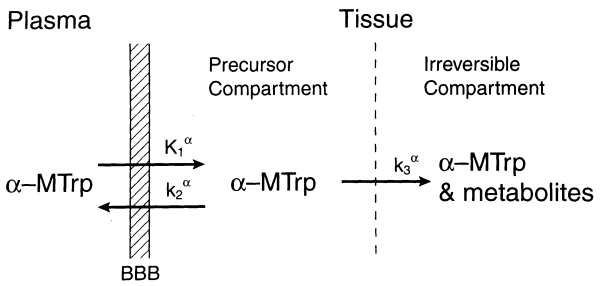

Fig. 1 Schematic presentation of the α-MTrp biological model. The compartments shown in the scheme are not necessarily distinct biological compartments. The rate constants responsible for the movement of the tracer between different compartments are assumed to be the first-order rate constants and have units of reciprocal time except K_1^{α}, which has units of mL/g/min.

The biological behavior of labeled α-MTrp in conditions where there is no inflammation in the brain permits us to construct a model that can aid us in deriving a mathematical procedure by which the brain-tissue uptake/trapping of the tracer is converted into a variable, 5-HT synthesis (TPH *in vivo* activity), to which we can assign a biological meaning. It must be emphasized that the model proposed is probably an approximation of the biological reality, as is the case with all biological modeling, regardless of how complex the model (Norwich 1977). In addition to the characteristics mentioned above, it has been shown that the blood-to-brain transport of labeled α-MTrp shares the same system as Trp (Diksic *et al*. 1991), and that there is a highly significant correlation (Table 1) in the rat brain between the permeability surface area (*PS*; mL/g/min) products for labeled α-MTrp with the *PS* for Trp (Diksic *et al*. 2000b). We have also collected data indicating that the brain trapping of labeled α-MTrp correlates with the metabolism of Trp via the 5-HT metabolic pathway in rat brain (Diksic *et al*. 2000b). However, there is no correlation (Table 1) between the *PS* products of either Trp or α-MTrp, and the trapping constant for α-MTrp or Trp (metabolism via 5-HT metabolic pathway). In addition there is no significant correlation between the uptake of α-MTrp and the incorporation of Trp into brain protein (Diksic *et al*. 2000b). The Pearson product moment correlation and probabilities (probabilities given in parenthesis) between the *PS* products and different brain uptake constants are presented in Table 1. These results suggest that the trapping (unidirectional uptake) constant of labeled α-MTrp correlates with the metabolism of Trp via the 5-HT metabolic pathway, but not with the blood–brain barrier (BBB) transport of Trp or Trp incorporation into proteins.

On the basis of those characteristics, the biological model for α-MTrp method, schematically shown in Fig. 1, has been proposed. Note that the compartments shown in the figure might not be distinct brain tissue compartments, and for that reason giving a distinct biological meaning to the individual rate constants is probably not advisable. It should be emphasized, as stated previously (Diksic *et al*. 1990b, 1991), that the α-MTrp model is based on the brain tissue trapping of labeled α-MTrp and/or metabolite, and not necessarily on its biological conversion to a metabolite (Diksic *et al*. 1990a,b, 1991; Diksic 2000). The proposed model also assumes that there is no loss of label from the tissue irreversible compartment during the time of the experiment. The rate constants shown are the first order rate constants k_2^{α} (1/min), for transport of tracer from tissue precursor pool to plasma, and k_3^{α} (1/min) for transport of tracer from precursor pool to an irreversible pool. The clearance constant K_1^{α} (mL/g/min) is defined as a product of the first order rate constant and the blood volume in the unit of brain (Diksic *et al*. 1990b; Diksic *et al*. 1991). Note that the free fraction of tracer is incorporated in the K_1^{α}. It should be noted that tissue trapping does not require the metabolic conversion of α-MTrp to its metabolite (Diksic *et al*. 1990b, 1991, 1999; Diksic 2000), as was suggested to be the case for the use of α-MTrp as a tracer for the measurements of brain 5-HT synthesis (Shoaf *et al*. 1998, 2000; Gharib *et al*. 1999).

This model representation allows us to write ordinary differential equations for the movements of the tracer between the different compartments, which can be solved by standard methods. This solution gives the tissue radioactivity of the tracer [$C_t^*(T)$; nCi/g] as a function of time (T, min) and those rate constants. Unfortunately, any biological data, including that obtained with labeled α-MTrp, has an unknown level of noise present, which complicates the fitting of the model equation to the data. In addition, the individual rate constants are highly correlated, resulting in solutions for the individual rate constants that might not be related directly to a biochemical process. Because of this, we believe that it is inadvisable to calculate individual rate constants, especially for the PET imaging data with α-MTrp as a tracer, or give them a biological meaning. However, if one expresses the tissue radioactivity concentration with K^{α} [mL/g/min; unidirectional uptake or trapping constant, which in the formulation is equal to the parameter $K_1^{\alpha}k_2^{\alpha}/(k_2^{\alpha} + k_3^{\alpha})$ in eqn 1; Diksic *et al*. 1991], the solutions for K^{α} are substantially more reliable and the fitting solutions are more stable. For this reason we would suggest that only K^{α} is calculated from the tissue–time activity curves of labeled α-MTrp.

$$DV(\Theta) = \frac{C_t^*(T)}{C_p^*(T)} = K^{\alpha}\cdot\Theta + \frac{K_1^{\alpha}k_2^{\alpha}}{(k_2^{\alpha} + k_3^{\alpha})}\int_0^T e^{(t-T)(k_2^{\alpha}+k_3^{\alpha})}\cdot\frac{C_p^*(t)\,dt}{C_p^*(T)}, \quad (1)$$

where DV is the distribution volume defined as the ratio between an amount of the tracer in the tissue (C_t^*; nCi/g)

and the plasma tracer concentration (C_p*; nCi/mL). To increase the stability of the fitting, one can approximate integral eqn 1 in Θ-space [$\Theta = \int_0^T C_p(t)\,ft/C_p(T)$ min; exposure time] with eqn 2, in which by definition the tissue volume of distribution is calculated by assuming that it is proportional to the plasma integral of the tracer (Cowles and Fenstermacher 1974; Eckmann *et al*. 1974). In this case one solves the integral to the exposure time Θ, using the fact that C_p by definition is equal to one (unit of radioactivity/mL) (the plasma integral is equal to the product of Θ and one). After integration, we obtain eqn 2, which should be more suitable for fitting data in Θ-space, and so K^α can be obtained without any need of assuming which part of curve can be represented by a straight line.

$$DV(\theta) = \frac{C_T{*}(T)}{C_p{*}(T)} = K^\alpha\theta + \frac{K_1^\alpha k_2^\alpha}{(k_2^\alpha + k_3^\alpha)^2}[1 - e^{-(k_2^\alpha + k_3^\alpha)\theta}] \quad (2)$$

Once the regional constant for the tissue unidirectional uptake constant for α-MTrp is known (K^α; mL/g/min), we are convinced that it can be converted into regional 5-HT synthesis. We have proposed that the appropriate way to make this conversion is by converting K^α into the uptake constant of Trp via the 5-HT metabolic pathway (K^T; mL/g/min). This is achieved by the division of K^α with the lumped constant (LC). In experiments with labeled Trp as tracer (Vanier *et al*. 1995; Diksic *et al*. 2000b) the K^T was calculated by fitting the tissue radioactivity representing Trp metabolism through the 5-HT metabolic pathway using eqn 3.

$$C_t(T) = \frac{K^T}{1 - k_{el}/V3}\int_0^T e^{-k_{el}(T-t)}\cdot C_p(t)\,dt + V2\int_0^T e^{-V3(T-t)}\cdot C_p(t)\,dt \quad (3)$$

Here $k_{el} = 0.017$ (min^{-1}) is the constant for the elimination of 5-HIAA from the brain (Burns *et al*. 1976), *V*2 (1/min) and *V*3 (1/min) are constant, consisting of the model first order rate constants for Trp (Vanier *et al*. 1995).

It is important to note that by the definition of the LC, the LC is equal to the ratio of K^α and K^T, the latter representing Trp metabolism via the 5-HT metabolic pathway (Vanier *et al*. 1995), and the former the unidirectional net uptake or trapping of α-MTrp tracer. This definition of the LC can also be related, assuming a particular meaning of the model rate constant (Diksic *et al*. 1999), to the LC expressed as a function of the Michaelis–Menten constants as described by Sokoloff *et al*. (1977) for 2-deoxyglucose, and by Phelps *et al*. (1979) for 2-fluoro-deoxyglucose models. Indeed, mathematically the two previously mentioned definitions of the LC can be converted from one to the other with certain assumptions (Diksic *et al*. 1999). However, the observation that there is a small quantity of labeled α-MTrp biochemically converted to a metabolite (Diksic *et al*. 1990b; Gharib *et al*. 1999; Shoaf *et al*. 2000) is probably related to the relative values of the Michaelis–Menten constants for Trp and tracer α-MTrp, and their relative concentrations in the tissue (the concentration of Trp is about three orders of magnitude greater than that of α-MTrp), as discussed in a previous publication (Diksic *et al*. 1999). If a proportionality between the ratios of the Michaelis–Menten constants and the constant for the irreversible step is preserved, and the constants for the unidirectional uptake of Trp and α-MTrp are proportional to the ratios of the K_m and V_{max}, then despite a small biological conversion, the ratios will probably remain the same; the LC would not be effected.

Trp is the only plasma amino acid partially bound to plasma proteins, mainly albumin (McMenamy and Oncley 1958), and this fact must be accounted for in any biological model. In our derivation of the α-MTrp model equation, the plasma-free fraction of the tracer is implicitly incorporated into the quantity K_1^α and K^α, and, as such, is implicitly incorporated into the value of the LC (Vanier *et al*. 1995; Diksic *et al*. 1999), when the previously mentioned definition is used (Diksic *et al*. 1999). Note that the free fraction of Trp in the plasma is incorporated into K^T in the same way when experiments are performed with Trp as the tracer (Vanier *et al*. 1995). When the LC is calculated from the *in vitro* measured values, as was the case in the report of Shoaf and Schmall (1996) and Gharib *et al*. (1999), the difference in the plasma-free fractions of Trp and α-MTrp must be used in the calculation (Nishizawa *et al*. 1998; Diksic *et al*. 1999) before the values can be compared with those reported in Vanier *et al*. (1995). Indeed, when the calculation is carried out as described by Diksic *et al*. (1999), to make the values reported in Gharib *et al*. (1999) directly comparable to those reported in Vanier *et al*. (1995), then the LC measurements carried out in very different ways, are the same across different rat brain structures (Bobillier *et al*. 1999; Diksic *et al*. 1999). Furthermore, the recalculated values of LC (Diksic *et al*. 1999) are not significantly different from the value obtained as a mean of two sets of experiments, performed by autoradiography (Vanier *et al*. 1995; Diksic *et al*. 1999). Similarly, the measurements of Shoaf *et al*. (1996), which were performed in anesthetized monkeys, when recalculated (Nishizawa *et al*. 1998) in order to make them directly comparable to the values in rats, suggest, assuming that anesthesia does not influence the measurements, that the LC in monkeys is about 1.5. This value is about three times grater than the values obtained in the brain of a conscious rat (Vanier *et al*. 1995; Gharib *et al*. 1999), which suggests that the LC might be species dependent. However it should be emphasized that deep anesthesia, as used in the monkey experiments, would probably have an effect on brain 5-HT synthesis, because neuronal transmission is likely to be greatly reduced under anesthesia (e.g. brain glucose utilization is much lower under anesthesia; Sokoloff 1971). All of the measurements

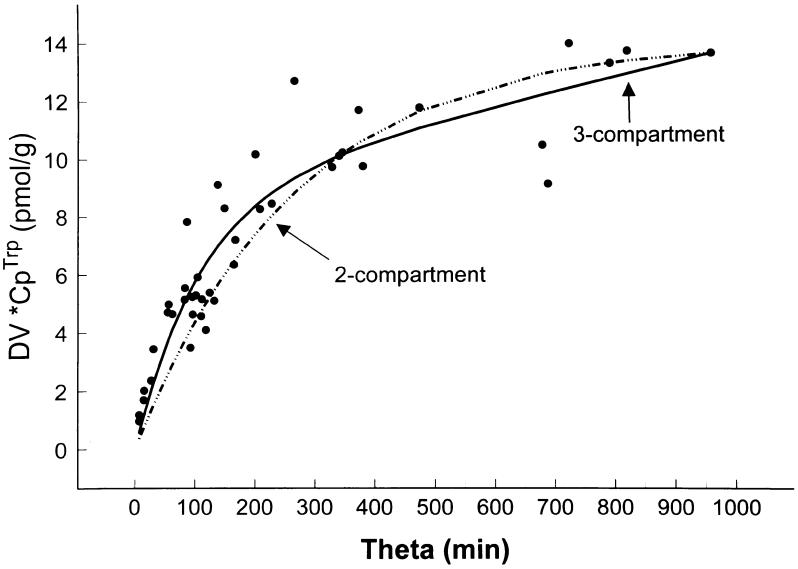

Fig. 2 An example showing the two- and three-compartment model fitting curves for rat caudate medial. Rats were injected with between 30 and 50 μCi of [α-^{14}C]MTrp, rats were killed between 5 min and 6 h after tracer injection, brains were cut into 30-μm slices, contacted with X-ray film, the film was developed and the images quantified using an image analyses system as described in our publications (e.g. Nagahiro *et al.* 1990b). The two- (using $k_3^{\alpha} = 0$ in eqn 1) and three-compartment models (eqn 1) were fitted to data, and the three-compartment model fitted significantly better as indicated by *F*-test on residuals ($F = 14.170$; $p < 5.2 \times 10^{-4}$; $n = 42$).

of the LC reported so far (Vanier *et al*. 1995; Shoaf and Schmall 1996; Diksic *et al*. 1999; Gharib *et al*. 1999) indicate that the LC is uniform throughout the brain. This characteristic is very important for the use of the α-MTrp tracer method in the study of regional brain 5-HT synthesis. This is not a great surprise because the LC is defined as the ratio between the unidirectional uptake constant for α-MTrp and that for Trp through the 5-HT metabolic pathway (Diksic *et al*. 2000b). As these two constants are highly correlated (Table 1), any changes in one would be offset by changes in the other, and as a result, there would be no change in their ratio, LC.

The presence of an irreversible compartment, in both rat and human tissue–time activity curves is exemplified by fitting curves shown in Figs 2 and 3, respectively. Even visual evaluation suggests that the data is fitted better by the three-compartment model than by the two-compartment model. In all cases examined so far the fitting is significantly better from a three-compartment structure.

As is the case in any biological modeling, the rate of a biological process [e.g. glucose utilization (Blomqvist *et al*. 1985) and protein synthesis (Smith *et al*. 1984; Kirikae *et al*. 1988; Jervic-Causevic and Diksic 1996)] can be calculated by the multiplication of the uptake constant for the process, *K*, with the plasma concentration of the precursor. The latter approach has been also used with labeled Trp as tracer (Lin *et al*. 1969). Using this principle, the rate of 5-HT synthesis (*R*; pmol/g/min) can be calculated as the product of K^{T} (mL/g/min) and plasma Trp concentration [C^{Trp} (pmol/mL)]: $K^{\mathrm{T}}\alpha C^{\mathrm{Trp}}$. As the plasma-free (non-albumin-bound) Trp

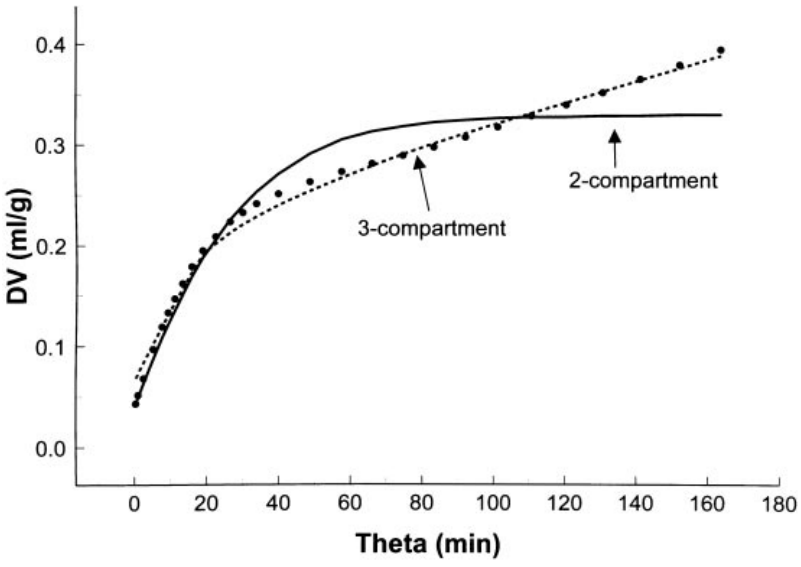

Fig. 3 An example of the fitting using two- and three-compartment structure models on PET data collected for 90 min in a normal volunteer. Data was fitted to eqn 1 (for the two-compartment structure $k_3^{\alpha} = 0$). Solid dots represent experimentally measured data points. The three-compartment structure fitted the data significantly better than than two-compartment strcture ($F = 85.8$; $p < 8 \times 10^{-9}$; $n = 30$).

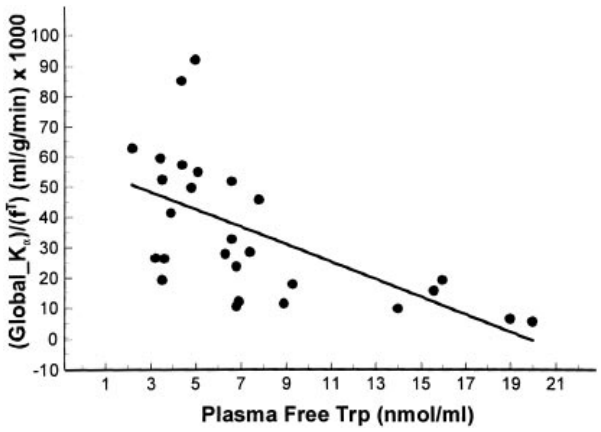

Fig. 4 Graphical representation of the functional relationship between ratio of K^{α} and f^{T} (free fraction of Trp in plasma) and the plasma-free Trp. There is a significant inverse correlation between these two quantities ($p < 0.001$), whereas there was no significant correlation between this ratio and the plasma total Trp, or between K^{α} and either free- or total-plasma Trp.

concentration is the one related to brain 5-HT synthesis (Bloxam and Curzon 1978; Salter *et al*. 1989; Takada et al. 1993), we have proposed that this concentration is used in the rates of 5-HT calculation (C^{Trp} in the above equation is the plasma-free Trp). Indeed, on the basis of experiments in rats performed under *in vivo* steady state conditions (Takada et al. 1993), unlike the experiments performed by the carotid artery injections (Pardridge and Oldendorf 1977; Smith *et al*. 1987), one can also conclude that only the plasma-free Trp, and not the albumin-bound tryptophan, exchanges with the brain Trp pool. From these equilibrium experiments, the K_{D} (μM) for Trp binding with plasma proteins (mainly albumin) was found to be 80 μM (Takada et al. 1993). Taking the plasma concentration of albumin to be 500 μM (Pardridge 1983), and applying these values to the equation derived by Pardridge [1983; $f^{\mathrm{T}} = K_{\mathrm{D}}/(K_{\mathrm{D}} + 500)$, where f^{T} is the free faction of Trp exchanging with the brain Trp], one finds the f^{T} to be about 13.6%. This value is very close to the value of the Trp-free fraction found in our rat experiments (Takada et al. 1993). Indeed this estimate agrees with the proposal of others (Bloxam and Curzon 1978; Salter *et al*. 1989). However, there is controversy on this topic, and some studies have reported that the brain Trp is related to total plasma Trp, not free-plasma Trp (e.g. Fernstrom *et al*. 1976). Possibly, free-plasma Trp is not the appropriate parameter to use in all biological situations. However, given that most of the data suggest that only the Trp-free fraction is available and required for the exchange with brain, and that there is no information on the particular circumstances in which albumin-bound Trp is available to the brain, we have used the free fraction in all situations. On the basis of these characteristics, a working method in rats has been proposed (Nagahiro *et al*. 1990b), in which rats are killed at two different times after tracer injections. Often this produces a reasonable spread of exposure times (Tsuiki *et al*. 1995c), which permits the fitting of a linear portion of the Patlak plot to the data (Patlak *et al*. 1983; Diksic *et al*. 1990a,b, Diksic *et al*. 1995).

Certainly, any linear relation between plasma Trp and brain 5-HT synthesis would be a valid approximation within the physiological range of the plasma-free Trp, as a result of the kinetic characteristics of TPH and the possible high-affinity uptake system for Trp on the serotonergic neurons (Mandell and Knapp 1977; Denizeau and Sourkes 1977). If this high-affinity neuronal uptake system controls the access of Trp to the enzyme inside the cells, the method, as proposed by us, would image that system. However, this system would then become the rate-limiting step in the brain synthesis of 5-HT, because it would control the access of the substrate to the enzyme, not the brain activity of TPH.

Recently, it has been suggested (Chugani and Muzik 2000) that the procedure described above should not be carried further than the calculation of K^{α}, which the authors described as 'an index of 5-HT synthesis'. However, if the brain uptake constant of the tracer α-MTrp (K^{α}) is an index of brain 5-HT synthesis, then it must be possible to convert it to brain 5-HT synthesis rates by applying an appropriate conversion/correction procedure. We have proposed to do this by first converting K^{α} to K^{T}, the constant for the unidirectional uptake (metabolism) of Trp via the 5-HT metabolic pathway, and then multiplying K^{T} by the plasma-free Trp concentration. The notion that this would be erroneous appears unsupported by the published experimental evidence, because the latter is equivalent to the procedure used when a natural substance is used as the tracer (see also above; Smith *et al*. 1984; Blomqvist *et al*. 1985; Kirikae *et al*. 1988; Jervic-Causevic and Diksic 1996).

The plasma-free fraction of α-MTrp (f^{α}), when used as a tracer and when the calculations are performed as described (Diksic *et al*. 1990b; Diksic *et al*. 1991; Muzik *et al*. 1997; Shoaf *et al*. 1998), is included in the value of K^{α}. Generally, in the α-MTrp experiments, the f^{α} is not measured, but the free fraction of Trp (f^{T}) is measured. Because of a highly significant correlation between f^{α} and f^{T}, in the rat experiments (Diksic, unpublished), one can remove this factor from the K^{α} by dividing it with f^{T}, if a comparison of the 'pure' K^{α} is desired. Although there is no significant correlation between K^{α} and the plasma Trp (either free or total), there is a highly significant negative correlation between K_{f} ($K_{\mathrm{f}} = K^{\alpha}/f^{\mathrm{T}}$) and the plasma-free Trp concentration (Fig. 4). There is no significant correlation between K_{f} and the total plasma Trp concentration. This observation again suggests that the plasma-free fraction of Trp is important and needs to be used in the calculations.

When the BBB transport of Trp is evaluated as a function of the plasma-free Trp, it is observed that as the free-plasma Trp increases the amount of Trp entering the brain also

increases (Takada et al. 1993). However, once the correction for the Trp-free fraction is made, the BBB transport constant (K_1^T in the model formulation) decreases as the plasma Trp increases (Takada et al. 1993), as would be expected on the basis of the competitive nature of the BBB transport (Pardridge 1983), in addition to diffusion through the BBB (Miller *et al*. 1985; Takada et al. 1993). It is possible to conclude, from these experiments, that the free fraction of Trp plays an important role in the transport of Trp into the brain, and that the extraction fraction is rather small ($< 10\%$). The small extraction fraction confirms that the brain uptake of Trp should not be dependent on the blood flow. This conclusion stems from the relationship between the *PS* product, blood flow, and the influx constant (Fenstermacher *et al*. 1981).

Plasma-free Trp levels will tend to vary throughout the day (Tagliamonte *et al.* 1974), which will result in alterations in the saturation of TPH and alterations in the rate of 5-HT synthesis. Thus, one advantage in expressing values as K^α is that this will normally give some index of the 5-HT synthesis capacity, and will probably be less subject to moment-to-moment fluctuations than the rate of 5-HT synthesis. However, if groups of subjects in which there is a large difference in the plasma-free fraction of Trp, and with it the free fraction of tracer, are compared, then comparisons between K^α (note that K^α has the free fraction of tracer implicitly incorporated in it) might yield misleading conclusions. In these instances comparisons should be performed with 5-HT synthesis rates. One of these situations might arise if the plasma Trp value was to rise greatly, the TPH would then become saturated with Trp and the rate of 5-HT synthesis would not increase any more (Young and Gauthier 1981). Thus, if Trp levels increase beyond this level, K^α should fall to account for the constant synthesis rate, as the plasma-free Trp rises. In this situation the capacity to synthesize 5-HT is not diminished, and the decline in K^α is misleading. Thus, in different circumstances there may be advantages and disadvantages in reporting either K^α or the rate of 5-HT synthesis. However, if the data is compared by using statistical parametric mapping (SPM) with proportional scaling then there is no need to calculate 5-HT synthesis rates because in the normalization procedure, differences related to, for example plasma Trp, are cancelled out (Friston *et al*. 1990). The SPM analysis will give the same results whether using K^α or the rate of 5-HT synthesis images.

Effect of serotonergic drugs on the uptake of α-MTrp in the brain: autoradiographic and PET studies

Uptake of α-MTrp influenced through serotonergic receptors

Selective 5-HT re-uptake inhibitors (SSRI) are used in the treatment of several mental disorders. Despite many investigations, the exact mode of action of SSRIs, and in particular the reason for the delay in the onset of their therapeutic action, is still not fully understood. The situation is complex because many of these drugs have biologically active metabolites (e.g. fluoxetine), which might have a somewhat different biochemical action than the parent compound. Some of the drugs and/or their metabolites may also influence the other monoamine neurotransmitters, which further complicates the issues. Acutely these drugs increase the extraneuronal concentration of 5-HT by blocking the re-uptake of released 5-HT (Wong *et al*. 1995). This results in additional action of 5-HT on autoreceptors and the firing rate of 5-HT neurons decreases. After several weeks of treatment the sensitivity of 5-HT autoreceptors is diminished and the firing rates of 5-HT neurons returns to normal (Piαeyro and Blier 1999).

In an attempt to further characterize the brain unidirectional uptake (trapping) of α-MTrp, we have carried out experiments in rats treated acutely or chronically with fluoxetine and the 5-HT releaser fenfluramine. In the experiments with fluoxetine, the α-MTrp autoradiographic measurements were performed after acute treatment with a dose of 10 mg/kg (Mück-Šeler *et al*. 1996) and 30 mg/kg (Tsuiki *et al*. 1995d). At 30 mg/kg, fluoxetine increased brain 5-HT synthesis, as measured by the α-MTrp method, in all brain areas. This increase could be explained only partly by a modest increase in free plasma Trp. Treatment with 10 mg/kg produced more modest increases in some terminal areas, with no change in some other terminal areas, and a reduction in the dorsal raphe nucleus. Eight days of treatment with fluoxetine (10 mg/kg once a day) produced a significant reduction in brain 5-HT synthesis in the raphe nuclei, and in many structures into which serotonergic neurons project (Mück-Šeler *et al*. 1996). However, in some terminal areas there was no significant reduction in brain 5-HT synthesis. Because there was no difference in the plasma-free Trp between the treated and control groups, the changes observed are most likely directly related to alterations in the activity of TPH induced by fluoxetine, as a result of the prolonged blockade of the 5-HT re-uptake.

Fenfluramine has been reported to work by stimulating the release (Mennini *et al*. 1985) and inhibiting the re-uptake of 5-HT (Davis and Faulds 1996). In our study rats were treated with D-fenfluramine (5 mg/kg, one injection per day) for 1 or 7 days (Yamane *et al*. 1999). In the acute experiment there was a similar profile in brain 5-HT synthesis as that obtained in the rats treated acutely with 10 mg/kg of fluoxetine; there was a reduction in synthesis in the dorsal raphe and an increase in some terminal areas. In the chronically treated rats there was also a decrease of the synthesis in the dorsal raphe with an increase in the terminal structures. The increase was more prominent in the brain structures predominantly receiving projections from the median raphe (e.g. hippocampus; Azmitia and Segal 1978),

suggesting that there might be an up-regulation of the synthesis in the serotonergic neurons not damaged by D-fenfluramine (Schuster *et al*. 1986; Ricaurte *et al*. 1991), and possibly in those with damaged terminals. This up-regulation of 5-HT synthesis (Tsuiki *et al*. 1995d) and of TPH mRNA (Ljubic-Thibal *et al*. 1999) occurs in neurons of rats lesioned in the dorsal hypothalamus with 5,7-dihydroxytryptamine. It may be that similar up-regulation occurs if the neurons are damaged by D-fenfluramine. Focal freezing lesions also results in an increase in 5-HT synthesis in cortical areas throughout the injured hemisphere (Tsuiki *et al*. 1995b).

We have also investigated the effect of (±)3,4-methylendioxymethamphetamine (MDMA) (Mück-Seler *et al*. 1998). When MDMA is given to rats, at first there is release of 5-HT resulting in a depletion of the neurotransmitter. The levels then start to return towards normal, but after 24 h start to decline again. This decline lasts many months, and is due to a distal axotomy of 5-HT neurons (Molliver *et al*. 1990; Green *et al*. 1995). In our experiments rats were treated with a total dose of 20 mg/kg: one group received two injections of 10 mg/kg 12 h apart, whereas the other group was injected four times, with a 5-mg/kg injection every 12 h for two days. The tracer measurements were performed about 12 h after the last injection of the drug. When the drug was given in smaller doses there was an increase in 5-HT synthesis, possibly stimulated by lowered stores of 5-HT, whereas the same total dose given in larger portions produced a decrease in the synthesis, possibly caused by damage to 5-HT neurons.

The results described above would not be expected if the tracer was measuring tryptophan transport into brain, but the results are consistent with what would be expected if the unidirectional uptake (trapping) of the α-MTrp tracer is related to the *in vivo* activity of TPH, and therefore to regional 5-HT synthesis.

Effect of reserpine on the brain uptake of α-MTrp

Reserpine inhibits the uptake of monoamines into vesicles, resulting in their metabolism by monoamine oxidase (Halaris and Freedman 1975; Long *et al*. 1983). Reserpine acts by binding to the vesicular uptake sites and has a half-life of about 16 h (Giachetti and Shore 1978). In addition to a long-lasting decline in the concentration of 5-HT in brain, there is an increase in TPH but no change in TPH mRNA (Park *et al*. 1993). The period required for metabolism to return to normal is from a few hours up to 10–12 days (Faith *et al*. 1968). Our studies were carried out in rats up to 4 days after a single injection of reserpine (10 mg/kg). Two hours after reserpine treatment there was a significant decrease (between 21% and 55%) in brain 5-HT synthesis in all brain areas, with no change in plasma-free Trp (Mück-Šeler and Diksic 1995). After 4 days there was an increase in 5-HT synthesis in many terminal areas, with no change in the dorsal and median raphe, and a decrease in the raphe magnus (Mück-Šeler and Diksic 1997). It is of interest to note that a differential uptake of the tracer was observed in the different brain structures, despite having the same level of plasma-free Trp in the control and reserpine-treated rats. This suggests that it is likely that the regional uptake of the α-MTrp tracer relates directly to 5-HT synthesis and also to *in vivo* activity of TPH.

Effects of buspirone treatment on the uptake of the α-MTrp tracer

Buspirone is a relatively selective 5-HT_{1A} receptor agonist reported to decrease neuronal activity in the raphe nuclei, as well as 5-HT release/turnover (Sharp *et al*. 1989), and 5-HT synthesis as measured by the accumulation of 5-hydroxytryptophan after the inhibition of AAAD with NSD-1015 (Hjorth and Carlsson 1982). As the 5-HT_{1A} receptor is an autoreceptor on the dendrites, it was hypothesized that an agonist action on those receptors should reduce 5-HT synthesis, and that the effects would be different in acute and chronic studies. Any differences between the acute and chronic action of buspirone may be relevant to its anxiolytic and antidepressant properties because these effects occur only after treatment lasting more than 2 weeks (Feighner *et al*. 1982; Jacobson *et al*. 1985). The autoradiographic measurements with α-MTrp as a tracer showed that acute treatment of rats with buspirone (10 mg/kg) reduced uptake of the tracer and 5-HT synthesis significantly in all brain areas. This reduction was between 29% and 59% (Okazawa *et al*. 1999). There was no influence on 5-HT synthesis in the pineal body, which does not have 5-HT_{1A} receptors. In the chronic experiment, the rats were treated with buspirone delivered by an osmotic pump for 14 days at the rate of 10 mg/g/day. The results obtained suggest that 5-HT synthesis was at control levels in most brain structures, except for the medial part of the caudate putamen, superior olive and raphe pallidus. In these experiments, there was no significant change in the plasma-free Trp between the treatment and respective control groups. The normalization of brain 5-HT synthesis after chronic treatment suggests that the serotonergic system has achieved a new steady-state in which the postsynaptic 5-HT_{1A} receptors are normosensitive (Haddjeri *et al*. 1998). The findings obtained with α-MTrp as a tracer are in agreement with the previous reports in which AAAD inhibition was used as an auxiliary treatment (Hjorth and Carlsson 1982), despite the fact that NSD-1015 can by itself have an effect on 5-HT synthesis (Mück-Šeler and Diksic 1995).

Effect of cycloheximide, a protein synthesis inhibitor

Recently we have performed experiments in rats treated with cycloheximide (Diksic *et al*. 2000a), an inhibitor of protein synthesis (Yeh and Shils 1969). These experiments were designed to evaluate the brain trapping of [α-^{14}C]MTrp after the blockade of protein synthesis with

cycloheximide. There was no significant difference in the brain trapping of [α-^{14}C]MTrp between rats treated with cycloheximide and those injected with saline. These results suggest that brain trapping of [α-^{14}C]MTrp was related to brain 5-HT synthesis and not to brain protein synthesis.

Measurements with ^{11}C-labeled α-MTrp and PET

Methodological considerations

^{11}C-Labelled α-MTrp has been used for PET studies in dogs (Diksic *et al*. 1991; Nishisawa *et al*. 1999), monkeys (Shoaf *et al*. 1998; Shoaf *et al*. 2000), and humans (Muzik *et al*. 1997; Nishizawa *et al*. 1997, 1998; Chugani *et al*. 1998a,b,c; Okazawa and Diksic 1998). Because of the 20-min half-life of [α-^{11}C]MTrp, the use of PET involves some compromises, as for all practical purposes in humans, scans longer than 100 min are not feasible. We (Nishizawa *et al*. 1997, 1998), along with others (Muzik *et al*. 1997), have carried out PET scans for 60 min, and have assumed that after 20–30 min of actual time (this is about 30–50 min of exposure time), that the *DV* (mL/g) is a linear function of the exposure time (Muzik *et al*. 1997; Nishizawa *et al*. 1997, 1998; Benkelfat *et al*. 1999; Diksic 2000). Strictly speaking this is not correct because the absolute values of the K^{α} estimates are dependent on the duration of the PET data collection, but the relative comparisons between groups were not affected by this dependence (Muzik *et al*. 1997; Nishizawa *et al*. 1998; Chugani and Muzik 2000). This linearity in the Patlak plot found in our human (Benkelfat *et al*. 1999; Diksic 2000) and dog experiments (Diksic *et al*. 1991; Nishizawa *et al*. 1999) is statistically highly significant, as it is in the experiments reported by Muzik *et al*. (1997), but might be somewhat different in the experiments with anesthetized monkeys (Shoaf *et al*. 1998, 2000). Despite the conclusion of Shoaf *et al*. (2000) that there is no irreversible compartment in the brain tissue uptake of [α-^{11}C]MTrp in the anesthetized monkey, their tissue uptake curves are fitted better ($p < 0.001$) by a model with an irreversible compartment (Diksic 2000; Shoaf *et al*. 2000). This would suggest that there is an irreversible trapping of labeled α-MTrp, which is in agreement with other observations (Diksic *et al*. 1990b, 1995; Muzik *et al*. 1997; Nishizawa *et al*. 1997; Benkelfat *et al*. 1999; Chugani and Muzik 2000), and illustrated in Figs 2 and 3. This is in accordance with the observation reported by Muzik *et al*. (1997). It is possible that in the anesthetized monkey, the size and/or half-life of the irreversible compartment is different from that in the conscious rat and human brain resulting in a greater loss of the label from an irreversible compartment. If this were the case then one might need to include the rate constant for the loss of the label from the brain into CSF, or possibly back into the precursor pool, as a part of the model formulation. The lack of linearity in a large part of the Patlak plot in the studies on anesthetized monkeys has led Shoaf *et al*. (1998, 2000) to suggest that [α-^{11}C]MTrp images only the uptake of Trp into brain tissue. On the basis of our experiments in rats (see above) as well as re-analysis (Diksic 2000) of curves presented in Shoaf *et al*. (2000), and our own data (Nishizawa *et al*. 1997; Benkelfat *et al*. 1999; Diksic *et al*. 2000b), this is unlikely. Indeed, if this were the case, one should be able to fit the experimental data with a two-compartment model better than with a three-compartment model. In humans, this is not the case reported by others (Muzik *et al*. 1997); in our experiments statistical analysis of residuals shows that the fitting a two-compartment model to the data is not satisfactory (Fig. 3). Note that by fitting with the full operational equation there is no need to make an assumption about which part of curve can be considered to be linear.

Chugani *et al*. (1998c) have also reported that the rank order of the regional *K*s are consistent with the rank order of the 5-HT content in the human brain. In addition we have observed a significant correlations between K^{α} and the constant for Trp metabolism via the 5-HT metabolic pathway (Table 1), but not with the constant for the transport through the BBB. This also points to a conclusion that K^{α} is related to brain 5-HT synthesis, not just the activity of the system the transport Trp into brain. Although there is a significant relationship between K^{α} and brain 5-HT content in different brain areas, it is most unlikely that there would be a 1 : 1 relationship. K^{α} represents a dynamic process, whereas brain 5-HT levels are a static measure. The tissue content of 5-HT might be influenced by different factors (e.g. storage and MAO activity) other than the rate of synthesis itself.

Studies on dogs

The first test of the α-MTrp method using PET was in the dog (Diksic *et al*. 1991). This study demonstrated the feasibility of measuring 5-HT synthesis with PET, and also demonstrated that, as expected, increased oxygen tension increased the rate of 5-HT synthesis. A study on the dog has also looked at the time-course of the effects of MDMA. Reports from recreational users indicated that soon after ingestion of the MDMA they have a euphoric reaction, which is subdued several hours later (Downing 1986; Cohen 1995). To mimic the effects of humans taking a dose, we infused 2 mg/kg of MDMA over 10 min (Nishisawa *et al*. 1999). One hour after the injection there was a large increase (six times) in α-MTrp trapping (5-HT synthesis), whereas 4 h later 5-HT synthesis was about one half of that at the baseline, and about 13 times lower than at the one hour mark after the MDMA injection. This suggests that the euphoriant effect in humans is associated with elevated 5-HT synthesis and presumably elevated release. A larger quantity of the extraneuronal 5-HT would eventually reduce 5-HT synthesis, as has been shown before with 5-HT agonists (Hjorth

and Carlsson 1982; VanderMaelen *et al*. 1986; Okazawa *et al*. 1999).

Studies on humans

The first study using ^{11}C-labeled α-MTrp and PET studied male/female differences and the effects of acute Trp depletion (Nishizawa *et al*. 1997). In this study, females were found to have a lower mean rate of 5-HT synthesis, an effect caused by lower free-plasma Trp rather than a lower value of *K*. This contrasts with the results of Chugani *et al*. (1998c), who reported that *K* was on average 15% higher in women, but did not report on free-plasma Trp or on the rate of 5-HT synthesis. It should be noted that a direct comparison of K^{α} (study of Chugani *et al*. 1998c) and our results, in which we calculated 5-HT synthesis rates, might not be straightforward (see above and Diksic 2000). The lower Trp levels in females in our study is consistent with other studies (e.g. Anderson *et al*. 1990b; Sarwar *et al*. 1991). However, plasma Trp is not necessarily lower in all circumstances. For example, plasma Trp tends to decline with age in males, but not in females (Caballero *et al*. 1991). On the other hand, plasma Trp declines in females but not in males when they are dieting (Anderson *et al*. 1990b). We have performed a baseline study in a second set of normal subjects (Okazawa *et al*. 2000), however, using a scanner with somewhat different characteristics, and found that there was no global difference in the brain 5-HT synthesis, but there were some specific regional differences. In addition, if both sets of data are combined we noticed a bimodal distribution in 5-HT synthesis rates. A difference in plasma Trp between males and females in our PET study may be related, in part, to a low-protein diet given the day before the PET scan. Thus, whether 5-HT synthesis rates are higher, lower or the same in women, relative to men, will probably depend on a variety of factors including the nutritional and metabolic state of the subjects.

The PET data showed that acute Trp depletion resulted in about a 90% decline in 5-HT synthesis in males, and at least a 95% decline in females (Nishizawa *et al*. 1997). This is consistent with the fact that acute Trp depletion can cause a lowering of mood (Young *et al*. 1985; Delgado *et al*. 1990), and that the effect seems to be greater in females than in males (Ellenbogen *et al*. 1996; Delgado *et al*. 1999).

Chugani *et al*. (1999a) looked at the development of 5-HT synthesis capacity (K^{α}). In normal children K^{α} was more than 200% of adult values until the age of five and then it declined towards adult values. In autistic children, K^{α} increased gradually between the ages two and 15 years to values of 1.5 times normal adult values. The same group also studied migraine patients during a headache-free interval (Chugani *et al*. 1999b). They found that K^{α} was increased in all brain areas relative to healthy controls, but this finding may be limited to patients without migraine aura.

Conclusions

We have outlined our understandings of the brain unidirectional uptake (trapping) of labeled α-MTrp when used as a tracer for the study of the brain 5-HT synthesis. This method is not perfect and might need refinements as new data becomes available. For example, it will not measure 5-HT synthesis when there is inflammation of brain tissue, and therefore induction of indoleamine-2,3-dioxygenase. Furthermore, because of the practical aspects of studying humans using PET scans, the PET methodology involves some compromises. Nonetheless, the results summarized above suggest that the method does supply useful information on brain 5-HT synthesis.

The model on which the method is based is complex, and understandably there have been different interpretations of the tissue uptake of the tracer and criticisms of the method of conversion of the brain uptake constant into 5-HT synthesis rates. This is a normal part of the development of any complex method. The major criticism has been that the method measures only the activity of the brain uptake system for Trp (BBB transport), and as a consequence of this the uptake constant should not be converted to 5-HT synthesis. Although there may be limited circumstances in which this is true, the evidence discussed above suggests that the method can be used to measure brain 5-HT synthesis in rats, dogs and humans. As we have emphasized, the unidirectional trapping of the labeled α-MTrp does not necessarily imply that all the trapped material is converted to α-M5-HT. It may be that TPH acts on a pool of trapped substrate, and that trapping in this pool is driven by the rate of 5-HT synthesis. This is one of many questions that needs further study. Another important methodological issue is the quantitation of the lumped constant for human brain regions. However, even though some methodological questions still exist, the results summarized above support the idea that the method is sufficiently developed to provide important information on brain 5-HT synthesis. During the next few years information will be available on the extent to which 5-HT synthesis is altered in neuropsychiatric illness and by psychotropic drugs.

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