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Response of Pituitary—Thyroid Axis to a Short-Term Shift in Deuterium Content in the Body

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We studied functional changes in rat pituitary—thyroid axis after a short-term shift in deuterium body content. Male Wistar rats consumed deuterium-enriched (500,000 ppm) or deuterium-depleted water (10 ppm) for 24 h. Rats of both experimental groups demonstrated elevated concentration of bound with transport proteins thyroxine and reduced level of thyroid-stimulating hormone in serum. No changes in the rate of thyroxine conversion to triiodothyronine were found. Thus, both the increase and reduction of deuterium body content produced similar changes in the function of the pituitary—thyroid axis with primary affection of the thyroid gland, indicative of its higher sensitivity to shift in deuterium levels.

Key Words: *thyroid; pituitary gland; deuterium; protium; thyroid hormones*

Deuterium is an abundant natural stable isotope. Deuterium content in organism mammals and humans exceeds the levels for many macroelements, such as potassium, sodium, and calcium [7]. The role of this hydrogen isotope in metabolism remains poorly understood. The most studied aspects are changes in the cell cycle, the rates of proliferation and apoptosis induced by shifts in deuterium content *in vitro* and *in vivo*, however, the mechanisms of these changes are not clear, and the data are often contradictory [4,5,9]. It is known that deuterium forms stronger chemical bonds with other biogenic atoms than protium, which, in turn, slows down chemical reactions and increases resistance of biopolymers, primarily proteins, to denaturation and decomposition [6]. Thus, changes in the deuterium and protium body content should affect the rate of anabolism and catabolism. Changes in function of endocrine glands produced by altered concentration of deuterium and protium in the internal environment of the body has not been actually studied.

The aim of the research was to assess pituitary—thyroid axis functioning after short-term changes in deuterium body content.

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MATERIALS AND METHODS

The experiment was performed on Male Wistar rats weighing 340-370 g were enrolled in the investigation. The rats were randomized into three groups: the control group (n=7) consumed distilled water with deuterium concentration [D]=146 ppm *ad libitum*, which corresponds to the deuterium content in tap water; the group consumed deuterium-enriched water [D]=500,000 ppm (n=6) manufactured by B. P. Konstantinov St. Petersburg Nuclear Physics Institute of National Research Center Kurchatov Institute; and the group consumed deuterium-depleted water [D]=10 ppm (n=6), manufactured by individual entrepreneur Selivanenko for 24 h. The deuterium content in distilled water and deuterium-depleted water was determined by T-LWIA-45-EP isotope analyzer (Los Gatos Research Inc.) that allows measuring deuterium content with an accuracy of 1 ppm. The amount of water consumed by rats for 24 h was measured, and the volumes of consumed water per 100 g of body weight were calculated. Serum concentrations of total and free thyroxine (T4 and fT4), total and free triiodothyronine (T3 and fT3), thyroid stimulating hormone (TSH) and thyroid hormone uptake were determined by ELISA (BioVendor, Monobind).

The data were processed using Statistica 7.0 software (StatSoft, Inc.). Normally distributed quantitative

data were expressed as mean. The relative values for the parameters of the thyroid status were calculated taking the mean values of the control group as 100%. Comparison of The groups were performed using one-way ANOVA test. The differences were significant at $p<0.05$.

RESULTS

The volumes of water consumed per unit of body weight for 24 h in rats of the experimental groups did not differ from the control (Fig. 1).

The rats that consumed deuterium-enriched water for 24 h showed an increase in the T4 concentration. The T3 level did not change. Some changes in the concentration of free fractions of thyroid hormones were also observed. The content of fT3 in the systemic circulation decreased, while fT4 did not undergo significant changed. The rate of thyroid hormone uptake did not differ from the control values. TSH concentration has been significantly reduced (Fig. 2).

In rats that consumed deuterium-depleted water, an increase in the concentration of total T4 and a decrease in the concentration of its free fraction were revealed. The content of T3 and fT3 in the bloodstream did not significantly changes. The rate of thyroid hormones uptake did not differ from the control values. The TSH concentration was also decreased (Fig. 2). No significant differences from the corresponding values of the group that consumed deuterium-enriched water were found.

The results of the study showed that the intake of water with different deuterium content corresponded to the parameters of the normal water intake of Wistar rats, which, as is known, exceeds their circulating blood volume [8]. Therefore, such an exposure should have led to a rapid shift in the deuterium content in the systemic circulation. It was shown that the consumption of water with a changed hydrogen isotopes content produces more pronounced shifts in their concentration in plasma than in inner organs [1]. The thyroid status of rats exposed to increase of deuterium body content indicates an up-regulation of the hormones production by the thyroid gland. A decrease in the level of TSH proves that these changes are due precisely to the reaction of the thyroid gland to the increased deuterium content in the internal environment of the body. An absence of increase in the T4 to T3 conversion indicates a lower sensitivity of peripheral deiodinases, primarily type I and II deiodinases, to an increase in the deuterium content in the organism [3]. Analysis of the thyroid status of rats subjected to a decrease in the deuterium body content revealed a similar development of primary hyperfunction of the thyroid gland without a decrease in the conversion of

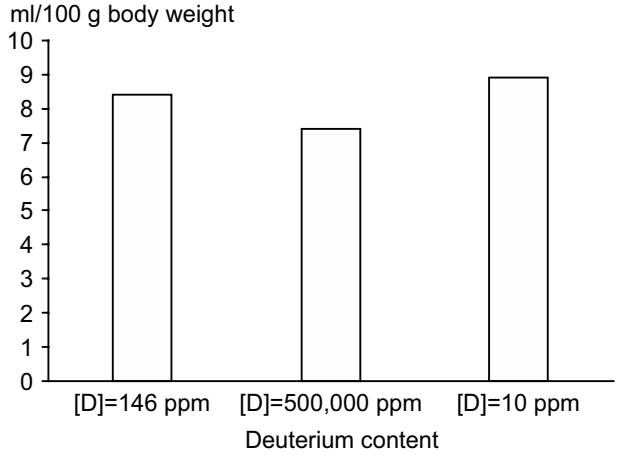

Fig. 1. Consumption of water with different deuterium concentration by rats over 24 h.

T4 to T3. Comparison of changes in the functioning of the pituitary—thyroid axis after increase and decrease in the deuterium body content found that differences are insignificant and associated with changes in the concentration of unbound fractions of T4 and T3. However, the uptake of thyroid hormones, reflecting the binding capacity of blood proteins, did not undergo changes, indicating that alterations in the thyroid status are primarily associated with functional activity of the thyroid follicular epithelium. The thyroid hormones production includes thyroglobulin synthesis and iodination, which requires high activity of redox processes and energy expenditure. It has been reported that changes in the concentration of deuterium *in vitro* does not affect the production of superoxide anion by complexes I, II, and III of the hepatocyte mitochondria respiratory chain, but reduces the efficiency of phos-

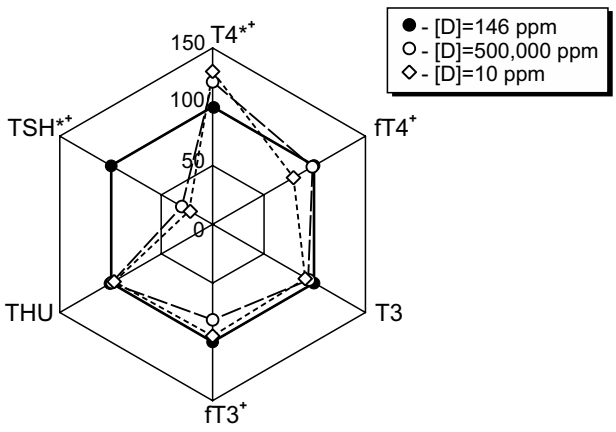

Fig. 2. Changes in concentration of thyroid hormones, TSH, and thyroid hormones uptake (THU) in rats drinking water with different deuterium concentration for 24 h. $p<0.05$ in comparison with the control: *for [D]=500,000 ppm group; +for [D]= 10 ppm group. The values in the control group ([D]=146 ppm) are taken as 100%.

phorylation [2]. At the same time, other researchers have demonstrated that an increase in the deuterium content in culture media of tumor cell lines shows its antioxidant, and a decrease, respectively, prooxidant activity [10,11]. Therefore, the opposite effects of increasing and decreasing the deuterium content on the activity of follicular thyrocytes may be assumed. However, our results show the presence of dualism, that is, the development of similar effects by opposite exposures.

Thus, a short-term increase and decrease in the deuterium body content led to similar changes in the functioning of the pituitary—thyroid gland axis with an increase in thyroxine production and a decrease in the secretion of TSH. These changes show that the thyroid gland is most sensitive to the shift in the balance of protium and deuterium part of the thyroid axis. An increase in the production of T4 without changes in its conversion to T3 indicates that short-term shifts in the balance of protium and deuterium do not affect the activity of deiodinases.

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