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Effect of repeated treatment with fluoxetine on tryptophan hydroxylase-2 gene expression in the rat brainstem

N.N. Dygalo *, G.T. Shishkina, T.S. Kalinina, A.M. Yudina, E.S. Ovchinnikova

Institute of Cytology and Genetics, Russian Academy of Science, Functional Neurogenomics Laboratory, Novosibirsk 630090, Russia

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Abstract

Selective serotonin (5-HT) reuptake inhibitors such as fluoxetine are widely used in the treatment of depression and anxiety; however, the mechanisms underlying their action and particularly the delay in therapeutic onset remain unclear. It is proposed that 5-HT reuptake inhibitors exert their therapeutic activity by increasing serotonergic neurotransmission; therefore, the aim of the present study was to investigate the effects of repeated treatment with fluoxetine (25 mg/kg/day p.o., 14 days) on expression of genes coding for proteins that involved in the synthesis and reuptake of 5-HT. Exposure of animals to plus-maze conditions on the first day of drug administration produced an increase in baseline anxiety on subsequent trial 2 weeks later. Fluoxetine strengthened the anxiogenic effects of maze experience. Two-week fluoxetine treatment also significantly reduced expression of tryptophan hydroxylase-2 (TPH2) and 5-HT transporter mRNAs as determined by RT-PCR in the brainstem. These changes were consistent with the decreased 5-HT levels and 5-HT turnover in the brain, and might contribute to the anxiogenic effects of the drug. The results also suggest that recently found association between treatment responses to fluoxetine and polymorphic variants of human TPH2 gene [Peters EJ, Slager SL, McGrath PJ, Knowles JA, Hamilton SP. Investigation of serotonin-related genes in antidepressant response. Mol Psychiatry 2004; 9:879–889] may be related to the drug effect on the TPH2 gene expression.

Keywords: Fluoxetine; Tryptophan hydroxylase-2; Serotonin transporter; Anxiety; Brain

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine have been shown to be effective in the treatment of depression and anxiety disorders (Handley, 1995; Birmaher et al., 2003); however, the precise mechanisms of their actions and particularly the delay in therapeutic onset of drug effects are not clear. During this delay, the development of neuroadaptive changes that are responsible for the antidepressant and anxiolytic effects may occur. Since it is generally proposed that the SSRIs including fluoxetine exert their therapeutic activity by increasing serotonergic function, these neuroadaptive changes may involve alterations in the expression of genes coding for proteins that are implicated in the synthesis and reuptake of 5-HT. Rate-limiting enzyme in the 5-HT biosynthesis tryptophan hydroxylase is of particular interest among candidate genes in the serotonergic pathway. Its major form expressed in the brain is tryptophan hydroxylase-2 (TPH2) (Walther et al., 2003; Zhang et al., 2004). Recently, the gene for this enzyme was suggested to be involved in determining the specificity of therapeutic response to fluoxetine (Peters et al., 2004) but little is known about the drug effect on its expression.

In the preclinical investigations of anxiety, the elevated plus-maze is one of the most popular animal models; however, this model produces contradictory data when evaluating serotonergic drugs. Anxiolytic (Griebel et al., 1999; Kurt et al., 2000), anxiogenic (Durand et al., 1999; Silva et al., 1999; Silva and Brandão, 2000; Kurt et al., 2000; Holmes and Rodgers, 2003) and negative effects (Durand et al., 1999; Griebel et al., 1999; Silva and Brandão, 2000) have been reported for acute and chronic treatment with fluoxetine. Since the initial aggravation in anxious symptoms is often observed in patients after SSRIs (Londborg et al., 2000), the reported inconsistencies may reflect the transition of an initial anxiogenic to a later anxiolytic effect during fluoxetine treatment. The duration of treatment for the

* Corresponding author. Tel.: +7 383 333 27 13; fax: +7 383 333 12 78.
E-mail address: dygalo@bionet.nsc.ru (N.N. Dygalo).

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development of anxiolytic effects may depend on multiple factors such as dosage, mode of treatment, animal genotype (species, strain), arousal state of the animal during investigation, amount and type of previous manipulations, and could vary from several hours to days (Kurt et al., 2000; Dulawa et al., 2004) and weeks (Griebel et al., 1999). Therefore, it could not be excluded that some contradictions in published fluoxetine effects on 5-HT transporter (5-HTT) gene expression, a decrease (Lesch et al., 1993; Neumaier et al., 1996; Oliva et al., 2005) or no changes (Koed and Linnet, 1997; Le Poul et al., 2000), may be related to the different behavioral steps of the drug action. Monitoring of behavior in investigations of the central mechanisms of fluoxetine action may help to overcome this problem.

To further identify neuroadaptive changes underlying the behavioral effects of repeated treatment with fluoxetine, we investigated in the rat the effect of the drug administration for 14 days on TPH2 and 5-HTT gene expression in the brain. To characterize neurobehavioral effects of fluoxetine, the tissue concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the brain, plasma corticosterone levels as well as behavior in the plus-maze were also assessed in these animals. In an attempt to reveal possible anxiolytic effects of repeated fluoxetine by means of the elevated plus maze, animals were maze-experienced on the first day of drug administration. It was previously found that the first exposure to plus-maze conditions produces an increase in baseline anxiety on subsequent trials (Almeida et al., 1993; Fernandes and File, 1996). It has been also demonstrated with this approach that relative to the behavior of maze-naive subjects, the form of anxiety-like behavior measured in maze-experienced animals better modeled the clinically observed short-term adverse effects of fluoxetine (Holmes and Rodgers, 2003).

2. Materials and methods

2.1. Animals and drug treatment

Male Wistar rats weighing 200–220 g at the first day of drug administration with free access to food and water were housed singly. Animals were housed under natural illumination. All animal use procedures conformed to international European ethical standards (86/609-EEC) and the Russian national instructions for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable data.

Fluoxetine hydrochloride was dissolved in water and gavaged into rats once daily (at 1000–1100) for 2 weeks at dose of 25 mg/kg. Control animals received similar volume of water by the same route. Body weights were recorded regularly throughout the period of fluoxetine administration. The dose of fluoxetine was chosen on the basis of previous studies demonstrating significant chronic drug effects on both behavior (Kurt et al., 2000; Dulawa et al., 2004) and neurochemistry (Caccia et al., 1992; Trouvin et al., 1993). Investigated groups consisted of 11–12 animals.

The animals were tested in the elevated plus maze for the first time 1 h after the first drug administration (trial 1). Half of the animals were sacrificed by decapitation at the end of the trial 1 for blood sampling. The other half of the animals that received fluoxetine or water for 2 weeks were retested in the elevated plus-maze 24 h after the last drug administration (trial 2) and decapitated immediately after the test. The following brain regions: the brainstem sample included block of tissue caudal to the colliculus superior, cortex that included tissue sections 1.5 mm thick cut from the upper surface of the frontal half of the hemispheres, hippocampus, amygdala and striatum (Pellegrino et al., 1979) were rapidly dissected out on a cooled plate and frozen in liquid nitrogen for determination of mRNA levels, 5-HT and 5-HIAA concentrations. Serum corticosterone was determined by competitive protein-binding assay using plasma from an adult female rat as the source of transcortin in blood sampled after decapitation (Murphy, 1967).

2.2. Analysis of 5-HT and 5-HIAA

Indoleamines were determined using electrochemical detection after separation on C4 column (4.6×150 mm; LabAlliance, USA) in the brain regions. Brain tissues were homogenized in an ice-cold solution of 0.4 perchloric acid containing 3,4-dihydroxy-benzylamine as an internal standard. Homogenates were centrifuged at 15,000×*g* for 15 min at 4 °C. Then supernatants were filtrated and 20 μl of each was injected into the HPLC system (Waters, USA). The mobile phase consisted of 0.1 M KH_2PO_4, 0.1 Na_2EDTA, octane sulfonic acid (300 mg/l) and 4% methanol. The flow rate through the system was 1.0 ml/min.

2.3. Analysis of mRNAs for TPH2 and 5-HTT

Semi-quantitative RT-PCR described previously (Shishkina et al., 2001) was used to detect mRNA for serotonergic markers. Total RNA was isolated from tissue by the acid guanidinium thiocyanate method and quantified by spectrophotometry. First strand cDNA was synthesized from 5 μg of each RNA sample with oligo(dT) primer and Superscript II reverse transcriptase (Gibco BRL, Life Technologies). PCR reactions were conducted using rat-specific primers for TPH2 forward (5′-TAAA TACTGGGCCAGGAGAGG-3′) and reverse (5′-GAAGT GTCTTTGCCGCTTCTC-3′) (Sugden, 2003); 5-HTT forward (5′-GTACCACCGAAACGGGTGCA-3′) and reverse (5′-TGGTGGATCTGCAGGACATG-3′) (Lai et al., 2003). To normalize TPH2 and 5-HTT data for semi-quantitative analysis, we performed RT-PCR on the same cDNA samples using beta-actin primers forward (5′-CGTGAAAAGATGACCCAGAT-3′) and reverse (5′-ATTGCCGATAGTGATGACCT-3′). PCR cycles were optimized to amplify each primer-specific RT-PCR product within a linear range of amplification as follows: denaturation for all pairs of primers was 94 °C 20 s: annealing for TPH2: 64 °C 20 s, 5-HTT: 66 °C 15 s, beta-actin: 62 °C 20 s; elongation for TPH2: 72 °C 15 s, 5-HTT: 72 °C 20 s, beta-actin: 72 °C 30 s. Thirty cycles were performed for TPH2 and 5-HTT, and 31 for beta-actin. Each pair of primers produced a single amplicon of an expected size: TPH2: 132 bp, 5-HTT: 300 bp and beta-actin: 411 bp. The PCR products were separated on ethidium bromide stained 1.5% agarose gel and quantified

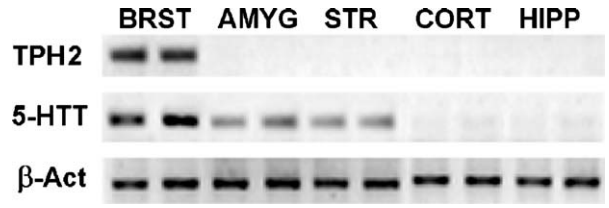

Fig. 1. Expression of tryptophan hydroxylase 2 (TPH2) and serotonin transporter (5-HTT) mRNAs in the brainstem (BRST), amygdala (AMYG), striatum (STR), frontal cortex (CORT) and hippocampus (HIPP) of intact adult male rats. Two samples for each mRNA (TPH2: upper, 5-HTT: the second, beta-actin: the third panels, respectively) presented in each of the five brain structures.

relatively to beta-actin mRNA by scanning densitometry (Biodoc II Video Documentation System, Biometra GmbH, Gottingen, Germany). The PCR parameters and detection procedures were estimated to provide a linear relationship between the amount of an input template and the amount of PCR product for all tested mRNAs. In addition, all cDNAs were also probed for TPH-2 PCR product on the plateau of amplification using 40 cycles.

2.4. Behavioral testing

For the measure of anxiety, animals were tested in the elevated plus-maze (Pellow et al., 1985) that consisted of two opposite open arms (45 × 10 cm) and two opposite enclosed arms (45 × 10 × 40 cm). All four arms were connected to a 10 × 10 cm center square. The maze was elevated 65 cm above the floor. The behavior of the animals was recorded on video for later analysis. Each rat was placed in the center of the maze facing an open arm. During the 5-min test, the number of entries into each arm, the time spent in each arm type and center square, the frequency of rearing and head-dipping were quantified. Before the next rat was introduced, the maze was cleaned with a water-wet sponge and then dried. Testing was performed between 1400 and 1600 h.

2.5. Data analysis

The effects of fluoxetine treatment were analyzed by comparing fluoxetine and water groups using a one-way ANOVA. Statistical differences between trial 1 and trial 2 were determined by an ANOVA with repeated measures followed by Fisher LSD post hoc test. Pearson's correlation analysis was used to evaluate relationships between 5-HTT and TPH2 mRNA levels and behavioral and neurochemical measures. The results were considered significant at probability level less than 0.05.

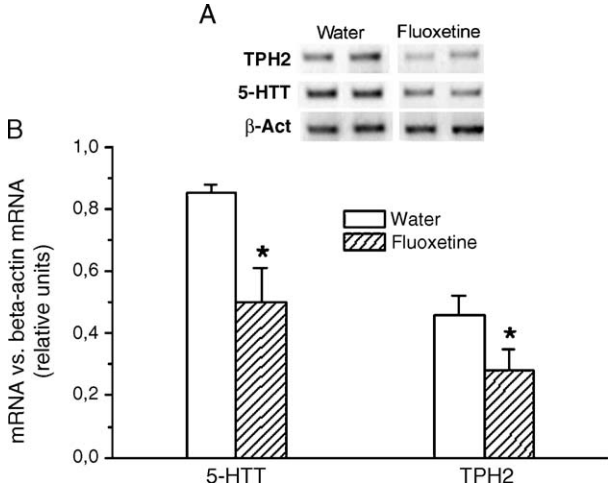

Fig. 2. Effects of repeated treatment with fluoxetine (25 mg/kg p.o. for 14 days) on 5-HTT and TPH2 mRNA levels in the rat brainstem. (A) Examples of RT-PCR analysis. Two samples for each mRNA (TPH2: upper, 5-HTT: the second, beta-actin: the third panels, respectively) in control (water) and fluoxetine groups. (B) Levels of 5-HTT and TPH2 mRNAs quantified relatively to beta-actin mRNA (β-Act: bottom panel on A) of the same cDNA sample. *$P<0.05$ vs. water controls.

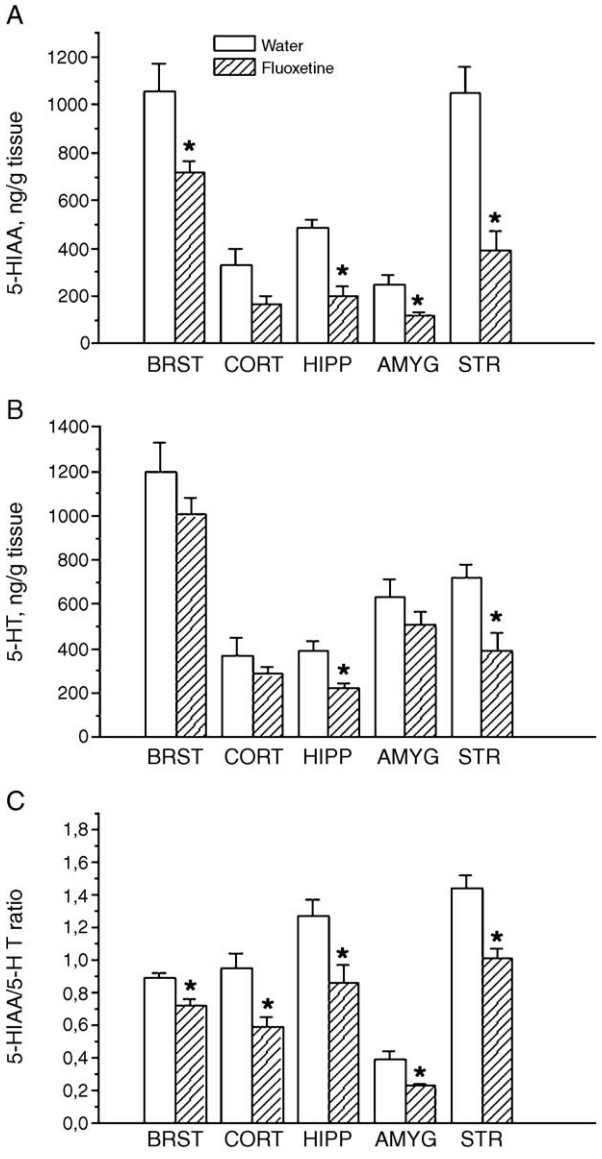

Fig. 3. Effects of repeated treatment with fluoxetine (25 mg/kg p.o. for 14 days) on concentrations of 5-HIAA (A), 5-HT (B) and 5-HIAA/5-HT ratios (C) in the rat brain regions. *$P<0.05$ vs. water controls.

3. Results

3.1. 5-HTT and TPH2 mRNAs

Qualitative differences were found in TPH2 mRNA expression in the brainstem and forebrain regions of intact rats. This transcript was detected in an RT-PCR analysis with 30 cycles of PCR only in the cDNA samples obtained from the rat brainstem (Fig. 1, upper panel). Moreover, using even up to 40 cycles of PCR, we could detect no convincing signal for TPH2 mRNA in the amygdala, striatum, frontal cortex or hippocampus (data not shown). 5-HTT messenger RNA also demonstrated region-specific distribution in the brain of intact animals (Fig. 1, the second panel). The main region of 5-HTT mRNA expression was the brainstem. The low, but detectable by RT-PCR, levels of this transcript were also found in other brain regions.

Administration of fluoxetine for 14 days significantly diminished mRNA levels of 5-HTT [$F(1,9)=11.137$, $P<0.01$] and TPH2 [$F(1,9)=7.048$, $P<0.05$] in the brainstem (Fig. 2).

3.2. 5-HIAA, 5-HT, 5-HIAA/5-HT

As shown in Fig. 3(A), 2-week administration of fluoxetine significantly reduced tissue concentrations of 5-HIAA in the brainstem [$F(1,9)=6.649$, $P<0.05$], hippocampus [$F(1,9)=23.410$, $P<0.001$], amygdala [$F(1,9)=9.690$, $P<0.05$] and striatum [$F(1,9)=19.963$, $P<0.01$]. In the frontal cortex [$F(1,9)=4.596$, $P=0.061$], the reduction did not reach statistically significant value. Fluoxetine also produced significant reductions in 5-HT (Fig. 3B) concentrations in some terminal brain regions, such as hippocampus [$F(1,9)=11.197$, $P<0.01$] and striatum [$F(1,9)=11.560$, $P<0.01$]. In all investigated brain regions, fluoxetine

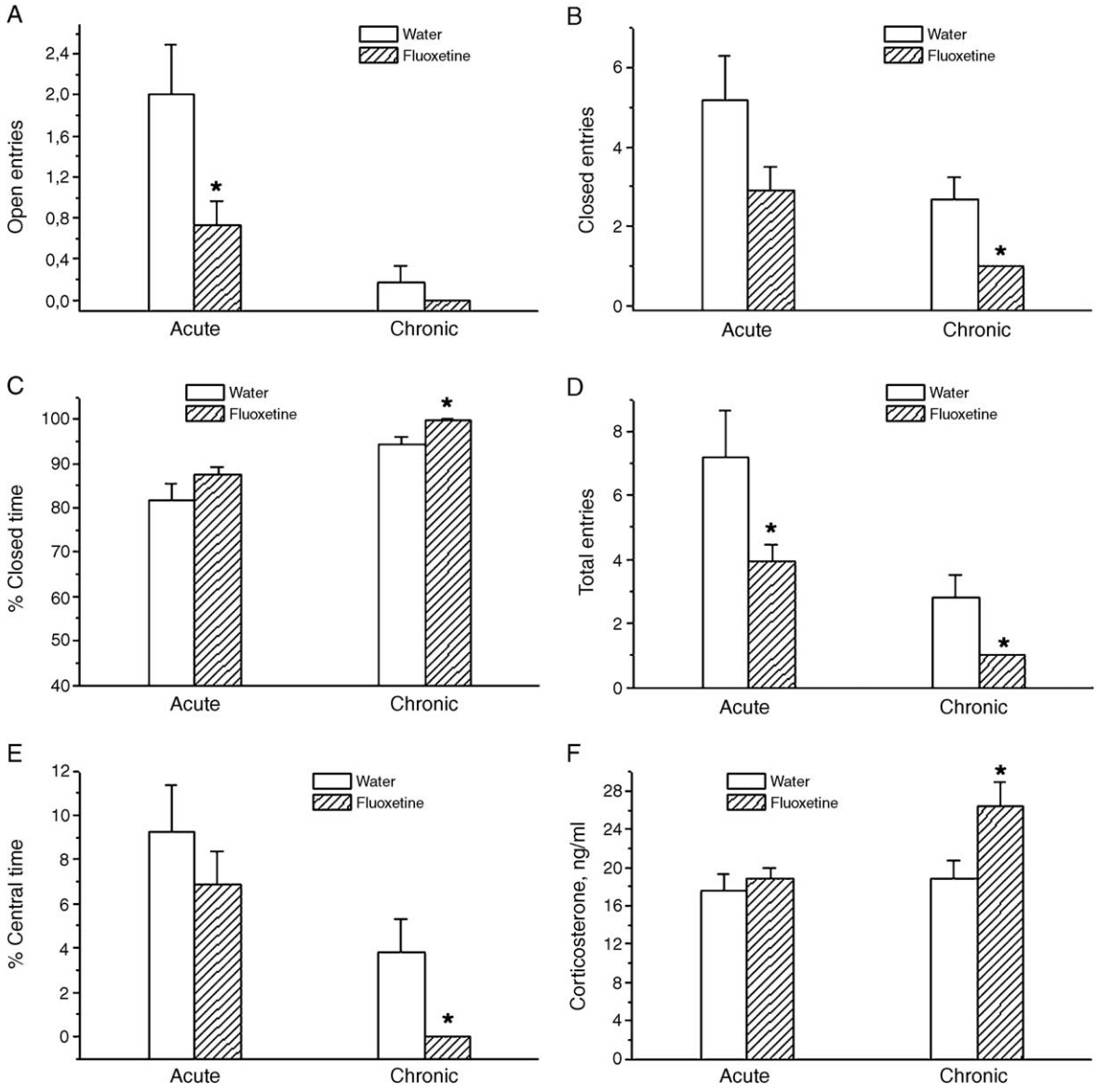

Fig. 4. Effects of acute and repeated treatment with fluoxetine (25 mg/kg p.o. for 14 days) on behavioral and hormonal profiles of rats tested on trial 1 (1 h after the first drug administration) and trial 2 (24 h after the last drug administration) of the elevated plus-maze. (A) The number of open arm entries, (B) the number of closed arm entries, (C) percent of closed time, (D) the number of total open and closed arm entries, (E) percent of central time, (F) the plasma corticosterone levels. *$P<0.05$ vs. water controls.

significantly reduced 5-HIAA/5-HT ratios (Fig. 3C): brainstem [$F(1,9)=11.450$, $P<0.01$], frontal cortex [$F(1,9)=10.777$, $P<0.01$], hippocampus [$F(1,9)=7.589$, $P<0.05$], amygdala [$F(1,9)=11.725$, $P<0.01$] and striatum [$F(1,9)=18.595$, $P<0.01$].

3.3. Plus-maze

Data on plus-maze behavioral and hormonal parameters are displayed in Fig. 4(A–F). Data for percent open entries, percent open time, head-dips and rears are not shown because no significant fluoxetine effects were found for these measures.

Acute fluoxetine resulted in a significant reductions in open entries [$F(1,21)=5.537$, $P<0.05$] (A) and total entries [$F(1,21)=4.547$, $P<0.05$] (D) that were observed on trial 1 one hour after the drug administration. A non-significant [$F(1,21)=3.503$, $P=0.075$] tendency to a decrease (−43%) in closed entries was noted (B). There were no effects of acute fluoxetine on percent open entries, percent open time, percent closed time (C), percent center time (E), head-dips and rears.

Retesting of animals 2 weeks later revealed a significant increase in behavioral indices of anxiety. Repeated-measures analysis of variance showed a significant reducing effect of retesting on open entries [$F(1,9)=13.520$, $P<0.01$] (A), percent open entries [$F(1,9)=19.150$, $P<0.01$], percent open time [$F(1,9)=8.343$, $P<0.05$], total entries [$F(1,9)=8.428$, $P<0.05$] (D), percent center time [$F(1,9)=5.580$, $P<0.05$] (E) and head-dips [$F(1,9)=11.267$, $P<0.01$]. A significant increase was found for percent closed time [$F(1,9)=7.925$, $P<0.05$] (C) on trial 2 compared to trial 1. Decreasing effect of retesting on closed entries and rears was only moderate [$F(1,9)=5.095$, $P=0.054$; $F(1,13)=4.598$, $P=0.051$, accordingly].

Repeated treatment with fluoxetine for 14 days produced an overall suppression of plus-maze behavior that was evaluated by trial 2. Animals did not enter in open arms (A) and central platform (E), but spent all time in closed arms (C). The data showed significant decreases in closed entries [$F(1,9)=7.305$, $P<0.05$] (B), total entries [$F(1,9)=5.562$, $P<0.05$] (D), percent center time [$F(1,9)=5.234$, $P<0.05$] (E) and significant increase in percent closed time [$F(1,9)=9.905$, $P<0.05$] (C). No significant between-group differences were seen in open entries, percent open entries, percent open time, head-dips and rears.

Corticosterone level (F) that was measured 1 h after the first fluoxetine administration and immediately after expose to trial 1 of the plus-maze was not differ from that in control animals [$F(1,10)=0.346$, NS]. Treatment with fluoxetine for 14 days significantly increased the corticosterone level after trial 2 of the plus-maze [$F(1,9)=6.347$, $P<0.05$].

3.4. Correlations between 5-HTT and TPH2 mRNA levels and neurobehavioral measures

Significant correlations between 5-HTT and TPH2 mRNA levels and behavioral and neurochemical measures are summarized in Table 1. For 5-HTT, correlations were found for 5-HIAA levels in the brain regions containing projections of the brainstem serotoninergic neurons: striatum and hippocampus, and a tendency for similar correlation was also detected in the cortex ($r=+0.58$, $P<0.06$). For TPH2, significant correlations were obtained for 5-HT level in the brainstem and for plus-maze behaviors: total arm entries and percent center time.

Table 1
Correlation coefficients between 5HTT and TPH2 mRNA levels in the brainstem and plus-maze behaviors and brain 5-HT and 5-HIAA concentrations in male Wistar rats ($n=11$ per group)

	Total arm entries	Percent center time	5-HT brainstem	5-HIAA striatum	5-HIAA hippocampus
5-HTT mRNA	0.52	0.40	0.23	0.64 *	0.73*
TPH2 mRNA	0.75*	0.66*	0.64*	0.31	0.49

*$P<0.05$.

4. Discussion

The main finding of this study is a reduction of the TPH2 mRNA level in the rat brainstem after a 2-week treatment with fluoxetine. To our knowledge, this is the first study demonstrating the effect of a repeated SSRI on the expression level of this enzyme that has been recently considered as a brain-specific for the 5-HT synthesis (Walther et al., 2003; Zhang et al., 2004). Our data also confirm that TPH2 is preferentially expressed in the brainstem of rats. We did not obtain an expression of TPH2 mRNA in other investigated brain regions that is in a good agreement with the results of in situ hybridization (Patel et al., 2004).

The reduction in TPH2 mRNA after repeated fluoxetine can contribute to a decrease in the brain concentration of 5-HT that has been demonstrated in this and previous studies (Caccia et al., 1992; Trouvin et al., 1993; Thompson et al., 2004). This interpretation is strengthened by a significant positive correlation between TPH2 mRNA level and 5-HT concentration and supported by a decrease in 5-HT synthesis in the rat brain regions after chronic fluoxetine (Mück-Šeler et al., 1996) and in the mouse brain after another SSRI, citalopram, treatment (Cervo et al., 2005).

Administration of fluoxetine for 2 weeks also produced robust 5-HIAA depletion in the rat brain. Primary mechanism for action of SSRIs including fluoxetine involves their binding to the 5-HTT and subsequent inhibition of uptake of extracellular 5-HT into serotonergic neurons (Murphy et al., 2004). The decrement in 5-HIAA content observed after fluoxetine treatment may reflect a decrease in metabolism of 5-HT to 5-HIAA by intraneuronal monoamine oxidase A. Significant positive correlations between 5-HTT mRNA content and 5-HIAA concentrations support this interpretation. The 5-HIAA content and 5-HIAA/5-HT ratio mirror long-lasting alterations in the serotoninergic system function and have been reported to remain significantly low for several days after the last drug administration during 1–3 weeks (Caccia et al., 1992; Trouvin et al., 1993).

Fluoxetine decreased or tended to decrease 5-HIAA and 5-HT levels in all brain regions studied. Quantitative regional differences in the drug effects may be related to region specificity of serotonergic neurotransmission, such as, for example,

neurochemistry and/or intensity of auto- and heteroreceptor regulation, as well as balance between this regulation and neurotransmitter release (Hervas et al., 2000), and to a lesser extent to the origin of the 5-HT afferents projecting to individual terminal areas (Hjorth et al., 2000). This notion is supported by differences in relative magnitudes of fluoxetine effects on 5-HIAA and 5-HT contents between cortex and striatum both getting most of their serotonergic projections from the dorsal raphe nucleus (DRN), and also by a very similar drug effects in the striatum and hippocampus irrespective of the different main sources of their serotonergic fibers, DRN for the former and the median raphe nucleus for the later (McQuade and Sharp, 1997). The similarity of fluoxetine effects on 5-HIAA to 5-HT ratio in various forebrain areas, regardless of the origin of serotonergic fibers, may result from direct action of fluoxetine on each serotonergic neuron because all these neurons express both the target for the drug and the resulting gene products, 5-HTT and TPH2, that influence 5-HIAA and 5-HT levels in all brain regions. However, since some second-order fluoxetine effects related to specific afferent inputs to different groups of 5-HT neurons could not be excluded, future analysis of raphe subregions would be important for a better understanding of the drug effects on the serotonergic gene expression in the brain.

The main region of 5-HTT mRNA expression is the brainstem. The low, but detectable by RT-PCR, levels of this transcript have been also reported previously in the striatum, cortex and hippocampus (Lesch et al., 1993). In addition to these structures, we also found 5-HTT mRNA in the amygdala. Data on 5-HTT gene expression after chronic fluoxetine treatment are conflicting. Some studies showed a decrease in 5-HTT mRNA levels in the rat midbrain after treatment with fluoxetine for 1–4 weeks (Lesch et al., 1993; Neumaier et al., 1996; Oliva et al., 2005), while other studies recorded no effects after the drug treatment at similar doses and duration in the same brain region (Koed and Linnet, 1997; Le Poul et al., 2000). Contradictory results were obtained by different groups, even if they used the same rat strains (Wistar or Sprague–Dawley) and methods to measure gene expression (RT-PCR or in situ hybridization). Because there is no evident basis for these discrepancies, an existence of some hardly controlled variable influencing effectiveness of fluoxetine treatment such as, for example, individual life history of the subject could not be excluded. In the present study, we found a significant reduction in 5-HTT mRNA levels in the brainstem of plus-maze-experienced rats after 2-week fluoxetine treatment.

An important question is whether the neurochemical changes caused by repeated fluoxetine could be linked to the behavioral effects of the drug. In accordance with rat studies (Almeida et al., 1993; Fernandes and File, 1996), retesting of animals in our work revealed a significant increase in anxiety on trial 2 compared with trial 1. This was supported by the lower percentages of open entries and open time on retest. Retested animals also spent more time in the closed arms and less time on the center platform, and show less head-dips. Present findings also show that a single prior experience of the plus-maze affects anxiety at least for 2 weeks. The dose of fluoxetine used in our study was approximately corresponding to those, which after repeated treatment either did not produce an axiogenic effect (Griebel et al., 1999) or even has led to some anxiolytic response in different tests (Dulawa et al., 2004) including the plus-maze in animals that were naive to this test (Kurt et al., 2000). However, in maze-experienced rats after repeated fluoxetine treatment, we found an overall suppression of plus-maze behavior that may indicate a strong anxiety-like response. Rats treated with fluoxetine for 2 weeks spent all time in the closed arms and had higher corticosterone levels in the elevated plus-maze test than control animals. The corticosterone levels could be related to behavioral effects of fluoxetine because high correlation had been obtained between the plasma corticosterone response to the elevated plus-maze and measures of risk assessment in this test (Rodgers et al., 1999), and repeated fluoxetine administration increased corticosterone response to open field exposure in Wistar–Kyoto rats (Durand et al., 1999).

All SSRIs have a delayed onset of therapeutic action that is considered to be due to the time required for adaptive alterations in serotonergic functions to occur (Handley, 1995). The majority of rodent studies also reported an anxiogenic response in the elevated plus-maze test after acute treatment with fluoxetine (Silva et al., 1999; Silva and Brandão, 2000; Kurt et al., 2000; Holmes and Rodgers, 2003) that is in keeping with the initial aggravation in anxious symptoms in some patients after SSRIs (Londborg et al., 2000). In the present study, a significant reduction in open entries and total entries was found in rats after 1 h of the first drug administration. In addition to possible non-specific fluoxetine effect on locomotor activity, the decrease in total entries may also reflect an increase in the level of anxiety (File, 2001). Acute anxiogenic-like effect of fluoxetine is associated with a rapid increase in the brain extracellular 5-HT concentrations resulting from the blockade of the 5-HTT-mediated reuptake of the neurotransmitter. As was shown by in vivo microdialysis studies, administration of fluoxetine dose-dependently elevated extracellular 5-HT concentrations in the raphe nuclei, prefrontal and frontal cortices, hypothalamus, thalamus, hippocampus, nucleus accumbens and striatum (Guan and McBride, 1988; Dailey et al., 1992; Perry and Fuller, 1992; Jordan et al., 1994; Malagie et al., 1995; Tao et al., 2002).

Anxiogenic-like effect of 2-week fluoxetine treatment observed in our work was accompanied by the decrease in 5-HTT mRNA level in the brainstem, depletion of 5-HT and 5-HIAA in brain regions as well as reduction in the brainstem TPH2 gene expression. All of these changes potentially could account for the behavioral effects of the drug. Stress sensitive animals are usually anxious and have decreased expression of 5-HTT in the dorsal raphe nucleus (Bethea et al., 2005). Besides, mice lacking 5-HTT showed increased anxiety-like behavior and inhibited exploratory locomotion in a battery of tests including elevated plus-maze (Holmes et al., 2003). However, if a decrease in the 5-HTT mRNA level is one of the mechanisms through which fluoxetine exerted its anxiogenic effects, this mechanism is indirect one. There were no correlations between this transcript level and the behavioral measures, while 5-HTT mRNA content and an immediate result of its protein function, 5-HIAA concentrations, were well correlated. Possible relation between the obtained 5-HT depletion and anxiogenic state of our animals after repeated fluoxetine is in consonance with

clinical reports suggesting that lowered 5-HT function is involved in anxiety disorders (Argyropoulos et al., 2004). Along this line, rats that were treated with neurotoxin 5,7-DHT exhibited a significant increase in anxiety-like behavior in the plus-maze together with a decrease (50-70%) in brain 5-HT levels (Hall et al., 1999). Significant correlations between TPH2 mRNA level and 5-HT concentration as well as plus-maze behaviors found in the present study suggest involvement of alterations in TPH2 gene expression in neurochemical and behavioral effects of fluoxetine. The suggested interrelation between this gene and the drug effects is also supported by an association of the therapeutic outcome of fluoxetine treatment to polymorphic variants of the human TPH2 gene (Peters et al., 2004) that might significantly differ in their activities (Zhang et al., 2005). Besides, recently was shown that mouse behavioral response to another SSRI, citolopram, depends on genotype-specific activity of TPH2 (Cervo et al., 2005). Our data demonstrate for the first time that interrelations between SSRIs and TPH2 could be extended to the drug effects on the enzyme gene expression. This fact may be important for the understanding of the mechanisms underlying the response to chronic SSRI treatment.

In conclusion, the results of the present study provide new evidences for fluoxetine modulation of the gene expression in the brain serotoninergic system. Two-week fluoxetine treatment induced a decrease in the TPH2 and 5-HTT gene expression that might contribute to the mechanisms mediating neurochemical and behavioral effects of the drug. Furthermore, these data provide possible functional basis for the suggested relation of therapeutic outcome of fluoxetine treatment to polymorphic variants of the human TPH2 gene (Peters et al., 2004).

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