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The effects of the perinatal treatment with 5-hydroxytryptophan or tranylcypromine on the peripheral and central serotonin homeostasis in adult rats

Dubravka Hranilovic [a,*], Sofia Blazevic [a], Nedjeljka Ivica [a], Lipa Cicin-Sain [b], Darko Oreskovic [b]

[a] Department of Animal Physiology, Division of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia
[b] Laboratory of Neurochemistry and Molecular Neurobiology, Department of Molecular Biology, Rudjer Boskovic Institute, Bijenicka 54, 10000 Zagreb, Croatia

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ABSTRACT

Serotonin (5HT) is a biologically active amine present in mammals in the brain and the peripheral tissues. Autism is a neurodevelopmental disorder in which 5HT homeostasis is disturbed both centrally and peripherally, but the relationship between the 5HT disturbances in the two compartments is not understood. In an attempt to explore the relationship between the disturbed peripheral 5HT homeostasis and central 5HT functioning, we exposed the developing rat brain to increased 5HT concentrations, by treatment of rats with subcutaneous injections of the immediate 5HT precursor 5-hydroxy-L-tryptophan (5HTP, 25 mg/kg), or the non-selective MAO inhibitor tranylcypromine (TCP, 2 mg/kg), during the period of the most intensive development of 5HT neurons – from gestational day 13 to post-natal day 21. The effects of the mentioned treatments on peripheral and central 5HT levels were then studied in adult rats. Platelet and plasma 5HT concentrations (measured by ELISA), as well as cortical and midbrain 5HT, tryptophan and 5-hydroxyindoleacetic acid levels (measured by HPLC) were determined in twelve 5HTP treated and eight TCP treated rats, and compared with the values measured in 10 control, saline treated rats. Treatment with 5HTP significantly raised peripheral but not central 5HT concentrations. At adult age, peripheral 5HT homeostasis was re-established, while modest decrease in 5HT concentration was observed in frontal cortex, presumably due to hyperserotonemia-induced loss of 5HT terminals during brain development. Treatment with TCP induced significant 5HT elevations in both compartments. At adult age, permanent changes in 5HT homeostasis were observed, both peripherally (as hyperserotonemia) and centrally (as altered 5HT metabolism with decreased 5HT concentrations). Further studies are planned in order to explore the nature of the different disturbances of 5HT homeostasis induced by the two compounds, and their results are expected to shed some light on the role of hyperserotonemia in autism.

1. Introduction

Serotonin (5-hydroxytryptamine, 5HT) is a biologically active amine present in mammals both, in the brain and the peripheral tissues.

In the periphery, 5HT mediates cardiovascular and gastrointestinal functions and platelet activation (Berger et al., 2009). Peripheral 5HT is synthesized from the amino acid tryptophan (Trp) mostly in enterochromaffin cells of the intestinal mucosa, by the action of tryptophan hydroxylase (Tph) and aromatic L-amino acid decarboxylase (Racke et al., 1995). 5HT is released into portal circulation in a receptor-mediated manner and actively accumulated into platelets via the serotonin transporter. The amine is catabolized primarily in lungs and liver by the action of the mitochondrial enzyme monoamine oxidase (MAO) into 5-hydroxyindoleacetic acid (5HIAA) and eliminated through kidneys. More than 99% of whole blood serotonin is contained in platelets in which its concentration is regulated either by the rate of peripheral 5HT synthesis and metabolism (i.e. its concentration in blood plasma), or the rate of its accumulation into- and release from the platelets (Cook and Leventhal, 1996).

In the brain, 5HT synthesizing neurons are located in discrete regions of the brain stem – *raphe nuclei*, but project their axons into various cortical and subcortical regions of the brain (Benes et al.,

Abbreviations: 5HT, 5-hydroxytryptamine; Trp, tryptophan; Tph, tryptophan hydroxylase; MAO, monoamine oxidase; 5HIAA, 5-hydroxyindoleacetic acid; 5HTP, 5-hydroxytryptophan; TCP, tranylcypromine; GD, gestational day; PND, post-natal day; WB, whole blood; PRP, platelet-rich plasma; PFP, platelet-free plasma; RN, raphe nuclei; FC, frontal cortex.

* Corresponding author. Tel.: +385 1 487 77 00; fax: +385 482 6260.
E-mail address: dubravka@biol.pmf.hr (D. Hranilovic).

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2000). In the developing brain, 5HT regulates serotonergic outgrowth and maturation of target regions (Whitaker-Azmitia, 2001), while later it assumes the role of a neurotransmitter modulating function and plasticity of the adult brain (Catalano, 2001; Lesch, 2001).

Central and peripheral 5HT compartments are two separated, yet closely related entities. On the one hand, enterochromaffin and neural cells use different Tph enzymes (Tph1 and Tph2, respectively) to synthesize 5HT (Walther et al., 2003), and the blood-brain barrier prevents serotonin entering from one compartment into the other. On the other hand, proteins that control 5HT function in both compartments are encoded by the same genes, have identical primary structures and follow the same kinetics (Chen et al., 1993; Lesch et al., 1993; Cook et al., 1994). Moreover, during the fetal and early postnatal development of the brain, the blood-brain barrier is not formed and the two compartments can freely communicate (Davies et al., 1996).

A disorder in which 5HT homeostasis seems to be disturbed both centrally and peripherally is autism, a neurodevelopmental syndrome with onset in early childhood, characterized by impairment in social interaction and communication, and by the presence of restricted and repetitive behaviors and interests (Owley et al., 2003). Elevated blood 5HT levels (hyperserotonemia) have been found in about one third of autistic patients of different ethnic and age groups (Cook and Leventhal, 1996; Owley et al., 2003; Mulder et al., 2004; Hranilovic et al., 2007). On the other hand, brain serotonin synthesis was found to be decreased in the cortex and thalamus of autistic children (Chugani et al., 1997). Despite decades of research, the mechanism of hyperserotonemia and its relation to central 5HT dysfunction are not fully understood. One explanation lies in possible alterations in the expression of one or more of the 5HT elements that could lead to the dysregulation of 5HT transmission in the brain (affecting so its early development and resulting in autistic behavioral symptoms), while it is at the same time reflected in the periphery as hyperserotonemia (Janusonis, 2005). Alternatively, dysregulation of the peripheral 5HT-homeostasis could happen first, leading to high concentrations of serotonin in blood. During fetal and early post-natal development, before the formation of the blood-brain barrier, these high 5HT levels could inhibit the development of 5HT neurons and lead to the anatomic and functional alterations of the brain, characteristic for autism (Whitaker-Azmitia, 2005).

We have recently started studies on the relationship between the disturbed peripheral 5HT homeostasis and central 5HT functioning on an animal model by pharmacologically inducing hyperserotonemia during the period of most intensive development of the 5HT neurons. For that purpose Wistar rats were treated subcutaneously with either the immediate 5HT precursor 5-hydroxytryptophan (5HTP, 25 mg/kg), or the non-selective MAO inhibitor tranylcypromine (TCP, 2 mg/kg), from gestational day (GD) 13 to post-natal day (PND) 21. In this study we report the effects of the mentioned perinatal treatments on peripheral and central 5HT levels in adult rats. Platelet and plasma 5HT concentrations, as well as cortical and midbrain 5HT, Trp and 5HIAA levels were measured in twelve 5HTP treated and eight TCP treated rats, and compared with the values measured in 10 control rats perinatally treated with saline.

2. Materials and methods

2.1. Breeding and housing of animals

Breeding procedure has been described in detail elsewhere (Blazevic et al., 2010). In short, eight nulliparous Wistar females from the animal facility of the Croatian Institute for Brain Research (University of Zagreb, Zagreb, Croatia), weighing 260–290 g, were randomly assigned to a "saline" (two), "5HTP" (three), or "TCP" (three) group, and mated with males of the same strain and age in 2:1 or 3:1 ratio, respectively. After gravidity was confirmed in all females, the male was removed from the cage. Females remained together until 2 days before parturition when they were separated and remained singly housed until weaning of the pups (at PND 21). After weaning, animals were kept 3–4 per cage. Animals were housed in polycarbonate cages under 12 h light:12 h dark conditions at a temperature of 22 ± 2 °C, with free access to rat chow and tap water.

All efforts were made to reduce the number of animals used and to minimize animal suffering. The study was approved by the Ethics committee of the University of Zagreb, and was conducted in accordance with the European Communities Council Directive (86/609/EEC) and the Croatian Animal Protection Law ("Narodne novine", 135/2006).

2.2. Pharmacological treatments

The experimental groups of pups were treated either with 2 mg/kg tranylcypromine (Sigma–Aldrich), or with 25 mg/kg of 5-hydroxy-L-tryptophan (Sigma–Aldrich), from GD 13 until birth by subcutaneous injections to pregnant females, and from PND1 until PND 21 by receiving subcutaneous injections of the same doses. 5HTP was dissolved in acidified saline. Before treatment, the solution was neutralized with NaOH and warmed to body temperature. TCP was dissolved in ethanol and saline. Before treatment, the solution was neutralized with HCl and warmed to body temperature. Solutions were delivered in volumes of 1.51 mL per kg of body mass to dams, in volumes of 3.3 mL per kg of body mass to pups until they reached 15 g, and in volumes of 5 mL per kg of body mass until the end of treatment. The control group was treated with saline in the same manner. All injections were performed between 2 and 3 pm. A 50 μL glass syringe (Hamilton) with disposable 30G needles (BD, Drogheda, Ireland) were used to treat the pups until they reached a body mass of 15 g, while disposable 0.5 mL plastic syringes with 30G needles (BD Micro-Fine Plus) were used to treat pregnant females and older pups.

2.3. Collection of tissue samples

Blood and brain samples were collected from 5 saline, five 5HTP and 3 TCP treated pups at the end of treatment (on PND 22), and from 10 saline (5 males, 5 females), twelve 5HTP (6 males, 6 females) and 8 TCP (4 males, 4 females) treated adult rats (on PND 70).

Under light ether narcosis, 800 μL of pup blood or 1.5 mL of adult blood was withdrawn from the jugular vein into syringes preloaded with 200 or 500 μL of 3.13% trisodium citrate anticoagulant, respectively. Animals were then decapitated and brains were removed from the sculls and briefly frozen at −20 °C in a freezer. A midbrain region containing serotonergic cell bodies of the dorsal and median raphe nuclei was obtained by a 3 mm thick coronal brain slice (plates 43 and 55 in the rat brain atlas, Paxinos and Watson, 2007), followed by a 3 mm diameter punch into the midbrain area. A 4 mm coronal cut was then made at the frontal lobes (plate 11) and cortex (all cortical areas anterior to bregma + 1.7 mm) was peeled off. Samples were weighted and frozen at −80 °C for later analysis.

2.4. Measurement of the peripheral 5HT concentrations

After thorough mixing, samples were transferred from syringes into microtubes. 5HT concentrations in whole blood (WB) of pups or in platelet-rich plasma (PRP) and platelet-free plasma (PFP) of

adult rats were determined using a commercial enzyme immunoassay kit (Serotonin ELISA kit, DRG Instruments GmbH, Germany), according to the kit instructions. A calibration curve was drawn based on the absorbances measured at 450 nm on the microplate reader (P-Lab IASON, Austria) and known concentrations of the standard solutions. Concentration values of samples were obtained by interpolating them to the calibration curve, using the 4-parameters non-linear regression curve fitting. Results were expressed in ng 5HT per mL of WB, PRP or PFP.

2.5. Measurement of brain Trp, 5HT and 5HIAA concentrations

The frozen samples were thawed and homogenized in 5 vol (w/v) of a solution of 0.1 M perchloric acid containing 0.2 mM EDTA and 0.4 mM $Na_2S_2O_5$. Tissue homogenates were then centrifuged at 14000*g* for 15 min at 0–4 °C, and aliquots of the clear supernatant were used for the high performance liquid chromatography analysis with electrochemical detection (HPLC-ED).

The HPLC system consisted of a delivery pump (Agilent 1100 Series, Agilent Technologies, U.S.A.), a sample injector (Rheodyne 7125, U.S.A.), a C18 reverse phase column (Agilent Technologies Zorbax SB-C8, 75 × 4.6 mm, 5 μm particle size), and a Guard column (4 × 4 mm, Agilent Technologies, U.S.A.). An electrochemical detector (HP-ED 1094A, Hewlett–Packard, U.S.A.) with a glassy carbon electrode was used at −0.55 V versus the reference electrode. All chromatograms were recorded and analyzed using the HPCore ChemStation Software.

Concentrations were determined from peak areas against external standards. The mobile phase contained 0.1 M Na_2HPO_4, 0.05 M citric acid, 5% methanol (v/v), 0.1 mM EDTA, and 1 mM KCl at pH 4.5. The flow rate was maintained at 0.8 mL per min at a pressure of 120 bars. Tissue concentrations of 5HT and 5HIAA were expressed as ng, and that of Trp as μg of substance per g of wet tissue.

2.6. Statistical analyses

Data were processed by the use of GraphPad InStat 3.01 software. Normality of distributions of the measured parameters was tested by Kolmogorov/Smirnov method. Mean values of normally distributed parameters were compared using unpaired *t*-test or one-way analysis of variance (ANOVA) with Tukey's post-test. Mean values of parameters that were not normally distributed were compared using Mann–Whitney test or using non-parametric Kruskal–Wallis method with Dunn's post-test. The level of significance was set to 0.05. The values were expressed as means (M) ± standard error of means (SEM).

3. Results

Direct effects of chronic treatment with 25 mg/kg 5HTP or with 2 mg/kg TCP on blood and brain 5HT levels were measured in pups at the end of treatment (Fig. 1). Both substances efficiently caused hyperserotonemia (KW = 8.65, $p = 0.003$), with 5HT concentrations being 874 ± 168 ng/mL in 5HTP treated and 669 ± 34.5 ng/mL in TCP treated pups, compared to 491 ± 59.5 ng/mL in saline treated pups. The effect of treatment on 5HT levels in frontal cortex was also significant (KW = 6.96, $p = 0.017$), although only TCP (917 ± 32.2 ng/g), and not 5HTP (393 ± 34.2 ng/g) caused considerable raise in 5HT concentrations compared to saline (346 ± 32.4 ng/g).

In order to check whether the perinatal treatment with 5HTP or TCP has permanently affected the peripheral 5HT homeostasis, 5HT concentrations in both PFP and PRP were measured in adult rats. One sample from the TCP treated group and one sample from the saline treated group were lost during processing. 5HT concentrations in PFP did not differ between male and female rats ($t = 0.011$, $p = 0.991$, 26 d.f.), and those in PRP were only indicatively higher in females (Welch corrected $t = 1.995$, $p = 0.061$, 19 d.f.). This allowed the results from males and females to be analyzed jointly (Fig. 2).

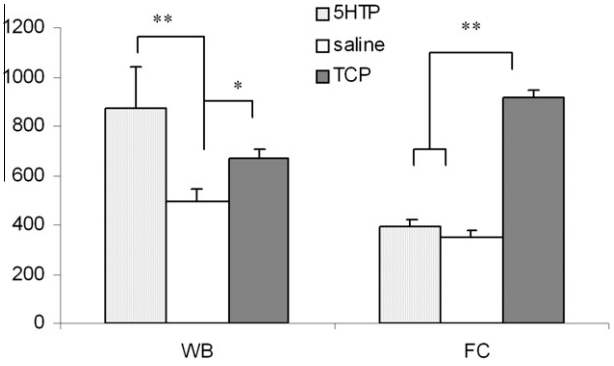

Fig. 1. The direct effects of chronic treatment with 25 mg/kg 5-hydroxytryptophan (5HTP) or 2 mg/kg tranylcypromine (TCP) on 5HT concentrations in whole blood expressed as ng 5HT per mL of blood (WB), and in frontal cortex expressed as ng 5HT per g of wet tissue (FC). $N = 5$ in saline and 5HTP treated, and $N = 3$ in TCP treated group. Values are expressed as M ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$; Dunn's multiple comparison after Kruskal–Wallis test.

The effect of treatment on 5HT concentrations was significant in both PFP ($F_{(2,27)} = 5.99$, $p = 0.008$) and PRP ($F_{(2,27)} = 6.89$, $p = 0.004$). Post-hoc analysis revealed significantly higher mean 5HT concentration in PRP of TCP treated rats (612 ± 57.8 ng/mL) than in PRP of saline treated (381 ± 24.6 ng/mL) or 5HTP treated (432 ± 42.6) animals, indicating the persistence of hyperserotonemia at adult age (Fig. 2B). On the other hand, mean 5HT concentrations in PFP of animals from 5HTP treated (5.08 ± 0.83 ng/mL) and TCP treated (3.92 ± 0.14 ng/mL) rats did not significantly differ from that of the control animals (4.67 ± 0.24 ng/mL), although they differed from each other (Fig. 2A).

We further searched for possible effects of the perinatal treatment with 5HTP or TCP on 5HT metabolism in the brain of adult rats by measuring 5HT, Trp and 5HIAA concentrations and their ratios in midbrain raphe region and frontal cortex. There were no gender influence on any of the measured parameters in either raphe region ($t = 0.186$, $p = 0.854$ for 5HT, $t = 0.252$, $p = 0.803$ for Trp, and $t = 0.641$, $p = 0.527$ for HIAA) or frontal cortex ($t = 0.260$, $p = 0.797$ for 5HT, $t = 0.554$, $p = 0.584$ for Trp, $t = 0.025$, $p = 0.980$ for 5HIAA) in an integral sample of ten 5HTP treated, 10 saline treated and 8 TCP treated adult rats (26 d.f.). Therefore, results obtained in males and females were pooled together for the analyses (Fig. 3).

There was a strong influence of treatment on 5HT concentration (Fig 3A) in both, the raphe region ($F_{(2,27)} = 73.69$, $p < 0.0001$) and frontal cortex ($F_{(2,27)} = 90.34$, $p < 0.0001$). While the mean 5HT levels of the TCP treated animals were markedly decreased compared to the controls in both regions (74.4 ± 6.11 ng/g vs. 306 ± 17.3 ng/g in RN, and 48.1 ± 1.20 ng/g vs. 185 ± 7.11 in FC), 5HTP treatment seemed to significantly lower only 5HT levels in the frontal cortex (155 ± 9.23 ng/g), but not in the raphe region (309 ± 16.2 ng/g).

The mean concentration of the 5HT precursor tryptophan (Fig 3B) was markedly increased in the raphe region of the TCP treated animals (18.9 ± 2.76 μg/g) in comparison to the saline treated (4.98 ± 0.70 μg/g) and 5HTP treated (4.39 ± 0.69 μg/g) rats (KW = 16.74, $p = 0.0002$). No significant differences among the mean values of Trp concentrations of 5HTP treated (4.29 ± 0.50 μg/g), saline treated (5.32 ± 0.63 μg/g) and TCP treated (6.32 ± 0.60 μg/g) rats were found in frontal cortices (KW = 5.70, $p = 0.058$).

The mean concentrations of the main 5HT metabolite 5HIAA (Fig 3C) were similar in raphe regions (663 ± 45.6 ng/g for 5HTP

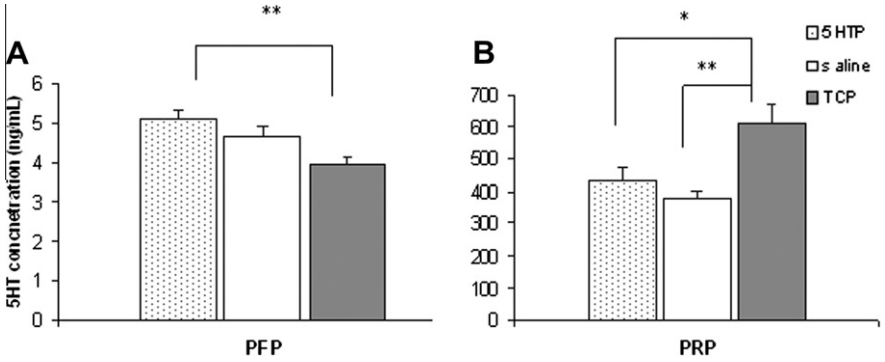

Fig. 2. Permanent effects of perinatal treatment with 5-hydroxytryptophan (5HTP) or tranylcypromine (TCP) on 5HT concentrations in (A) platelet-free plasma (PFP) and (B) platelet-rich plasma (PRP). $N = 12$ in 5HTP treated, $N = 9$ in saline treated, and $N = 7$ in TCP treated group. Values are expressed as M ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$, Tukey–Kramer multiple comparisons after one-way ANOVA.

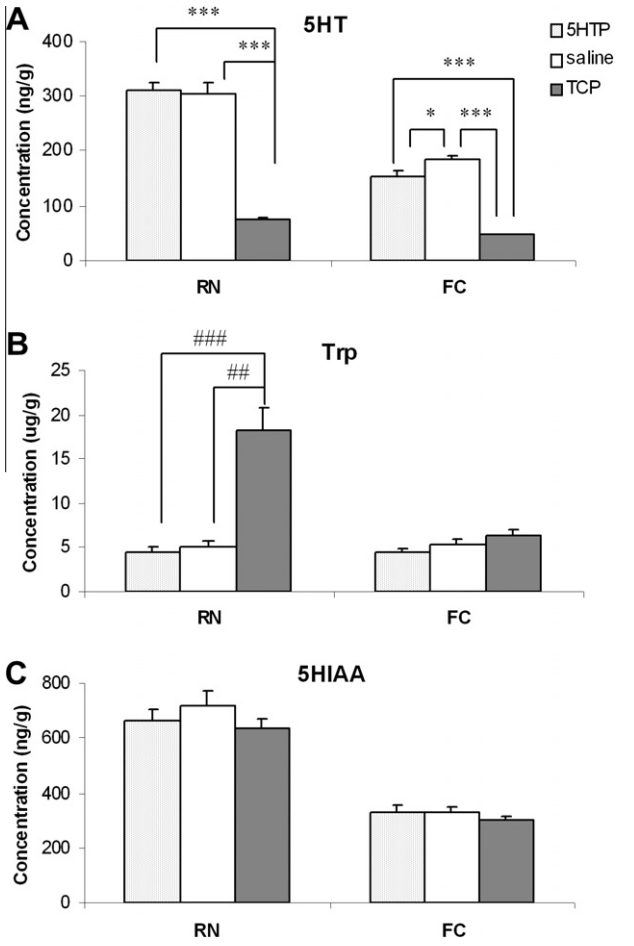

Fig. 3. Concentrations of (A) serotonin (5HT), (B) tryptophan (Trp), and (C) 5-hydroxyindol acetic acid (5HIAA) in midbrain raphe nuclei (RN) and frontal cortices (FC) of 5-hydroxytryptophan (5HTP) treated ($N = 10$), saline treated ($N = 10$) and tranylcypromine (TCP) treated ($N = 8$) rats at PND 70. Values are expressed as M ± SEM. $^{*}p < 0.05$, $^{***}p < 0.001$, Tukey–Kramer multiple comparison after one-way ANOVA. $\#\#p < 0.01$, $\#\#\#p < 0.001$, Dunn's multiple comparison after Kruskal–Wallis test.

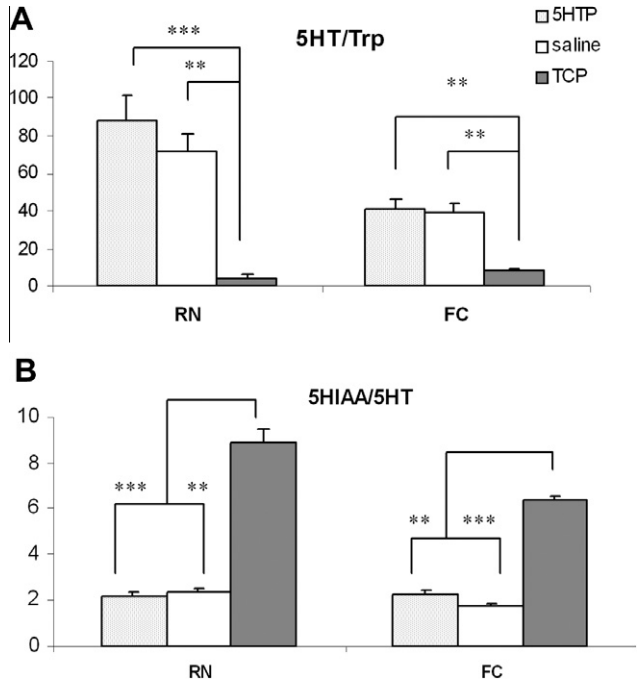

Fig. 4. (A) 5HT/Trp ratio ($\times 10^{-3}$), and (B) 5HIAA/5HT ratio in midbrain raphe nuclei (RN) and frontal cortices (FC) of 5-hydroxytryptophan (5HTP) treated ($N = 10$), saline treated ($N = 10$) and tranylcypromine (TCP) treated ($N = 8$) rats at PND 70. Values are expressed as M ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, Dunn's multiple comparison after Kruskal–Wallis test.

treated, 716 ± 60.3 ng/g for saline treated, and 638 ± 36.0 ng/g for TCP treated animals) as well as in frontal cortices (330 ± 21.8 ng/g for 5HTP treated, 328 ± 18.5 ng/g for saline treated, and 303 ± 11.9 ng/g for TCP treated animals) of the investigated groups ($F_{(2,27)} = 0.625$, $p = 0.543$, and $F_{(2,27)} = 0.602$, $p = 0.556$, respectively).

Finally, we determined the 5HT/Trp ratio, which could indicate the activity of Tph, and the 5HIAA/5HT ratio, which indicates the rate of 5HT degradation, in both brain regions (Fig. 4). 5HT/Trp ratio (Fig 4A) was significantly influenced by the treatment in both the raphe region (KW = 16.7, $p = 0.0002$) and the frontal cortex (KW = 16.6, $p = 0.0003$). Post-hoc analysis revealed no significant differences in the 5HT/Trp ratio between 5HTP treated and saline treated animals in either the raphe region ($87.6 \times 10^{-3} \pm 14.3 \times 10^{-3}$ and $71.9 \times 10^{-3} \pm 9.22 \times 10^{-3}$, respectively), or the frontal cortex ($40.9 \times 10^{-3} \pm 5.53 \times 10^{-3}$ and $39.5 \times 10^{-3} \pm 4.77 \times 10^{-3}$, respectively). On the other hand, the 5HT/Trp ratio of TCP treated animals was markedly reduced in both regions ($4.87 \times 10^{-3} \pm 1.10 \times 10^{-3}$ in RN and $8.09 \times 10^{-3} \pm 1.08 \times 10^{-3}$ in FC). The rate of 5HT degradation (Fig 4B) was also significantly influenced by treatment in both brain regions (KW = 16.98, $p = 0.0002$ for RN and KW = 17.86, $p = 0.0001$ for FC). The observed significance was the result of considerable increase in the 5HIAA/5HT ratio of the TCP treated rats in comparison with 5HTP treated

and saline treated rats in both, the raphe region (8.83 ± 0.64 vs. 2.18 ± 0.16 and 2.35 ± 0.16, respectively) and frontal cortex (6.31 ± 0.21 vs. 2.21 ± 0.20 and 1.78 ± 0.08, respectively).

4. Discussion

The aim of our study was to investigate in which way does the perinatal exposure to elevated 5HT levels influence peripheral and central 5HT homeostasis in adult rats. Two different 5HT enhancers were used for this purpose: the immediate 5HT precursor, which affects only the 5HT system and elevates 5HT levels without acting directly on the key regulators of serotonergic transmission (rate-limiting enzymes, transporters, receptors), and a non-selective MAO inhibitor, which inhibits the main enzyme involved in 5HT degradation and also affects other monoaminergic systems.

5HTP is the intermediate in the synthesis of serotonin from its precursor tryptophan. Unlike Trp which is an essential amino acid with many functions in the body (mainly a precursor in protein synthesis), 5HTP is only found in the serotonin synthesis pathway and is quantitatively converted to 5HT (Udenfriend et al., 1957; Magnussen and Nielsen-Kudsk, 1980; Birdsall, 1998). The advantage of using 5HTP over 5HT itself, is that it readily crosses the placental barrier (Birdsall, 1998), which is crucial for the prenatal part of the treatment. Administration of 5HTP allowed us to elude the rate-limiting step in the synthesis of serotonin and to mimic the effect of increased serotonin synthesis through a chosen 5HTP dose. According to our experience with adult rats, we have chosen a dose of 25 mg/kg 5HTP which was quite effective in raising blood 5HT concentrations, while causing only a slight reduction in body weight and no signs of brain toxicity, during the two weeks of treatment (Jernej and Cicin-Sain, 1986).

TCP is an irreversible MAO A and MAO B inhibitor which inhibits oxidative deamination – an essential step in the catabolism of exogenous amines and monoamine neurotransmitters, including serotonin (Billett, 2004; Frieling and Bleich, 2006). Although the two isoenzymes have different substrate affinities under normal physiologic conditions (MAO A preferentially oxidizes serotonin and norepinephrine, MAO B preferentially oxidizes phenylethylamine, while dopamine and tyramine represent substrates for both isoenzymes), both can catabolize the same compounds and are able to take over when the function of the other is compromised through pharmacological inhibition. Accordingly, more pronounced effects on rat 5HT metabolism were observed after the inhibition of both isoforms than after the sole inhibition of MAO A (Johnston, 1968; Green and Youdim, 1975; Sleight et al., 1988; Celada and Artigas, 1993). Significant effects of TCP were measured in the brain and the periphery of adult rats, after acute or chronic administration at doses of 0.5–15 mg/kg, as a reduction in MAO A activity (Celada and Artigas, 1993), an increase in 5HT concentrations (Green and Youdim, 1975; McKim et al., 1983; Malyszko et al., 1993; Ferrer and Artigas, 1994), or a decrease in 5-hydroxyindolacetic acid levels (Celada and Artigas, 1993; Malyszko et al., 1993). We have chosen a dose of 2 mg/kg, which was expected to effectively block most, but not all of the 5HT degradation.

4.1. The effects of 5HTP

Several pups were sacrificed at the end of treatment to check for the direct effects of 5HTP on blood and brain 5HT concentrations. Although transient increases in 5HTP, 5HT and 5HIAA content were reported in rat serotonergic neurons after a single oral or intraperitoneal dose of 5HTP (Sémont et al., 2000; Lynn-Bullock et al., 2004), it seems that the chronic treatment with 5HTP used in our experiment significantly raised 5HT levels only in blood but not in the frontal cortex. It is possible that, at the administered way and dose, 5HTP was more efficiently converted to 5HT and/or stored in the periphery than in the brain, or that the neuronal compensatory mechanisms were much more efficient in compensating for the excess of newly synthesized 5HT than the peripheral ones. In any case, given the results of its direct effects, we could assume that, under our experimental conditions, the long lasting effects of 5HTP in the adult brain would primarily result from hyperserotonemia during the perinatal period, and to a lesser extent from the increased brain 5HT levels. This condition corresponds to the theory that the excessive 5HT, which causes alterations in brain development, originates from blood.

The effect of 5HTP on blood 5HT levels seemed to be only temporary as the peripheral 5HT homeostasis was established at adult age (presumably after a wash-out period).

Perinatal administration of 5HTP did not seem to affect 5HT levels or metabolism in the serotonergic cell bodies of the adult rats. However, in the region of serotonergic terminals there was modest but significant decrease in 5HT concentration without any changes in 5HT synthesis or degradation rate. This indicates intact expression/activity of the 5HT metabolizing enzymes but suggests possible reduction in number of serotonergic terminals in frontal cortex. This would be in line with the reported inhibitory effects of serotonin on 5HT terminal outgrowth in tissue culture (Whitaker-Azmitia and Azmitia, 1986) as well as on animal models using pharmacological treatment with the 5HT receptor agonist 5-methoxytryptamine (Shemer et al., 1991), 5HT precursor tryptophan (Huether et al., 1992), combination of selective MAOA and MAOB inhibitors (Whitaker-Azmitia et al., 1994), and 5HT reuptake inhibitors (Cabrera-vera et al., 1997).

4.2. The effects of TCP

Measurements of blood and cortical 5HT levels at the end of treatment revealed that TCP, besides inducing hyperserotonemia in the periphery, significantly raised 5HT concentration in the brain, due to efficient inhibition of MAO isoenzymes in both 5HT compartments. This is in line with the findings of Ferrer and Artigas (1994) that chronic treatment with low doses of tranylcypromine increases extracellular 5HT concentration in frontal cortex and dorsal raphe nuclei. Therefore, it would be expected that the developing brain of a TCP treated animal would be exposed to the high levels of 5HT not only from blood but from serotonergic neurons as well. This situation complies more with the theory of simultaneous 5HT dysregulation in the brain and the periphery as a neurobiological basis of autism.

TCP treated animals remained hyperserotonemic at adult age. It is important to note that although a mean 5HT concentration in platelets was increased by about 60%, concentration of 5HT in plasma was indicatively lower than that of the control animals. The lack of parallelism between plasma and platelet 5HT changes point to a long-lasting (or permanent) increase in activity/expression of the 5HT transporter on platelet membranes (Anderson et al., 1987) which probably happened during treatment to compensate for the excess of 5HT left in circulation after the inhibition of the degrading enzyme.

The effect of TCP on brain 5HT levels and metabolism in adult animals was impressive. Compared to the control animals, 5HT concentration and 5HT/Trp ratio in both, raphe nuclei and frontal cortex were significantly decreased while 5HIAA/5HT ratio was significantly increased. In addition, in the raphe region there was an almost fourfold increase in Trp concentrations. We suppose that, in analogy to our results obtained with 5HTP and to the earlier mentioned reports, reduction in number of serotonergic terminals also occurred in brains of TCP treated animals, but the additional consequence of TCP treatment appeared to be permanent changes in 5HT metabolism. We could speculate that, while such low levels of released 5HT in the terminal region have in-

duced increased Trp uptake into the nerve cell bodies, this Trp was either not sufficiently converted to 5HT or the synthesized 5HT was not efficiently protected from MAO activity. A reason for that might lie in the long-lasting/permanent downregulation of tryptophan hydroxylase activity and/or 5HT storage, induced by high 5HT levels during the chronic inhibition of 5HT degradation. Another long-lasting consequence of TCP treatment might be the upregulated degradation of 5HT, since 5HIAA levels reach those of the control animals despite considerably lower 5HT concentrations. Of course, possible effects of increased concentrations of other monoamines during brain development (Andersen, 2003), and of consequential compensatory mechanisms, complicate interpretation of the obtained results and should be kept in mind.

5. Conclusion

Through chronic pharmacological treatments during the perinatal period, we have exposed the developing rat brain to increased 5HT concentrations in two different manners. Simple increase in the availability of the immediate 5HT precursor significantly raised peripheral but not central 5HT levels, leading to decreased 5HT concentration in the cortex of adult animals. On the other hand, inhibition of the 5HT-degrading enzyme simultaneously elevated 5HT levels in the brain and the periphery, and caused permanent changes in 5HT homeostasis of adult animals, both peripherally (hyperserotonemia) and centrally (altered 5HT metabolism with decreased 5HT concentrations). Further anatomic and expressional studies are planned to explore the mechanisms of the different disturbances in 5HT homeostasis induced by the two compounds. We expect that the obtained results will bring some answers to the question whether hyperserotonemia is a cause or a marker of the central 5HT-alterations seen in autism.

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