Effects of Polyphenol Compounds from *Alchemilla vulgaris* on Morphofunctional State of Thyroid Gland in Rats Exposed to Low Temperature

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In control rats and animals receiving polyphenol compounds extracted from *Alchemilla vulgaris,* intense cooling stimulated synthesis of thyroid hormones and promoted their peripheral deiodination. In control rats, the period of recovery after cooling (readaptation period) was characterized by a compensatory reduction in thyroid function. In rats receiving test preparation, this period was characterized by significant activation of thyroid hormone synthesis and by further growth of reserve follicles that appeared during the cooling.

Key Words: *polyphenol compounds; thyroid gland; morphometry; thyroid hormones; cooling*

Negative anthropogenic factors of modern life reduce individual and population health capacities, increase the incidence of specific pathology, and contribute to the development of new diseases caused by ecological stress [1]. Treatment with plant preparations is one of the most efficient ways of increasing body resistance [4-7,9]. Active components of many phytopreparations are represented by polyphenol compounds (PPC), characterized by a wide spectrum of biological activities and low toxicity [2]. Neurohumoral mechanisms and, in particular the effects of thyroid hormones play an important role in increasing the body resistance to nonspecific stress factors [5].

In this study we investigated the effects of PPC extracted from the overground part of *Alchemilla vulgaris* on the functional activity of the thyroid gland in rats exposed to extreme cooling.

MATERIALS AND METHODS

The PPC preparation from the overground part of *Alchemilla vulgaris* was prepared in the Laboratory of Phytochemistry of the Central Siberian Botanic Garden, Russian Academy of Sciences.

Experiments were carried out on mature male Wistar rats weighing 180-220 g. Experimental rats received 10 mg/kg of the preparation daily as an aqueous solution administered through an intragastric tube. Control rats received the same volume of water. After 7 days the rats were subjected to daily 22-h cooling at -10°C for 7 days and decapitated after the last session. In parallel, treated and untreated rats were returned to home cages after cooling and kept at 20-22°C for the next 7 days without treatment (recovery period, readaptation).

The left TG lobe was fixed with Tellesnitskii fluid and embedded in histoplast. Serial 7-μ sections were stained with hematoxylin and eosin. The structural components of TG were measured by point-counting system and their absolute volumes were calculated [3].

The right TG lobe was homogenized in 1.5 ml 0.01 M phosphate buffer and centrifuged for 20 min at 3000 rpm. The contents of thyroxin (T_4), triiodothyronine (T_3), and thyroglobulin in the supernatant and serum were measured by radioimmunoassay using RIA-T4-ST, RIA-T_3-ST (Beloris), and Rio-TG-^{125}I (Institute of Bioorganic Chemistry, Belarus) kits, respectively.

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RESULTS

Two-week treatment with *Alchemilla vulgaris* preparation elevated the blood content of T_4 (Fig. 1, *a*), which could be explained by enhanced incretion of the hormone into circulation rather than its activated synthesis, since the content of T_4 in TG was reduced (Fig. 1, *b*). Activated secretion was confirmed by morphological changes: more than 2-fold decrease in the volume of the colloid in TG follicles and moderate dilation of surrounding capillaries and interlobular venules (Fig. 2). The lowered contents of T_3 and thyroglobulin in the TG (Fig. 1, *b*) also imply a tendency for a decrease in synthetic activity. Interfollicular epithelial islets were enlarged in comparison with the control (Fig. 2) and more mitoses were seen in these structures.

As demonstrated by both morphological and functional changes in TG (Fig. 1, *b*), extreme cooling activated the synthesis of hormones in control rats, which was accompanied by a sharp reduction of T_4 content in the blood and an increase in the T_3:T_4 deiodination ratio (Fig. 1, *a*). These findings agree with previously reported data [10].

In rats receiving PPC from *Alchemilla vulgaris*, extreme cooling also lowered the blood level of T_4 and increased the deiodination ratio to a greater extent than in control rats (Fig. 1, *a*). The content of thyroid hormones and thyroglobulin in the TG increased in comparison with the baseline values (Fig. 1, *b*). Therefore, PPC also stimulated synthesis and peripheral deiodination of thyroid hormones. Morphometric analysis revealed a decrease in the volume of TG and its follicles and a slight dilation of the vascular bed (Fig. 2). A small quantity of colloid was found between epitheliocytes in the center of interfollicular islets, which indicate the presence of synthetic activity in the thyroid epithelium and the formation of new follicles.

It can be suggested that cooling-induced activation of the thyroid hormone synthesis in experimental animals resulted from acceleration of synthetic processes with the involvement of reserve structures rather than from functional hypertrophy of secretory epithelium. The newly-synthetized hormones were not stored but rapidly increted into the circulation, as evidenced by the low content of colloid in TG follicles (Fig. 2).

In control rats, blood content of T_4 remained low during readaptation. This period was also characterized by slow peripheral deiodination (Fig. 1, *a*) and reduced TG synthetic activity (Fig. 1, *b*). These shifts are typical of the stress-response phase, when compensatory relaxation of previously overstrained endocrine mechanisms occurs after abolition of the stress-inducing factor [8]. In rats receiving *Alchemilla vulgaris* preparation, readaptation was accompanied by significant activation of hormone synthesis as demonstrated by elevated contents of hormones in the TG (Fig. 1, *b*), by increased volumes of TG, follicles, follicular epithelium, and vascular bed, and by further growth of reserve follicles formed during cold exposure (Fig. 2). Peripheral deiodination of T_4 was suppressed (Fig. 1, *a*), which can be explained by reduced demand for stimulating effects of thyroid hormones (especially T_3, physiologically more active hormone) after cessation of cooling.

The question arises, why functional strain persisted in experimental rats for a long time, while in control animals TG function decreased one week after

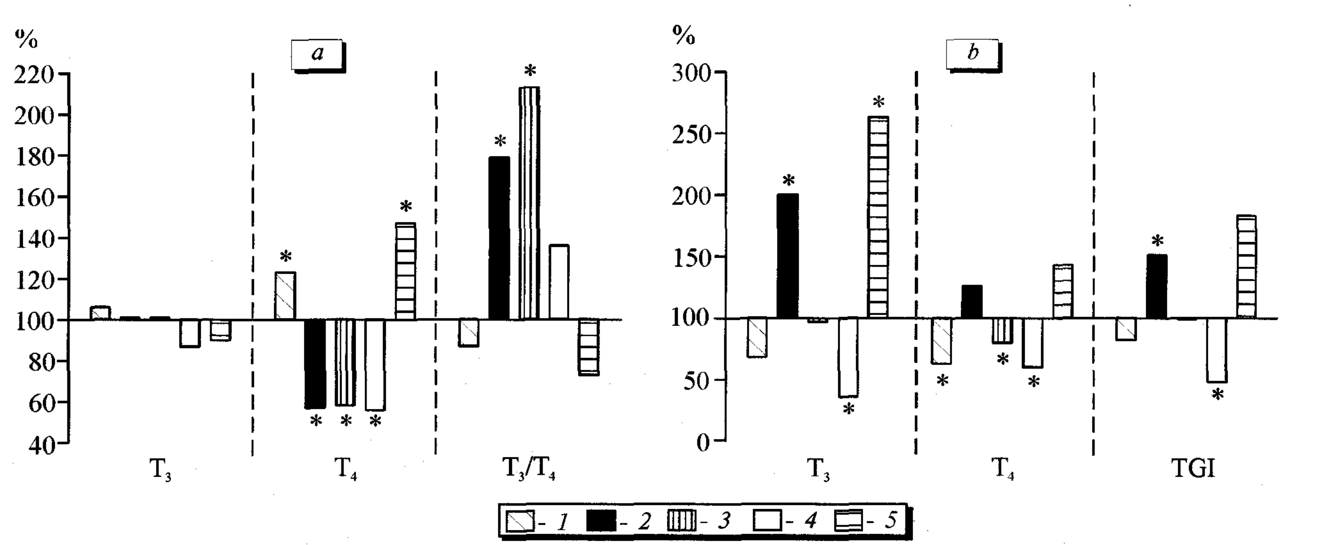

Fig. 1. Changes in the contents of thyroid hormones and thyroglobulin in the blood (*a*) and thyroid gland (*b*) of experimental rats expressed as percentage of control values (100%). T_3, triiodothyronine; T_4, thyroxin; TGI thyroglobulin. Here and in Fig. 2; *1*) *Alchemilla vulgaris*; *2*) cold; *3*) *Alchemilla vulgaris*+cold; *4*) readaptation; *5*) *Alchemilla vulgaris*+readaptation; *$p<0.05$ in comparison with the control.

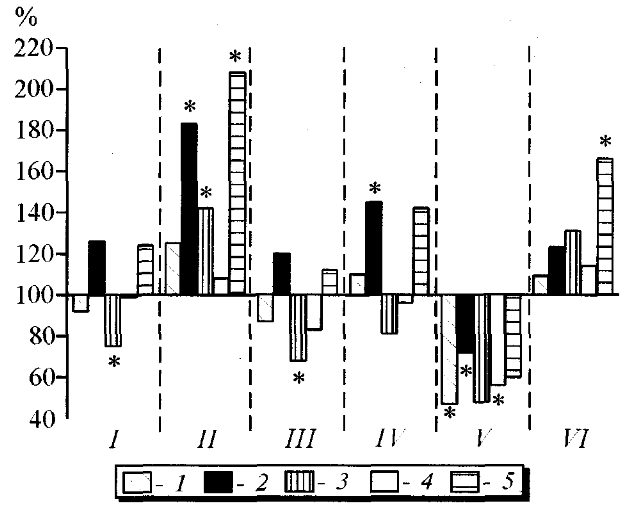

Fig. 2. Structural changes in the thyroid gland of experimental rats expressed as percentage of control parameters (100%). *I*) left lobule of the thyroid gland; *II*) interlobular blood vessels; *III*) follicles; *IV*) follicular epithelium; *V*) colloid; *VI*) interfollicular epithelium.

cessation of cooling. Our findings suggest that PPC from *Alchemilla vulgaris* activate proliferative processes in the interfollicular islets consisting of poorly differentiated thyreocytes and representing a reserve compartment of TG. During cold exposure these reserve structures are mobilized and participate in the thyroid hormone synthesis, thus optimizing functional strain. These morphofunctional changes persisted for a long time after cold exposure improving the functional capacities of TG.

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