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Reduction in serotonin synthesis following acute and chronic treatments with paroxetine, a selective serotonin reuptake inhibitor, in rat brain: An autoradiographic study with α-[^{14}C]methyl-L-tryptophan

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Abstract

Serotonin (5-HT) synthesis rates were calculated on the basis of the assumption that trapping of α-[^{14}C]methyl-L-tryptophan (α-[^{14}C]MTrp) is directly related to brain 5-HT synthesis. In the first series of experiments, an acute intraperitoneal injection of paroxetine (10 mg/kg) produced a significant reduction in 5-HT synthesis in brain structures containing serotonergic cell bodies (the dorsal, median, and pallidum raphe nuclei), as well as in most projection areas: the ventral tegmental area, median forebrain bundle, hippocampus CA3 region, and nigrostriatal structures (substantia nigra, lateral and medial caudate nuclei). The reductions in the projection areas were greater (between 25 and 53%) than in those areas containing serotonergic cell bodies (between 18 and 23%). In the cerebral cortex, 5-HT synthesis rates were not modified by acute paroxetine treatment. In a second series of experiments, rats were treated with paroxetine (10 mg/kg/day, s.c., delivered by osmotic minipumps) for 14 days. There was a marked decrease (39–69%) in 5-HT synthesis in every structure examined. In conclusion, the present data suggest that the effects of paroxetine on 5-HT synthesis in the cerebral cortex are different from its effects in the cell body area of the brainstem.

Keywords: Paroxetine; α-[^{14}C]Methyl-L-tryptophan; Autoradiography; Serotonin synthesis rate; Tryptophan hydroxylase

1. Introduction

An autoradiographic method for measuring the regional 5-HT synthesis rate was developed using labeled α-[^{14}C]MTrp, a Trp analogue, as a tracer [1,2]. It has been shown that α-[^{14}C]MTrp is, in part, metabolically converted into α-[^{14}C]M5HT in the rat dorsal raphe [1,3], and that both get irreversibly trapped in the brain tissue [1–7]. It was therefore proposed that this method could be used to assess 5-HT synthesis rates because labeled α-MTrp is trapped in brain tissue in direct proportion to 5-HT synthesis [2,4,8–11]. In addition, the rate of trapping is influenced by drugs known to influence the brain serotonergic system. An increase in blood oxygen concentration, without any change in the plasma Trp level, increases the trapping of α-[^{11}C]MTrp in the dog brain [12]. The increase in 5-HT synthesis in the dog brain was similar to the increase reported in the brains of rats where accumulation was measured after inhibition of aromatic amino acid decarboxylase [13]. In addition, when Trp was used as a tracer and the rate of Trp metabolism through the 5-HT metabolic pool was calculated, the values for 5-HT synthesis were in agreement with those calculated from the trapping of α-[^{14}C]MTrp [2,9].

The aim of the present study was to assess whether the SSRI paroxetine could affect 5-HT biosynthesis as calculated from the trapping constant of α-[^{14}C]MTrp. The assessments were done following acute and chronic treatments with paroxetine. This antidepressant drug was chosen because of its high affinity and selectivity for the 5-HT transporter and also because it does not have active metabolites, unlike some other SSRIs (e.g. fluoxetine).

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Abbreviations: 5-HT, serotonin, 5-hydroxytryptamine; α-[^{14}C]MTrp, α-[^{14}C]methyl-L-tryptophan; SSRI, selective serotonin reuptake inhibitor; and α-[^{14}C]M5HT, α-[^{14}C]methyl-5-HT.

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2. Materials and methods

2.1. Animals

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

Sprague–Dawley male rats (Charles River), weighing between 200 and 230 g, were used in the acute studies. The rats were housed in the animal facility (room temperature of 22° with a 7:00 a.m. to 7:00 p.m. day–night cycle) for at least 3 days before being used in the experiments and had free access to solid chow and drinking water. Fourteen rats were injected with a 10 mg/kg dose of paroxetine (Smith-Kline Beecham Pharmaceuticals) dissolved in distilled water, and twelve rats received the same amount of vehicle via intraperitoneal injection. The tracer was injected 60 min after the paroxetine or water injection. For chronic treatments, osmotic minipumps (type 2ML2; Alza) were implanted subcutaneously in male rats (130–150 g); these minipumps delivered 10 mg/kg/day of paroxetine for 14 days based on the mean weight of the rats during the treatment. The drug was dissolved in a 50% ethanol:water solution, and control rats were implanted with a minipump containing the vehicle. For the chronic experiments, the drug was delivered until decapitation. All implantation procedures were carried out under halothane (1.0 to 2.0%) anesthesia using sterile techniques.

2.2. Experimental procedure

A detailed experimental procedure has been described in previous publications [2,4]; hence, only a short summary is given here. Before the autoradiographic studies, the rats were fasted overnight with water provided *ad lib.* Fasting was necessary to obtain a stable concentration of plasma amino acids, including Trp [2]. The rats were always killed between 2:00 and 4:00 p.m. The physiological parameters [pH, $PaCO_2$ (arterial blood partial pressure of CO_2 in mm Hg), PaO_2 (arterial blood partial pressure of O_2 in mm Hg), hematocrit, body weight] for the experimental groups were within the range of previously published values in saline-treated rats [2]. Under light halothane (1.0 to 2.0%) anesthesia, plastic catheters were inserted into a femoral artery (for blood sampling) and a vein for tracer injection. The rats were then allowed to wake up before tracer injection. Thirty microcuries of α-[^{14}C]MTrp (specific activity of about 55 mCi/mmol; synthesized in house using a procedure described previously [14]) was injected intravenously in 1 mL of saline over 2 min, using a constant infusion injection pump. With the beginning of the tracer injection, arterial blood samples were taken at progressively increasing time intervals up to the time the rats were killed. The blood samples were centrifuged at 12,500 *g* for 3 min at room temperature, and 20 μL of plasma was taken for liquid scintillation counting to measure plasma ^{14}C radioactivity (input function). At the beginning, midpoint, and end of each experiment, an additional 50 μL of plasma was deproteinized with 25 μL of 20% trichloroacetic acid, to be used for the determination of the total Trp concentration in the plasma. The plasma concentration of free Trp was measured in an ultrafiltrate of plasma (MW cutoff of 10,000; details provided in Refs. 1 and 2). The rats were guillotined 1 or 2.5 hr after tracer injection. The brains were removed, frozen in isopentane, and cut into 30 μm slices with a cryostat at –20°. The brain sections were exposed to x-ray film along with ^{14}C-polymer standards (American Radiolabel) for 2 weeks to obtain autoradiograms.

2.3. Measurement of α-[^{14}C]MTrp trapping and calculation of the trapping constant and its conversion for the determination of the 5-HT synthesis rate

Images were digitized using a microcomputer-based image analysis system (MCID; Imaging Research Inc.) consisting of a video camera, a frame grabber, and an IBM-AT compatible computer. Optical densities were converted into tissue radioactivity concentration (nCi/g) using calibration ^{14}C-standards. Tissue concentrations of tracer were measured separately in thirty-five brain structures. Averages of six readings (bilaterally) in each brain structure in three consecutive sections were obtained. Different brain areas were identified using the Paxinos and Watson rat brain atlas [15]. The tissue radioactivity concentrations were converted into volume of distribution (VD*; mL/g) by dividing tissue concentrations [$C^*_t(T)$; nCi/g] by the plasma tracer concentration [$C^*_P(T)$; nCi/mL] at the end of the experiment. There is a linear relationship between VD* and θ (min) [$\theta = {}_0\int^T C^*_P(t)dt/C^*_P(T)$]; it has a slope equal to K* (mL/g/min; trapping constant for α-[^{14}C]MTrp) as described before [2,16]. The slope of this line was calculated by a least-squares method, which yields also the standard deviation (SD) of K* [2,16]. In the calculation of the 5-HT synthesis rate, the first trapping constant for α-[^{14}C]MTrp is converted into a constant for the metabolism of Trp through the 5-HT metabolic pathway (K^T; mL/g/min). This conversion is accomplished by dividing K* with the lumped constant (LC), because the LC is by definition the ratio of K* and K^T [9]. The rate of 5-HT synthesis (R; pmol/g/min) is then calculated by multiplying the calculated K^T by the plasma free (non-protein-bound) Trp (Cp; pmol/mL); $R = Cp \cdot K^*/LC = Cp \cdot K^T$. The use of plasma free Trp in this calculation is based upon reports indicating that this plasma fraction is in dynamic equilibrium with brain Trp [17–19]. On the basis of the observations mentioned above, and the use of the tracer procedure (which permits a calculation of the rate at which a biological process is occurring from the rate at which Trp is metabolized via the 5-HT pathway), it was possible to calculate the rate of 5-HT synthesis in the brain, assuming that the plasma free Trp is the appropriate plasma Trp concentration in this calculation. The LC was measured

Table 1
Physiological parameters of rats used in acute (10 mg/kg, i.p., 60 min before tracer) and chronic (10 mg/kg/day, s.c., for 14 days; osmotic pump) treatments with paroxetine

Parameters	Acute treatment		Chronic treatment	
	Control (N = 12)	Treated (N = 14)	Control (N =17)	Treated (N = 19)
Weight gain (g)	NA	NA	92.2 ± 12.0	71.3 ± 14.9[a]
pH	7.45 ± 0.02	7.43 ± 0.02	7.46 ± 0.02	7.46 ± 0.02
$PaCO_2$ (mm Hg)	40.2 ± 1.3	41.2 ± 2.2	38.3 ± 1.7	39.9 ± 2.0
PaO_2 (mm Hg)	95.7 ± 6.5	95.0 ± 3.3	90.3 ± 5.5	90.8 ± 5.8
Plasma free Trp (nmol/mL)	9.3 ± 3.5	10.0 ± 4.3	12.0 ± 1.6	9.4 ± 2.4[b]
Plasma total Trp (nmol/mL)	94.8 ± 23.8	89.3 ± 9.6	79.5 ± 10.4	76.0 ± 12.7
Fraction of free Trp	0.10 ± 0.05	0.11 ± 0.05	0.15 ± 0.02	0.13 ± 0.04

Data are presented as means ± SD. NA = not applicable.
[a] Weight gain after 14-day treatment was significantly lower than in the control group ($P < 0.001$ by ANOVA).
[b] Significantly ($P < 0.05$) different from the concentration in the respective control group. There was no comparison done between variables in the chronic treatment group and those in the acute treatment group.

in vivo and found to be 0.42 ± 0.07 and uniform throughout the rat brain [9]. In the present experiments, all the linear relationships between θ and VD* had a significant ($P < 0.05$; F-statistics) and positive slope [2,20].

2.4. Statistical analysis

All values are expressed as means ± SD of the mean. This standard deviation was calculated from the standard deviation of K* obtained from the least-squares fit [2,16]. The 5-HT synthesis rates in the treatment groups and respective controls were first evaluated for the main effect by comparing the ratios to one with a standard deviation of zero using a one-sample, two-tailed *t*-test. If there is no difference between the treatment group and its respective control, the ratio should not be significantly different from one with a standard deviation of zero (null-hypothesis). Note that this method does not provide regional synthesis rates for the individual rats, thus precluding the use of more conventional statistical methods. After the main effect was found to be significant, the synthesis rates in different structures were compared by one-way ANOVA. A $P < 0.05$ was regarded as a significant difference. It should be noted that comparisons in the present experiments, in which multiple variables were compared between the control and the treated groups, do not represent classical multicomparisons, because comparisons of multiple dependent variables are carried out and not comparisons of multiple independent groups or variables. Since the distribution volumes and probably the 5-HT synthesis rates in different structures are highly correlated ($P < 0.00001$) within each group, no straightforward adjustment for a Type I error is possible [21]. Note that because the method does not provide the rates for the individual rats, the synthesis rates cannot be formally tested for a correlation. Assuming that there is also a significant correlation between the regional 5-HT synthesis rates (dependent variables) in different brain structures, as for the distribution volumes, the univariate F values of ANOVA are not independent, and no straightforward adjustment of a Type I error rate is possible [21]. To overcome this drawback, at least in part, the significance levels are presented on the basis of the univariate F values and, in addition, a statement is made that evaluation of the pooled within-group correlations among dependent variables indicates a high significant correlation that should be taken into account in the interpretation of differences, as suggested in the literature [21]. Statistical analyses were done using SYSTAT-9 (SPSS Inc., 2000) and SigmaStat-2.03 (SPSS Inc., 1999). Note that in all experiments, respective controls underwent exactly the same procedure.

3. Results

There were no significant differences in the physiological parameters, e.g. pH, $PaCO_2$ (arterial carbon dioxide tension), PaO_2 (arterial oxygen tension), and hematocrit between the controls and the treated group. Rats treated with the vehicle had a significant weight gain during the 14-day treatment (controls: F = 963; N = 17; treated: F = 392; N = 19; $P < 0.001$; repeated measure ANOVA). However, the weight gain in rats treated with paroxetine for 14 days (71 ± 15 g; N = 19) was significantly lower (F = 18.8; $P < 0.001$; one-way ANOVA) than that in controls (92 ± 12 g; N = 17) (Table 1). The plasma concentration of free Trp (10.0 ± 4.3 nmol/mL) in rats acutely treated with paroxetine (10 mg/kg) was not significantly different (by ANOVA) from the mean concentration in the control (saline-treated) group (9.3 ± 3.5 nmol/mL). In the 14-day experiment, the free Trp levels in the plasma were 12.0 ± 1.6 and 9.4 ± 2.4 nmol/mL in the control and paroxetine-treated rats, respectively. These values were significantly ($P < 0.05$; ANOVA) different from each other (Table 1).

Two sets of representative autoradiograms obtained in the brain of control and treated rats killed 1 and 2.5 hr after tracer injection are shown in Figs. 1 and 2. No specific attempt was made to match the levels in the brain slices, but rather the slices were selected to illustrate the distribution of

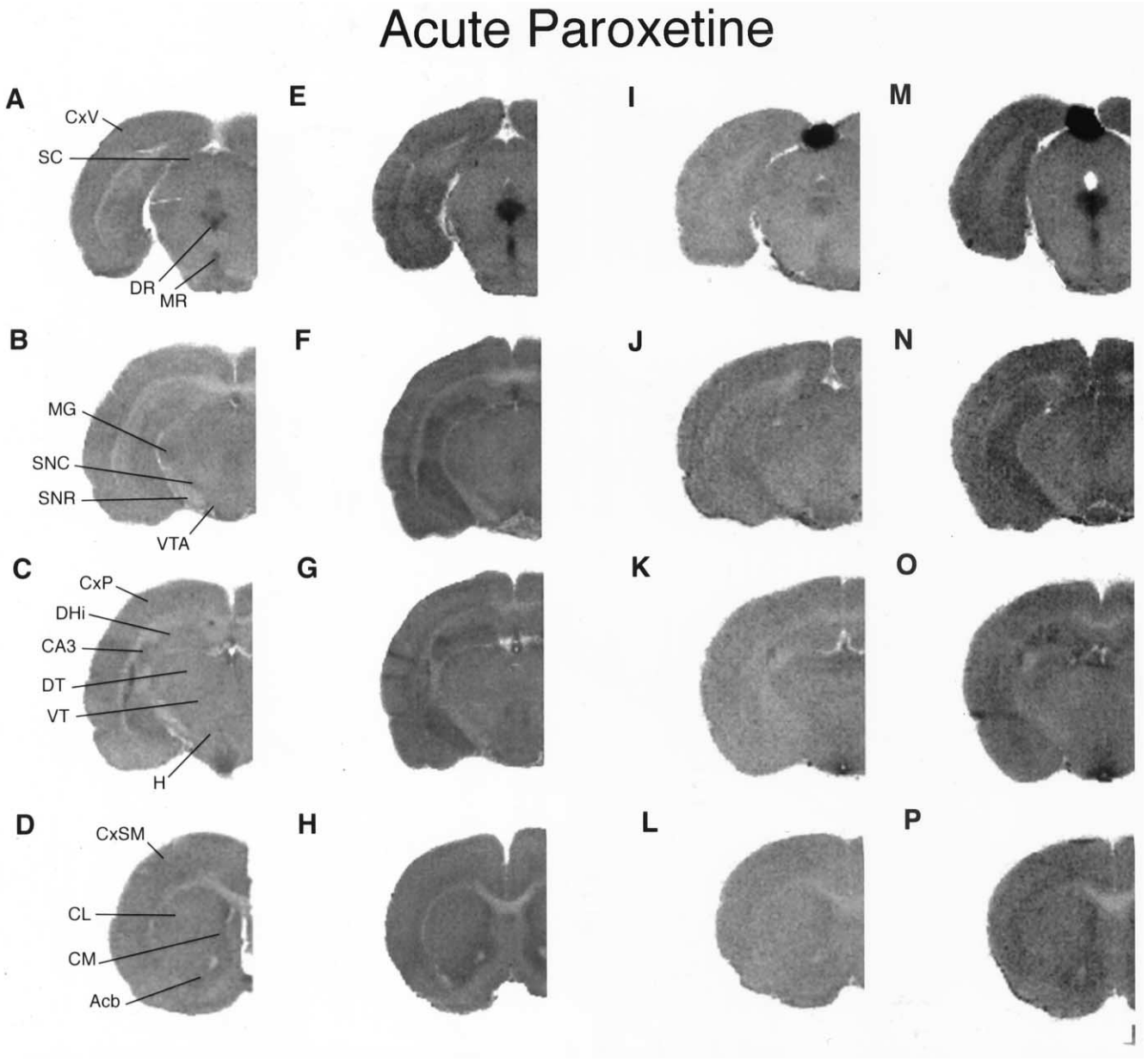

Fig. 1. Representative autoradiograms obtained in rats used in the acute paroxetine experiments: (A-H) control rats and (I-P) rats treated with paroxetine (10 mg/kg/day, s.c.; see experimental procedure in "Materials and methods"). Autoradiograms obtained at 1 hr (A–D and I–L) and at 2.5 hr (E–H and M–P) after injection of 30 μCi of α-[^{14}C]MTrp. The brain slices were 30 μm thick, and the x-ray films were in contact with them for 3 weeks. No special attempt was made to get slices from the same levels in the rats killed at different times. The numerical values for the 5-HT synthesis in different brain structures are given in Table 2. See Table 2 for structure abbreviations. DHi = dorsal hippocampus.

the tracer in the entire brain of the rat. The autoradiograms in Fig. 1 show the distribution of the tracer in controls and paroxetine-treated rats from the acute experiment. Even with mere visual examination, it was easy to identify the structures known to contain a high concentration of 5-HT-producing cells (e.g. pineal body, dorsal and median raphe). The autoradiograms shown in Fig. 2 were obtained from rats in the 14-day paroxetine treatment group. It can be seen that there was a greater tracer concentration on the images prepared from rats killed 2.5 hr after injection, indicating a greater tracer VD*, whose rate of change is related to the 5-HT synthesis rate.

In rats treated acutely, the rate of 5-HT synthesis was decreased significantly in most of the cerebral structures when compared with the controls (Table 2). There was a significant global effect of treatment (mean ratio = 0.72 $\pm$ 0.14; t = 10.4; $P < 0.001$; N = 26). Since there was no significant change ($P > 0.05$) in the plasma free Trp concentration between the control and the treated groups, one can state that there was a decrease in the K^T in the acutely

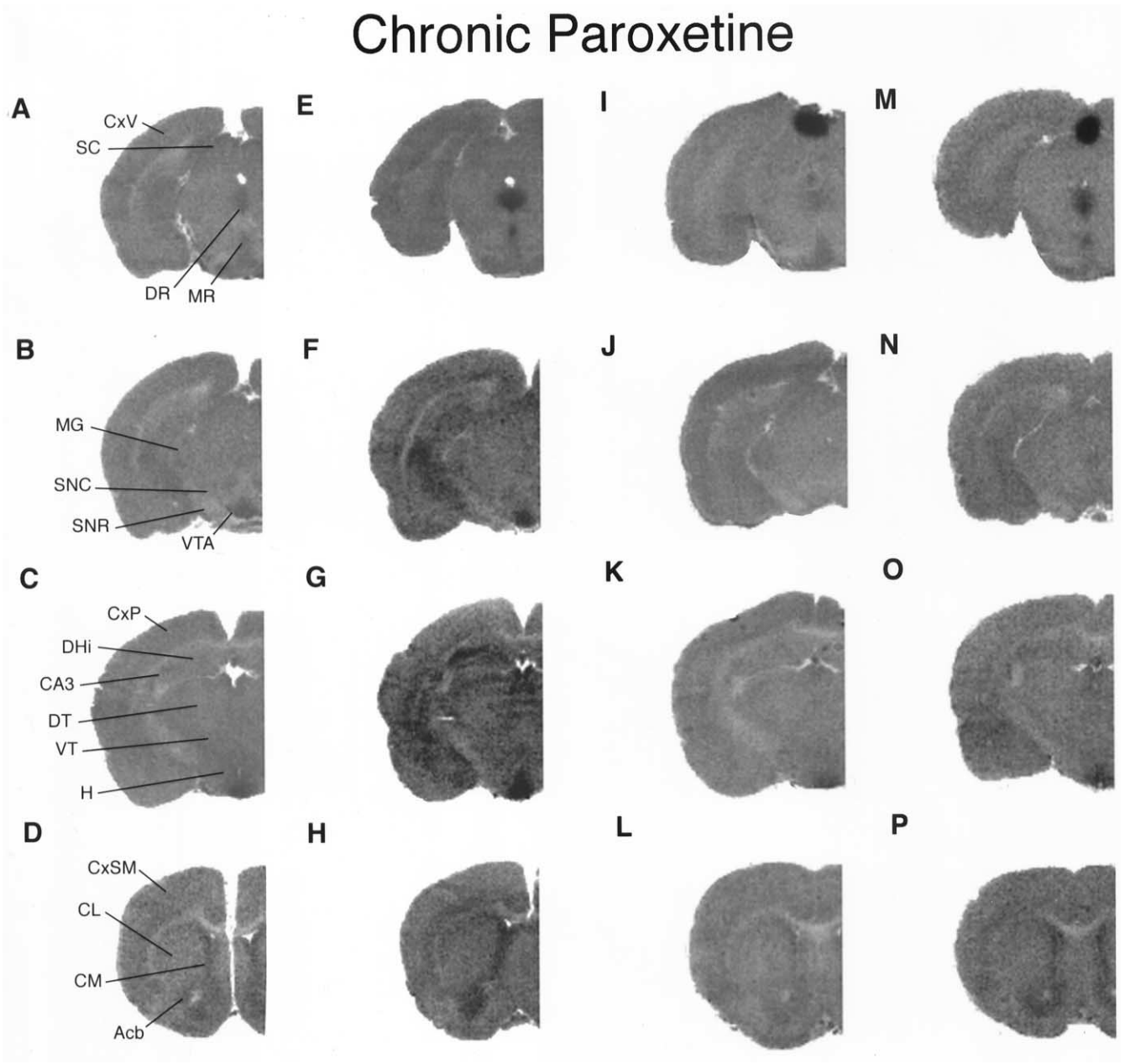

Fig. 2. Representative autoradiograms obtained in rats used in the 14-day treatment protocol: (A–H) control rats and (I–P) paroxetine-treated rats. Rats were injected with tracer like those shown in Fig. 1 and killed 1 hr (A–D and I–L) and 2.5 hr (E–H and M–P) later. The experimental procedure is described in "Material and methods." The brain cross-sections were done at levels similar to, but not the same as, those in Fig. 1. The numerical values for the 5-HT synthesis rates are given in Table 3. See Table 3 for the structure abbreviations. DHi = dorsal hippocampus.

treated rats in comparison to the respective control group. To aid in the interpretation of the structures identified to be different between the two groups, by the univariate F values of ANOVA, the pooled within-group correlations among different brain structures (dependent variables) were examined. Highly significant ($P < 0.00001$) correlations were found between dependent variables. The most pronounced decrease was observed in the inferior colliculus (64%) and the substantia nigra pars reticulata (53%). Although somewhat smaller, a significant decrease was also observed in serotonergic cell bodies giving rise to ascending projections (dorsal and median raphe), descending axons (pallidum and magnus raphe), as well as the hippocampus CA3 region, substantia nigra, hypothalamus, and medial caudate nucleus. These changes were between 18 and 50%. There were no significant changes anywhere in the cerebral cortex.

In the chronic paroxetine treatment group (Table 3), there was a significant global effect of treatment on the 5-HT synthesis rates (mean ratio = 0.52 ± 0.07; t = 36.6; $P < 0.001$; N = 26). Since there was a significant ($P < 0.05$) decrease in the plasma free Trp in the chronic paroxetine treatment group (25%), and a greater reduction in the

Table 2
Rates of serotonin synthesis in rat brains after an acute treatment with paroxetine (10 mg/kg, i.p., 60 min before tracer)

Structure[a]	Rate[b] (pmol/g/min)	
	Controls (N = 12)	Treated (N = 14)
Raphe nucleus obscurus	45 ± 9	34 ± 10[c]
Magnus raphe nucleus	36 ± 10	28 ± 10
Median raphe nucleus (MR)	86 ± 13	68 ± 18[c]
Dorsal raphe nucleus (DR)	107 ± 15	88 ± 18[c]
Superior olive	35 ± 10	24 ± 11[c]
Locus coerulus	32 ± 13	23 ± 12
Inferior colliculus	25 ± 14	9 ± 6[c]
Superior colliculus (SC)	34 ± 13	17 ± 13[c]
Substantia nigra reticulata (SNR)	34 ± 11	16 ± 9[c]
Substantia nigra compacta (SNC)	41 ± 12	22 ± 11[c]
Medial geniculate body (MG)	42 ± 13	23 ± 12[c]
Lateral geniculate body	43 ± 13	32 ± 11[c]
Hypothalamus (H)	44 ± 12	31 ± 10[c]
Thalamus ventral (VT)	37 ± 12	27 ± 10[c]
Thalamus dorsal (DT)	36 ± 11	29 ± 10
CA3	57 ± 15	43 ± 12[c]
Caudate lateral (CL)	46 ± 12	31 ± 10[c]
Caudate medial (CM)	54 ± 13	38 ± 12[c]
Accumbent nucleus (Acb)	62 ± 12	46 ± 12[c]
Visual cortex (CxV)	34 ± 13	30 ± 12
Auditory cortex	35 ± 12	29 ± 13
Parietal cortex (CxP)	31 ± 11	27 ± 11
Sensory-motor cortex (CxSM)	35 ± 10	28 ± 12
Frontal cortex	37 ± 10	33 ± 13
Median forebrain bundle	40 ± 12	29 ± 10[c]
Ventral tegmental area (VTA)	47 ± 13	23 ± 12[c]

Rates were calculated on the assumption that regional trapping of α-[^{14}C]MTrp relates directly to 5-HT synthesis. Control rats were treated with a single i.p. injection of distilled water, the solvent for paroxetine.

[a] Abbreviations are given for some structures; the same abbreviations are used in Fig. 2 for easier cross-reference with these data.

[b] Rates are expressed as mean ±SD. The number of rats tested is given in parentheses.

[c] After the global effect was found to be significant (see details in the text), the synthesis in the individual structures was compared by one-way ANOVA. These values are significant at the $P < 0.05$ level using univariate F-statistics. Since in these experiments it is not possible to use a simple multiple comparisons correction (see "Materials and methods"), these results of the univariate tests should be interpreted together with the pooled within-groups correlations [24] between individual brain structures, which were highly significant.

Table 3
Effects of long-term paroxetine treatment (10 mg/kg/day, s.c., × 14 days; delivered by osmotic pump) on the 5-HT synthesis rate, calculated on the basis of the assumptions that regional trapping of α-[^{14}C]MTrp relates to 5-HT synthesis, as described in the "Materials and methods"

Structure[a]	Rate[b] (pmol/g/min)	
	Controls (N = 17)	Treated[c] (N = 19)
Raphe nucleus obscurus	88 ± 8	33 ± 7
Magnus raphe nucleus	67 ± 10	34 ± 8
Median raphe nucleus (MR)	132 ± 15	64 ± 9
Dorsal raphe nucleus (DR)	175 ± 12	89 ± 9
Superior olive	43 ± 11	23 ± 7
Locus coerulus	41 ± 10	21 ± 6
Inferior colliculus	30 ± 6	9 ± 7
Superior colliculus (SC)	41 ± 6	21 ± 6
Substantia nigra reticulata (SNR)	42 ± 5	16 ± 5
Substantia nigra compacta (SNC)	50 ± 7	23 ± 6
Medial geniculate body (MG)	51 ± 6	23 ± 6
Lateral geniculate body	49 ± 6	25 ± 7
Hypothalamus (H)	48 ± 7	26 ± 8
Thalamus ventral (VT)	44 ± 7	27 ± 9
Thalamus dorsal (DT)	42 ± 7	20 ± 7
CA3	76 ± 7	41 ± 7
Caudate lateral (CL)	54 ± 8	29 ± 8
Caudate medial (CM)	65 ± 9	37 ± 8
Accumbent nucleus (Acb)	77 ± 9	43 ± 8
Visual cortex (CxV)	50 ± 6	24 ± 8
Auditory cortex	52 ± 6	24 ± 7
Parietal cortex (CxP)	42 ± 6	20 ± 7
Sensory-motor cortex (CxSM)	45 ± 8	20 ± 9
Frontal cortex	46 ± 11	21 ± 8
Median forebrain bundle	42 ± 6	21 ± 7
Ventral tegmental area (VTA)	63 ± 6	28 ± 6

Controls were injected with the solvent used for paroxetine, which contained 50% ethanol.

[a] Some structures are also identified by an abbreviation that is also used in Fig. 2 for an easier cross-reference with these data.

[b] Rates are expressed as mean ± SD; the N stands for the number of rats.

[c] The global effect was highly significant for this treatment (see details in the text). After finding the global effect highly significant the syntheses in the individual structures of the control and the treated groups were compared by one-way ANOVA. There was a significant difference in all brain structures ($P < 0.001$). There were also highly significant pooled within-group correlations between different structures.

synthesis in many structures, it can be concluded that, in addition to the decrease in the plasma free Trp concentration, there was also a decrease in the K^T of the rats in the treatment group when compared with the respective control group. Significance in the 5-HT synthesis between different groups should be interpreted with the aid of the pooled within-group correlations as described in "Materials and methods." The rates of 5-HT synthesis were decreased significantly in every structure examined, including all subdivisions of the cerebral cortex, in contrast to the lack of change in the acute experiments in that brain region. The greatest decreases were observed in the inferior colliculus (70%), substantia nigra pars reticulata (62%), raphe obscurus (63%), dorsal thalamus (52%), medial geniculate bodies (55%), median raphe (52%), and sensory-motor cortex (56%).

4. Discussion

The α-MTrp method used in this work permits an estimation of the *in vivo* 5-HT synthesis rates in a large number of brain structures with good anatomical resolution (approximately 0.1 mm), without the use of any other pharmacological agent. Previous studies of the effects of the SSRIs citalopram [22,23] and zimelidine [24] on 5-HT synthesis rates done with an aromatic amino acid decarboxylase in-

hibitor have indicated a reduction in 5-HT synthesis after acute administration, whereas chronic treatment with citalopram [22,23] increased, and with zimelidine decreased, 5-HT synthesis. It is important to emphasize here that these measurements were done using a methodology that by itself affects the rate of 5-HT synthesis [4].

Shoaf *et al.* [25,26] and Gharib *et al.* [3] have criticized our [1,2,9,12,16] approach on the grounds that they did not find a significant correlation between 5-HT synthesis, calculated from the trapping of α-[^{11}C]MTrp in anesthetized monkeys, and 5-hydroxyindolacetic acid measured in the CSF taken at different times. In addition, it was deemed problematic that there was no substantial conversion of labeled α-MTrp to its metabolite. These issues have been addressed before [27–30], and on the basis of the experimental evidence summarized here and that presented in the referenced publications, the notion that the trapping of labeled α-MTrp relates to brain 5-HT synthesis remains tenable. In particular, Chugani and Muzik [7] also concluded that such trapping represents an index of 5-HT synthesis. The issues related to the above-mentioned points have been discussed in our recent publications [27,28,30].

A significantly smaller weight gain (Table 1) in rats treated for 14 days with paroxetine (compared with controls) is in general agreement with data generated with another SSRI, fluoxetine [8]. The reduction in plasma free Trp has not been reported in the experiments with other SSRIs, but since paroxetine was dissolved in a solvent containing 50% ethanol, a confounding effect of a pharmacological interaction between paroxetine and ethanol might be present. However, as of now, there is no information that would support this possibility. It is unlikely that a slight increase in the plasma ratio between tracer and Trp, resulting from the plasma reduction in Trp concentration, would have a substantial effect on the trapping of α-MTrp. Because of the fact that the plasma input function has a maximum of about 600 nCi/mL, and assuming that approximately the same amounts of the tracer were injected (30 μCi over 2 min with the specific activity of the tracer being 55 mCi/mmol), the tracer would represent an additional 13.7% of Trp concentration in the control group and 14% of Trp concentration in the treated group. However, this increase lasts for only a short period of time (30–40 sec). The tracer concentration falls to about one-half of that concentration 5 min after injection and then to approximately one-quarter of that value 60 min after injection. It should be noted that Trp shares the transport system into the brain with that of other large neutral amino acids, and it also has a substantial diffusion constant. Therefore, the addition of this tracer is expected to have a negligible influence on the estimation of 5-HT synthesis. Actually, in the chronic treatment experiments the ratio between tracer (α-MTrp) and tracee (Trp) is greater in the treated group (30/9.4 = 3.19) than in the control group (30/12 = 2.5). Because of all these considerations, it is suggested that a reduction in the plasma Trp concentration, as such, should not influence trapping of this tracer. Indeed, if there were any influence, then one would expect that the reduction in synthesis after chronic paroxetine treatment (Table 3) would actually be underestimated.

After acute administration of an SSRI, the firing of the raphe neurons is decreased through the action of enhanced synaptic availability of 5-HT in the vicinity of 5-HT$_{1A}$ autoreceptors [31,32]; thus, the SSRI acts as an indirect agonist. In agreement with the present study, a reduction in 5-HT synthesis in these cell bodies has been reported with the use of a 5-HT$_{1A}$ agonist [11]. A similar observation has been reported in fluoxetine-treated rats [8]. The present data indicate that the SSRI paroxetine, using a single dose of 10 mg/kg, also induced a decrease in 5-HT synthesis in axon terminal areas like the ventral tegmental area, hippocampus CA3 region, substantia nigra, hypothalamus, and medial caudate nucleus (Table 2). The nigrostriatal structures are indeed known to receive relatively dense 5-HT projections [33]. In addition, there are especially important projections from the dorsal raphe to the extrapyramidal and limbic basal ganglia.

In the present investigation, there was no change in 5-HT synthesis in the cerebral cortex after acute paroxetine administration. This finding is not consistent with that of a previous report in which a decrease in 5-HT synthesis in the cortex was found after citalopram treatment [23]. It has been reported that following acute systemic SSRI treatment, extracellular 5-HT levels in the raphe region are increased markedly, but cortical 5-HT levels are often either unaffected or increased only marginally [34,35]. The former change is probably due to an increased activation of the cell body 5-HT$_{1A}$ autoreceptor because the extracellular concentration of 5-HT is much greater in the raphe than in the cortex. In contrast, the lack of change in the cortex could be attributable to a near perfect balance between an expected decrement, resulting from a suppression of the impulse flow in 5-HT axons, and a compensation being exerted by the 5-HT transporter and terminal 5-HT autoreceptor in the cortex. Indeed, the acute effects of the 5-HT$_{1A}$ agonist buspirone on 5-HT synthesis [11] are in general agreement with the results obtained in the acute paroxetine experiments. There was an overall reduction in brain 5-HT synthesis after acute buspirone and paroxetine administration with the exception of the cerebral cortex. Considering all of these results together, one may conclude that terminal 5-HT autoreceptors in the cerebral cortex exert a greater influence to dampen elevations of synaptic 5-HT levels in the cortex than in other projection fields, but that it is lost upon long-term treatment. The exact relationship between synthesis modulation and sustained terminal 5-HT autoreceptor activation will have to come, however, from studies examining the effects of direct agonists on this parameter.

Two recent investigations of the effects of acute fluoxetine administration on 5-HT synthesis using the same autoradiographic method indicated that a single injection of fluoxetine actually increased the 5-HT synthesis in nerve

terminal areas [8,36]. It is important to note that fluoxetine differs in many respects from other SSRIs. For instance, it has an optical isomer that is active on another 5-HT neuronal element: it is a 5-HT_2 receptor antagonist [37]. In addition, the desmethylated metabolite of fluoxetine, norfluoxetine, can also release 5-HT from its neuronal storage [38]. Furthermore, it has been reported that fluoxetine inhibits MAO-B, albeit at high concentrations [39]. All of these factors probably contribute to the capacity of fluoxetine to alter 5-HT synthesis differently than other SSRIs do, given that they do not have these additional characteristics.

The lack of normalization of 5-HT synthesis after a chronic treatment of paroxetine stands in contrast with the normalization observed with the 5-HT_{1A} agonist buspirone after chronic treatment of the same duration. These divergent results might appear quite surprising as both types of agents, SSRI and 5-HT_{1A} agonists, induce a desensitization of 5-HT_{1A} autoreceptor after long-term administration [32]. However, it should be noted that SSRIs also desensitize in the raphe area a 5-HT_{1D} autoreceptor that is involved in controlling impulse flow-independent release of 5-HT from the cell body of 5-HT neurons [40]. In addition, SSRI, but not 5-$HT_{1A,}$ agonists desensitize the terminal 5-HT_{1B} autoreceptor. Such differential adaptative mechanisms could account, at least in part, for the divergent effects of the two classes of agents. It should be noted that the observed reduction in 5-HT synthesis was consistently greater in almost every structure examined after long-term paroxetine treatment than after acute treatment.

Another possible reason for the decrease in 5-HT synthesis following chronic treatment with paroxetine is a reduction in the plasma free Trp concentration (Table 1). However, the 50% decrease in 5-HT synthesis was substantially more than that expected from the decrease in the free Trp concentration (25%). As mentioned above, this reduction in the plasma Trp level would actually increase the ratio between the tracer and Trp, possibly resulting in a somewhat greater (not smaller) uptake of the tracer. These observations, as stated in Results, also suggest that K^T was decreased in the paroxetine treatment group in comparison with the respective controls.

In conclusion, the present data clearly showed a decrease in 5-HT synthesis following long-term treatment with paroxetine. It seems likely that short- and long-term treatments with paroxetine decrease 5-HT biosynthesis in different ways among the various brain structures. Still, there are major issues that remain unsolved, such as the precise contribution of 5-HT_{1B} autoreceptors on 5-HT synthesis alone and during 5-HT reuptake inhibition. These questions are presently under investigation in our laboratories.

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