GERONTOLOGY

The Role of the Serotoninergic System in the Regulation of Thyroid Function in Old Rats

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The effect of serotoninotropic substances with positive and negative effects on serum concentrations of thyrotropic hormone, total triiodothyronine, and total thyroxin was studied in old male rats. Chronic treatment with L-tryptophan and citalopram reduced serum level of total thyroxin in old intact animals and serum concentration of thyrotropic hormone in old thyrectomied rats. DL-p-chlorophenylalanine increased the serum concentrations of total thyroxin in old intact rats and in thyroidectomied animals treated with triiodothyronine. Chronic methysergide therapy was associated with reduction of serum concentration of thyrotropic hormone in old intact and old thyroidectomied rats and of total triiodothyronine in old intact animals.

Key Words: *thyroid hormones; hypothalamic—pituitary—thyroid system; serotonin; enzyme immunoassay*

Close interactions between the serotoninergic neurotransmitter and hypothalamic—pituitary—thyroid systems were recently demonstrated. It was reported that serotonin (5-HT) is involved in the regulation of the hypothalamic—pituitary—thyroid system [6]. Thyroid hormones, in turn, modulate serotonin synthesis and turnover. Experimental hypothyrosis in rats is associated with reduction of 5-HT concentration in the hemispheric cortex, midbrain, and diencephalon [11] and with an increase of its turnover in the hippocampus [4]. Injection of thyroid hormones to rats with hypothyrosis and to euthyroid animals led to an increase of cortical 5-HT concentration [8]. In addition, thyroid hormones modify sensitivity and expression of 5-HT receptors [4].

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Hence, changes in the functioning of the serotoninergic neurotransmitter system can lead to changes in the production of thyrotropin releasing hormone (TRH), thyrotropic hormone (TTH), and thyroid hormones. Dysfunction of the hypothalamic—pituitary—thyroid system, in turn, can involve disorders in the serotoninergic neuronal transmission.

We studied the impact of serotoninotropic substances with positive and negative effects for the serum concentrations of TTH and thyroid hormones in old male rats.

MATERIALS AND METHODS

The study was carried out on 150 old male Wistar rats (400-450 g; 22-24 months) from Rappolovo Breeding Center. The animals were kept at standard day/night regimen on standard diets. Experimental hypothyrosis was induced by total thyroparathyroidectomy by the standard method. All thyroidectomied (TE) rats daily

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received 10% calcium chloride solution throughout the experiment. The postoperative period was 14 days.

The following preparations were used in the study: triiodothyronine (T_3; Berlin-Chemie AG), injected intraperitoneally to TE rats in a daily dose of 70 μg/kg for 14 days (first as monotherapy, then in combination with serotoninotropic agents); 5-HT precursor L-tryptophan (Ferrak) in a daily dose of 100 mg/kg intraperitoneally for 7 days; selective inhibitor of 5-HT re-uptake citalopram (cipramyl; H. Lundbeck Company) in a daily dose of 4 mg/kg intraperitoneally for 10 days; 5-HT receptor antagonist methysergide (Sandoz) in a daily dose of 1 mg/kg intraperitoneally for 7 days; 5-HT synthesis inhibitor DL-p-chlorophenylalanine (DL-p-CPA; Sigma) injected intraperitoneally daily for 5 days (300 mg/kg on day 1 followed by maintenance doses of 100 mg/kg). Controls were injected with saline in an equivalent volume.

In order to evaluate the effects of serotoninotropic substances on the hormonal status under conditions of experimental thyroid disease, the animals were divided into groups of 10-12 rats. Group 1 comprised intact rats injected with saline throughout the experiment (control 1). Group 2 consisted of intact rats injected with saline for 7 days and then a serotoninotropic agent in the above dose. Group 3 included TE rats injected with saline (control 2). Group 4 were TE rats receiving T_3 throughout the experiment. Group 5 were TE rats injected with saline for 7 days and then a serotoninotropic agent. Group 6 were TE rats injected with T_3 daily for 7 days and then T_3 in combination with a serotoninotropic agent in the above doses.

After decapitation, the blood was collected from the cervical vessels into clean tubes and centrifuged at 3000 rpm (15 min). Serum concentrations of T_3, total thyroxin (T_4), and TTH were measured by solid phase EIA with commercial kits (Vector-Best). Optical density was measured on an AIFR 01 enzyme immunoassay analyzer (PIKON). Before the analysis, the reagents were titered in order to select the optimal dilution of the serum. The concentration of the antigen in the samples was calculated using calibration curves.

The data were statistically processed using SPSS 12.0 for Windows software by ANOVA unifactorial analysis of dispersions [2]. The differences were statistically significant at $p<0.05$.

RESULTS

Thyroidectomy was associated with reduction of serum concentrations of total T_3 ($p<0.01$), total T_4 ($p<0.01$), and an increase of TTH ($p<0.01$) in comparison with the control (Table 1). Chronic treatment of TE rats with T_3 led to a reduction of serum TTH concentration ($p<0.05$; Table 2).

Serum concentration of total T_4 decreased in intact rats injected with L-tryptophan ($p<0.01$) in comparison with control group 1 (Table 1). Chronic treatment with L-tryptophan led to a reduction of blood TTH content in intact animals ($p<0.05$). L-Tryptophan reduced serum concentration of TTH in TE rats ($p<0.01$) in comparison with control group 2 and did not modify the hormonal status of TE rats treated with T_3 (Table 2).

Chronic treatment of intact rats with citalopram reduced serum concentration of total T_4 ($p<0.05$) *vs.* control group 1 (Table 1). The levels of total T_3 and TTH in intact animals treated with citalopram did not differ from the values in control group 1. Citalopram reduced serum TTH concentration in TE rats ($p<0.05$) in comparison with control group 2 and did not modify TTH level in the blood of TE rats treated with T_3 (Table 2).

Treatment with DL-p-CPA increased serum concentrations of total T_4 ($p<0.01$) and TTH ($p<0.05$) in intact rats in comparison with control group 1 (Table 1). The levels of total T_3, total T_4, and TTH in TE rats treated with DL-p-CPA did not differ from those in

TABLE 1. Serum Concentrations of Thyroid Hormones in Old Rats Treated with Serotoninotropic Substances (M±m)

Group	Serum hormone concentrations		
	Total T_3, ng/ml	Total T_4, ng/ml	TTH, mU/liter
Intact (control 1)	1.25±0.09	79.88±4.42	0.91±0.12
Intact+L-tryptophan	1.58±0.13	50.13±1.89*	0.43±0.13*
Intact+citalopram	1.29±0.15	52.28±9.77*	0.92±0.14
Intact+DL-p-CPA	1.53±0.14	107.08±7.12*	1.58±0.15*
Intact+methysergide	0.84±0.04*	154.56±5.05*	1.02±0.11

Note. *$p<0.05$ compared to control group 1.

control group 2 (Table 2). Chronic treatment of TE rats receiving T_3 with DL-p-CPS was associated with an increase of serum TTH concentration ($p<0.05$).

Serum concentrations of total T_3 and TTH reduced ($p<0.05$ and $p<0.05$, respectively) in intact animals during chronic methysergide therapy in comparison with control group 1 (Table 1). Methysergide reduced serum TTH concentration in TE rats in comparison with control group 2 (Table 2). No appreciable differences in the hormonal status of TE rats receiving a combination of T_3 and methysergide and those receiving T_3 monotherapy were detected.

Hence, serotonin precursor L-tryptophan and selective inhibitor of serotonin re-uptake citalopram reduced total T_4 concentration in the serum of intact rats and serum concentration of TTH in TE rats. In addition, L-tryptophan reduced serum TTH concentration in intact animals. Serotonin synthesis inhibitor DL-p-CPA elevated the serum concentrations of total T_4 in intact animals. The level of TTH increased in response to DL-p-SPA in the sera of intact and TE rats treated with T_3. Chronic treatment with 5-HT receptor nonselective antagonist methysergide was paralleled by a reduction of the serum concentration of TTH in intact and TE rats and of total T_3 level in intact animals.

The therapeutic effect of L-tryptophan is due to the increase of serotonin content in the brain. On the other hand, it was shown that 5-HT is involved in the regulation of the hypothalamic—pituitary—thyroid system functioning by inhibiting the TRH secretion [6,12]. Reduction of TRH release by the positive feedforward mechanism inhibits the production of TTH by the anterior pituitary lobe β-cells with subsequent reduction of thyroid hormone synthesis in thyrocytes. The effect of L-tryptophan on activity of deiodinase-2 (highly sensitive to treatments causing changes in neuronal activity [5,7]) is also possible. The reduction of serum T_4 concentration during treatment with the above serotoninergic agent in this case can be caused by stimulation of T_4 transformation into T_3 in the CNS. The mechanism underlying the effect of citalopram on serum levels of TTH and thyroid hormones in rats is presumably similar to the effect of L-tryptophan.

Importantly that L-tryptophan and citalopram caused more significant changes in serum concentrations of TTH in animals with hypothyrosis and did not change the hormonal status of TE rats treated with T_3. Hence, the effects of these serotoninergic drugs depended on the initial hormonal status of animals.

The increase of serum TTH concentration during DL-p-CPA treatment was presumably due to leveling of the negative effect of serotonin on the secretory activity of TRH-synthesizing neurons in the hypothalamus and presumably of the anterior pituitary β-cells. On the other hand, treatment with this drug was also associated with a drop of dopamine concentration in the brain [9] (it is known that dopamine reduces TTH concentration in the pituitary, modulating the thyrortropocytes through type 2 dopamine receptors [2]). In addition, this neurotransmitter stimulates the release of somatostatin inhibiting TTH secretion [2]. It was found that secretion of TRH decreased under the effect of dopamine [10]. Hence, the stimulatory effect of DL-p-CPA on TTH secretion can be caused by reduced synthesis of 5-HT and by reduction of dopamine concentration in the brain of rats of different age. The effect of this serotoninergic agent on activity of TRH-producing neurons is also possible.

The described effects of methysergide can be explained by its effect on the serotoninergic and dopaminergic systems. The dopaminergic neurotransmission is stimulated in response to methysergide treatment. An increase of dopamine concentration in response to this drug can be explained by canceling of the inhibitory effect of 5-HT on the secretory activity of dopaminergic neurons. On the other hand, it is known that dopamine reduces TTH secretion by the anterior pituitary lobe β-cells [2,10]. The reduction of total T_3 concentration in the blood of intact animals in response to methysergide can be also caused by its effect on the rate of 5'-deiodination of T_4 in the CNS and/or peripheral tissues.

Comparison of our findings with previous data demonstrates the selective nature of the effects of various therapeutic combinations in animals of different age groups. According to a previous report [3], chronic citalopram treatment of young animals led to

TABLE 2. Serum Concentrations of TTH in TE Rats Treated with Serotoninotropic Drugs (*M*±*m*)

Group	TTH, mU/liter
TE (control 2)	2.69±0.23
TE+T_3	0.55±0.15*
TE+L-tryptophan	1.40±0.36*
TE+T_3+L-tryptophan	0.61±0.12*
TE+citalopram	1.48±0.11*
TE+T_3+citalopram	0.51±0.12*
TE+DL-p-CPA	2.90±0.35
TE+T_3+DL-p-CPA	1.03±0.09*+
TE+methysergide	1.33±0.29*
TE+T_3+methysergide	0.53±0.17*

Note. $p<0.05$ compared to *control group 2, +TE+T_3.

a reduction of serum TTH concentration. On the other hand, in our study citalopram caused no changes in serum TTH level. Hence, we can speak about more pronounced effect of citalopram on functional activity of the hypothalamic—pituitary—thyroid system in young *vs.* old animals.

Chronic methysergide treatment of old intact animals was associated with reduction of total T_3 and increase of total T_4 concentrations in the serum. Previous studies have demonstrated that methysergide treatment of young animals caused no appreciable changes in the serum concentrations of thyroid hormones [4]. In old animals methysergide treatment caused no statistically significant shifts in TTH levels. On the other hand, all scientists note a reduction of serum TTH concentrations in young intact rats in response to this drug [3]. The decrease of TTH concentration under the effect of methysergide was observed in old TE rats but not in young TE rats, as was shown by the same team of scientists [3]. Changes in serum concentrations of T_3 and T_4 in response to methysergide in old animals exclusively can indicate a more pronounced effect of this drug on the rate of 5'-deiodination in rats of this age group. The reduction of total T_3 concentration in parallel with an increase of total T_4 level can indicate inhibition of 5'-deiodinase-1 and/or -2 under the effect of methysergide.

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