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Altered Thyroid Hormone Production Induced by Long-Term Exposure to Low Doses of the Endocrine Disruptor Dichlorodiphenyltrichloroethane

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Abstract—Endocrine disruptors are exogenous substances that enter the body and exhibit hormone-like action thus disrupting the homeostatic action of endogenous hormones. The most wide-spread disruptor is dichlorodiphenyltrichloroethane (DDT). The aim of this study was to investigate changes in thyroid hormone production induced by prolonged exposure to low doses of DDT. The experiment was performed on male Wistar rats treated with daily doses of DDT 1.89 ± 0.86 μg/kg and 7.77 ± 0.17 μg/kg for six and ten weeks. After six weeks there was a dose dependent increase of serum total thyroxine, total triiodthyronine, and thyroid peroxidase. Subsequently, concentration of free thyroxine decreased. These data indicate that impaired production of thyroxine by follicular thyrocytes, rather than decreased deiodinase activity and blood transport proteins is the major cause of altered thyroid status induced by DDT.

Keywords: endocrine disruptors, thyroid gland, thyroid hormones, DDT

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INTRODUCTION

Endocrine disruptors are exogenous substances found in soil, water, air, food and some industrial products, which enter the body and exhibit hormone-like effects, which impair homeostatic mechanisms of regulation by endogenous hormones of vitally important processes in living organisms. It is known that certain endocrine disruptors can persist in the environment for a long time and even accumulate in living organisms, constantly affecting them and disrupting their hormonal regulatory mechanisms. One of the wide-spread endocrine disruptors existing in the environment is the pesticide dichlorodiphenyltrichloroethane (DDT). During many years DDT was widely used as an effective tool against transmitters of malaria, typhus and pests with norms of soil treatment of 0.3–2.0–11.2 kg/hectare. It is believed that under normal conditions DDT in soil can persist for up to 12 years. However, the process of its natural decomposition is accompanied by formation of some metabolites, which exhibit the biological action even higher than that of DDT. During repeated treatment with DDT the concentration of its metabolites in the soil can persist up to 20 years [1]. After a long ban, in 2006, the World Health Organization issued the permission to use DDT as an insecticide to control malaria transmitters in Africa and Asia. It is well known that various concentrations of DDT are detected in the seas and oceans, soil; it is accumulated in various products of plant and animal origin. During prolonged use of foodstuff containing DDT, it can accumulate in different amounts in human and animal organs: liver, brain, thymus, testes, and to a greater extent in adipose tissue [2, 3].

The study of the disrupting action of DDT on the functioning of the endocrine glands is one of the urgent problems of modern medical science. The International Society of Endocrinology repeatedly expressed the need to study the relationship between changes induced by disruptors at the molecular level, and the development of pathological changes and clinical manifestations of endocrine disorders [4]. The complexity of this kind of research is related to the need of the use of very low doses of the disruptor in vivo, as the use of higher doses results in the development of toxic effects, complicating identification of the molecular mechanisms mediating the DDT action as an endocrine disruptor.

One of the least studied aspects of the problem is the effect of low doses of DDT on the metabolism of thyroid hormones.

The aim of this study was to identify changes in thyroid hormone production during prolonged exposure to low doses of the endocrine disruptor DDT.

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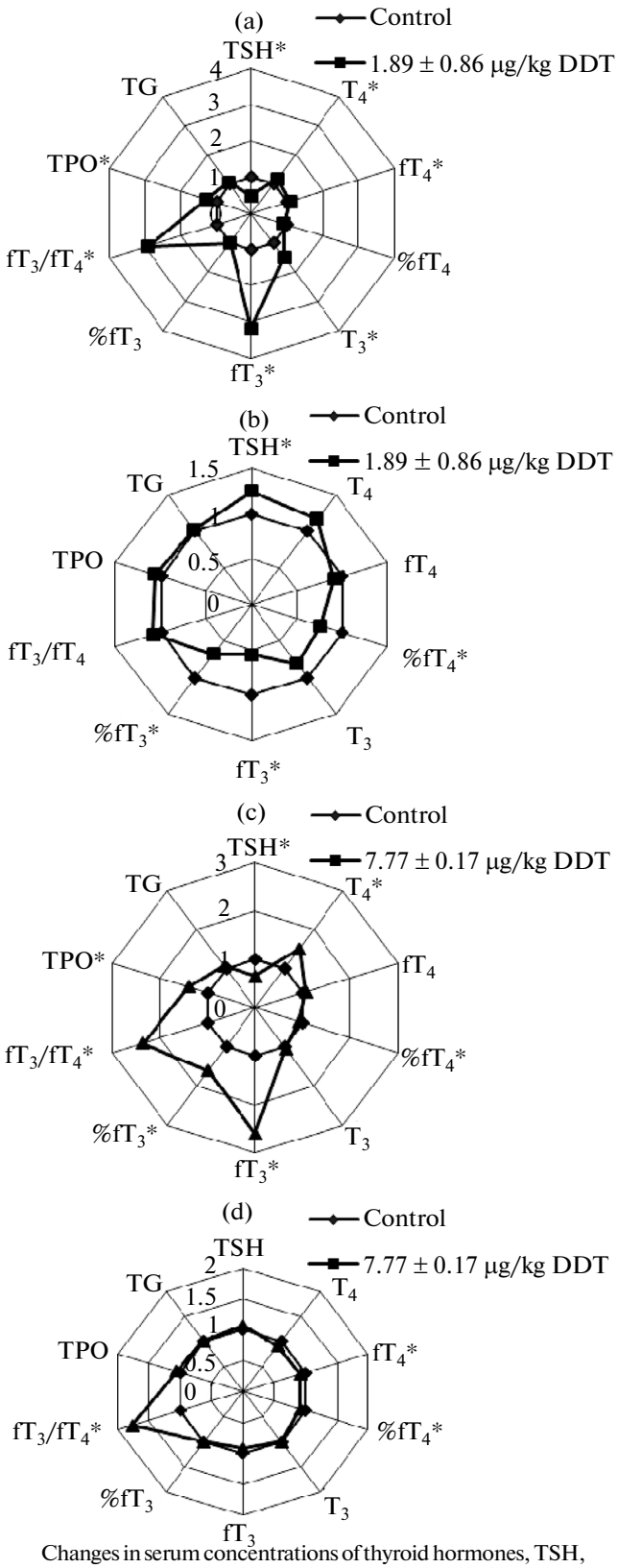

Changes in serum concentrations of thyroid hormones, TSH, thyroperoxidase (TPO), and thyroglobulin (TG) in rats treated with DDT at the daily dose of 1.89 ± 0.86 μg/kg for 6 weeks (a) and 10 weeks (b) and with DDT at the daily dose of 7.77 ±0.17 μg/kg for 6 weeks (c) and 10 weeks (d). Values in the control group were defined as one unit; the asterisk (*) shows statistically significant changes versus control group.

MATERIALS AND METHODS

Male Wistar rats ($n = 64$) weighing 80–100 g were used in experiments. The consumed doses of DDT was calculated using the requirements of National Toxicology Problem (USA) [5] to the definition of low-dose determination, taking into consideration the thresholds for the low doses of DDT (50 μg/kg/day) [6] and the regulations of the DDT content in food products in the Russian Federation [7]. The experimental animals received (instead of water) the solutions [*o*,*p*]-DDT (Sigma, USA) at a concentration of either 20 μg/L ($n = 22$) or 80 μg/L ($n = 20$) for 6 and 10 weeks. The average daily intake of DDT was 1.89 ± 0.86 μg/kg and 7.77 ± 0.17 μg/kg, respectively. The control animals ($n = 20$) received tap water. The absence of DDT, its metabolites, and related organochlorine compounds in tap water and chow of the laboratory animals was confirmed by gas-liquid chromatography. The first half of the experimental and control animals were taken from the experiment after 6 weeks, and the second half after 10 weeks by zoletil overdose. The experiment was conducted in accordance with the rules of the work with experimental animals, approved by order of the USSR Ministry of Public Health no. 577 from 08.12.1977. Concentrations of serum thyroid stimulating hormone (TSH), total thyroxine (T_4), free thyroxine (fT_4), total triiodothyronine (T_3), free triiodothyronine (fT_3), thyroperoxidase (EC 1.11.1.8) and thyroglobulin were detected by means of the enzyme-linked immunosorbent assay based on monoclonal antibodies available in the commercial kits (Cusabio Biotech, China, Monobind, USA). Also we calculated proportions of free T_4 and T_3 forms in the total concentration of T_3 and T_4 (fT_4% and % fT_3) and the fT_3/fT_4 ratio.

Statistical treatment was performed using the Statistica 7.0 package (Statsoft Inc., USA). The central tendency and dispersion of quantitative traits, with approximately normal distribution, were presented as the mean and standard error of the mean. On their basis, we recalculated the absolute values to the relative ones, defining the values in the control group as one unit. Comparison of independent groups by variables was performed using Student *t*-test taking into consideration the Levene's test on equality of variances, and the Mann–Whitney test. Differences were considered as statistically significant at $p < 0.01$.

RESULTS AND DISCUSSION

After 6 weeks of DDT consumption at the daily dose of 1.89 ± 0.86 μg/kg the treated rats were characterized by changes in serum concentrations of thyroid hormones and TSH (figure, a). There was increased production of T_4 by follicular thyrocytes, as evidenced by elevation of total ($p = 0.019$) and free ($p = 0.045$) T_4 concentrations in the systemic circulation. The proportion of fT_4 remained unchanged compared to the

values of the control group. Increased production of T_4 by follicular thyrocytes occurred under conditions of increased concentration of serum thyroperoxidase, one of the key enzymes involved in the processes of iodine organification in thyroid cells ($p = 0.000002$) (figure, a). We did not observe any statistically significant increase in the concentration of thyroglobulin in the systemic circulation (figure, a). However, there was a significant increase in the concentration of total ($p = 0.023$) and free T_3 ($p = 0.000001$). Enhanced production of thyroid hormones was expectedly accompanied by a decrease in the secretion of pituitary TSH.

After 6 weeks of DDT consumption at the daily dose of 7.77 ± 0.17 μg/kg similar changes in the T_4 production by follicular thyrocytes ($p = 0.0034$) were found (figure, c). However, in contrast to the treatment with the lower dose, the content of T_3 bound to blood albumin and globulins insignificantly differed from the control group, while the concentration of free T_3 was higher than in the control group ($p = 0.00001$), but was lower than in the compared group ($p = 0.0060$). As in the previous group, there was an imbalance in the ratio of fT_3 and fT_4 in the direction of fT_3 prevailing. Another difference consisted in the higher content of serum thyroperoxidase ($p = 0.020$). The concentration of thyroglobulin as in the previous group has been increased as compared with the control group, but these differences did not reach statistical significance (figure, c).

After 10 weeks of DDT consumption at the daily dose of 1.89 ± 0.86 μg/kg serum T_4 concentration in the treated rats insignificantly decreased as compared to the previous period of observation ($p = 0.10$) and control values for the control group of this period of observation ($p = 0.094$) (figure, b). On the contrary, the concentration of free T_4, significantly decreased, as compared with the previous period of observation ($p = 0.023$) and nearly reached the control values. Consequently, the proportion of fT_4 significantly decreased both in comparison with the previous period of observation ($p = 0.0022$) and also with control values ($p = 0.0012$). There was a reduction of T_4 to T_3 conversion, which was manifested by a decrease in the content of fT_3 ($p = 0.000003$) in the systemic circulation and its proportion of the total T_3 ($p = 0.00061$). These parameters were significantly lower also in comparison with control values for this period of the treatment ($p = 0.00035$ and $p = 0.0067$, respectively). The ratio of free fractions of T_3 and T_4 normalized. Serum concentrations of thyroperoxidase and thyroglobulin insignificantly differ from the control values (figure, b). Reduced production of thyroid hormones resulted in the increased secretion of TSH ($p = 0.0018$) and its serum concentration as compared with the values of the control group ($p = 0.021$).

After 10 weeks of DDT consumption at the daily dose of 7.77 ± 0.17 μg/kg changes in the serum thyroid profile basically corresponded to those observed after the previous period of observation in rats treated with a lower dose of DDT (figure, d). There was a decrease in production of T_4 and T_3 and their free fractions. The greatest changes were found for the proportion of fT_4 ($p = 0.0081$), and fT_3 ($p = 0.041$). There was a pronounced decrease in fT_4 and the proportion of fT_4, and therefore their values became lower than in the control group ($p = 0.012$ and $p = 0.039$, respectively). Comparison of serum concentrations of T_4 and fT_4 in this group of rats with the corresponding values obtained for the experimental group treated with the lower dose of DDT showed a more pronounced decrease in the concentration of T_4 bound with transport proteins and proportion of fT_4 with the increase in DTT consumption ($p = 0.036$, $p = 0.0064$, respectively). On the contrary, comparison of serum T_3 and fT_3 revealed a significant difference in the concentration of the free fraction, but its value in the group consuming the higher DDT dose was higher ($p = 0.00036$). The imbalance between the free fractions of the hormones persisted. Concentration of thyroperoxidase decreased and did not differ from the control group as well as the comparison group. The level of thyroglobulin in the systemic circulation remained unchanged and corresponded to the values of the control group (figure, d).

The effect of DDT on synthesis of hormones in various endocrine glands has been examined in many studies [8, 9]. However, conclusions about the impact of DDT on the production of thyroid hormones are largely contradictory. Different authors reported that individuals exposed to different doses of DDT had reduced production of T_4 and increased production of T_3 as well as lack of any changes in thyroid status [10–13].

This study showed that changes in the metabolism of thyroid hormones are biphasic. The first stage is characterized by increased organification of iodine in the thyroid gland, as evidenced by an increase in the systemic circulation of thyroperoxidase concentration and increased proteolysis of thyroglobulin; this is consistent with the increase in the serum concentration of T_4 at unchanged concentration of serum thyroglobulin. Exposure of animals to the higher dose of DDT was accompanied by the increase in production of thyroperoxidase and T_4, followed by lower conversion of T_4 to T_3. This suggests that at low doses DDT disrupts iodine transport into follicular thyrocytes, and to reactive dose-dependent increase in secretion of TSH, synthesis of thyroperoxidase and T_4 and its conversion to T_3, exhibiting reciprocal dependence on T_4 production. Such changes in thyroid status are typical for the early stages of endemic goiter associated with iodine deficiency [14].

During prolonged consumption of DDT production of thyroid hormones reduced. Differences found at this stage are associated with changes in the rate of the two main processes of formation and maintenance

of the thyroid status: T_4 production by follicular thyrocytes and its conversion into T_3. The rate of the decrease in serum concentrations of T_4 and its free fraction increased with the increase of DTT doses, while T_3 and its free fraction attenuated. This caused an imbalance in fractions of free hormones and formation of the thyroid status with relative prevalence of T_3.

Certain evidence exists that one of the mechanisms responsible for reduced T_4 concentrations in the systemic circulation induced by exposure to DDT consists in displacement of the hormone from its complexes with blood albumin and globulins [15–17]. It is known that the affinity to T_3 to binding proteins is very low [18], so that the ratio of free and bound fractions of T_4 is a more reliable parameter. This study has shown that the increase in blood T_4 concentration induced by exposure to different doses of DDT was accompanied by a proportional increase in its free fraction and maintenance of the proportion of fT_4 at the control level; consequently, at this stage we have not observed any reduction in the binding capacity of blood proteins. At a later stage there was a decrease, rather than an increase in the proportion of fT_4. However, in rats consuming a large dose of DDT, this parameter was significantly higher. Comparison of these data with results of other studies employing much (hundreds or thousands times) higher doses of DDT suggests that the decrease in the binding capacity of blood transport proteins may occur after consumption of large DTT doses exhibiting toxic effects on hepatocytes. This leads to a decrease in synthesis of albumin and globulins. Since after 10 weeks of consumption of low doses of DDT, the decrease in fT_4 concentrations represented the main change in thyroid status, this indicates impaired T_4 production by follicular thyrocytes.

CONCLUSIONS

(1) Changes in thyroid hormone production induced by low doses of DDT persisted in the environment, are biphasic.

(2) Changes observed during the first stage are similar to those observed in early stage of iodine deficiency and include reactive increase in the synthesis of thyroid-stimulating hormone, thyroxine, triiodothyronine, and thyroperoxidase.

(3) Subsequent depletion of reactive changes leads to decreased thyroxine production and impaired thyroxine/triiodothyronine ratio.

(4) The dose-dependent effect of DDT results in increased synthesis of TSH, thyroxine, and thyroperoxidase during the first stage and decreased production of thyroxine in the second stage; this is evidence for the leading role of impaired thyroxine production by follicular thyrocytes, rather than decreased deiodinase activity and blood transport proteins concentration in the changes of the thyroid status induced by DDT.

(5) Exposure to low doses of DDT should be considered as a complicating factor in the pathogenesis of endemic goiter and other types of hypothyroidism.

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