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On association between cortical 5-HT_{2A} receptors and behavior in rats with experimental thyroid disturbances

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Abstract

Thyroid hormones (TH) were hypothesized to affect behavior via neurotransmission alterations. The present study was aimed to reveal effects of chronic TH deficit and excess on some types of adaptive behavior (catalepsy, acoustic startle reflex, open-field performance), sexual arousal and cerebral 5-HT_{2A} serotonin receptors of adult Wistar rats. Administration of thyroxine synthesis inhibitor, propylthiouracil (PTU, 50 mg/l, 28 days), in drinking water produced substantial decrease in plasma thyroxine level and body weight gain, attenuated significantly acoustic startle reflex amplitude, sexual motivation and plasma testosterone surge in response to receptive female introduction, increased predisposition to catalepsy without considerable effects on open-field performance. L-thyroxine treatment (T4, 0,5 mg/l, 28 days) caused significant plasma thyroxine augmentation, somatic growth retardation and disturbances in sexual but not in other types of behavior studied. TH dysfunctions markedly increased number of DOI-induced wet dog shakes reflecting high functional activity of 5-HT_{2A} receptors without any effect on cortical 5-HT_{2A} receptor mRNA level. The involvement of cerebral 5-HT_{2A} receptors alterations at posttranslational level in mechanisms of TH effects on sexual arousal was suggested. The data attract particular attention to undesirable effects of PTU and L-thyroxine treatment on behavior.

Keywords: Thyroid hormones; Catalepsy; Startle reflex; Sexual arousal; 5-HT_{2A} receptors; Rat

1. Introduction

Numerous clinical observations suggest a close link between disturbances in hypothalamic–pituitary–thyroid axis and depressive disorders (Joffe and Levitt, 1993; Musselman and Nemeroff, 1996; Kirkegaard and Faber, 1998). However, the available data are rather contradictory. Some investigators point to association of depression with hypofunction (Cleare et al., 1995; Cleare et al., 1996; Prange, 1996) while other with hyperfunction (Joffe and Levitt, 1993; Musselman and Nemeroff, 1996; Kirkegaard and Faber, 1998) of the hypothalamic–pituitary–thyroid system. Neurobiological mechanisms of the association still remain obscure.

Thyroid hormones (TH) are supposed to play neurotrophic role in mature CNS (Muller and Clos, 1997). There are experimental data indicating the involvement of TH into neurotransmission modulation (Mason et al., 1993; Dratman and Gordon, 1996). Recently, the role of brain serotonin system as a mediator between TH disturbances and depression was hypothesized (Kirkegaard and Faber, 1998; Duval et al., 1999). Cortical serotonin 5-HT_{2A} receptors attract particular attention as the most probable molecular nexus between TH and depression. Indeed, some studies revealed alterations in 5-HT_{2A} receptor density (Arora and Meltzer, 1989; Hrdina et al., 1993) and functional activity (Cleare et al., 1995; Cleare et al., 1996) in the brains of depressive patients. The most antidepressant treatments decrease cortical 5-HT_{2A} receptor density (Lafaille et al., 1991; Maj et al., 1996). On the other hand, chronic triiodothyronine treatment was found to downregulate 5-HT_{2A} receptors in mouse (Heal and Smith, 1988) and rat (Sandrini et al., 1996) brain. Thyroidectomy was also shown to decrease 5-HT_{2A} receptor density (Kulikov et al., 1999) and expression (Kulikov et al., 2002a) in the frontal cortex of rats.

At the same time, the involvement of cerebral 5-HT_{2A} receptors in the regulation of some forms of animal behavior,

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such as catalepsy (excessive freezing) (Kulikov et al., 1995; Popova and Kulikov, 1995; Kulikov et al., 2002b), acoustic startle reflex (Popova et al., 1999) and sexual motivation (Popova and Amstislavskaya, 2002) has been demonstrated. However, an association between TH, brain 5-HT_{2A} receptors and animal behavior is still scantily studied.

Catalepsy (freezing reaction) defined as a pronounced immobility with a plastic muscle tonus is a syndrome of grave psychopathology in humans (Abrams et al., 1979; Singerman and Raheja, 1994; Dixon, 1998). Considerable role of serotonergic system in catalepsy regulation was revealed (Klemm, 1989; Popova and Kulikov, 1995; Wadenberg, 1996). Selective breeding of rats for high predisposition to catalepsy had resulted both in genetically defined hypothyroidism (Barykina et al., 2002) and in decreased density (Kulikov et al., 1995; Popova and Kulikov, 1995) and expression (Kulikov et al., 2002b) of 5-HT_{2A} receptors in brain of catalepsy-prone rats. Importantly, thyroidectomy-induced TH deficiency produced both a decrease in 5-HT_{2A} receptor density and expression (Kulikov et al., 1999; Kulikov et al., 2002a) and a significant augmentation in predisposition to catalepsy in rats (Barykina et al., 2002; Kulikov et al., 2002c).

The acoustic startle reflex is a rapid sensorimotor response elicited by a sudden and intense auditory stimulus and mediated by a relatively simple neuronal circuit located in the lower brainstem. Prepulse inhibition of startle reflex (PPI) is evoked by preceding signal introduction and reflects processes of sensorimotor gating. The PPI disturbances are implicated in psychopathology (Davis, 1984; Koch, 1999). Neonatal hypothyroidism was shown to reduce the amplitude of startle reflex response (Schneider and Golden, 1987; Goldey et al., 1995; Goldey and Crofton, 1998), whereas administration of 5-HT_2 receptor antagonists was found to influence both startle reflex amplitude and its prepulse inhibition in rats and mice (Popova et al., 1999).

Sexual behavior is regarded as one of the most biologically essential forms of behavior. Many human psychical disorders are accompanied with sexual dysfunctions. There are clinical evidences of association between sexual dysfunctions and thyroid disorders (Wortsman et al., 1987; Jaya Kumar et al., 1990). The disturbances in copulatory behavior in rats with congenital (Jiang et al., 2000), pharmacological or thyroidectomy-induced hypothyroidism (Tohei et al., 1998) were shown. On the other hand, the participation of 5-HT_{2A} receptors in regulation of different components of sexual behavior was demonstrated for rats and mice (Gorzalka and Hanson, 1998; Brotto and Gorzalka, 2000; Popova and Amstislavskaya, 2002).

Thus, the numerous data suggest the key role of brain 5-HT_{2A} receptors and TH in the control of behavior. However, experimental proof is needed to reveal whether TH exert their effects on behavior via mechanisms including regulation at the level of 5-HT_{2A} receptors. The present study was aimed to study simultaneously under the same experimental conditions the effects of pharmacological decrease or increase of plasma T4 level on (1) acoustic startle reflex response, catalepsy and sexual arousal and (2) functional activity and mRNA level of 5-HT_{2A} receptors in rats.

2. Methods

The experiments were carried out on 65 male Wistar rats. At the beginning of experiment all animals were four weeks old and weighed 73.7±2.2 g. The rats were housed in groups of 5 to 6 per cage (60×40×20 cm) under standard conditions (temperature: 18–22 °C; relative humidity: 50–60%). The animals of the control group were given water for 28 days (Control, $n=21$). Rats of the second group (PTU, $n=22$) received propylthiouracil (2-thio-4-hydroxy-6-*n*-propyl-pyrimidine, PTU, Sigma, USA) dissolved in drinking water to the concentration of 50 mg/l (0.005%) for 28 days. Animals of the third group (T4, $n=22$) were treated with L-thyroxine (Berlin-Chemie, Germany) dissolved in drinking water to the concentration of 0.5 mg/l for 28 days. Thyroxine was given in drinking water containing bovine serum albumin (100 mg/l). All animals were weighed once a week. Body weight gain was calculated as the difference between weight of an animal at the beginning of experiment and after 28 days of treatment. In two days before testing rats were isolated in individual cages of the same size.

Catalepsy was tested over two successive days. To induce catalepsy, the rat was lifted by its forepaws with a stick in a corner of a cage, then the stick was removed. The period of time (s) during which a rat maintained the imposed vertical posture was recorded. The procedure was repeated three times in each of two test sessions. Animals, which maintained the forced vertical posture for three times for at least 10 s, were considered as cataleptics. Catalepsy was estimated by mean immobility time in two test sessions and percentage of cataleptics (Barykina et al., 2002).

The acoustic startle response was measured in SR-Pilot system (San-Diego Instruments, San Diego, CA, USA). The system consisted of a weight-sensitive platform inside a 14×21×23 cm sound- and light-attenuated chamber. The chamber had a peephole on its front wall, allowing continuous observation. Body movements produced by startled animal were transformed into analog signal by a piezoelectric unit attached to the platform. The signals were digitized and recorded by interfaced microcomputer. A loudspeaker inside the chamber was used to produce a background white noise of 65 dB during the entire session. An animal was placed in the chamber for 3 min for adaptation. The test session included two components: pulse trails (P) in which a 40 ms, 115 dB broadband noise burst was presented and prepulse+pulse trails (PP) in which the 40 ms, 85 dB broadband noise (prepulse) preceded the onset of the 115 dB pulse by 100 ms. Each animal received 10 acoustic stimuli (5 P and 5 PP). Each P trail alternated with PP trail with an interval of approximately 15 s. An animal was exposed to testing signals while showing no activity, with four paws resting on the platform. Prepulse inhibition (PPI) of the acoustic startle response was calculated in percent by formula: PPI=[(amplitude of pulse − amplitude of prepulse+pulse)/amplitude of pulse]×100%. Magnitudes

of acoustic startle response amplitude and prepulse inhibition were estimated by mean values of 5 P and 5 PPI, respectively (Maslova et al., 2002).

To exclude the possibility that the effects observed in other tests were linked to alterations in locomotion, *the open field test* was performed in a device (140 × 70 × 45 cm) made of Perspex and colored white. The floor, brightly lit (300 lux), was divided into 98 squares (10 × 10 cm each). Each rat was placed at a wall of the arena and its behavior was videotaped for 6 min. The number of crossed squares and rearings was recorded and used as the index of horizontal and vertical locomotor activity, respectively.

To evaluate the behavioral component of *female-induced sexual arousal*, each rat was placed in a 52 × 33 × 20 cm experimental cage divided by a perforated transparent partition into two compartments for three days for adaptation prior to experimenting. The perforated transparent partitions were designed so that the males could smell, see and hear receptive females but were not able to establish any physical contact associated with mating; the holes in partition were 10 mm in diameter and the partition thickness was about 3 mm. Test sessions were run at 20:00–22:00 h under red light. The animals were given 5 min for adaptation to novel lighting conditions, their spontaneous activity at the partition was assessed during the subsequent 10 min. Then each animal was exposed to a receptive female of the same strain. The amount of time a test male had spent actively exploring the partition over 10 min (partition time) and the number of approaches it had made to the partition over this interval were recorded. The time and approaches were counted whenever male contacted the partition with its nose or one or two forepaws. In females estrus was induced by estradiol administered subcutaneously at 48 h before testing with a dose of 100–150 μg/animal and progesterone administered subcutaneously at 4 h before testing with a dose of 750 μg/animal (Popova and Amstislavskaya, 2002; Amstislavskaya and Popova, 2004).

For randomly chosen 22 rats (by seven of controls and T4-treated and eight of PTU-treated) *testosterone concentration*, the hormonal characteristic of sexual arousal, was determined in tail blood samples drawn under a 1-min ether anesthesia at 20 min of exposure to a receptive female in the neighboring compartment (i.e. after 35 min of total exposure to red light). The assay of resting testosterone concentrations was carried out in blood samples drawn from tails of the animals at night time after 35-min exposure to red light. Collected blood was heparinized and centrifuged, the final plasma was frozen and kept at −65 °C. Testosterone levels were estimated by radioimmunoassay using a highly specific antiserum and [^{3}H] testosterone (Amersham, UK). The percentage of cross-reactions shows specificity of antiserum used for testosterone (100%), dihidrotestosterone (62%), androstenediol, androstenedione, androsterone, progesterone, estradiol, cortisol (0% to 0.5%); CV% intraassay = 8; interassay = 6 (Amstislavskaya and Popova, 2004). In previous studies partition time and testosterone surge were demonstrated to reflect sexual interest rather than social curiosity (Amstislavskaya and Popova, 2004), these parameters were taken as the main indices of sexual arousal.

2.1. Functional activity of 5-HT$_{2A}$ receptors

To induce wet dog shakes regarding as an index of functional activity of cerebral 5-HT$_{2A}$ receptors (Green and Heal, 1985), rats were administered 5-HT$_{2A}$ receptor agonist, DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) (Sigma, USA), dissolved in saline in a dose of 1 mg/kg, intraperitoneally at 30 min before testing. The test was carried out in animal home cage, the number of wet dog shakes for each rat was recorded for 30 min. To exclude possible effect of DOI injection on 5-HT$_{2A}$ receptor mRNA level, DOI-treated animals were not used in the further experimental procedures.

2.2. Tissue preparation

In two days after the last test randomly chosen 28 rats (by 9 of controls and PTU-treated and 10 of T4-treated) were decapitated, trunk blood was collected from each animal into Eppendorf tube containing 20 μl of EDTA (1 M) and then centrifuged at 6000 ×*g* for 10 min (+4 °C). The final plasma was frozen and kept at −20 °C until assay of total thyroxine concentration. The brain was removed on ice, frontal cortex was dissected and frozen with liquid nitrogen, and then stored at −65 °C until RNA extraction. Prostate gland and testes of the animals were removed and weighed.

Total thyroxine concentration (nM) was estimated in plasma by an immunoenzymatic technique using the thyroidIEA-thyroxine KIT (Alkor-Bio, Russia) (Barykina et al., 2002).

2.3. RT-PCR assay of 5-HT$_{2A}$ receptor mRNA

For RT-PCR of 5-HT$_{2A}$ receptor mRNA expression, the method described in detail earlier (Kulikov et al., 2005) was used. Total RNA was isolated by guanidinium thiocyanate–phenol–chloroform extraction (Chomczynski and Sacchi, 1987), its concentration was determined by absorbance at 260 nm and its purity was estimated by 260/280 ratios. The RNA integrity was evaluated by electrophoresis in 0.8% agarose gel. Only RNA samples with clearly visible 28S and 18S rRNA bands were used. The absence of DNA contamination in RNA preparations was checked by PCR analysis using primers for β-actin and 1 μl of total RNA diluted five times. No sign of genomic β-actin fragment was detected by 32 cycles of amplification.

Total RNA, 8 μl (1 μg), was mixed with 180 ng of random hexamer primer and 2.25 μmol of sterile KCl in the volume of 16 μl, denatured at 94 °C for 5 min, annealed at 41 °C for 15 min, and then 15 μl of mixture containing reverse transcriptase M-MLV (200 U) (Biosan, Russia), Tris HCl (pH 8.3, 0.225 μmol), dNTP mixture (0.015 μmol of each) (Sibenzyme, Russia), dithiothreitol (0.225 μmol) and $MnCl_2$ (0.03 μmol) were added. The mixture (at the final volume of 31 μl) was incubated at 41 °C for 60 min. The synthesized cDNA was stored at −20 °C.

An aliquot of 2 μl of cDNA was mixed with 2 μl of PCR ×10 buffer, 1 μl of dNTP mixture, 0.03 μmol of $MgCl_2$, 5 μl of mixture of appropriate sense and antisense primers (250

pmol of each; 5-HT_{2A} primers: 5′-agaagccaccttgtgtgtga-3′ and 5′-ttgctcattgctgatggact-3′, β-actin primers: 5′-cggaaccgctcattgcc-3′ and 5′-acccacactgtgcccatcta-3′), 1 U of Taq polymerase (Sibenzyme, Russia) and sterile water to the final volume of 20 μl. PCR was carried out in Hybaid Omn-E (UK) thermocycler in the following conditions: (i) 5 min at 94 °C; (ii) 40 s at 94 °C, 40 s at 58 °C (5-HT_{2A}) or 61 °C (β-actin), 40 s at 72 °C, 32 (5-HT_{2A}) or 20 (β-actin) cycles; (iii) 4 min at 72 °C.

The control tubes containing 2 μl of external exogenous standard DNA (100 ng/μl for β-actin or 10 ng/μl for the receptor) instead of cDNA and the same reagent mixture were amplified together with the corresponding sample tubes. We used rat genomic DNA as the external standard containing 200 copies of the target genes per 1 ng. Negative controls included the same reagent mixture except for cDNA or DNA were omitted.

The sample PCR products with the corresponding standards and the negative controls were then resolved by electrophoresis in the same 2% agarose gel, stained with ethidium bromide and scanned with Biometra^{TI3} system. The band intensities for samples and standards were analyzed using Scion Image software (Scion Corporation, http://www.scioncorp.com). The band intensities for the samples were calibrated by corresponding standard DNA, and the number of copies of 5-HT_{2A} and β-actin per 1 μl of cDNA was calculated. Expression of 5-HT_{2A} receptor mRNA was evaluated by the number of copies per 100 copies of respective β-actin mRNA.

2.4. Statistics

Data on body weight gain, T4 plasma concentration, quantitative behavioral indices and 5-HT_{2A} receptor mRNA level were expressed as MEAN±SEM and analyzed by one-way ANOVA with subsequent post hoc comparison according to Neuman–Keuls test. Since acoustic startle amplitude and weight of organs usually depend on body weight, the data on these indices were expressed as MEAN±SEM and compared with analysis of covariance (ANCOVA) taking the body weight values as covariables. Number of cataleptic animals was presented as percentage of total number of animals in each group and compared with χ^2-test. Data on sexual motivation and testosterone level were analyzed by repeated measures ANOVA with subsequent post hoc comparison according to Newman–Keuls Multiple Range Test.

All experimental procedures were in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

3. Results

Substantial effect of experimental dysthyroidism on T4 plasma level was found ($F(2,24)=37.53$; $p<0.001$). The plasma thyroxine level in control rats was 48.78±3.21 nM and did not differ from that demonstrated earlier in our studies (Barykina et al., 2002; Kulikov et al., 2002c) or by other researchers (Escobar-Morreale et al., 1996; Redei et al., 2001). Chronic PTU treatment significantly decreased plasma hormone level in comparison with control group (32.91±3.06 nM; $p<0.05$). The mild decrease in total T4 level (33%) in response to chronic administration of 0.005% PTU solution is in a good agreement with previous data (Hood et al., 1999). Chronic L-thyroxine treatment augmented plasma hormone concentration up to 89.87±6.69 nM ($p<0.001$ vs. control).

Dramatic impact of thyroid disturbances on body weight gain was revealed ($F(2,62)=21.69$; $p<0,001$). Both pharmacologically induced hypo- and hyperthyroidism reduced body weight gain in rats (38.5±3.07 g ($p<0.001$) and 59.5±3.26 g ($p<0.01$), respectively) in comparison with control animals (76.95±5.68 g). It is well known that thyroid deficiency and excess decrease body weight and growth rate (Schneider and Golden, 1987; Dratman, 1993). Thus, the sufficiency of drug doses used and treatment duration to produce the significant effects on hormonal and somatic characteristics of adult rats can be concluded.

Experimental groups differed substantially in immobility time ($F(2,61)=4.39$; $p<0.05$) (Fig. 1A) and the number of cataleptic animals (Fig. 1B). Chronic PTU administration led to the significant augmentation of cataleptic animal percentage compared to control ($\chi^2=5.5$; $p<0.05$) and hyperthyroid group ($\chi^2=4.06$; $p<0.05$). Duration of cataleptic freezing was also significantly higher in PTU-treated rats than in control ($p<0.05$) or T4-treated rats ($p<0.05$). On the other hand, T4-treated rats did not differ significantly from control rats in cataleptic animal percentage ($\chi^2=0.47$; $p>0.05$) and time of cataleptic freezing ($p>0.05$).

Plasma T4 level had a significant effect on the startle reflex amplitude ($F(2,61)=10.03$; $p<0.001$). The magnitude of startle response was lower in hypothyroid group than in control ($p<0.001$) or hyperthyroid group ($p<0.001$), while hyperthyroid rats did not differ from control ones in this index ($p>0.05$) (Fig. 2A). Prepulse inhibition did not vary between groups ($F(2,62)=2.11$; $p>0.05$) (Fig. 2B).

No differences in the numbers of crossed squares and rearings in the open-field test between groups were shown (Table 1).

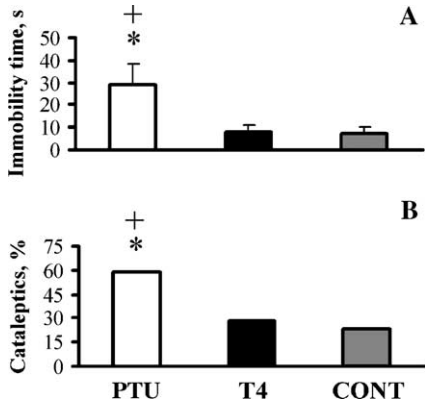

Fig. 1. The effect of chronic PTU (50 mg/l, 28 days) and L-thyroxine (T4) (0.5 mg/l, 28 days) treatment on the mean immobility time (s) (A) and percentage of cataleptic animals (B) in rats. Data are presented as MEAN±SEM. *$p<0.05$ vs. control group. +$p<0.05$ vs. hyperthyroid group (T4).

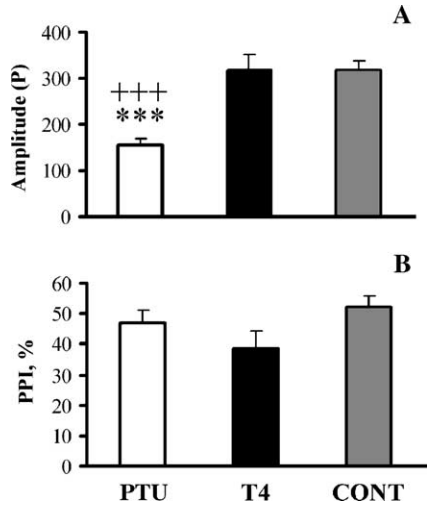

Fig. 2. The mean startle reflex amplitude (A) and prepulse inhibition (%) (B) in rats received PTU (50 mg/l, 28 days) and L-thyroxine (T4) (0.5 mg/l, 28 days). Data were analyzed taking into consideration the body weight values as covariables. Each bar represents MEAN±SEM. ***$p<0.001$ vs. control group. +++$p<0.001$ vs. hyperthyroid group (T4).

A significant influence of both thyroid function ($F(2,46)=3.6$; $p<0.05$) and presence of a receptive female ($F(1,46)=384.5$; $p<0.001$) on the partition time was found. The interaction between these factors ($F(2,46)=3.6$; $p<0.05$) indicates that variation in the partition time of males with different plasma thyroxine levels depends upon the presence or absence of a receptive female behind the partition. At the same time, the number of approaches was significantly affected only by the presence of a receptive female ($F(1,47)=84.0$; $p<0.001$), while thyroid status did not seem to have effect on this characteristic ($F(2,47)<1$), the interaction between these two factors was not shown either ($F(2,47)=1.3$; $p>0.05$). Spontaneous activity at the partition was similar in hypo-, hyper- and euthyroid rats (control group). Although a receptive female introduction caused a considerable increase of the corresponding parameters vs. spontaneous activity in all groups, the partition time in presence of a receptive female was found to be significantly lower in hypo- ($p<0.05$) and hyperthyroid ($p<0.01$) groups compared to control (Fig. 3A and B).

A significant influence of presence of a receptive female ($F(1,19)=12.49$; $p<0.01$) but not thyroid function ($F(2,19)=3.11$; $p>0.05$) on testosterone level was detected, the interaction between these two factors was not significant ($F(2,19)=2.14$; $p>0.05$). Resting testosterone level was similar in hypo-, hyper- and normothyroid males (control group). An exposure to receptive female caused an increase of testosterone concentrations vs. resting values in all groups, in control group this augmentation was significant ($p<0.01$). Testosterone level in presence of a receptive female was shown to be significantly lower in hypothyroid group ($p<0.05$) compared to control but not hyperthyroid group ($p>0.05$) (Fig. 3C).

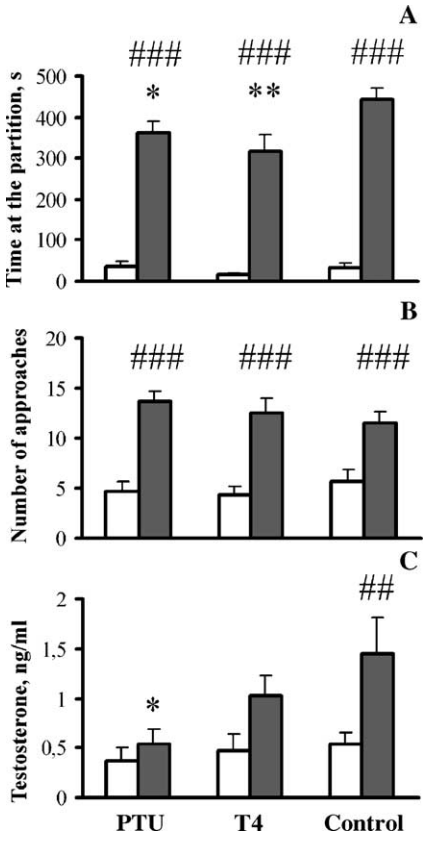

Fig. 3. The effect of chronic PTU (50 mg/l, 28 days) and L-thyroxine (T4) (0.5 mg/l, 28 days) administration on sexual arousal induced by the presence of a receptive female behind the partition in male rats. Time spent at the partition (s) (A) and number of approaches to the partition (B) separating empty compartment and resting testosterone levels (ng/ml) (C) are displayed in open bars, the alterations in behavioral activity and plasma testosterone concentrations after a receptive female introduction are presented in hatched bars. Data are expressed as MEAN±SEM. ## $p<0.01$, ### $p<0.001$ vs. behavioral activity towards empty compartment or resting testosterone levels. *$p<0.05$, ** $p<0.01$ vs. control group.

No significant influence of thyroid function on the weight of testicular and prostate glands was found (Table 2).

Functional activity of 5-HT_{2A} receptors was found to be affected by TH dysfunction ($F(2,17)=5.04$; $p<0.05$). Number of wet dog shakes was higher in hypo- ($p<0.05$) and hyperthyroid ($p<0.05$) rats than in control ones (Fig. 4C).

No differences between groups were revealed in number of 5-HT_{2A} receptor mRNA copies per 100 copies of β-actin mRNA in the frontal cortex of experimental animals ($F(2,18)=1.09$; $p>0.05$) (Fig. 4B).

Table 1
The effect of chronic PTU (50 mg/l, 28 days) and L-thyroxine (T4) (0.5 mg/l, 28 days) administration on the indices of horizontal and vertical locomotor activity in open field test performance in rats

Parameter	PTU	T4	Control	F; p-level
Number of crossed squares	26.45±13.91	40.0±17.24	46.0±13.08	$F(2,25)<1$
Rearings	5.89±3.28	4.3±1.63	6.89±1.69	$F(2,25)<1$

Data are expressed as MEAN±SEM.

4. Discussion

Earlier it was demonstrated that severe hypothyroidism produced by thyroidectomy decreased the expression of 5-HT_{2A} receptors in cortex (Kulikov et al., 2002a). However,

Table 2
The weight of reproductive organs in male rats treated with PTU (50 mg/l, 28 days) and L-thyroxine (T4) (0.5 mg/l, 28 days)

Weight, mg	PTU	T4	Control	F; p-level
Testes	1271.22±96.63	888.9±155.96	1604.22±198.93	$F(2,24)=2.92$; $p>0.05$
Prostate gland	31.11±2.92	35.4±4.5	47.11±5.7	$F(2,24)<1$

Data were analyzed taking into consideration the body weight values as covariables. Each cell represents MEAN±SEM.

mild TH deficit after chronic PTU treatment appeared to be insufficient to affect 5-HT_{2A} receptor mRNA level in frontal cortex. Moderate excess of T4 caused by chronic L-thyroxine administration also failed to modify expression of 5-HT_{2A} receptors. At the same time, both hyper- and hypothyroid rats were characterized by the increased number of DOI-induced wet dog shakes indicating elevation of 5-HT_{2A} receptor functional activity. Thus, even moderate thyroid disturbances were able to alter the functional activity of 5-HT_{2A} receptors. Such unidirectionality in effects of hypo- and hyperthyroidism on 5-HT_{2A} receptors agrees with previous data. Indeed, thyroidectomy (Kulikov et al., 1999) as well as administration of triidothyronine (T3) (Sandrini et al., 1996) was demonstrated to reduce the density of 5-HT_{2A} serotonin receptors in the frontal cortex. The observed increase in functional activity of the receptors may reflect compensatory response to alterations in serotonin synapse induced by thyroid disbalance or the direct posttranslational receptor regulation by TH. The present findings widened the concept of interaction between TH and cerebral 5-HT_{2A} receptors (Kulikov et al., 1999).

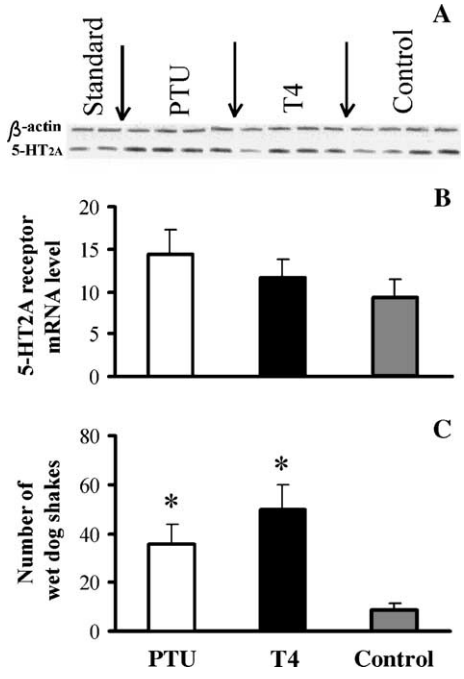

Fig. 4. 5-HT_{2A} receptor mRNA level in the frontal cortex and functional activity in Wistar rats given PTU (50 mg/l, 28 days) and L-thyroxine (T4) (0.5 mg/l, 28 days). A—the negatives of the PCR product bands of external exogenous standard (Standard) and frontal cortex samples of hypo-(PTU), hyper-(T4) and euthyroid (Control) rats; B—the level of 5-HT_{2A} receptor mRNA evaluated by the number of copies per 100 copies of respective β-actin mRNA; C—the number of DOI-induced wet dog shakes reflecting functional activity of cerebral 5-HT_{2A} receptors. Data are presented as MEAN±SEM. *$p<0.05$ vs. control group.

Since cerebral 5-HT_{2A} receptors were known to participate in the regulation of sexual motivation (Popova and Amstislavskaya, 2002), catalepsy (Kulikov et al., 1995, 2002b; Popova and Kulikov, 1995) and acoustic startle reflex (Popova et al., 1999), it might have been expected that the elevated 5-HT_{2A} receptor activity produced by chronic treatment with PTU and T4 would modify these types of behavior.

Sexual arousal is the initial component of sexual behavior in males. While the disturbances in copulatory behavior in rats with congenital (Jiang et al., 2000), pharmacological or thyroidectomy-induced hypothyroidism (Tohei et al., 1998) had been already shown, the effect of experimental thyroid dysfunctions on sexual arousal was first revealed in the present study. The time a male spent at the partition attempting to penetrate to receptive female (partition time) is considered as the main index of sexual motivation (Amstislavskaya and Popova, 2004). Specificity of sexual interest is proved by accompanying increase in the plasma testosterone level in a male exposed to a receptive female. It should be noted that testosterone concentration remained unchanged for the first 10 min and increased only thereafter (Popova and Amstislavskaya, 2002). Thus, the behavioral changes which are observed from the first minutes of interaction reflect genuine sexual motivation associated with activation of neuromediating brain systems triggered by female pheromones, whereas increase in plasma testosterone marks later stage of sexual arousal associated with hypothalamo–pituitary–testicular axis activation (Amstislavskaya and Popova, 2004). We found that both behavioral and hormonal components of sexual arousal were modified by TH dysfunctions. It was shown that PTU-treated and T4-treated males spent significantly less time at the partition in the presence of a receptive female compared to control (untreated) males. The observed decrease in partition time was unlikely to be caused by reduction of total locomotor and exploratory activities as far as pharmacological hypo- and hyperthyroidism did not affect the number of approaches to the partition and the partition time towards empty compartment. Moreover, either PTU or T4 treatment failed to alter the number of squares crossed and the number of rearings in the open-field test. In hypothyroid rats hormonal response on receptive female introduction was significantly blunted compared with untreated control animals. The decreased sexual motivation and the blunted testosterone level in response to receptive female exposure in hypo- and hyperthyroid rats did not seem to result from a direct TH influence on male reproductive system. Indeed, adult male gonad is known to be irresponsive to thyroid hormones (Jannini et al., 1995). Our findings confirmed this supposition. Neither experimental TH deficit nor TH excess modified the resting testosterone level and weight of testes and prostate gland. Therefore, both deficiency and

abundance of TH did produce an attenuation of interest towards the receptive female; additionally, pharmacological hypothyroidism induced a simultaneous decrease in hypothalamo–pituitary–testicular axis activation.

The data obtained suggested the involvement of 5-HT_{2A} receptors in the mechanisms of TH effects on sexual arousal. Truly, both hypo- and hyperthyroid rats exhibited decreased sexual arousal with simultaneous increase in number of wet dog shakes. These findings are consistent with data of other authors indicating reciprocal relations between number of wet dog shakes and sexual behavior expression, namely, chronic treatment with corticosterone inhibited sexual behavior and increased wet dog shakes in rats (Gorzalka and Hanson, 1998) while melatonin facilitated sexual behavior and reduced functional activity of 5-HT_{2A} receptors (Brotto and Gorzalka, 2000).

Catalepsy and startle reflex were sensitive to deficit but not excess of TH. Catalepsy (freezing reaction) in response to lifting the animal into vertical posture may be regarded as a behavioral abnormality, a disturbance of normal avoidance behavior (Kolpakov et al., 2004). Chronic PTU administration produced a significant augmentation of cataleptic animal percentage and duration of cataleptic freezing. This fact agrees with the data of previous experiments showing a close association between TH deficiency and predisposition to catalepsy in rats. Indeed, rats of GC strain selectively bred for 50 generations for high predisposition to catalepsy happened to have a decreased total T4 level (Barykina et al., 2002). Furthermore, thyroidectomy sharply increased predisposition to catalepsy in Wistar rats (Barykina et al., 2002; Kulikov et al., 2002c). These findings taken together provide a strong evidence of the significant role of thyroid hormone deficiency in predisposition to catalepsy in rats. Earlier, chronic T4 treatment was found to decrease catalepsy manifestation in GC rats (Kulikov et al., 2002c). However, in the present study no effect of chronic T4 treatment on catalepsy in Wistar rats was revealed. The increased hormone level failed to produce any reduction in percentage of cataleptics in Wistar rats with weakly expressed catalepsy. Hence, thyroid state seems to be substantial but not the only factor of catalepsy regulation in rats.

In our previous studies an association between TH deficit, 5-HT_{2A} receptor decrease and predisposition to cataleptic freezing was suggested. Rats of GC strain selectively bred for high predisposition to catalepsy were characterized by the decrease in activity (Popova et al., 1985), density (Kulikov et al., 1995; Popova and Kulikov, 1995) and expression (Kulikov et al., 2002b) of brain 5-HT_{2A} receptors. Moreover, a severe hypothyroidism produced by thyroidectomy increased catalepsy (Barykina et al., 2002; Kulikov et al., 2002c) and decreased the receptor density (Kulikov et al., 1999) and expression (Kulikov et al., 2002a). It was supposed that TH deficiency predisposed rats to catalepsy via decreasing the activity of 5-HT_{2A} receptors. The present data did not support this hypothesis. Indeed, pronounced catalepsy in the hypothyroid PTU-given rats was accompanied with the increase in the receptor activity without alteration in the cortical 5-HT_{2A} receptor mRNA level. Although, both PTU-induced hypothyroidism and T4-produced hyperthyroidism activated 5-HT_{2A} receptors, only the former predisposed rats to catalepsy. We can conclude that the interaction between TH, 5-HT_{2A} receptors and catalepsy is more complicated. Catalepsy is also known to be regulated by dopaminergic, cholinergic and GABAergic systems (Klemm, 1989) and further studies should be carried out to evaluate the precise role of these systems and their interaction in the mechanisms of hypothyroidism-induced catalepsy.

PTU treatment diminished significantly the amplitude of startle reflex in rats. This attenuation of startle reflex amplitude in hypothyroid rats did not seem to be caused by the decrease of the body weight. Indeed, a significant reduction of startle magnitude in hypothyroid rats was shown even after correction of the amplitude by body weight with ANCOVA. Moreover, hyperthyroid animals with reduced body weight displayed similar to control rats startle reflex response. Hence, the observed decrease of startle response amplitude did indicate the specific effect of hypothyroid state induced by PTU treatment on this trait. Earlier, it was demonstrated that rats of GC strain with genetically determined hypothyroidism (Barykina et al., 2002) had increased startle reflex amplitude compared to Wistar rats (Popova et al., 1999). It might be hypothesized that the increase in startle reflex amplitude in GC rats was not associated with hypothyroidism. On the other hand, the discrepancy in effects of genetically determined and pharmacologically induced hypothyroidism on startle reflex response may be explained by PTU effects on the auditory system. Neonatal hypothyroidism was also found to reduce the magnitude of startle reflex response, and in that case the effect was caused by hearing loss (Schneider and Golden, 1987; Goldey et al., 1995; Goldey and Crofton, 1998). We cannot completely exclude the possibility that the attenuation in startle reflex response of adult hypothyroid rats might have resulted from the disturbances in auditory system; nevertheless, the absence of differences in prepulse inhibition between groups suggests some other pathophysiological mechanisms. No link between the activation of 5-HT_{2A} receptors in PTU- and T4-treated rats and startle reflex amplitude was found. While both PTU and T4 activated the receptors, only PTU modified startle reflex amplitude.

The open-field test is usually used to study locomotor and exploratory activities. Some authors disclosed an increase of locomotor activity in rats with neonatal (Tamasy et al., 1986; Akaike et al., 1991) or adult hypothyroidism (Fundaro, 1989), while others did not reveal any effect of thyroid hormone deficit on locomotion (Gordon, 1997; Redei et al., 2001; Barykina et al., 2002; Sala-Roca et al., 2002). We found no differences in the indices of vertical (number of rearings) and horizontal (number of crossed squares) activities in the open-field test between control and hypothyroid rats. Hyperthyroid state also failed to produce any significant changes in locomotor and exploratory activities in rats. Therefore, mild adult dysthyroidism accompanied with activation of 5-HT_{2A} receptors does not appear to play the key role in the regulation of locomotion and exploratory behavior in rats, and we can exclude the possibility that the effects observed in other tests

were linked to general alterations in locomotion and exploratory behavior.

Thus, the present data showed that even moderate hypo- and hyperthyroidism were able to increase significantly the activity of 5-HT_{2A} receptors as well as to modify behavior. Good association between the TH dysfunctions, 5-HT_{2A} receptors and sexual arousal in rats was demonstrated. The behavioral effects of T4 excess were less pronounced compared to those produced by the hormone deficit. Although TH deficiency markedly altered catalepsy expression and startle reflex amplitude, no direct link of these traits with 5-HT_{2A} receptors was shown.

Thyroid dysfunctions are very frequent human disorders, and inhibitors of T4 biosynthesis and L-thyroxine are widely used to treat hyper- and hypothyroidism, correspondingly. However, possible consequences of the treatment on behavior and psychic state are poorly investigated. The present study experimentally proves the substantial influence of chronic treatment with T4 synthesis inhibitors as well as exogenous T4 on behavior and attracts particular attention to possible undesirable effects on patient behavior and psychic functions.

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