Hormonal Changes in Rats Poisoned by Herbicide 2,4-DA.

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Abstract

One of the major chloroorganic pollutants of environment is the phenoxy herbicide 2,4-D derivatives containing microdoses of dioxins. Effects of 4-week and 16-week poisoning of male rats with 2,4-D in total doses of 2.5 and 25 milligrams / kg on serum hormones level were studied. In 4-week poisoning the serum concentration of total T4 (tT4) significantly reduced, though the quantity of free hormone grew up. The concentration of total T3 (tT3) was also slightly increased, as the changes of free T3 were close to normal level. The tT3 / tT4 ratio was increased according to negative correlation between the levels of T3 and T4. Effect of the toxicant in 16-week experiment resulted to more expressed and unidirectional dose-dependent decrease of free and total T4 and phase fluctuations of total and free T3 levels. Increase of cortisol content found out in 4-week experiment may be a part of non-specific general stress reaction to chemicals, whereas 16-week experiment was resulted in dose-dependent suppression of serum cortisol level. The content of insuline was significantly increased in 4-week intoxication. Serum testosterone level was significantly reduced in our experiments; at 16-week intoxication this effect was dose-dependent. The content of progesterone was also considerably decreased, and the level of estradiol, in opposite, was increased. It resulted in androgens / estrogens ratio decrease, i.e. relative estrogenisation was observed. We believe the disturbances of testicular function are secondary and connected first of all with the changes of hypothalamic-pituitary regulation and periferic reception of gonadotropic hormones, as well as interference 2,4-DA in androgen synthesis. The changes of hormonal spectrum found out in rats in experimental 2,4-DA action testify to disorders of endocrine function as a whole and can generally reflect metabolic processes.

Introduction

A problem of accumulation of chlorinated organic compounds and dioxins in environment gains the significant importance last time. Numerous researches [1, 2, 3] are devoted the toxic effects of it. The action of polychlorinated aromatic substances upon the population as a result of inevitable contacts with chemical products is the persistant problem all over the world.

One of the major chloroorganic pollutants is the phenoxy herbicides included effective and widely used in agriculture 2,4-D. During its manufacture (particular at Khimprom chemical plant in Ufa, Russia) undesireable contacts with toxic substances of workers and population living around may occur, and chloroorganics contaminated with

dioxins accumulate in environment (soil, water, food). This is also promoted by emergency throw out of semi-products and waste derivatives at chemical enterprises [1].

Dioxin-content ecotoxicants cumulate in tissues and express the toxic action on organism functional systems. It results to metabolic disorders, development of carcinogenic, teratogenic and mutagenic effects as well as occurrence of reproductive system diseases [4, 5]. The endocrine organs are inevitably involved in these processes [4, 5, 6, 7, 8]. As the dioxins and 2,4-D themselves have hormone-like effect [4] they can specifically influence upon metabolic pathways at intoxication. However the detailed damage mechanism of chlorinated aromatic compounds on pituitary-testicle-tissue and liver-pituitary-thyroid axes and the level where the primary changes occur seem to be not sufficiently clear.

Hormonal disturbances, despite latent character in most cases, play a leading role in development of various diseases and intoxications. Without its evaluation the objective analysis of pathochemical changes is impossible.

Experimental Methods

170 male rats of 150-200 grams body weight were used in experiment. Meaning that about 90 % of ecotoxicants enter the organism with food, for modelling of subchronic poisoning rats were daily received the water solution of 2,4-D-ammonium salt (2,4-DA) during 4 weeks in total doses 25 and 2,5 milligrams/ kg (equivalent to LD_{50} and 1/10 LD_{50}). The commercial preparation of herbicide contained chlorophenols (about 2 %) and other chloroorganic compounds (summary about 1,5 %) including dioxins (about 30 nanograms / kg, according to Khimprom analytical laboratory data). The chronic poisoning was modelled by 16-week daily administration of 2,4-DA in total doses 1/100 LD50, 1/10 LD50 and LD50 for all period. Control animals were received sodium chloride solution.

Upon the termination of the experiment rats were decapitated, sera collected, frozed and stored at -20C^0 before investigation of hormones. Radioimmunoassay of free and total thyroxine (T4) and triiodothyronine (T3), testosterone, estradiol, progesterone, cortisol and insuline was carried out using standard test kits "Beloris" (Belarus) and "Immunotech" (Czech - France) with ^{125}I label.

Statistic data processing was performed using Student t-criterion.

Results and Discussion

As indicated in the tables, the disorders of the thyroid status in rats were developed in both subchronic and chronic intoxication by 2,4-DA. In conditions of 4-week poisoning the serum concentration of main thyroid hormone - total T4 (tT4) - was significantly reduced, though the quantity of free hormone grew up to 138 - 139 % of control. The concentration of total T3 (tT3) was also slightly increased, as the changes of free T3 were close to normal level. The tT3 / tT4 ratio was increased according to negative correlation between the levels of T3 and T4. Probably, the main reason of T4 level decrease was the acceleration of bile and urine excretion of T4-glucuronide conjugate owing to activation of microsomal oxidation and glucuronation in liver under the influence of chlorinated organic herbicides and technological microimpurity of dioxins [8, 9, 10]. The same effect can also be caused by infringement of thyroxin linkage to blood transporting proteins [9, 10], as changes of tT4 / free T4 ratio also testify.

In conditions of relative T4 deficiency some increase of more active T3 can be compensational, therefore at intoxication by 2,4-DA the clinical and metabolic signs of

hypopthyreosis can be absent. This question is of special interest meaning that the action of thyroxine in cells-targets realises via its preliminary deiodination to triiodthyronine and other iodinated thyronines. Besides it a part of T4 may be conversed to metabolically inactive reversed T3, which can be glucuronated and excreted even faster than T4 or T3, under the influence of dioxins. On the other hand, observed changes of tT3 and tT4 serum levels may be a result of thyroid gland hyperstimulation and overtension in conditions of 2,4-DA and its metabolites action resulting in infringement of iodination of the thyroid hormones predcessors.

Effect of the toxicant in 16-week experiment resulted to more expressed and unidirectional changes of thyroid hormones content, first of all significant dose-dependent reduction of total and free T4. We suppose that it was caused by infringement of hormone synthesis in thyroid gland and amplification of T3 and T4 periferic metabolism. Changes of tT3 / tT4 ratio were much smaller than at 4-week experiment. Phase fluctuations of total and free T3 levels could also be a result of hormone iodination disorders in thyreocytes and disturbances of T4 metabolic deiodination in periferic organs and tissues.

The changes of cortisol content found out in 4-week experiment may be a part of non-specific general stress reaction to chemicals (increase of "adaptation hormones" content evoked by LD_{50} of the toxicant). In opposite,16-week experiment was resulted in dose-dependent suppression of serum cortisol level under the influence of 2,4-DA. This may be both a toxic effect of 2,4-DA to synthesis of steroids in adrenals as well as consequence of hypothalamo-pituitary regulation disorders and decrease of adrenal cells sensitivity to ACTH at long action of dioxin-content substances [11, 12]. In their turn, the changes of glucocorticoids content can influence first of all upon carbohydrates and lipids metabolism, modulating processes of gluconeogenesis and lipolysis.

Hyperglykaemia as a result of the evoked gluconeogenesis influences the contents of other hormones regulating blood glucose level, insuline first of all. The activation of lipolysis can therefore result in stimulation of lipid peroxidation and cause additional destruction of cell membranes. This effect can be considered as a reply to hyperproduction of glucocorticoids and hyperglykaemia developed [11]. As we suppose no insulinase synthesis depression was observed in our experiments as specific hepatic protein synthesis function was not significantly damaged. So the increase of serum insuline level observed could be connected with its hyperproduction in pancreatic beta cells. Another way, hyperinsulinaemia with parallel hyperglykaemia usually indicates depressed sensitivity of cells-targets to insuline. Probably as a result of long-duration action of 2,4-DA and products of its metabolism as well as dioxins on insuline receptors the disruption of its structure and function may occur. The supression of thyroid hormones genetically supervising the synthesis of most regulative receptor proteins in norma, does not contradict this statement.

The serum level of testosterone - the main androgen - in our experiments was significantly reduced; in 16-week intoxication this effect was dose-dependent. These changes can be connected with the increase of hypothalamus-pituitary system sensitivity to steroids and depression of gonadotropins production [5, 13] effected by dioxin-content chlorinated organic substances (that was later confirmed in our experiments), as well as possible direct interference of toxicants to synthesis of testosteron and realization of its action in cells-targets [4, 13]. Significant decrease of testosterone level and simultaneous absolute or relative increase of estradiol content can be the consequence of pathologic acceleration of estrogen way of testosterone metabolism in conditions of sharp, probably specific, hyperactivation of microsomal P_{450}-dependent oxydases in liver at long effect of 2,4-DA and dioxins. Shakhoud [14] has determined the minimal concentration of pure

dioxins causing the damage of Leydig cells and reduction of testosterone synthesis - 1 microgram/ kg. In our experiments the total dose of dioxins was 100-1000 times less, so, as we suppose, the disturbances of testicular function are secondary and connected first of all with the changes of hypothalamic-pituitary regulation and periferic reception of gonadotropic hormones, as well as the 2,4-DA interference in androgen synthesis (phenoxy herbicides may inhibit the cholesterol and steroid synthesis) [13].

The changes of hormonal spectrum found out in rats in condition of experimental 2,4-DA action testify the disorders of endocrine function as a whole and can generally reflect metabolic processes.

Literature Cited

(1) Fedorov, L.A. *Dioxins as ecologic danger:* Nauka; Moscow; **1993**; p. 161.
(2) Violante, S.; Lodi, V.; Mandes, A.; Raffi, D. *La difesa delle piante* **1989**, 3, 37-40.
(3) Shearer, R.; Halter, M. *Literature reviews of four selected herbicides: 2,4-D, dichobenil, diquat and endothall*; Municipality of Washington; US Metropolitan Seattle; 1980.
(4) Pozniakov, S.P.; Rumak, V.S.; Umnova, N.V. *Zhurnal Ecol. Chem.* **1993**, 3, 189-199.
(5) Moore, R.; Bookstaff, R.; Mably, T.; Peterson, R. *DIOXIN 91;* NC; **1991;** p. 31.
(6) Feeley, M.M. *Environ. Health Perspect.* **1995,** 103(2), 147-150.
(7) Birnbaum, L.S. *Environ.Health Perspect.* **1994,** 102(8), 676-679.
(8) Sewall, C.H.; Flagler, N.; Van den Heuvel, J.P.; Clark, G.C.; Tritscher, A.M.; Maronpot, R.M.; Lucier, G.W. *Toxicol. Appl. Pharmacol.* **1995,** 132(2), 237-244.
(9) Brouwer, A.; Morse, D. *DIOXIN 91;* **1991**; NC; p. 56.
(10) Lans, M.; Brouwer, I.; De Winden, P.; Brouwer, A. *DIOXIN 93;* Vienna; **1993**; p. 137-140.
(11) Rozman, K.; Lebofsky, M.; Stahl, B.; Weber, L. *DIOXIN 90;* NY; **1990**; p.133.
(12) Bestervelt, L.L.; Pitt, J.A.; Nolan, C.J.; Piper, W.N. *Neurotoxicol. Teratol.* **1993**, 15(6), 371-376.
(13) Kleeman, J.M.; Moore, R.W.; Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1990**; 106(1); p. 112-125.
(14) Chahoud, I.; Hartmann, J.; Rune, G.M.; Neubert, D. *Arch.Toxicol.* **1992**, 66(8), 567-572.

Table 1. Serum hormones concentration in rats poisoned by different doses of herbicide 2,4-DA in 4-week experiment, M ± m.

Hormone / 2,4-DA dose	Total T4 nM / l	Free T4 pM/100 ml	Total T3 nM / l	Free T3 pM/100 ml	Cortisol nM / l	Testostero-ne nM / l	Estradiol pM / l	Progestero-ne nM / l	Insuline mcEd/ml
Control	22.57±5.61	1.07±0.19	1.30±0.11	3.55 ± 0.17	26.3±5.3	15.6±3.8	203.8±20.5	1.95±0.31	15.8±3.4
1/10 LD50	14.94±2.53	1.48±0.24	1.48±0.12	3.18 ± 0.22	25.6±3.4	4.2±0.3*	318.6±49.3	1.15±0.31	28.8±2.7*
% of control	66	138	114	89	98	27	157*	59	182
LD50	9.5±1.53 *	1.49±0.49	1.34±0.16	3.79 ± 0.43	40.6±4.2*	7.6±1.1*	268.4±34.3	0.97±0.10*	28.6±3.2*
% of control	42	139	107		154	49	132	50	181

* statistically significant changes (P<0.05)

Table 2. Serum hormones concentration in rats poisoned by different doses of herbicide 2,4-DA in 16-week experiment, M ± m.

Hormone / 2,4-DA dose	Total T4 nM / l	Free T4 pM/100 ml	Total T3 nM / l	Free T3 pM/100 ml	Cortisol nM / l	Testosterone nM / l	Estradiol pM / l	Progesterone nM / l
Control	28.46 ± 1.85	3.27 ± 0.45	1.18 ± 0.11	3.66 ± 0.26	25.3 ± 2.6	17.2 ± 1.9	190.1 ± 23.8	2.43 ± 0.43
1/100 LD50	27.82 ± 1.91	2.32 ± 0.38	1.24 ± 0.08	4.10 ± 0.57	23.3 ± 3.3	13.0 ± 1.5	176.5 ± 26.4	0.84 ± 0.15*
% of control	98	71	105	112	92	76	93	35
1/10 LD50	22.23 ± 1.03*	1.89 ± 0.45*	1.17 ± 0.08	4.41 ± 0.47	18.3 ± 2.0*	10.2 ± 2.1*	233.4 ± 21.4	1.10 ± 0.39*
% of control	78	58	99	121	72	59	123	45
LD50	16.90 ± 2.42*	1.73 ± 0.21*	0.96 ± 0.21	4.34 ± 0.56	13.6 ± 1.3*	9.5 ± 1.8*	174.8 ± 24.1	0.50 ± 0.11*
% of control	59	53	81	119	54	55	92	21

* statistically significant changes (P<0.05)