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The inhibition of tryptophan hydroxylase, not protein synthesis, reduces the brain trapping of α-methyl-L-tryptophan: an autoradiographic study

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Abstract

The effects of the tryptophan hydroxylase (TPH) inhibitor *p*-chlorophenylalanine (PCPA; 200 mg/kg; 3 days), and of the protein synthesis inhibitor cycloheximide (CXM, 2 mg/kg), on regional serotonin (5-HT) synthesis were studied using the α-[^{14}C]methyl-L-tryptophan (α-[^{14}C]MTrp) autoradiographic method. The objectives of these investigations were to evaluate the changes, if any, on 5-HT synthesis, as measured with α-MTrp method, following the inhibition of TPH by PCPA, or the inhibition of proteins synthesis by CXM. The rats were used in the tracer experiment approximately 24 h after the last dose of PCPA was administered, and in the CXM experiments, they were used 30 min following a single injection of CXM. In both experiments, the control rats were injected with the same volume of saline (0.5 ml/kg; s.c.) and at the same times as the drug injections. The results demonstrate that trapping of α-MTrp, which is taken to be related to brain 5-HT synthesis, is drastically reduced (40–80%) following PCPA treatment. The inhibition of protein synthesis with CXM did not have a significant effect on the global brain trapping of α-MTrp and 5-HT synthesis. These findings suggest that the brain trapping of α-[^{14}C]MTrp relates to brain 5-HT synthesis, but not to brain protein synthesis.

Keywords: Serotonin synthesis; α-Methyl-L-tryptophan; *p*-Chlorophenylalanine; Tryptophan hydroxylase

1. Introduction

Serotonin (5-hydroxytryptamine) is a neurotransmitter and/or neuromodulator implicated in many brain disorders (Frezer and Hensler, 1995). 5-HT neurons innervate forebrain structures from the raphe nuclei, located in the brain stem (Dahlström and Fuxe, 1964; Steinbusch and Nieuwenhuys, 1983; Descarries et al., 1982). The biosynthesis of 5-HT from an essential amino acid, Trp, is catalyzed by tryptophan hydroxylase (EC 1.14.16.4; Lovenberg et al., 1967), producing, at first, 5-hydroxy-tryptophan. The decarboxylation of 5-hydroxy-tryptophan into 5-HT is catalyzed by the aromatic amino acid decarboxylase (EC 1.1.4.28). The in vivo activity of TPH is considered to be the rate limiting step in 5-HT biosynthesis. Measurements of in vivo 5-HT synthesis of the brain have not been very easy to obtain because of the lack of a specific and selective tracer for in vivo measurements. We have described a method (Diksic et al., 1990, 1991; Nagahiro et al., 1990) using labeled α-MTrp. However, there have been some criticisms of our approach. It has been suggested that the trapping of labeled α-MTrp relates to Trp incorporation into proteins (Gharib et al., 1999), and that the trapping is only an indication of the brain uptake of Trp (Shoaf et al., 2000). Some issues relating to the criticisms have been discussed in more detail in recent publications (Diksic et al., 1999; Benkelfat et al., 1999; Diksic, 2000, 2001; Diksic and Young, 2001). It should be noted that from the modeling point of view (Diksic et al., 1990, 1991, 1999), the actual conversion of the tracer (labeled α-MTrp) to labeled α-methylserotonin (α-M-5-HT) does not need to occur, as long as the tracer enters an irreversible compartment and remains there for a length of time which is "long enough" to permit a good estimate of the trapping constant. The appropriateness of this approach is also supported by experiments with drugs known to affect both 5-HT synthesis and serotonergic transmission (e.g. fluoxetine,

Abbreviations: 5-HT, 5-hydroxytryptamine or serotonin; α-MTrp, α-methyl-L-tryptophan; Trp, tryptophan; TPH, tryptophan hydroxylase; CXM, cycloheximide; PCPA, *p*-chlorophenylalanine; VD, volume of distribution

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fenfluramine, reserpine, lithium, buspirone). These experiments demonstrated that the effect of these drugs on 5-HT synthesis is different in the brain areas innervated by the serotonergic terminals than that in the structures containing cell bodies (Nagahiro et al., 1990; Mück-Šeler and Diksic, 1995, 1996; Mück-Šeler et al., 1996; Tsuiki et al., 1995). These results also demonstrate that the effects are different in the acute treatment studies than those observed in the chronic treatment studies, and in some instances, the effects are opposite in the cell bodies and the projection areas.

p-Chlorophenylalanine has been used for some time as a drug which specifically reduces brain 5-HT through an irreversible inhibition of TPH (Koe and Weissman, 1966, 1968; Weitzman et al., 1968; Jéquier et al., 1967; Gal and Whitacre, 1982; Berger et al., 1989). The inhibition is a result of an incorporation of tyrosine in the TPH active site in the place of alanine (Gal and Whitacre, 1982). It has been shown that 5-HT levels in the brain are drastically reduced in rats following the injection of PCPA. This reduction persists for a long time, suggesting that 5-HT synthesis must also be reduced. Similarly, cycloheximide has been shown to be a very effective inhibitor of brain protein synthesis between 30 min and approximately 4 h following s.c. administration of 0.6 mg/kg (Pavlik and Teisinger, 1980), permitting an evaluation of the brain trapping of labeled α-MTrp, and the 5-HT synthesis calculated from that trapping, following an inhibition of protein synthesis with CXM.

The objective of the studies reported here was to measure the rate of trapping, and the calculation of brain 5-HT synthesis rates from the trapping constant following the inhibition of TPH with PCPA, or protein synthesis with CXM. There were two hypotheses tested: (1) is there a change in 5-HT synthesis as measured by α-MTrp following TPH inhibition with PCPA? and (2) is there a change in 5-HT synthesis following the inhibition of protein synthesis with CXM?

2. Materials and methods

2.1. Animal treatment

All animal use procedures were approved by the Institutional Animal Care Committee and were done according to the guidelines of the Canadian Council of Animal Care.

In both sets of experiments, male Sprague–Dawley rats weighing approximately 200 g were used. Drug doses and injection schedules were based on previously reported data (Lovenberg et al., 1967; Deguchi and Barchas, 1972, 1973; Pavlik and Teisinger, 1980). Rats in the PCPA experiments were injected subcutaneously (s.c.) once a day with 200 mg/kg of PCPA (PCPA methyl ester hydrochloride; Aldrich Chemical Co.) dissolved in normal saline for 3 days. The main reason for injecting PCPA s.c. was to ensure prolonged plasma concentration of the drug, and with it a larger exposure of the brain to PCPA, by reducing the so called “first pass” metabolic degradation. The rats used in the CXM (Tocris Cookson Inc., Ballwin, MO, USA) experiments were injected with 2 mg/kg (s.c.) of CXM 30 min before the injection of the tracer. The dose of 2 mg/kg of CXM and the time at which the tracer was injected thereafter were selected because of the results of an earlier study (Pavlik and Teisinger, 1980). This study reported that an administration of 0.6 mg/kg (s.c) produced a large inhibition of protein synthesis between 30 min and 4 h following the CXM administration. It should be noted that the rats in our experiment were killed approximately 1.5 and 3.5 h following the CXM injection. The controls were injected with the same volume and on the same injection schedule of saline (0.5 ml/kg). The controls for the PCPA experiment were injected once a day over 3 consecutive days, while the controls for the CXM group were injected with saline only once. The animals in the PCPA group were used in the tracer experiments approximately 24 h after the last injection of PCPA or saline. To confirm that CXM treatment significantly decreased protein synthesis, 1-[^{14}C]valine incorporation was measured in three rats and compared to the rats injected with saline. The rats were injected with 1-[^{14}C]valine 30 min after the CXM injection and were killed 75 min after the 1-[^{14}C]valine injection. The brains were extracted, dissected, weighed, homogenized in four volumes of 20% trichloroacetic acid, and the supernatant was separated by centrifugation at $10{,}000 \times g$ for 10 min. The supernatant was removed and the radioactivity of the 1-[^{14}C]valine not incorporated into the proteins was determined by liquid scintillation counting. The residue was liquified by adding 1.5 ml of protosol (DuPond, Boston, MA). After the residue was liquified, the scintillation cocktail was added and the radioactivity of the protein incorporated with 1-[^{14}C]valine was determined. Our measurements essentially confirmed results reported by (Pavlik and Teisinger, 1980) confirming that it can be taken that CXM in the present experiments inhibited protein synthesis.

The rats were surgically prepared as previously described (Mück-Šeler and Diksic, 1995; Nagahiro et al., 1990) and injected with approximately 30 μCi of α-[^{14}C]MTrp over 2 min. A prolonged injection was used to reduce the danger of violating the tracer kinetics of α-MTrp or the effect on the steady state of Trp metabolism as previously discussed (Diksic et al., 1990). The tracer was synthesized as described by Mzengeza et al. (1993). The tracer had a specific activity of approximately 55 mCi/mmol, and a radiochemical and chemical purity of above 96%. The rats were killed 60 or 150 min after the beginning of the tracer injection (Nagahiro et al., 1990). The blood gases were monitored and there was no significant difference in the physiological parameters of the blood between the treated and untreated rats. The blood samples (about 50 μl each) were taken at progressively increased time intervals to obtain the plasma input function (Diksic et al., 1990). In addition, five samples (50 μl each) were taken at different times to determine the plasma free (ultrafiltrate using 10,000 molecular weight cut-off) and total tryptophan. These were determined using the high performance liquid chromatography (HPLC)

method with a reverse phase column and a fluorescence detector as described earlier (Mück-Šeler et al., 1996; Diksic et al., 1990). The brains were cut to 30 μm slices and co-exposed with standards to an X-ray film for 3 weeks. The autoradiographic images were quantified with the aid of the Image Analysis System (MCID-4; Imaging Research Inc., St. Catherines, Ont., Canada), using standards calibrated to the tissue equivalent. The tracer concentration in each brain region was determined on the left and right sides of the brain, and in at least three consecutive slices. The average values were used in further calculations.

2.2. Calculation of the brain trapping constant for α-[^{14}C]MTrp and the 5-HT synthesis rates

The basis of the biological model used in the calculation, along with the assumptions on which it is based, have been previously described (Diksic et al., 1990, 1991, 1995, 1999; Nagahiro et al., 1990; Diksic, 2000). The K^{α} (ml/g min), the trapping constant, was calculated from a linear relationship between $\Theta = \int_0^T C_p^*(t)\,dt / C_p^*(T)$ (min) ($C_p^*(t)$ is the plasma tracer concentration as a function of time), and the VD (ml/g; the volume of distribution of the tracer in the brain (Nagahiro et al., 1990; Diksic et al., 1990, 1995)) for the individual brain structures. The VD values were calculated as a ratio between the tissue tracer concentration and the plasma tracer concentration at the end of the experiments (Diksic et al., 1990; Nagahiro et al., 1990). On the basis of our experiments with rats (Diksic et al., 1990, 1995; Diksic, 2000), for the measurements obtained between 60 and 150 min (exposure time, Θ between approximately 100 and 280 min), one can apply the linear relationship between VD and Θ.

The α-[^{14}C]MTrp trapping constants (K^{α}) were converted into the 5-HT synthesis rates as described earlier (Diksic et al., 1990; Nagahiro et al., 1990; Mück-Šeler and Diksic, 1995). The calculation of the 5-HT synthesis rates was done by applying a conversion factor named the lumped constant (LC) and the following equation: $R = C_p K^{\alpha}/\mathrm{LC}$. In the present work in vivo measured LC = 0.42 was used (Vanier et al., 1995). This calculation is performed in an analogous way, as earlier proposed, for the use of labeled Trp in brain 5-HT synthesis measurements and the separation of different Trp metabolites (Lin et al., 1969). The plasma free Trp concentration (C_p, pmol/ml) was used in this calculation, because there is a good correlation between the plasma free Trp and brain Trp, and because the plasma free Trp relates to 5-HT synthesis in the brain (e.g. Bloxam and Curzon, 1978; Salter et al., 1989; Takada et al., 1993). However, this does not mean that the plasma Trp concentration will be appropriate for this calculation in all situations (Diksic and Young, 2001).

The images shown in Fig. 1 exemplify the brain 5-HT synthesis rates calculated by taking an average value for the intercept in the linear relationship (Diksic et al., 1995). However, the 5-HT synthesis rates presented in Tables 1 and 2 were calculated using individual values as described in detail in Nagahiro et al. (1990). The S.D. given in Tables 1 and 2 were calculated on the basis of the variance matrix of the regression line (Bevington, 1969).

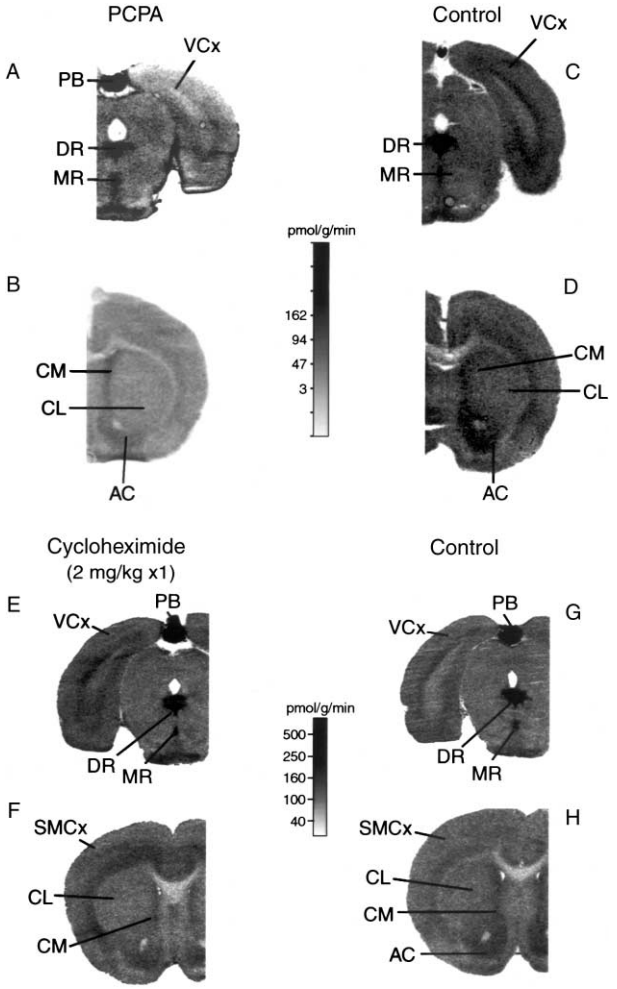

Fig. 1. Set of autoradiograms exemplifying the brain 5-HT synthesis as calculated from the trapping constant for α-[^{14}C]methyl-L-tryptophan in different areas of the rat brain, in both the control and PCPA treated rats (A–D). Note a different calibration bar for the two sets of images. The experiments done with CXM and respective control (E–H). DR: dorsal raphe; MR: median raphe; PB: pineal body; VCx: visual cortex; SMCx: sensory-motor cortex; CL: caudate-lateral; CM: caudate-medial; AC: accumbens. It is easy to notice a large difference in the synthesis between rats treated with PCPA (A and B) and respective control (C and D), while there is no significant difference between rats treated with CXM (E and F) and respective control (G and H).

The global influence of these drugs was evaluated by comparing the ratios between 5-HT synthesis in the control and treatment rats of the same experimental protocol. The ratios were used to test a null-hypothesis having the ratios equal to 1 (no effect) with a S.D. of 0, by using one sample two-tailed *t*-test. Once a global effect was found to be significant, the differences in the individual structures between the treatment and control groups were evaluated using the two-tailed *t*-test. The significance was evaluated at $P < 0.05$. The weight gain/loss in the rats treated with PCPA was evaluated

Table 1
The values of the α-[^{14}C]MTrp trapping constants (K^{α}) and the 5-HT synthesis rates (R) calculated from K^{α} as described in Section 2[a]

Structures	α-[^{14}C]MTrp trapping constant, K^{α} ($\times 10^3$ ml/g min)		5-HT synthesis rates, $R \pm$ S.D. (pmol/g min)[b]		Percent of control in PCPA treated
	Controls ($N = 12$)	PCPA ($N = 12$)	Controls ($N = 12$)	PCPA ($N = 12$)	
Raphe magnus	1.52 ± 0.36	0.78 ± 0.30	38.4 ± 9.1	21.1 ± 8.2	55
Median raphe	5.5 ± 1.1	1.07 ± 0.37	139 ± 29	29 ± 10	21
Dorsal raphe	6.88 ± 0.91	1.33 ± 0.43	174 ± 23	36 ± 12	21
Pineal body	7.6 ± 1.5	3.1 ± 0.7	192 ± 37	83 ± 19	43
Medal forebrain bundle	1.25 ± 0.37	0.82 ± 0.32	31.6 ± 9.4	22.2 ± 8.7	70
Locus coeruleus	1.17 ± 0.48	0.65 ± 0.34	30 ± 12	17.6 ± 9.3	59
Substantia nigra	1.22 ± 0.35	0.69 ± 0.31	30.7 ± 8.9	18.8 ± 8.4	61
Medial geniculate	1.27 ± 0.40	0.56 ± 0.34	32 ± 10	15.3 ± 9.1	48
Lateral geniculate	1.10 ± 0.30	0.68 ± 0.34	27.8 ± 7.7	18.4 ± 9.2	66
Hypothalamus	1.33 ± 0.51	0.56 ± 0.34	34 ± 13	15.1 ± 9.2	44
Thalamus ventral	0.96 ± 0.34	0.70 ± 0.37	24.2 ± 8.6	19 ± 10	79
Hippocampus ventral	1.55 ± 0.35	0.61 ± 0.36	39.1 ± 8.7	16.7 ± 9.7	43
Hippocampus dorsal	1.24 ± 0.38	0.76 ± 0.33	31.2 ± 9.6	20.7 ± 8.9	66
Caudate lateral	1.30 ± 0.34	0.69 ± 0.32	32.8 ± 8.5	18.8 ± 8.8	57
Caudate medial	1.33 ± 0.42	0.78 ± 0.36	34 ± 11	21.3 ± 9.8	63
Accumbens	1.86 ± 0.49	0.73 ± 0.42	47 ± 12	20 ± 12	43
Visual cortex	1.10 ± 0.39	0.53 ± 0.29	27.9 ± 9.8	14.5 ± 7.7	52
Auditor cortex	1.17 ± 0.31	0.57 ± 0.32	29.5 ± 7.7	15.5 ± 8.6	53
Parietal cortex	0.95 ± 0.37	0.60 ± 0.30	24 ± 9.2	16.3 ± 8.3	68
Sensory-motor cortex	1.02 ± 0.31	0.56 ± 0.31	25.7 ± 7.8	15.3 ± 8.4	60
Frontal cortex	1.22 ± 0.38	0.56 ± 0.30	30.9 ± 9.7	15.2 ± 8.0	49

[a] Values were obtained from the rats treated with *p*-chlorophenylalanine (200 mg/kg; 3 days and once a day) and from the control rats injected with the same volume of saline.

[b] The number of rats in the particular groups is identified by *N*, S.D. stands for a standard deviation. In all of the structures, there was a significant ($P < 0.05$; two-tailed *t*-test) reduction in 5-HT synthesis in the PCPA treated group when compared to the control group.

Table 2
The values of the α-[^{14}C]MTrp trapping constants (K^{α}) and the 5-HT synthesis rates (R) calculated from K^{α} as described in Section 2[a]

Structures	α-[^{14}C]MTrp trapping constant, $K^{\alpha} \pm$ S.D. ($\times 10^3$ ml/g min)[b]		5-HT synthesis rates, $R \pm$ S.D. (pmol/g min)[b]	
	Controls ($N = 14$)	CXM treated ($N = 13$)	Controls ($N = 14$)	CXM treatment ($N = 13$)
Raphe magnus	2.68 ± 0.34	2.30 ± 0.35	103 ± 13	88 ± 13
Median raphe	5.00 ± 0.39	4.47 ± 0.41	192 ± 15	171 ± 16
Dorsal raphe	8.70 ± 0.81	6.66 ± 0.63	334 ± 31	255 ± 24[c]
Pineal body	12.3 ± 3.3	13.9 ± 3.3	461 ± 127	535 ± 196
Medial forebrain bundle	1.60 ± 0.32	1.44 ± 0.36	61 ± 12	55 ± 14
Locus coeruleus	1.45 ± 0.39	1.50 ± 0.34	56 ± 15	57 ± 13
Substantia nigra	1.31 ± 0.29	1.34 ± 0.34	50 ± 11	40 ± 11
Medial geniculate	1.46 ± 0.30	1.23 ± 0.35	56 ± 11	47 ± 14
Lateral geniculate	1.53 ± 0.25	1.50 ± 0.38	58.7 ± 9.6	58 ± 15
Hypothalamus	1.58 ± 0.25	1.52 ± 0.34	60.5 ± 9.5	58 ± 13
Thalamus ventral	1.51 ± 0.25	1.51 ± 0.36	57.9 ± 9.5	58 ± 14
Hippocampus ventral	2.33 ± 0.28	2.38 ± 0.41	89 ± 11	91 ± 16
Hippocampus dorsal	2.15 ± 0.27	2.20 ± 0.40	83 ± 10	83 ± 15
Caudate lateral	1.33 ± 0.26	1.52 ± 0.34	51 ± 10	58 ± 13
Caudate medial	1.75 ± 0.26	1.79 ± 0.39	67 ± 10	69 ± 14
Accumbens	2.11 ± 0.26	2.05 ± 0.34	81 ± 10	78 ± 13
Visual cortex	1.52 ± 0.30	1.46 ± 0.36	58 ± 12	56 ± 14
Auditory cortex	1.54 ± 0.26	1.49 ± 0.38	59 ± 10	57 ± 15
Parietal cortex	1.39 ± 0.29	1.35 ± 0.35	53 ± 11	52 ± 13
Sensory-motor cortex	1.16 ± 0.29	1.26 ± 0.35	44 ± 11	48 ± 13
Frontal cortex	1.34 ± 0.28	1.43 ± 0.37	51 ± 11	55 ± 14

[a] Values were obtained from the rats treated with cycloheximide (2 mg/kg; 30 min before tracer) and from the control rats injected with the same volume of saline.

[b] The number of rats in the particular groups is identified by *N*, S.D. stands for a standard deviation.

[c] There is a significant difference ($P < 0.05$; two-tailed *t*-test) in this structure, however since there is no global difference ($P > 0.05$, one sample two-tailed *t*-test on ratios), and this is only 1 structure out of 21 which shows a significant difference it is possible that this difference occurred by chance alone.

by repeated measures ANOVA. Statistical evaluations were done with SYSTAT-9 (SPSS Inc., 2000) or SigmaStat-2.03 (SPSS Inc., 1999) programs. No comparison between the controls used in the PCPA and CXM experiments was done, because the animals were differently treated and can only be considered controls for a particular experimental group. The difference in trapping and 5-HT synthesis calculated therefrom probably only relates to the differential handling, which was not the object of this investigation.

3. Results

The physiological variables (e.g. pO_2, pCO_2) of the animals during the experimental procedure were within normal levels, and did not vary from those of the other animals used in the laboratory for experiments. The rats treated with PCPA had significantly lower weight gain than the respective controls. These rats actually lost weight during the 3 days of treatment. The weight of the rats entering the PCPA treatment was 214.9 ± 9.3 g (S.D.) and after 3 days of treatment, the weight was 202 ± 11 g (S.D.). This loss in weight is significant ($P < 0.05$). The control rats weighed 223 ± 11 g upon entry and 229 ± 10 g after 3 days of saline injections. While the PCPA treated rats had lost, on average, 12.9 g, the control rats gained an average of 6 g in body weight. The plasma concentration of free or total Trp was not significantly different in the PCPA treated rats (11.4 ± 2.5 nmol/ml free and 70 ± 10 nmol/ml total; $N = 12$) from that found in the controls (10.6 ± 1.9 nmol/ml free and 63.9 ± 9.7 nmol/ml total; $N = 12$). In the CXM experiment, the plasma concentration of free Trp was 16.1 ± 5.9 nmol/ml in the controls and 16.1 ± 7.6 in the CXM treatment group. The total plasma Trp was 43 ± 10 nmol/ml in the controls and 59 ± 14 nmol/ml in the CXM treated rats. There were no significant differences between the plasma-free Trp concentrations between the CXM treatment and the respective control groups, but there was a significant difference ($P < 0.005$) in the plasma total Trp between the CXM and control groups. The Trp concentrations in both control groups were within the range found in the other experiments performed in the laboratory.

Fig. 1(A–D) represent a set of representative autoradiographic images is provided to visualize the differences in the brain 5-HT synthesis rates in the control and PCPA treated rats. The images are provided at two representative cross-sections of the rat brain and for a direct comparison, the same scale is used for the control and treatment rats. A large difference in 5-HT synthesis between the two groups is obvious. Fig. 1(E–H) exemplify the 5-HT synthesis rates in the CMX treated rats and the respective controls. The gray scales in Fig. 1 (note a different scales for PCPA and CXM images) provide the comparison between the different levels of the gray and corresponding 5-HT synthesis rates. A visual inspection of these images suggest that while there is a large difference in 5-HT synthesis in PCPA treated rats and the respective controls, there is no difference between the CXM treated rats and the respective controls. The quantitative data is provided in Tables 1 and 2 for 21 brain structures, both for the 5-HT synthesis rates and the trapping constants. Percent reduction, relative to the synthesis in the control group, of 5-HT synthesis in the PCPA treated rats is given in the last column of Table 1. The trapping constants are also provided to make it easier for the readers to obtain an understanding of the effects on the trapping of α-MTrp. It is easy to see (Fig. 1) that there is a greater rate of 5-HT synthesis in the control rats than in the PCPA treated rats. There was a reduction in 5-HT synthesis, both in the cell body structures as well as in the terminal areas. The reduction is rather obvious, but to be consistent with a significance test, it was tested, and the ratio of the synthesis between the control and PCPA treated rats was significantly greater than 1 (ratio $= 2.06 \pm 0.96$; $t = 5.08$; $P < 0.00003$; $N = 21$). This indicated that the PCPA treatment has a significant effect on brain 5-HT synthesis, resulting in a significant global reduction in brain 5-HT synthesis. The largest reduction in 5-HT synthesis was seen in the raphe nuclei (about 80%), while reductions in the terminal regions were somewhat smaller (about 40%).

In contrast to the effect produced by the PCPA treatment, there was no global significant effect of CXM (Table 2) on the regional rates of 5-HT synthesis, which is also depicted in Fig. 1(E–H). The significance of the difference between the CXM treated rats and the respective controls was evaluated by comparing the ratio of 5-HT synthesis between the control and CXM treated rats to one (ratio $= 1.04 \pm 0.10$; $t = 2.08$; $P > 0.05$; $N = 21$). The synthesis in the control rats is not significantly different from that found in the CXM (protein synthesis inhibitor) treated rats (Table 2). It is possible that the reductions observed in the cell body structures are real, but because there is no global effect, they should be ruled out as occurring by chance alone. It is also evident that in all of these images, 5-HT synthesis is substantially greater in the structures known to have a high density of serotonergic cell bodies (e.g. dorsal raphe) than in the projection areas (e.g. cortex; caudate). One should also note a non-uniformity in 5-HT synthesis throughout the brain. The medial parts of the caudate have a higher value of 5-HT synthesis than the lateral parts and the VI-layer of the cortex has a higher value of 5-HT synthesis than the rest of it.

A comparison of the brain trapping constants in the control groups used in the PCPA and CXM experiments indicate that these are somewhat greater in the controls used in the CXM experiments. At the same time it should be noted that these controls have a fraction of the plasma-free Trp which is more than double that found in the controls in the PCPA experiment, and that the controls were handled differently than the rats used in the PCPA experiment.

4. Discussion

The reduction in the weight of the PCPA treated rats accords with earlier reports (Koe and Weissman, 1966,

1968; Gross et al., 1999). The present observation of the weight reduction indirectly confirms that the PCPA treatment produced the expected pharmacological effect in the treatment rats. It was also reported that the PCPA treatment does not have any effect on the BBB transport of Trp (Koe and Weissman, 1968). This observation is also supported by the results which show that the changes in the brain Trp parallel the changes in the plasma Trp in the PCPA treated rats (Tagliamonte et al., 1971). In the experiments reported here, there was no significant change in the plasma Trp by the PCPA treatment. On the basis of the mentioned reports and plasma tryptophan concentrations in the control and PCPA groups presented here, we assume that, since α-MTrp and Trp share the same brain uptake system (Diksic et al., 1991), the PCPA treatment should not have any direct influence on the transport of the tracer into the brain.

The experiments reported here clearly show that there is a reduction in 5-HT synthesis calculated from the trapping constant of α-[^{14}C]MTrp in the rats treated with PCPA as hypothesized on the basis of the irreversible inhibition of TPH (Koe and Weissman, 1966, 1968; Weitzman et al., 1968; Gal et al., 1970, 1975; Gal, 1972; Gal and Whitacre, 1982). This reduction in 5-HT synthesis concurs with the findings that there is a reduction of the amount of the enzyme (Richard et al., 1990), and its activity in the brain tissue measured ex vivo (Carlsson et al., 1972; Hamon et al., 1972; Macon et al., 1971). It has also been shown that several days following the treatment with PCPA, there was still a reduction in the actual amount of the enzyme. The in vivo activity of TPH was also drastically reduced following PCPA treatment. The correlation between the trapping of labeled α-MTrp observed here, and the expected reduction of TPH activity reported previously (Carlsson et al., 1972; Hamon et al., 1972; Macon et al., 1971) strongly suggests that the brain trapping relates to brain 5-HT synthesis. The reduction in 5-HT synthesis is between 40% (in the terminal areas) and 80% in the raphe nuclei. This reduction in 5-HT synthesis is in the general range of reduction in the levels of TPH in the rat brain reported by others (Jéquier et al., 1967; Miller et al., 1970). A reduction in 5-HT synthesis of 57% in the pineal body can be compared to a 75% (Lovenberg et al., 1973) and a 43% (Deguchi and Barchas, 1973) reduction in the ex vivo TPH activity using the same treatment protocol. However, the smaller reduction in the in vivo measurement of synthesis rates can be, at least in part, a result of an upregulation of the remaining TPH after the absolute amount of the enzyme has been reduced. Note that the trapping measurements as performed in this report relate to the in vivo activity of TPH and not necessarily its amounts. The half-life of the TPH recovery has been estimated to be between 1.43 days (Richard et al., 1990) and 2.5 days (Meek and Neff, 1972), which corresponds reasonably well to our measurement of 1.25 days (Ljubic-Thibal, Diksic et al., unpublished). On the basis of these values for the half-life of the enzyme recovery, and the fact that the rats were injected for 3 consecutive days with PCPA, it is reasonable to assume that the in vivo activity of TPH was significantly reduced in the rats when the brain 5-HT synthesis rates were measured in the present experiments.

In the experiments in which protein synthesis was inhibited with CXM, the regional 5-HT synthesis was the same in the treatment and control groups. It should be noted that the CXM experiment was an acute experiment and the TPH protein synthesis inhibition produced by the CXM would not have any noticeable effect on the amount of protein present, as TPH has a half life of between 1.25 and 2.5 days. These results suggest that an acute inhibition of protein synthesis does not affect 5-HT synthesis as measured by α-MTrp method, unlike the inhibition of TPH. No effect of CXM on brain 5-HT synthesis is in accordance with the data reported previously by Renson (1973). It is interesting to note that a reduction in 5-HT synthesis in the raphe nuclei of between 11 and 21% agrees well with the reduction of 18% reported in the ex vivo TPH activity measurement (Renson, 1973). The observation that the CXM treatment does not affect brain 5-HT synthesis is not surprising, as we recently reported that the constant for the metabolic conversion of Trp into proteins does not correlate with the constant of Trp conversion to 5-HT (Diksic et al., 2000). In those experiments, we also demonstrated that there is a correlation between the constant for α-[^{11}C]MTrp trapping and Trp conversion to 5-HT, but not with Trp incorporation into proteins. A correlation between the uptake of labeled Trp via the neuronal high affinity Trp uptake system and its conversion into 5-HT was previously reported (Mandell and Knapp, 1977). There is, therefore, a possibility that the tissue trapping of α-[^{14}C]MTrp, at least in some situations and to some extent, is related to this high affinity Trp uptake system. Indeed, if this system controls the access of Trp to the enzyme, it could be hypothesized that it also controls the neuronal 5-HT synthesis as well. Furthermore, results of the PCPA and CXM experiments, taken together, suggest that the tracer is trapped proportionately to brain 5-HT synthesis and not proportionally to the brain incorporation of Trp into proteins.

As stated in Section 2, no comparison was done between the trapping or 5-HT synthesis in the different control groups. However, we would like to comment that the trapping constants in some structures differ quite a bit between the two control groups while others are quite similar. Because the control animals in the two sets of experiments were not handled (one can say “treated”) in the same manner, there is a possibility that this has a different effect on the 5-HT synthesis in different brain structures, which then produces these apparent differences. In addition, the S.D. of these values are substantial, which also suggests the possibility of rather large statistical fluctuations in those values. In addition, we could state that the controls used in the CXM experiment had a plasma fraction of the free Trp which was almost double, which could in part explain the difference in the trapping constants. The measured trapping constant has incorporated the free fraction of the tracer in the plasma (Diksic et al., 1990, 1991; Diksic, 2000) which is highly

correlated with the plasma free fraction of Trp (Diksic, unpublished). It should be noted that the controls used in the CXM experiments were handled for the first time when the tracer experiment was done, while the PCPA controls were handled on three occasions before the tracer experiment. This, by itself, could produce a differential stress in the two sets of controls, and could be responsible, in part, for the different rates of 5-HT synthesis between the two control groups.

In conclusion, we can state that the data presented here suggests that the brain trapping of the tracer, α-[^{14}C]MTrp, and 5-HT synthesis rate, in the rats treated with PCPA, are reduced because of the reduction of TPH activity due to the drug treatment (Koe and Weissman, 1966, 1968; Weitzman et al., 1968; Jéquier et al., 1967; Miller et al., 1970). In addition, CXM treatment, which produces protein synthesis inhibition (Pavlik and Teisinger, 1980), does not have any significant effect on brain 5-HT synthesis. It is of interest to note that the greatest reduction, as a percentages of the control values, in 5-HT synthesis, occurred in the raphe nuclei, which also have the greatest concentration of serotonergic cell bodies. From these results, as well as from the data reported on the effects of drugs and the correlations between transports into different tissue pools of Trp and α-[^{14}C]MTrp (Diksic et al., 2000; Diksic, 2001), we can conclude that the tissue trapping of α-[^{14}C]MTrp is related directly to 5-HT synthesis, and that the conversion of the brain trapping of labeled α-MTrp to the 5-HT synthesis rates, as previously reported (Diksic et al., 1990, 1991, 1995; Nagahiro et al., 1990; Diksic, 2001; Diksic and Young, 2001) is appropriate and the measurements with the α-MTrp method could provide important information on regional 5-HT synthesis.

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