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Role of Glucocorticoids in Regulation of Iodine Metabolism in Thyroid Gland: Effects of Hyper-And Hypocorticism

Liliya Nadolnik

Additional information is available at the end of the chapter

1. Introduction

A close relationship between the key bodily regulatory systems, hypophysis-adrenal and hypophysis-thyroid systems, is fairly well-known.

However, the mechanisms of their interaction at different levels have not been conclusively established. This is of considerable interest due to glucocorticoids and thyroid hormones playing a key role in regulation of the most important systems of vital activity and adaptation. The role of glucocorticoids in regulation of thyroid cell function is interesting due to marked growth of thyroid pathology in different world's regions, along with considerably improved iodine prevention [1], as well as an increased level of environmental stressogenicity. One should also note an increased tension in life of the individual and the society on the whole (psychological, social and other types of stress). The development of the society has actually created a new human environment with a raised level of stressogenic factors. The chronic stress –induced development of hypercorticism can play a significant pathogenetic role in the changed thyroid function which does not only depend on bodily iodine allowances.

Thyroid-stimulating hormone (TSH) [2, 3, 4], iodine [4, 5], thyroglobulin (ThG) [6], estrogens [7], cytokines [8] and other biologically active molecules play an important role in regulation of thyroid cell functions. It is interesting that deficiency of iodine, the key substrate for synthesis of thyroid hormones, decreases the activity of the HPA-axis. It was found [9] that rats with chronic iodine deficiency showed the absence of a normal circadian rhythm of corticosterone secretion and a weakened secretory rise of a corticosterone level under stress

that remains to be diminished in amplitude during a month following restoration of the iodine status.

Thyroid cell function can be regulated by glucocorticoids via changes in the concentrations of the pivotal bioregulators: thyroid-stimulating hormone, TSH, iodine and thyroglobulin. The mechanisms and effects of these interactions call for further studies. Thyrocytes express glucocorticoid receptors, alpha (GR-alpha) and beta (GR-beta), which seem to play an important role in differentiation of thyroid cells since cells of thyroid adenoma demonstrated a decrease of mRA GR-alpha and an increase in GR-beta [10].

1.1. Relationships between regulatory effects of hypothalamic and hypophyseal hormones of hypothalamo-hypophyseal-adrenal and hypothalamo-hypophyseal-thyroid axes

Relationships between the hypothalamo-hypophyseal-adrenal (HHA) and hypothalamo-hypophyseal-thyroid (HHT) systems were established at different regulatory levels. Administration of a thyrotropin-releasing hormone (TRH) was accompanied by a decreased adrenocorticotropic hormone (ACTH) level in blood serum of stressed rats [11]. Corticotropin-releasing hormone (CRH) increased plasma TSH and T4 [12]. Banos C. et al. [13] demonstrated that administration of 2 mg ACTH to healthy volunteers decreased the TSH response to TRH. These results characterize certain antagonism between TSH and ACTH.

1.2. Effects of glucocorticoids on TRH and TSH levels

TSH synthesis is determined by balance of positive regulation and negative regulation by TRH and triiodothyronine (T3), respectively; in addition, somatostatin and dopamine also exert inhibitory control (Diagram 1). Glucocorticoids decreased serum TSH in animals and humans. Administration of a high dose of dexamethasone not only suppressed TSH but also decreased the TSH response to TRH administration [14]; the suppressive effect of dexamethasone on TSH decreased in elderly people [15].

Administration of a single dose of hydrocortisone (500 mg) increased both TSH production and stimulation by TRH [16]; only long-term hypocorticism (Cushing's disease) may be a cause for decreased TSH level. The earlier recovery (up to control values) of the diurnal rhythm of TSH than that of cortisol suggests that the TSH rhythm is not under the direct control of circulating cortisol [17]. In adrenalectomized rats the TSH level decreased in serum but not in the pituitary gland [18]. Glucocorticoids decrease blood serum TSH concentrations in humans and animals. Dexamethasone administration to hypothyroid rats decreased serum TSH; dexamethasone augmented a T3-induced decrease of TSH. However, changes in pituitary TSH α- and β-subunit mRNA concentrations were not found [19].
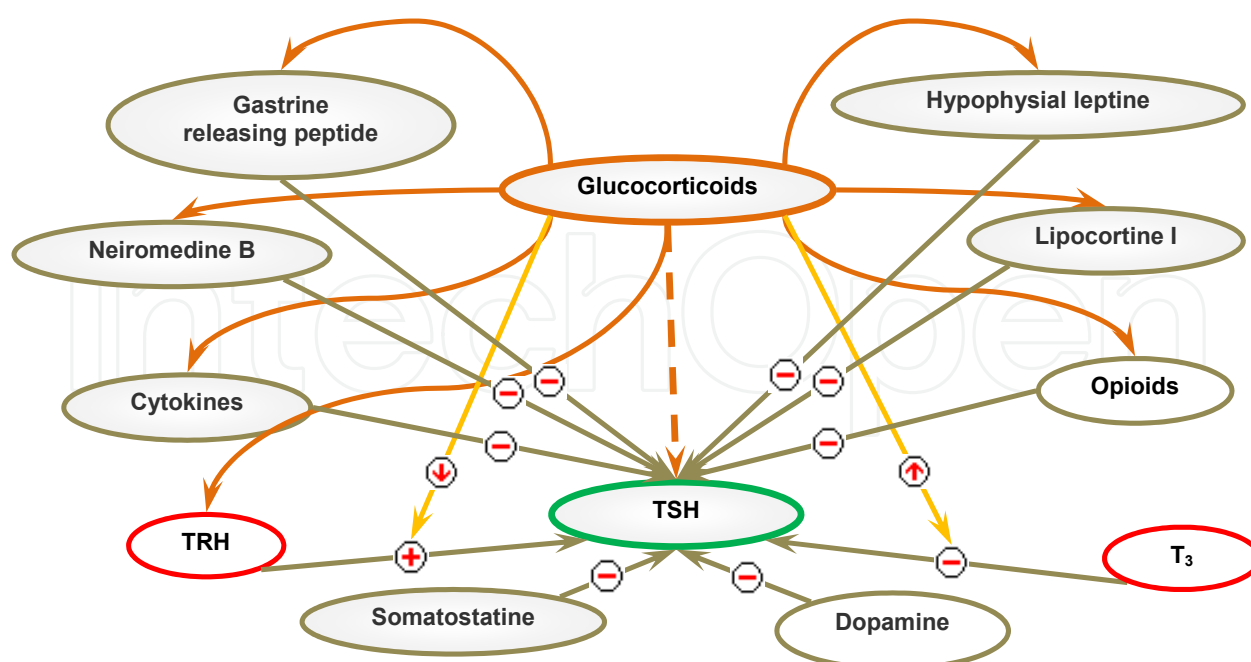
Scheme 1. Effect of glucocorticoids on TSH. +, stimulatory effect; -, inhibitory effect; ↓– weakening of stimulatory effect; ↑– enhancement of inhibitory effect

Kakucska I. et al. obtained clearer results on the effects of glucocorticoids on the hypothalamo-pituitary-thyroid axis [20]. In the paraventricular hypothalamic nuclei of adrenalectomized rats, an increase in corticotropin releasing hormone (CRH) mRNA occurred in parallel to the increase (68.3%) in pro-TRH mRNA. On the contrary, administration of corticosterone or dexamethasone caused a marked decrease in CRH mRNA and pro-TRH mRNA by 43.2 and 73.3%, respectively. Insignificant changes in pro-TRH mRNA were found in the lateral hypothalamus.

Mechanisms of the stress-induced decrease in TRH/TSH secretion possibly involve glucocorticoids, cytokines, and opioids. Recently, a new regulatory mechanism, involving pituitary neuromedin B, gastrin-releasing peptide, and pituitary leptin, acting as local inhibitors of TSH release, has been proposed [21]. In vitro studies have shown that the lipocortin-1 (LC1) protein is a mediator of the glucocorticoid-induced suppression of TSH secretion by the anterior pituitary [53]. Treatment of anterior pituitary cells with 0.1 μM dexamethasone significantly increased the amount of LC1, associated with the outer surface of the pituitary cells and decreased the intracellular content of LC1. Addition of an N-terminal LC1 fragment (residues 1-188) decreased TSH release mediated by vasoactive intestinal peptide and forskolin, but failed to influence those initiated by 10 μM BAYK 8644, the calcium channel stimulator. The inhibitory action of dexamethasone was substantially reversed by a specific monoclonal anti-LC1 antibody [22]. The inhibitory effect of dexamethasone was used for monitoring of subclinical hypothyroidism in obese patients. Administration of TRH after dexamethasone increased the TSH level only in hypothyroid patients but not in euthyroid obese patients [23].

1.3. Effect of glucocorticoids on iodine uptake by the thyroid gland

Iodine uptake is the most important function of thyroid cells; it is controlled by TSH, which stimulates ^{131}I uptake in vivo and in vitro and also expression of sodium-iodide symporter (NIS) in the culture of human thyrocytes [24]. Sodium-iodide symporter (NIS) is located on the apical membrane of thyrocytes; its activity is coupled to Na+,K+-ATPase. TSH influences transcription of NIS gene through Pax-8 and factors activated by intercellular interaction during folliculogenesis [25]. High iodine doses directly inhibit iodide uptake by influencing regulation of NIS protein and mRNA expression [26, 27].

Immobilization stress and also ACTH administration to rats with pituitary damages increased ^{131}I uptake by the thyroid gland in vitro [28]. Cultivation of FRTL-5 thyrocytes under hypoxic conditions was accompanied by increase iodide uptake [29]; heat stress (15 min at 45°C) eliminated this effect. Using culture of ewe thyroid gland follicles it was found that combination of TSH and 10 nM cortisol was optimal for stimulation of iodide uptake without additive and synergistic effects; this effect was also reproduced by combination of TSH with dexamethasone [30]. In addition, the stimulating effect of TSH was potentiated by physiological concentrations of insulin and insulin-like growth factors (IGF I and IGF II). Subsequent studies demonstrated a direct biphasic effect of hydrocortisone on metabolism of thyroid gland cells. Physiological concentrations of hydrocortisone (1—1000 nM) in a dose-dependent manner stimulated TSH- and 8-bromo-cAMP-induced iodide uptake, realized via increased production of cAMP and activation of cAMP-dependent metabolic pathways in the primary cultures of porcine thyrocytes [31]. The stimulating effect of hydrocortisone in combination with TSH was inhibited by the glucocorticoid antagonist RU486; the specific hydrocortisone effect appears to be mediated by a thyrocyte glucocorticoid receptor.

It is suggested that the stimulating effect of glucocorticoids on ^{131}I uptake may be used for treatment for breast cancer [32] and prostate cancer [33]. Incubation of NP-1 cells with dexamethasone (10^{-8}–10^{-6} M) caused a 1.5-fold increase in iodide uptake, and a 1.7-fold increase in expression of Na^+/I^- simporter (NIS) mRNA and protein concentration; NP-1 cell death increased from 55 to 95%, thus suggesting increased cytotoxicity of [131]I. These studies (employing clonogenic assay and nonradioactive proliferation assay) also revealed that treatment of NP-1 cells decreased proliferation of prostate cancer cells. Thus, stress (at least acute stress) may be considered as a factor activating iodide content in the thyroid gland; however, univocal solution of this problem requires further investigations because of multilevel effects of glucocorticoids on thyroid homeostasis

1.4. Effects of glucocorticoids on iodine oxidation and organification in thyrocytes

Single reports on the effect of stress or glucocorticoids on iodide oxidation by thyroperoxidase (TPO), thyroglobulin iodination and subsequent thyroid hormone secretion

are available in the literature. Corticosterone administration for 10 days in three different doses (25, 50, 100 mg per 100 g of body weight) inhibited thyroid gland TPO of juvenile female turtles [34], but the mechanism of the inhibitory effect was not studied. Studies in this direction are especially important due to the key role of TPO in thyroid hormone biosynthesis.

The electron microscopy study of thyrocytes revealed accumulation of colloidal droplets in follicle cytoplasm; this suggests that prednisone may decrease basal secretion of thyroid hormones by inhibiting lysosomal hydrolysis of colloid in the follicular cells [35].

1.5. Role of glucocorticoids in the regulation of thyroid hormone receptors

It is known that most of T3 effects are realized via nuclear receptors of thyroid hormones. T3 and glucocorticoid hormones synergistically interact in biosynthesis of growth hormone in the rat pituitary and in the T3-induced metamorphoses in amphibians. Glucocorticoid hormones potentiated metabolic effect of T3 [36]. Dexamethasone increased rat liver specific receptor binding of thyroid hormones. Dexamethasone administration to adrenalectomized rats increased the concentration of protein and mRNA of beta 1 receptor [36]. Molecular studies employing transfection of COS-7 cells revealed that dexamethasone increased transcription activity of thyroid hormone receptor beta 1 promoter [36].

1.6. Effect of stress on peripheral metabolism of thyroid hormones (deiodinase activity in target tissues)

Brain, liver, kidney, heart, muscles, and immune system are the most important targets for thyroid hormones. It is possible that glucocorticoids control tissue levels of T3. Acute stress (footshock) increased the brain T3 content in male and female rats by 12—19% [37]. Two days of total water and food deprivation as stress increased the thymus lymphocyte T3 content in weanling and adult female rats [38], which was normalized after 48 h [39]. It is known that thyroxine (T4) is the main hormone produced by the thyroid gland, however, since it does not exhibit biological activity and therefore thyroxin may be considered as a prohormone or a plasma storage form of thyroid hormones, which plays an important physiological role. A family of selenocysteine oxidoreductases known as iodothyronine deiodinases (D) plays the major role in T4 activation. Three types of these enzymes (mainly determining realization of the hormonal effect of thyroid hormones) have been identified. Their localization and activity are tissue-specific (Scheme 2, 3).

Glucocorticoids exhibit differentiated tissue- and age-specific effects on various tissue deiodinases [40, 41]; they also regulate deiodinases during embryogenesis. Dexamethasone administration to pregnant ewes increased activity of DI in the fetal liver and decreased DIII activity in fetal kidneys [42]. In 20-day-old fetuses, glucocorticoids had no effects on circulating thyroid hormone levels despite their clear decrease in the activity of hepatic and renal deiodinases and an increased activity in the brain, thereby indicating that in this age
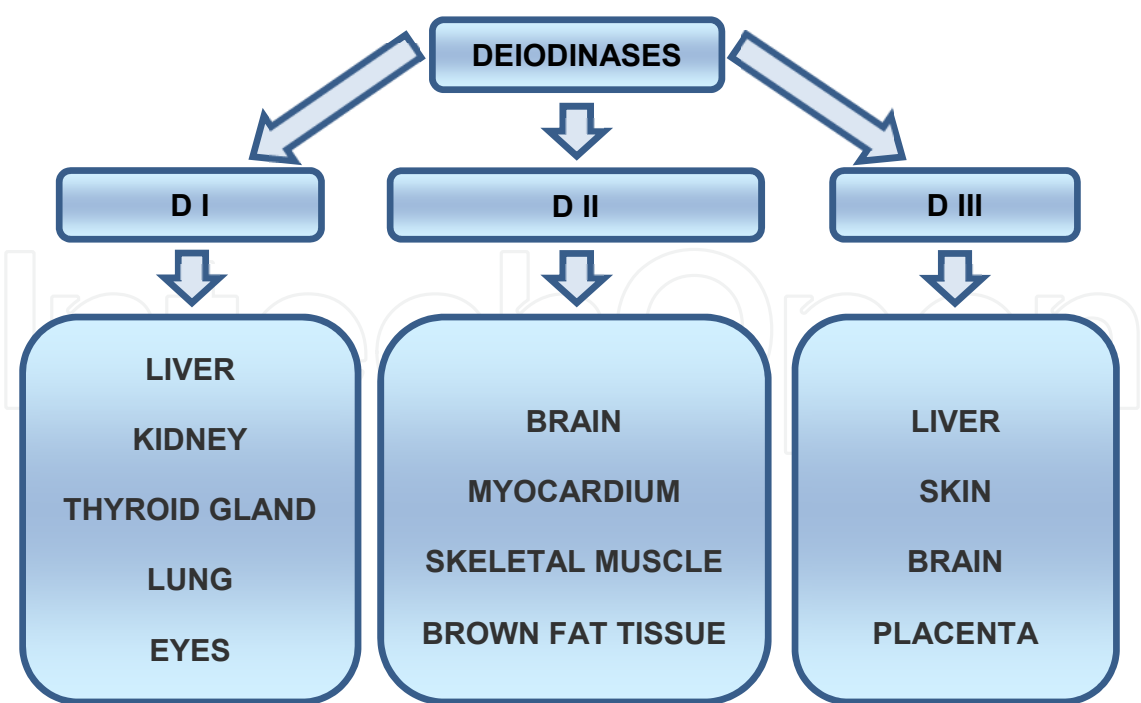
Scheme 2. Tissue distribution of deiodinases
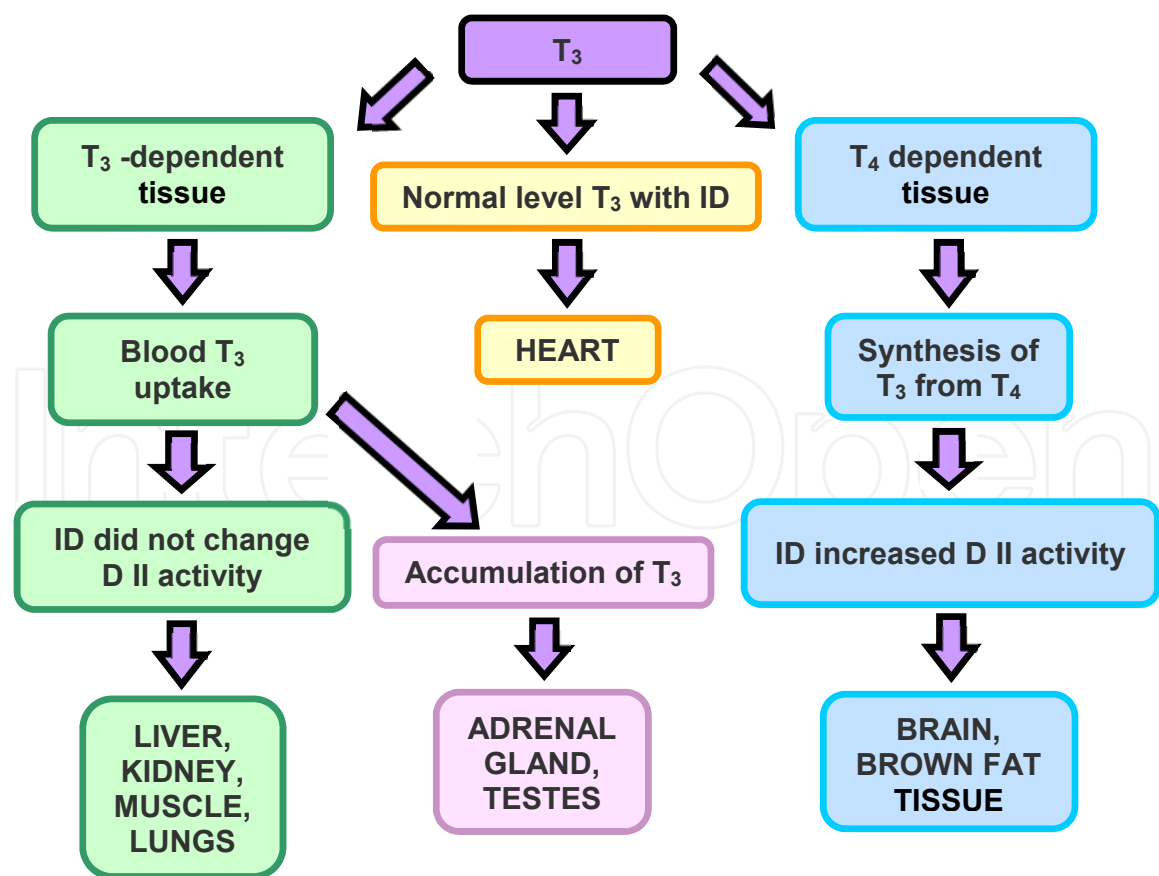
Scheme 3. Forms of thyroid hormone utilization by various

Types of deiodinases	Target tissues	Effect	Reference
DI	fetal liver, ewes	↑	[42]
DIII	fetal kidneys, ewes	↓	[42]
DIII	5-day-pups liver, rat	↑	[43]
DIII	5-day-pups kidneys, rat	↑	[43]
DIII	5-day-pups brain, rat	–	[43]
DIII	brown adipose tissue, rat	↓	[58]
DII	brain, rat	↑	[44]
DI	liver, rat	↓	[51]
DI	kidneys, rat	↓	[52]
DI	hepatocytes in vitro	↑	[54]

Table 1. Effect of GC and stress on activities of various types of deiodinases in rat tissues (↑– stimulatory effect; ↓ inhibitory effect; – no effect)

thyroid circulating thyroid hormone levels are more dependent on thyroidal secretion than on peripheral deiodination. In 5-day-pups, dexamethasone increased blood T3 and T4 and DII activity in the liver, kidney but not in the brain; however, in 12-day-old pups, the dexamethasone effects were maintained only on liver and kidney DIII activity [43].

Effects of stress on deiodinase activity in various tissues still require better elucidation. The most significant effect of glucocorticoids was found on brain DII activity. Even mild, short-term stress (intraperitoneal injection of saline, intragastric intubation, and two different forms of handling (being grasped as for intraperitoneal injection and being moved from one cage to another, and a 2-h period spent in a slowly rotating drum) caused a significant increase in brain DII activity [44], this was accompanied by a 300%-increase in T3 concentration. These effects were not found in the liver and no changes of DI activity were found in the brain and liver. Dexamethasone caused up-regulation of DII activity [45]. Administration of steroidogenesis inhibitors (aminoglutethimide and metyrapone) to rats decreased adrenal DII activity both in physiological rest and under stress [46]; this suggests normal corticosterone levels required for a deiodinase response to the stress treatment. It appears that the glucocorticoid regulation of DII is the most differentiated. Recent in vitro data obtained using mouse and rat pituitary cells demonstrated that addition of glucocorticoids increased the activity of this enzyme and its mRNA [47], whereas the opposite effect was obtained in mouse mammary gland epithelial cells [48]. In the AtT-20 mouse pituitary tumor cells, glucocorticoids and CRH stimulated expression of mRNA and activity of DII [49]. Effects of glucocorticoids, found in experiments on cultivated hypophyseal cells, confirm their important stimulatory role in the metabolism of thyroid hormones in the CNS.

A decrease in blood thyroid hormones and TRH mRNA seen in fasting and food deprivation was accompanied by the increase DII activity and DII mRNA. Studies of

mechanisms of DII activation during fasting revealed that the decrease in leptin levels plays a permissive role during glucocorticoid-induced regulation of the DII enzyme [50].

There are contradictory data on the effects of glucocorticoids on DI activity in various tissues. Cold stress of rats either for 24 h or 28 days (as well as that combined with immobilization) significantly reduced DI activity in the liver [51]. Immobilization of rats for 6 - 8 h was accompanied by the decrease in DI activity in the liver and kidneys; this was attributed to the decrease in the enzyme activity rather than to decreased substrate availability because serum T4 concentration remained unchanged [52]. In adult rats, glucocorticoids decreased DI activity in the liver [53]. In vitro studies on the cultured rat hepatocytes revealed the opposite effects: glucocorticoids increased DI activity and expression of DI mRNA [54]. In kidney NRK 52E cells, dexamethasone increased DI activity and expression of DI mRNA, while in cultured pituitary tumor cells, glucocorticoids did not influence DI mRNA [55]. In the fish *Nile tilapia*, dexamethasone decreased activity of DI and DII in the liver; long-term administration of this hormone increased availability of circulating T3 [56].

The decrease in plasma T3 and the increase in rT3 concentrations observed in stress may be associated with glucocorticoid stimulation of DIII [57]. Regulatory mechanisms of effects of thyroid hormones in various tissue cells have not been conclusively established. Glucocorticoids decreased DIII expression in rat brown adipose tissue [58]. The study of deiodinase activities in human cell lines revealed that estradiol increased DIII activity in ECC-1 cells, dexamethasone inhibited DIII in WRL-68 cells only in the presence of fetal calf serum in the medium [59]. Dexamethasone in a dose-dependent manner decreased the stimulatory effect of T3 on ICAM-1 protein in human ECV 304 cells [74].

All these results indicate that glucocorticoids modulate effects of thyroid hormones by influencing deiodinase activity in various target tissues. They cause significant increase of DII activity in the brain (and thus increase brain T3 level); stress exhibited inhibitory effect on DI activity in the liver and kidneys. Nevertheless, mechanisms underlying glucocorticoid regulation of T4 deiodination in various tissues require further investigation.

1.7. Thyroid gland function under impaired adrenal functions

Taking into consideration the multilevel effects of glucocorticoids on the thyroid status and peripheral metabolism of thyroid hormones, a study of functional activity of the thyroid gland under conditions of adrenal impairments appears to quite reasonable.

Adrenalectomy in rats increased thyroid gland stimulation by TSH and its secretory activity [60]. In patients with adrenal insufficiency cessation of replacement glucocorticoid therapy resulted in an increase of T3 and a decrease of (reversive triiodothyronine) rT3 concentrations, whereas the level of T4 and TSH remained basically unchanged [61].

There are clinical case reports on impairments of thyroid function in patients with hypercorticism before and after adrenalectomy and with adrenal insufficiency. The state of the pituitary-adrenal axis mainly determines the thyroid status in humans. Under hypercorticism in patients with Cushing's syndrome there were decreased serum concentrations of thyroid hormones and TSH; in addition, in 56.2--66.6% there was a prevalence of thyroid nodular disease; this was significantly higher than in the control group [63]. Long-term hypercorticism in patients with Cushing's syndrome was accompanied by inhibition of basal and TRH-stimulated TSH secretion [62]. These patients had an attenuated pituitary response to TRH administration and there was a negative correlation between plasma levels of TSH and cortisol (but not T3); after convalescence the reaction to TRH normalized [64]. There was a single case report on the development of Graves's disease characterized by pronounced hyperthyroidism after a successful surgical operation in a patient with Cushing's syndrome [65]. Authors suggest that suppression of hypercorticism activated latent autoimmune processes in the thyroid gland. Graves's disease with hyperthyroidism manifestations was diagnosed 9 months after unitaleral adrenalectomy in a woman with Cushing's syndrome [66]. In some patients subjected to surgical adrenalectomy for hypercorticism transitory dysfunction of the thyroid gland with symptoms of hypo- or hyperthyroidism developed [67]. Silent thyroiditis developed in a female patient after unilateral adrenalectomy for treatment of Cushing's syndrome followed by a gradual tapering of replacement dose of prednisolone to 5 mg/day; thus thyroiditis was characterized by low TSH, increased thyroid hormone levels, extremely low iodine uptake and increased titers of antimicrosomal and antithyroglobulin antibodies [68]. Recent observations have demonstrated that secondary hypothyroidism and hypercalciemia are consequences of the glucocorticoid deficiency developed after adrenalectomy for Cushing's syndrome [69].

In 103 patients with ACTH deficiency Murakami T. et al. [70] found signs of hypothyroidism (a decrease in free T3 and T4 concentrations, high TSH) and characteristic symptoms of clinical manifestations of thyroid insufficiency (cold intolerance, muscle rigidity, loss of interest in life). After hydrocortisone therapy all signs of impairments of the pituitary-thyroid axis disappeared in more than 70% cases; this suggests that glucocorticoid insufficiency is one of reasons underlying thyroid dysfunction. A high TSH level was found in patients with Addison's disease; administration of glucocorticoids caused dose-dependent inhibition of TRH-induced stimulation of TSH secretion; it is possible that glucocorticoids regulate pituitary sensitivity to TRH [71].

Moderate hypothyroidism is a consequence of exogenous or endogenous hypercorticism. In prepubertal children with nonclassical congenital adrenal hyperplasia (NCCAH) TSH and cortisol were secreted in a pulsatile and circadian fashion with a clear nocturnal TSH surge; daytime TSH levels were lower in the NCCAH group than in control children. The cross-correlation analysis of the 24-h raw data demonstrated that TSH and cortisol were negatively correlated, with a 2.5-h lag time [72].

Adrenalectomy not only reduced plasma corticosterone levels to almost zero, but also decreased plasma T3 and T4 levels, but diurnal rhythms of the HPT axis did not depend on rhythms of the HPA axis [73]. In pregnant female rats adrenalectomized on gestation day 8 there was a decrease in TRH mRNA, increase in serum TSH, and a decrease of T3 only in females [74]; it appears that maternal glucocorticoids determine the development of the hypothalamic-pituitary-thyroid axis in progeny.

Conclusion. The analysis of the literature data shows that the role of glucocotricoids in regulation of iodine metabolism in thyroid cells as well as their effects on the HHT system have not been conclusively established. Very few data are available on early changes in thyrocyte iodine metabolism induced by psychoemotional stress which characterize triggering of adaptation in metabolic systems. The idea is very important of the mechanisms of iodine oxidation and organification and the function of the key enzyme in thyroid hormone biosynthesis, TPO, with the activity governing synthesis of thyroid hormones. This seems to be especially topical in relation to increased levels of stressogenic factors in human environment and functions of all the systems under the stress of hypocoticism.

The goal of the above research is to assess the effects of glucocorticoids on the activities of the main steps of thyroid iodine metabolism and to study the features of iodine metabolism under exposure to short-term and chronic psychoemotional stresses.

2. Materials and methods

Experimental animal models. All the experiments were carried out on Wistar female rats (160-180g body weight) which were fed on a standard laboratory diet. Control and experimental groups contained 10-12 animals.

Acute unavoidable psychoemotional stress. This model was aimed at simulation of negative emotions in rats (fear, alarm, anger and aggression). To this end, we used the modified techniques of Desiderato O. [75] and Tolmachev D.A. [76]. The rats were exposed to a combined stress (each animal was placed in an individual box) in a special chamber whose metallic floor was penetrated with 5-mA electric current. The mild painless irritation of the low extremities was accompanied by interrupted noise (electric bell) and light (100-Wt electric bulb) during 20 min or 5-60 min singly. Stress was always given at the same time from 9.00 to 10.00 o'clock in the morning. No manipulations were carried out before placing the animals to penal cells and taking them out. The number of animals in the groups was 8 to 10.

Short-term repeated psychoemotional stress was simulated using the modified techniques of Desiderato O. [75] and Tolmachev D.A. [76] but the exposure was repeated: 20 min daily during 28 days. Animals with normal thyroid status were subjected to multiple exposures to psychoemotional stress.

Simulation of hypocorticism in rats. To simulate glucocorticoid deficiency, the animals were subjected to bilateral adrenalectomy (AE) (n=10). The surgery was performed by a conventional method [30] under ester anaesthesia. After the surgery, the animals were fed on the standard laboratory diet and received a 0.9 sodium chloride solution as a drinking

fluid. The animals were selected for experimental groups after a 3-day recovery period following the surgery.

Administration of high doses of potassium iodide to animals with normal and reduced glucocorticoid status.

Single administration. A KI solution was administered by a gastric tube at doses of 0.7; 7.0 and 70.0 mg/kg B.W. (which corresponds to 10, 100 and 1000 daily KI doses [77] or 0.54; 5.35; 53.51 mg iodide/kg B.W.) in the volume of 0.4-0.6 ml. The control rats received distilled water (0.5 ml). The animals were decapitated after 24 h following the administration.

Multiple administration. Potassium iodide was administered by a gastric tube at doses of 0.07, 0.21, 0.70 and 7.0 and 35 mg/kg body weight (which corresponds to 1, 3, 10, 100 and 500 daily doses of potassium iodide or 0.05, 0.16, 0.64, 5.35 and 27.76 mg iodine / kg body weight) in a volume of 0.4-0.6 ml at 9 o'clock daily over 14 days. The control rats received distilled water in a volume of 0.5 ml. After 24 h following the last (14th) administration of KI, the animals were decapitated.

Studies on thyroid iodine metabolism. Determination of total (It), protein-bound (Ib) and free iodine (If) in rat thyroid tissue. The method for determination of total iodine and its protein-bound and free fractions in thyroid tissue was developed directly for this research applying a commonly used catalytic cerium-arsenite method for measurement of iodine in the urine [78]. To determine the total iodine content, 0.125 ml of thyroid homogenate (1:2000) was placed to a test-tube and 0.3 ml of concentrated $HClO_3$ and $HClO_4$ (5:1) was added. The samples were incubated at 110°C for 60 min. They were cooled to a room temperature and 1 ml of a 0.5% sodium arsenite solution was added. The samples were shaken, and after 20 min, 0.5 ml of 1.2% cerium ammonium sulfate was added. Optical density was measured after 20 min at a wavelength of 400 nm. Iodine concentration was calculated by a calibration curve. To construct the calibration curve, we used KIO_4 at concentrations of 0, 20, 50, 100, 150 and 200 μg/l.

To measure the contents of protein-bound and free iodine in the thyroid homogenate, the proteins were sedimented with 5.2% perchloric acid and 0.125 ml of the supernatant was used to determine free iodine concentration, the sediment was used to measure protein-bound iodine concentration. After the separation of the iodine fractions, the procedure of measurement corresponded to that described above (I total).

Determination of urinary total iodine concentration in rats. To determine urinary iodine concentration, 0.125 ml of urine from the morning portion (collected between 7 and 9 a.m.) was used. At high iodine concentrations, the samples were diluted 5-, 10-fold and over. Then the urine was burned in a mixture of concentrated $HClO_3$ and $HClO_4$ and the sample was assayed for iodine content as was described above.

Determination of TPO activity. To determine TPO activity, we used the method based on reactions of iodine enzymatic oxidation [79]. 2.8 ml of 0.05 M of sodium phosphate buffer,

0.05 ml of 0.6 M KI and 0.1 ml of thyroid homogenate (1:80) or its microsomal fraction were placed in a 1-cm thermostatically controlled cell. The reaction mixture was stirred and incubated for 15 min at temperature of 28°C. The reaction was started by addition of 0.05 ml of 12 μM H_2O_2. The reaction rate was recorded for 1 min at a wavelength of 353 nm using a Cary-100 spectrophotometer. The TPO activity was calculated using the molar extinction coefficient of ε =22900 M^{-1} x cm $^{-1}$ for the product formed [77]. Enzyme activity was expressed as μmol/min x g protein.

Determination of thyroid hormone concentration in blood serum. The concentrations of total T_4 and total and free blood serum T_3 were measured radioimmunologically using RIA-T4 –CT and RIA-T3-CT kits (Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Republic of Belarus).

Determination of total corticosterone concentration in rat blood serum. Blood serum total corticosterone concentration was measured by high performance liquid chromatography (HPLC). The assay was carried out using 0.2 ml of blood serum which was placed to a test-tube with a ground stopper, 1.0 ml of chloroform and 10 μM dexamethasone solution were added (as an internal standard) followed by addition of 40 μl of fresh 1.0 M solution of sodium hydroxide. Corticosteroids were extracted for 1 min. The test-tubes were centrifuged for 3 min at 600 g and then the lower (chloroform) fraction was carefully collected. The chloroform fraction was evaporated to dryness in a nitrogen flow. The samples were dissolved in 20 μl of the mobile phase and applied on a column. The steroids were separated on KAX-1-64-3 columns (2x64 mm) filled with a Silasorb -600 (LC) normal-phase sorbent with the particle diameter of 5 μm (Lachema, Czech Republic). The mixture of hexan-chloroform-methanol in the volume ratio of 7:1:1 was used as the mobile phase [80]. A Milikhrom liquid microcolumn chromatograph (Russia) was used for detection in a UV-detector at a wavelength of 246 nm. The rate of the eluent supply was 200 μl/min. Steroids were identified from the retention time. Corticosterone concentration was calculated from the calibration curve and expressed as nM. A corticosterone (Sigma) solution was used to construct the calibration curve and a dexamethasone (Sigma) solution was used as an internal standard.

Statistical analysis. The data were processed statistically using Mann-Whitney's U-test. The results are presented as means (M) ± standard deviation of the mean. * P<0,05; ** P<0,01; *** P<0,001. The critical value for the significance level was taken to be 5%.

3. Results

3.1. Studies on iodine metabolism under hypercorticism (stress and post-stress periods)

Under psychoemotional stress, the corticosterone content was most elevated (405.8-447.7%) for 15-60 min. It was decreased following 2 hours after stress cessation (2.9-fold) and

increased after 6 hours (2.1-fold) at the post-stress period (Fig. 1). Analysing the wave-like dynamics of changes in corticosterone concentration at the post-stress period, we should note that the rats were stressed in the morning (9.00 to 10.00 a.m.) and the rise in corticosterone concentration at the post-stress period was not related to its circadian rhythm (since the circadian rhythm of corticosterone is characterized by maxima per 20.00 hour). The corticosterone concentration was observed to increase in the afternoon (16.00 p.m.) after 6 hours following the post-stress period, and this elevation of serum corticosterone is a characteristic manifestation of a regulatory feedback mechanism. As a response to a marked reduction of corticosterone concentration after 2 hours following stress, the ACTH concentration elevated, which induced a new wave in increasing blood and adrenal corticosterone concentration that is a manifestation of the adaptation syndrome.

(a)

(b)
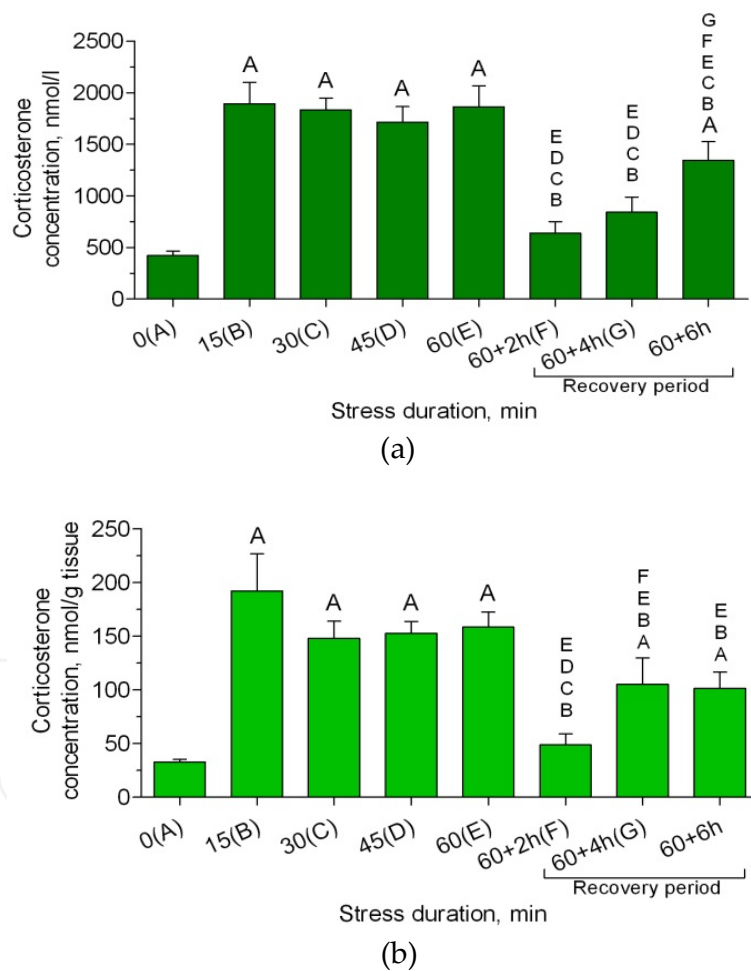
Figure 1. Blood serum (A) and adrenal (B) corticosterone concentrations in rats at acute stress and post-stress periods, after exposure to psychoemotional stress (n=8).

A, B, C, D, E, F, G are groups of animals, respectively. The letters under each column indicate statistically significant changes in the parameter (p<0.05) compared to the corresponding group (e.g., in Fig.1A, the parameter for Groups 15B, 30C, 45D, 60E is statistically significant compared to Group A). The same designations are in Figures 2-4.

The dynamics of changes in the parameters characterizing thyroid iodine metabolism was of a wave-like pattern, which indicates a pronounced response of the rat thyroid to stress. This was most pronounced for changes

in the index If, which is quite explicable. During 15-30 min of stress the thyroid total iodine concentrations remained unchanged (176.9-234.9 µg/g tissue). However, after 60 min, its content was 39.3% decreased in comparison with 15-and 30-min stress (Fig. 2A). During the acute stress phase (15-30 min), intensification of iodide organification was noticed: the concentration of its protein-bound fraction was 37.6% elevated, and the ratio of protein-bound I to total I was 1.2-fold increased (Fig. 2B). The 70.5% elevation of free iodide concentration in the thyroid gland (Fig. 2C) was probably due to activation of proteolytic processes in thyroglobulin and thyroid hormone formation. We cannot also exclude activation of iodine uptake with consideration for the absence of iodine supply to the body during stress, which can be due to increased activities of tissue deiodinases. Along with this, in spite of the evidence for Na^+/I^-- simporter expression in some cells (salivary and mammary glands) the literature lacks information about other iodine depots in addition to the TG. After 60-min stress, the thyroid showed diminished concentrations of free and protein-bound iodine, which seemed to be a consequence of highly active secretory processes and inhibition of iodine organification, TPO activity (Fig. 3) remained at a level of control values during 30-min exposure to stress, decreasing by 34.8% after 45 min, which was accompanied by a 16.8% reduction of protein-bound I concentration. The stress-induced drop in TPO activity can be due to changed kinetic parameters of the enzyme. TPO was found to be sensitive to elevation of ROS concentrations and aldehyde products of lipid peroxidation in thyroid cells [81]. Moreover, an important role in this case can be played by a decreased TSH level that regulates key processes in the TG. Taking into consideration the antagonistic relations between ACTH and TSH, one can suggest the metabolic changes in the TG to be caused by a stress-induced increase of the ACTH level which can induce a decrease of TSH production.

The correlation analysis of the results did not show a correlation between thyroid TPO activities and glucocorticoid levels in the blood serum and adrenal glands. After 60-min stress, a negative correlation was found between the total thyroid iodide and adrenal corticosterone (r= –0.952, p=0.003). In the control group, the content of adrenal corticosterone positively correlated with the protein-bound I to total I ratio (r=0.955, p=0.01), which indicates involvement of glucocorticoids in regulation of iodine homeostasis in the TG.

The decrease in corticosterone concentration after 2 h following the stress exposure was followed by activation of TPO (3.6-fold) as opposed to 60-min stress and control (3.4-fold). The TPO activation at the post-stress period suggests the presence of regulatory mechanisms for its activity which are related to a corticosterone level since it is at that period that its blood and adrenal concentrations were diminished most appreciably. The subsequent elevation of corticosterone concentrations in 4 and 6 h within the recovery period was followed by a dramatic decrease of thyroid TPO activity.

(a)

(b)

(c)
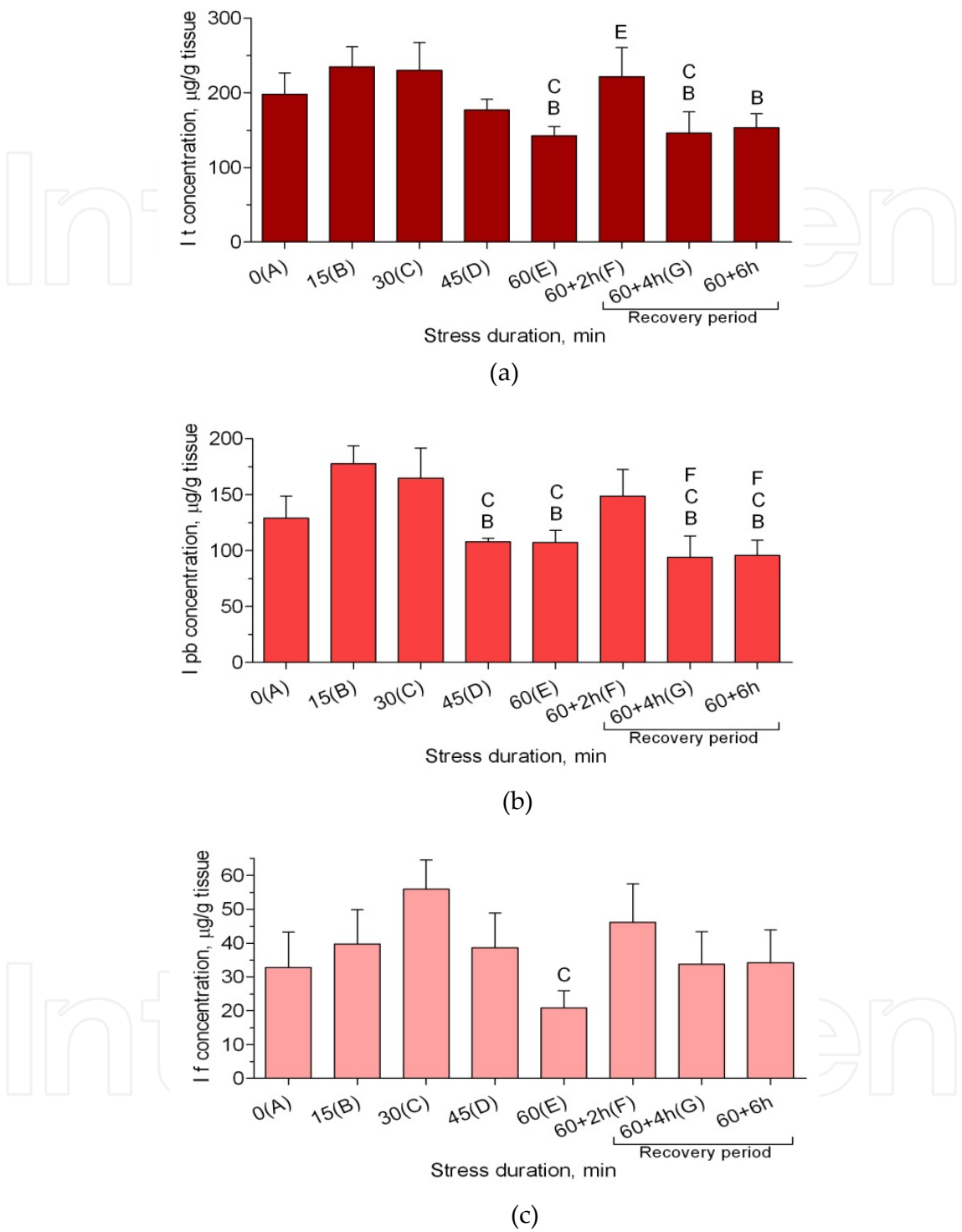
Figure 2. Rat thyroid total (A), protein-bound (B), and free (C) concentration of iodine during acute stress and post-stress periods.

A, B, C, D, E, F, G represent respective designations for groups of animals.

B, C, E. F represent statistically significant change in the parameter (p< 0.05) compared to the corresponding group
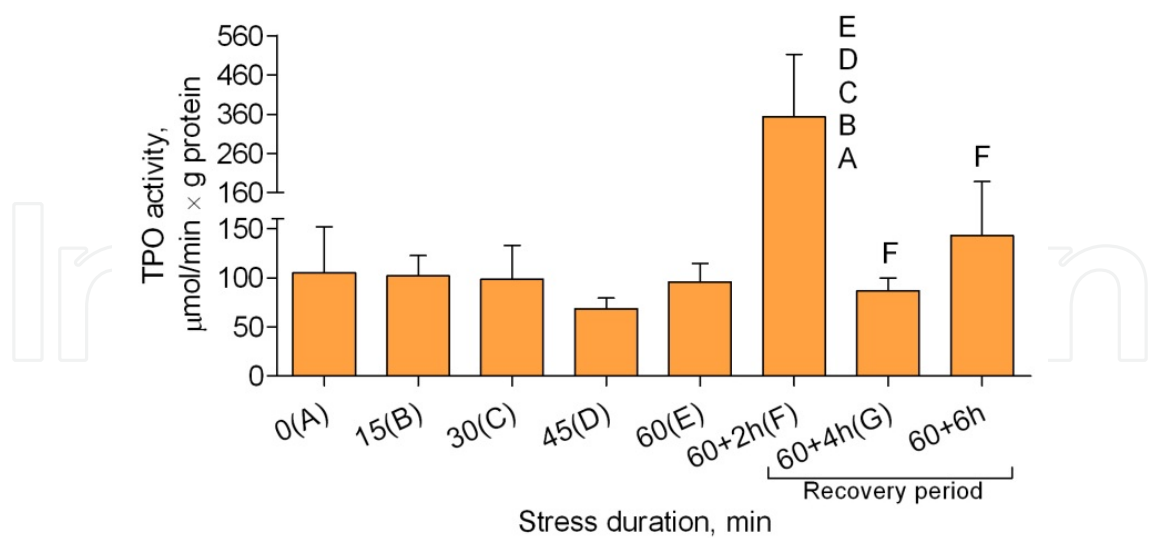
Figure 3. Rat thyroid TPO activity at acute stress and post-stress periods.
A, B, C, D, E, F, G represent corresponding designations for animal groups.
A, B, C, D, E represent statistically significant changes in parameter ($p<0.05$) compared to the corresponding group.
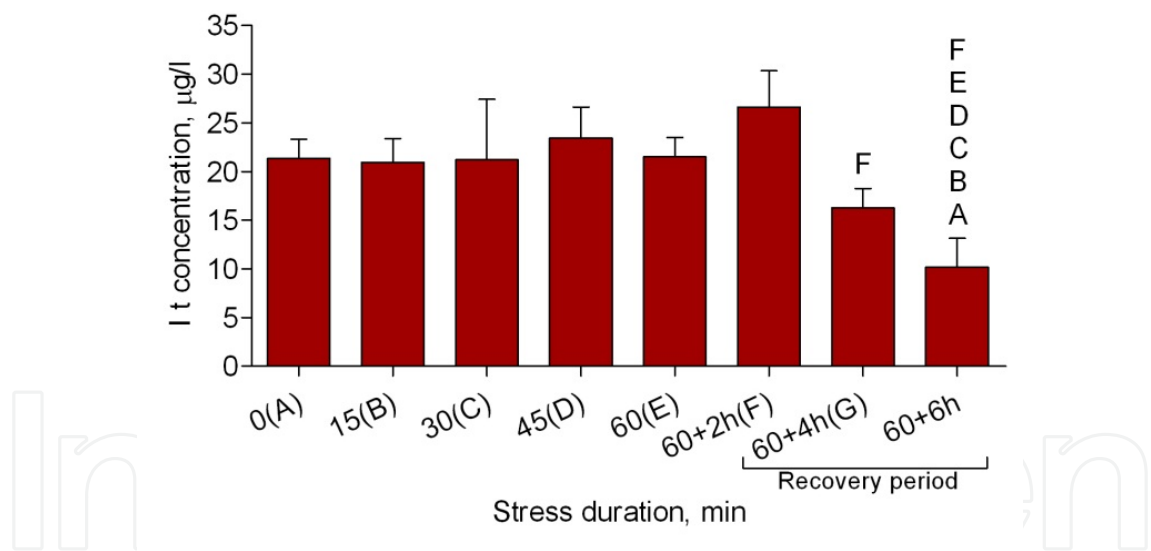
Figure 4. Rat blood iodine concentration at acute stress and post-stress periods.
A, B, C, D, E, F, G represent corresponding designations for animal groups.
A, B, C, D, E, F represent statistically significant changes in the parameter ($p<0.05$) compared to the corresponding group.

The iodine status restoration after the 2- h post-stress period is characterized by elevated concentrations of total I, protein-bound I and free I (55.5, 38.3 and 40.8%, respectively). A marked restoration to the control values of all the thyroid parameters studied was noticed after 4-6 h following the cessation of stress exposure. Under physiological conditions, the blood serum iodine content was not high (approx. 20 μg/l). However, acute stress

diminished its level (52.3%) at the post-stress recovery period (after 6 h following stress), which can be a consequence of restoration of the iodine status in the thyroid (Fig. 4).

	Control	Stress, 15 min	Stress, 30 min	Stress, 45 min
Groups	A	B	C	D
T$_4$ total, nM	59.4±4.1	60.6±3.6	60.5±2.4	59.09±4.4
T$_3$ free, nM	2.9±0.22	2.9±0.21	2.3±0.12	2.3±0.20^{A,B}

	Stress, 60 min	Stress, 60 min + 2-hour post-stress periods	Stress, 60 min + 4-hour post-stress periods	Stress, 60 min + 6-hour post-stress periods
Groups	E	F	G	H
T$_4$ total, nM	58.7±3.5	59.1±2.2	64.8±6.0	51.6±4.5
T$_3$ free, nM	2.1±0.21 ^{A,B}	2.7±0.29	2.1±0.13^{A,B,F}	2.0±0.26^{A,B,F}

^{A,B,F} P<0.05 compared to control.

Table 2. Rat blood T$_4$ and T$_3$ concentrations at acute stress and post-stress periods

The stress exposure did not produce significant changes in the concentration of blood serum total T$_4$. However, the free T$_3$ content lowered at the 30th minute of stress and remained to be 18.6 to 28.5% lowered throughout the experiment. It was not until 2 hours later that it increased up to the control values (Table 1).

Our findings show involvement of the TG in adaptation of the body to acute stress. We should note the thyroid ability to a rapid recovery of the iodine status at the post-stress period. Throughout a short period of time (15-30 min), the acute stress induced activation and uptake of iodide and thyroid hormone secretion.

However, oxidation of iodide was inhibited and the contents of total I, protein-bound I and free I were decreased after 45 and, significantly, after 60 min.

The 60-min exposure to psychoemotional stress revealed a negative correlation between the concentration of total I in the thyroid and the corticosterone concentration in the adrenals (r= –0.952, p=0.003). This shows that overproduction of glucocorticoids under stress induces a decrease of thyroid iodine content, resulting in a negative iodine balance at the post-stress period. The 2-hour recovery period is characterized by a pronounced activation of thyroid iodine metabolism (TPO activity rose over 3-fold), and the partial restoration of the thyroid iodine status (after 4-6 hours) was accompanied by a decreased blood serum iodine content.

The following correlations were established at the post-stress recovery period:

- after 4 hours, the blood serum iodide concentration negatively correlated with the corticosterone concentration (r= –0.831, p=0.040);
- after 6 hours, there was a highly significant correlation (r=0.937, p=0.006) between the blood corticosterone level and the ratio of protein-bound I to total I;

The data for the recovery period demonstrate that the blood corticosterone level can be viewed as a factor inducing a decrease of blood iodine concentration in rats.

Thus, the short-term stress (5-30 min) induced activation of biosynthesis and secretion of thyroid hormones. The most important regularity of the post-stress period is restoration of the thyroid iodine status due to activation of iodine uptake and organification as well as the presence of a close negative correlation between the thyroid concentration of I total and the adrenal corticosterone concentration (r= –0.956, p=0.003). After 6 h of the recovery period, the concentration of blood corticosterone was positively correlated to the ratio of protein-bound I/total I in the TG (r=0.937, p=0.006). A close correlation found between the levels of corticosterone and iodine in the thyroid gland may primarily show possible regulatory effects of glucocorticoids on iodine uptake. But no effects of glucocorticoids on TPO were found, which definitely indicates the absence of direct interactions. However, elevation of thyroid iodine concentration, induced by glucocorticoids, can activate TPO.

The above findings show that the exposure to stress induced a marked imbalance in the thyroid iodine status which was rapidly recovered at the post-stress period due to the decreased blood serum iodine concentration and that the restoration of the thyroid iodine status is most closely related to the glucocorticoid status.

3.2. Studies on the effect of acute exposure to stress on the kinetics of iodine metabolism in rats after administration of physiological potassium iodide doses

We studied the effects of 30-min psychoemotional stress on the iodine metabolism after administration of three daily doses of potassium iodide (KI was administered directly before the exposure to stress). The administration of three daily doses of KI increased 4.3-fold the blood iodine level within 6 hours. This concentration was decreased to the control values after 24 h (Fig. 5). In the group of rats subjected to stress, the iodine content also increased (296.7%) after 30 min following the administration of 3 daily doses of KI. In contrast to the control rats, the stressed rats showed a pronounced maximum of blood iodine concentration after 6 h (839.4% elevation, 170.7μg/l). After 24 h, the level of blood iodine in the stressed rats did not differ from that in the controls. The stress-induced changes in the kinetics of blood iodine concentration are a consequence of a disturbed regulation of iodine homeostasis. The dramatic, over 800%, elevation of blood iodine concentration can be due to an imbalance in the activity of its uptake: lowering of uptake in the TG and activation of uptake in the gastrointestinal tract at the post-stress period. It should be noted that it is at that period that the rat blood showed an increase in the corticosterone concentration (Table 2). A comparative examination of the curves characterizing changes in thyroid iodine concentrations in two animal groups (Fig. 5 B) shows that after 24 h, the thyroid iodine concentration elevated 1.7-fold in the control rats and remained essentially unchanged in the stressed rats (1.2-fold increase).

The 30-min psychoemotional stress leveled off the increase in the thyroid iodine status after administration of 3 daily KI doses. The changed concentrations of thyroid protein-bound I

and free I (Fig. 5, B and C) reflect changes in TPO activity in the thyroid gland (Fig. 5D). The administration of 3 daily KI doses was accompanied by activation of its organification in the group of control rats within 1 h (the level of protein-bound I was increased by 54.1%) and elevation of its concentration by 74.3% after 24 h.
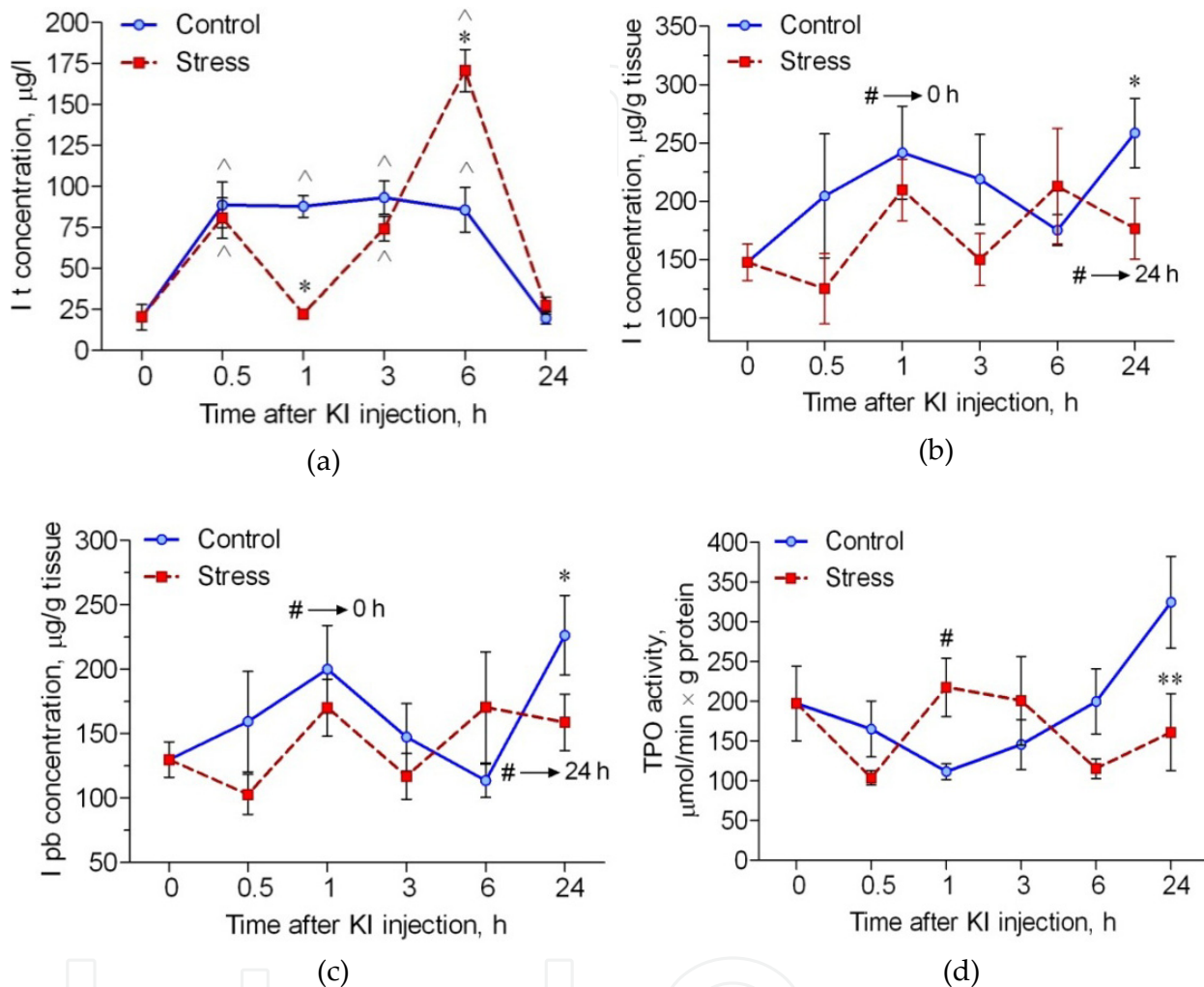
Figure 5. Effect of 30-min exposure to psychoemotional stress on iodine content in rat blood serum (A), total iodine (B), protein-bound iodine (C), activity of TPO (D) in rat thyroids after administration of 3 daily doses of KI within 24 h of the post-stress period (^ *P<0.05 compared to the initial level (0 h); * P<0.05 when comparing the indices in control and stressed rats; $ P<0.05; #→0 – 0.1<p<0.05 compared to group of 0 h; #→24 – 0.1<p<0.05 when comparing the indices in stressed rats to controls (24 h).*

The dynamics of changes in TPO activity in the stressed animals treated with 3 daily doses of KI had an essentially opposite character in comparison with the controls (Figure 5D). The post-stress increase in TPO activity after 1 h was accompanied by 41.5% decrease of its activity by 6 h as opposed to the initial level. As compared to the control animals, the activity of TPO in the thyroid of the stressed rats diminished over 2-fold , whereas the concentration of protein-bound I decreased 1.4-fold after 24 h following the administration of 3 daily KI doses.

The data obtained indicate that the 30-min exposure to stress after the administration of 3 daily KI doses changed the kinetics of iodine metabolism in rats within 24 h of the post-stress period. These data reflect complex relationships between the regulatory effects of the pituitary-thyroid and pituitary-adrenal systems as well as the whole complex of metabolic stress changes in the organism in respect to the key steps in thyroid iodine metabolism. Stress enhances the iodine inhibitory effect.

	Before KI administration	After KI administration				
		30 min	1 h	3 h	6 h	24 h
Blood corticosterone, nM	302.8±28.5	1279.1± 101.6*	1580.4± 118.9*	2135.8± 260.7*	1778± 194.9*	472.7± 47.4
	Before stress	After administration of KI and exposure to stress				
		30 min	1 h	3 h	6 h	24 h
Blood corticosterone, nM	302.8±28.5	2571.6± 282.7*	867.8± 104.5*	664.3± 100.5*	1661.8± 272.5*	697.7± 75.9*

Table 3. Effect of 30-min stress exposure on corticosterone concentration in rat blood after administration of 3 daily doses of KI within 24 h of post-stress period

The most pronounced stress-induced changes in iodine metabolism after administration of physiological KI doses (3 daily doses) are characterized by:

- abnormal kinetics of changes in blood iodine concentration within 24 h after administration of KI, which was manifested by accumulation of blood iodine (839.4% elevation) after 6 h at the post-stress period;
- changes in the kinetics of iodine uptake and oxidation in the TG, which results in a decreased content of total I and protein-bound I in thyroids of stressed rats after administration of 3 daily doses of KI as opposed to the control group which showed an increase of these parameters.

3.3. Effect of unavoidable repeated short-term psychoemotional stress on the functional activity of the rat thyroid

A research was carried out into a short-term stress effect (daily, over a long period of time) on the activities of the key steps in iodine metabolism in the rat thyroid. The data obtained indicate that daily 20-min exposure to stress (4 weeks) induced pronounced changes in thyroid iodine metabolism.

Figure 6 shows that the total thyroid iodine content in stressed animals was elevated 1.97-fold as opposed to controls and amounted to 491.8±15.5 µg/g tissue. The contents of its protein-bound and free fractions corresponded to 329.9±8.3 µg/g tissue and 161.8±18.4 µg/g tissue, which was 1.6-and 3.1-fold higher compared to the controls. The increased thyroid iodine concentration was accompanied by a changed ratio of its various fractions (Table 3). The 2-fold elevated free I/protein-bound I ratio and the lowered protein-bound I/total I ratio

(1.18-fold) are indicative of a lowered efficiency of thyroid iodine organification under stress.
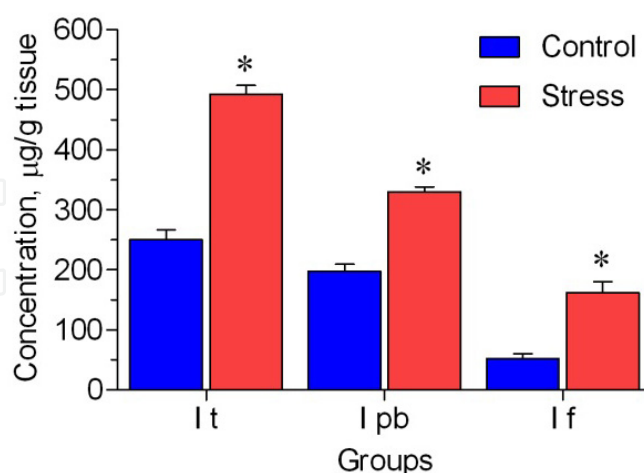
*statistically significant changes vs control group (p<0.05)

Figure 6. Effect of 4-week psychoemotional stress (20 min, daily) on contents of total I, protein-bound I and free I in the rat thyroid

Indices	Control	Stress
Free I/protein-bound I	0.26±0.034	0.50±0.066*
Protein-bound I/total I	0.79±0.021	0.67±0.028*
Urinary I, µg/l	17.9±2.29	22.2±1.94

* P<0.05 compared to control.

Table 4. Effect of short-term daily psychoemotional stress on the ratio of different rat thyroid iodine fractions and urinary iodine excretion

Indices	Control	Stress
T4 total, nM	49.2±2.82	51.7±3.34
T3 total, nM	1.2±0.06	1.3±0.07
TPO, µmol/min x g tissue	23.4±2.70	20.9±2.91
Thyroid weight, mg	15.7±0.63	13.3±0.47*
Thyroid cytosolic protein, mg/g tissue	158.5±3.6	137.9±5.3*

* P<0.05 compared to control.

Table 5. Effect of short-term daily psychoemotional stress on the concentration of blood thyroid hormones, TPO activity, thyroid weight and thyroid protein concentration

No changes were found in the activity of TPO, the key enzyme of thyroid hormone biosynthesis (Table 5). The thyroid weight in stressed rats was lowered by 18%, whereas the protein concentration in the thyroid cytosolic fraction – by 13%. The blood thyroid hormone

content at the post-stress period was maintained at the level of control values (Table 5), the level of corticosterone was increased by 32.8% (Table 6) and the weight of the adrenal glands rose by 13%.

Indices	Control	Stress
Blood serum corticosterone, nM	383.2±65.9	509.2±90.0#
Adrenal corticosterone, nmol/g tissue	152.8±17.9	176.2±30.8
Adrenal weight, mg	46.6±1.9	52.7±2.5*

* $P<0.05$; # $P<0.1$ compared to control.

Table 6. Effects of short-term daily psychoemotional stress on adrenal weight, blood corticosterone concentration and corticosterone concentration in rat adrenals.

As our data show, stress caused multidirectional changes in the activities of the key steps of thyroid iodine metabolism. The elevated content of the total and free iodine is a consequence of stimulation of its absorption at the post-stress period [28]. The decreased efficiency of iodine organification may be due to TPO inhibition and lowering of thyroglobulin concentration. The stress-induced lowering of thyroid TPO activity was shown earlier. As Table 7 demonstrates, the repeated exposure to short-term stress during 7 days and over was accompanied by a decrease of thyroid TPO activity both directly after exposure to stress (46.9-56.6%) and after 24 h following its cessation (59.2-60.7%).

Index	Control	Stress, 7 days		Stress, 14 days	
		А	Б	А	Б
TPO, μmol/min g protein	153.5±15.2	81.4±21.43*	60.2±4.9*	66.6±9.4*	62.6±18.22*

Group A animals were decapitated directly after the last exposure to stress; Group B animals were decapitated 24 h after the last exposure to stress. * $P<0.05$ compared to control.

Table 7. Effect of short-term (20 min) psychoemotional stress (daily, 7, 14 days) on thyroid TPO activity

Effects of stress on iodine oxidation and organification in thyroid cells have not been virtually investigated. We found only one study on female tortoises. Thyroid TPO activity in young female tortoises was lowered after ten-fold administration of corticosterone (25, 50, 100 μg/100 g body weight) [34]. Nothing has been known of the effect of stress on thyroglobulin biosynthesis. However, the diminished level of thyroid protein-bound I can be stipulated by its impaired biosynthesis. Moreover, a consequence of stress was a 13%-decreased total protein concentration in the thyroid cytosolic fraction. This certainly applies to thyroglobulin, taking into consideration that it amounts to 75-80% and up of the total thyroid protein.

The main regulator of TPO and thyroglobulin synthesis is TSH whose secretion is inhibited by glucocorticoids [20], which can induce depression of thyroid hormone synthesis. Stress is suggested to cause a decrease of TSH production via pituitary neuromedin B, gastrin-releasing peptide and pituitary leptin acting as local inhibitors of TSH release under stress [21]. It was found that lipocortin -1 is a mediator of glucocorticoid-induced suppression of TSH secretion by the anterior lobe of the pituitary gland [21].

The inhibitory effect of stress seems to be followed by activation of thyroid metabolism at the post-stress period and the restoration of thyrocyte function is related to activation of thyroid hormone secretion, which is confirmed by resorption of colloid and depletion of thyroid follicules. These conditions disturb the thyroglobulin synthesis/secretion balance. As a result, the thyrocytes and follicular lumen accumulate a considerable amount of non-organified iodine, which is confirmed by our findings. Stress decreases thyroid weight, which can be both a consequence of its hypersecretion and destructive processes; the mechanism of this change is certainly interesting.

The experimental findings show that a consequence of the repeated exposures to psychoemotional stress are pronounced structural and metabolic changes in the TG that are characterized by an elevated iodine content, a decreased extent of its organification, development of oxidative stress and lymphocyte infiltration along with the impaired thyroid follicular structure. The mechanisms of the regularities found call for detailed research and are of great interest to disclose the pathogenesis of autoimmune thyroiditis, thyroid carcinoma as well as the contribution of the thyroid component to development of endemic and nodular goiters.

There are presently no unambiguous data on the role of stress in induction of thyroid pathology in humans. Individual cases have been described of autoimmune thyroiditis developed after surgical treatment of hypercorticism (Cushing's syndrome) [65]. A pronounced stress effect can be an onset of Graves' disease [82]. There were reports about relationships between stress and Hashimoto's thyroiditis [83]. According to Polish researchers [84] secondary adrenal deficiency can be a cause of autoimmune thyroid diseases in humans: stress affects the immune system, and immunologic modulations are considered to be a factor inducing autoimmune thyroiditis in genetically prone individuals [85]. Stress hormones, affecting antigen-presenting immune cells, can influence the differentiation of bipolar T-helpers from Th1 to Th2 phenotype, which causes suppression of cellular immunity and enhancement of humoral immunity. Stress is likely to contribute to the development of Graves' disease by shifting the Th1/Th2 ratio from Th1 to Th2. Recovery after stress or immunosuppressive effect of pregnancy can induce a "reverse shift" in Th2 → Th1, causing autoimmune (sporadic) thyroiditis [85].

Stress-induced impairment of thyroid function characterized by development of oxidative and iodine stress is likely to be viewed as a main mechanism of thyroid ageing in humans and, consequently, to be a cause of diseases of age related to thyroid deficiency [86, 87]. Further studies are needed to disclose the mechanisms of stress-induced impairment of thyroid functions.

3.4. Studies on rat thyroid iodine metabolism under hypocorticism (after adrenalectomy)

The above findings confirm that stress considerably changes thyroid iodine metabolism, affecting its uptake and organification. Since all the experimental studies were carried out using models characterizing hyperfunction of the adrenal glands (stress), a comparative investigation of iodine metabolism in rats with adrenal deficiency should be done in order to establish the biochemical mechanisms.

The glucocorticoid status in rats was assessed by the level of corticosterone which was lowered 4.4 to 6.4-fold in adrenalectomized (AE) rats compared to controls. Adrenal deficiency was a cause of 44.2 % - decreased thyroid TPO activity (Fig. 7B). The administration of 1000 daily doses of KI (a dose=70 mg/kg) decreased thyroid TPO activity in the TG of the control rats and elevated it in the glucocorticoid-deficient animals with to the control values. The administration of 1000 daily doses of KI was accompanied by increases in thyroid total I (42.2%), protein-bound I (19.1%) and free I (90.6%) in AE rats (Fig. 8). This indicates that under hypocorticism the regulatory mechanisms for thyrocyte functions can be disturbed by high iodine doses.
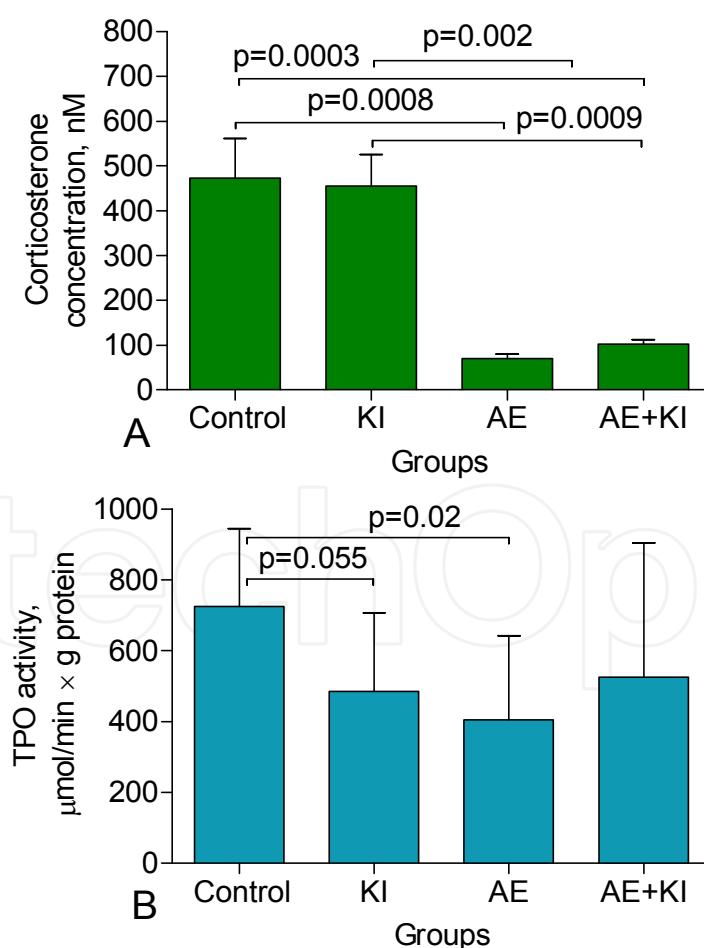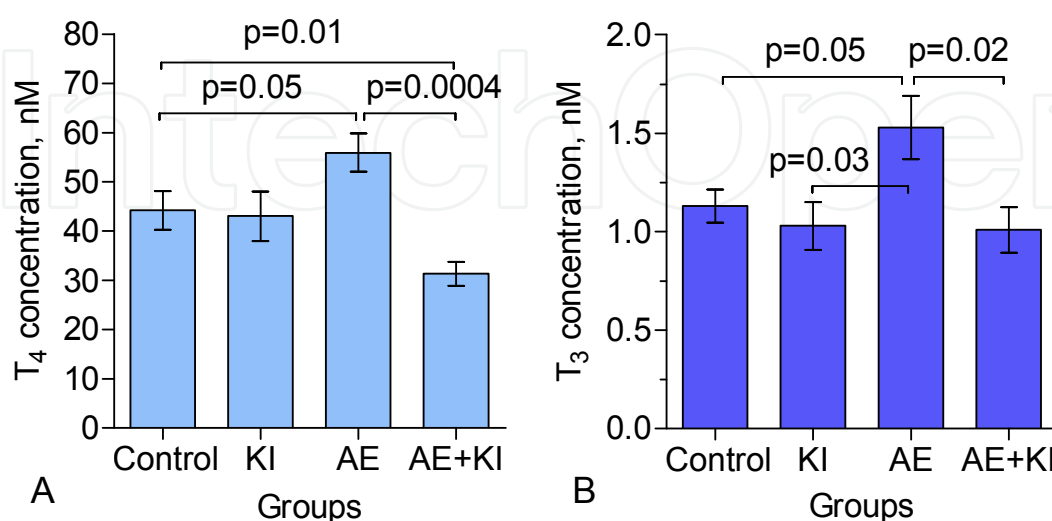

Figure 7. Effect of single administration of 1000 daily doses of KI on concentration of blood serum corticosterone (nM) (A) and TPO activity (μmol/min x g protein) in thyroids of intact and AE rats (B)

(a)

(b)

(c)
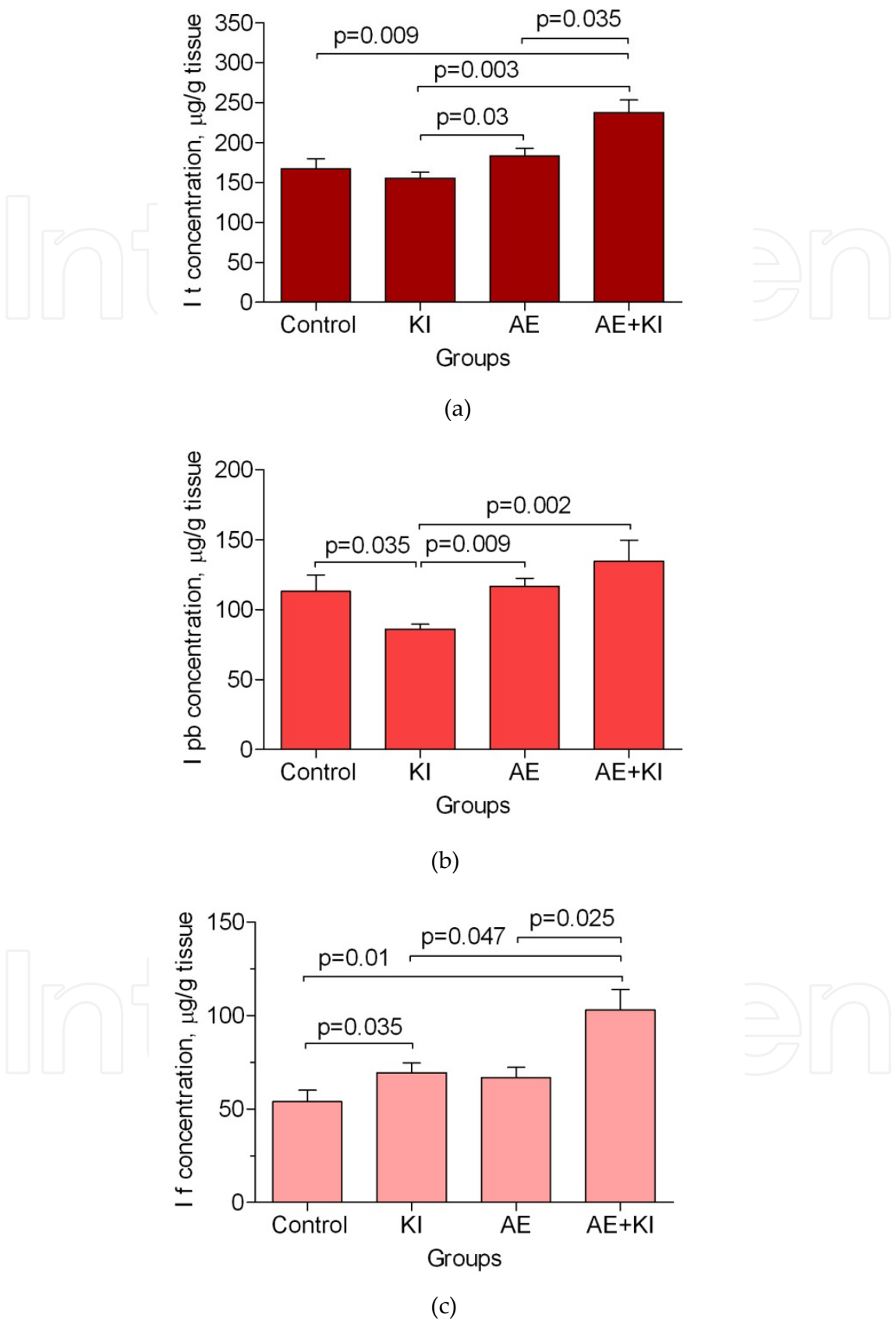
Figure 8. Effect of single administration of 1000 daily KI doses on contents of total I, protein-bound I and free I in thyroids of rats with normal and decreased glucocorticoid statuses

The blood thyroid hormone levels in AE rats were above the control values (27% for T_4 and 35% for T_3), but administration of KI lowered the concentrations of T_4 by 41.1% and T_3 by 34% compared to the AE animals. T_4 was 29% decreased even in comparison with the controls (Fig. 9).

Figure 9. Effect of single administration of high KI dose on concentrations of total blood serum T4 and T3 in rats with normal and decreased glucocorticoid statuses

Index	Control	1000 daily doses of KI	AE	AE+1000 daily doses of KI
	A	B	C	D
TBARS, nmol/g tissue	131.2±8.9	212.5±22.9A	150.4±15.0	115.5±9.6B,C
Catalasa, μmol/min×g protein	36.8±1.3	40.0±1.6	32.2±0.8A,B	33.7±1.7B
SOD, activity u./min×g protein	43.2±4.2	56.8±1.4A	51.1±2.0A,B	48.9±2.9B
GR, μmol/min×g protein	24.0±1.2	22.7±1.0	26.7±1.0B	25.5±1.5

Table 8. Effect of single administration of 1000 daily doses of KI on TBARS levels and antioxidant enzyme activities in thyroids of rats with normal and decreased glucocorticoid statuses

In contrast to the rats with the normal glucocorticoid status, in which the administration of KI inhibited the thyroid function and induced activation of oxidative processes (62.0% elevation of TBARS concentration, 54.3% activation of SOD), the adrenalectomized rats did not show activation of lipid peroxidation (the level of TBARS was decreased by 23.2%, Table 8).

The AE animals demonstrated elevated concentrations of T_3 and T_4 in the blood serum (Fig. 9). These changes seemed to be caused by alterations in thyroid iodine metabolism since the contents of its different fractions did not change under decreased glucocorticoid status (Fig. 8). It was found earlier that AE caused enhancement of liver thyroxin-binding globulin

synthesis and its binding capacity in the blood [88] as well as inhibition of the peripheral metabolism of thyroid hormones. Enhancement of deposition of blood thyroid hormones and, consequently, inhibition of their metabolism may cause elevation of thyroid hormone concentrations.

Most interesting changes were found after administration of high iodine doses to rats. In contrast to control animals with the characteristic acute Wolff – Chaikoff's effect, we did not observe inhibition of iodide organification in this group. Moreover, a pronounced lowering of T_4 and T_3 concentrations in the blood serum of AE rats after the administration of high iodine doses suggests that the cause of the absence of the Wolff-Chaikoff's effect under hypocorticism can be impaired maturing of the prohormone, thyroglobulin, and abnormal secretion of thyroid hormones to the blood, which provides for elevated concentration of protein-bound I in this group. Elevated TSH concentrations and enhanced NIS expression and, consequently, increased uptake of iodide absorption by the TG are also possible.

Our findings show that the effects of the single administration of the high KI dose on the activity of hormonogenesis in thyroids from normal and AE animals are multidirectional. Thyroids from the intact rats show inhibition of iodide organification accompanied by induction of oxidative stress, whereas the hypocorticoid rats demonstrate a reverse effect: activation of iodide uptake and organification as well as a decrease in the intensity of lipid peroxidation. These results are of a considerable interest in relation to some clinical studies which prove that impairments in the glucocorticoid status can be linked to development of autoimmune thyroid diseases. Lowering of the functional activity in the hypophyseal link (ACTH) and/or the adrenal (cortisol) link was noted in patients with autoimmune tyroiditis [89, 90]. It was shown that autoimmune tyroiditis and diabetes mellitus are developed on the average 7 years after autoimmune damage of the adrenal glands [91]. In patients suffering from hypercorticism of different genesis, AE contributes to development of autoaggression in their thyroids [68, 66, 92]. It is suggested that puerperal thyroiditis, as a consequence of a temporary decrease of the glucocorticoid status in females at the puerperal period [93], is due to ACTH-releasing hormone inhibition of the synthesis and secretion of maternal hypothalamic ACTH-releasing hormone and that this inhibition is of a placental origin. It should be mentioned that impaired functional activities of the pituitary-adrenal axis are also noted in other autoimmune diseases [94]. The mechanisms of the regularities found certainly require further studies since the literature lacks information on this problem.

3.5. Excess administration of iodine induces development of hyperthyroiditis in rats with glucocorticoid deficiency

Glucocorticoid deficiency is a cause for impairments of the adequate regulation of the thyroid status and thyroid iodine metabolism. It was interesting to study the properties of the iodine metabolism after its repeated administration to rats with adrenal deficiency.

After 2 weeks following AE, the blood serum thyroid hormone concentrations in operated animals were partially restored and amounted to 23.7-42.3% of the control values (Fig. 10)
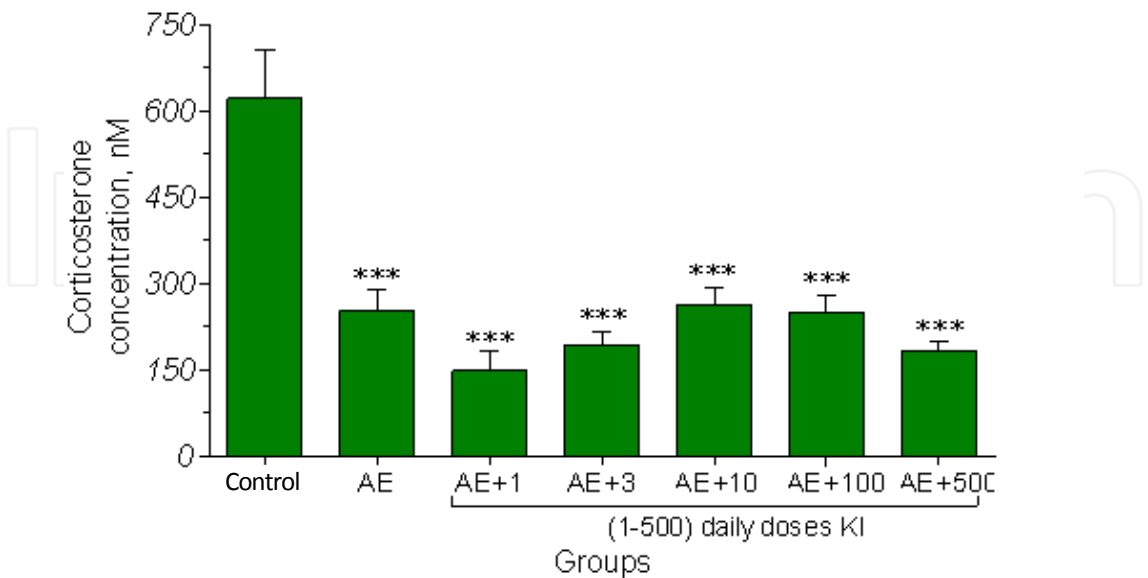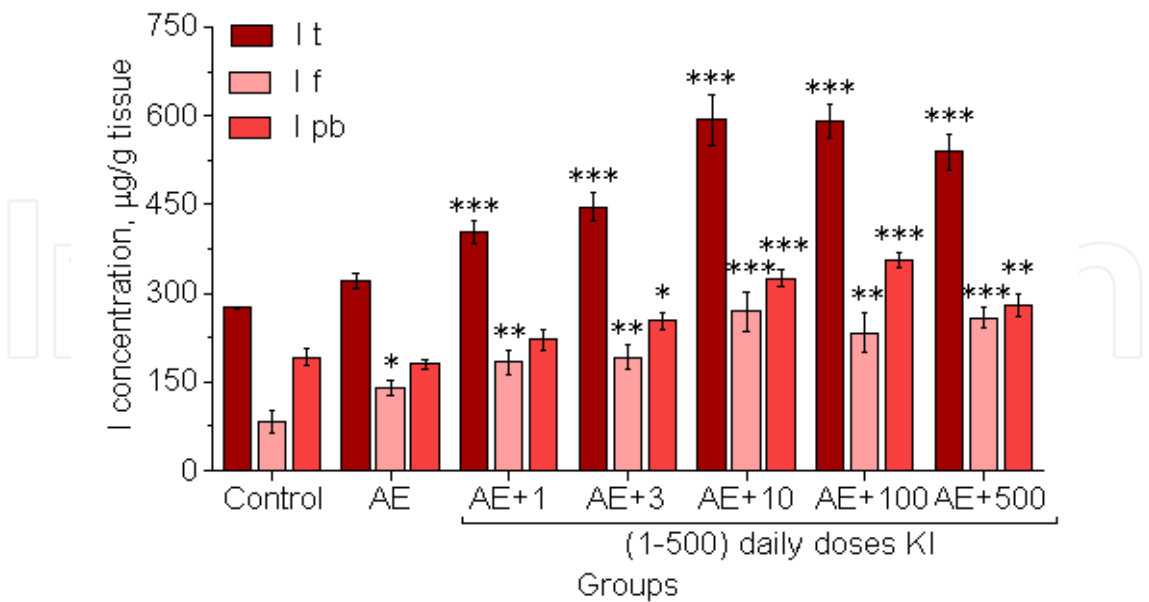

*** statistically significant changes vs control group (p<0.001)

Figure 10. Effect of 14-day administration of 1-500 daily doses of KI on blood serum corticosterone concentration in AE rats.

Studies on the thyroid iodine metabolism showed that repeated administration of high KI doses resulted in 46.9-115.7% increased concentrations of total I and caused 120.4 to 223.9% elevations of free I in all the experimental groups (Fig. 11). Glucocorticoid hormones are likely to inhibit iodide uptake by erythrocytes since the levels of nonorganified iodine were 1.2-fold increased after AE in rats which did not receive supplementary KI. One more confirmation is a more considerable growth of free I concentrations in thyroids of rats with hypocorticism (120.4-223.9%, Fig. 11) compared to controls (94.8-128.0%) after administration of KI at the same doses. Iodine organification in AE rats was enhanced by 32-86% in rats treated with 3 to 500 daily doses of KI (Fig. 11). TPO activity in AE rats was 29.4% elevated and 2.4, 3.9 and 3.7-fold increased (Table 9) after administration of 3, 100 and 500 KI daily doses.

	Control	Daily doses of KI administered to AE animals				
		No administration	1	3	100	500
TPO, µmol/min× g protein	173.9±22.5	193.8±10.3	275.4±77.4	387.9±78.4*	616.5±178.1*	579.7±120.6*

Table 9. Effect of 2-week administration of 1 to 500 daily doses of potassium iodide on TPO activity (µm/min×g tissue) in thyroids of AE rats

*statistically significant changes vs control group (p<0.05), **p<0.01; ***p<0.001

Figure 11. Effect of 14-day administration of 1-500 daily doses of KI on the concentrations of total I, protein-bound I and free I in thyroids of AE rats
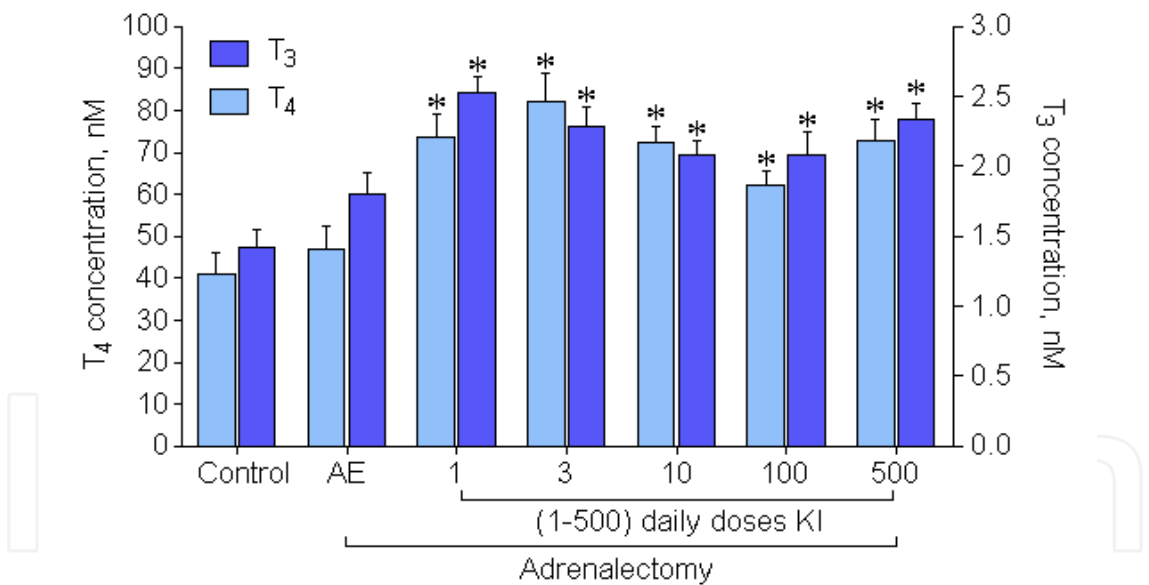
* statistically significant changes vs control group (p<0.05)

Figure 12. Effect of 14-day administration of 1 to 500 daily doses of potassium iodide on the concentrations of total T_4 and T_3 in the blood serum of rats with normal and lowered glucocorticoid statuses

The repeated and single administrations of excess potassium iodide to rats with hypocorticism were characterized by activation of iodide metabolism in the TG, which was followed by development of pronounced hyperthyroidism in AE animals. The blood serum total T_4 concentration (Fig. 12) was increased by 52-100% in rats with adrenal deficiency

treated with KI for 14 days compared to control animals. The T_3 concentration (Fig. 12) reached 145.5-177.5% of the control level.

In this situation, a pronounced disturbance in the regulatory mechanisms of the pituitary-thyroid axis may be observed, which is accompanied by development of hypothyroidism and indicates a permissive (coordinating) role of glucocorticoids in regulation of thyroid homeostasis.

Our findings indicate that regulation of iodide uptake is very closely related to the state of the pituitary-adrenal system. Excess iodine intake under hypocorticism causes development of hyperthyroiditis.

4. Conclusion

1.   Short-term stress (5-30 min) induced activation of biosynthesis and secretion of thyroid hormones. The most important established regularity of the post-stress period is restoration of the iodine thyroid status due to activation of uptake and organification of iodine as well as a negative correlation between the total thyroid concentration and adrenal corticosterone concentration (r= –0.952, p=0.003), which indicates participation of glucocorticoids in regulation of iodine thyroid homeostasis.
2.   The most pronounced stress-induced changes in iodine metabolism after the treatment by physiological KI doses (3 daily doses) are characterized by:
     -   disturbed kinetics of blood iodine content within 24 h following the KI treatment, which was characterized by accumulation of blood iodine (826%) after 6 hours following the post-stress period;
     -   changed dynamics of thyroid uptake and oxidation of iodine, which caused a decrease in the concentrations of total I and protein-bound I in thyroids of stressed rats after the treatment with 3 daily doses of KI in contrast to the control group which showed elevation of these indices.
3.   It was shown that repeated exposure to short-term psychoemotional stress (for 4 weeks) induced pronounced structural and metabolic changes in the thyroid gland that were characterized by elevated iodine content, as well as a decrease of the extent of its organification and development of oxidative stress.
4.   The lowered glucocorticoid status in rats is characterized by increased blood thyroid hormone concentrations and decreased TPO activity (44.2%). In contrast to the animals with normal glucocorticoid status, the AE rats did not show any inhibitory effect of high iodine doses (Wolff-Chaikoff's effect) after the single administration of 1000 daily doses of KI, and activation of thyroid iodide uptake and organification was observed.
5.   The 2-week administration of KI (1-500 daily doses) to rats with glucocorticoid deficiency increased the levels of free iodine (120-224%) and protein-bound iodine (32-86%) as well as thyroid TPO activity. In contrast to controls, this was followed by development of pronounced hyperthyroiditis (T_4 amounts to 152-200% and T_3 – 145 to

177% of the control values), which is a consequence of impairments in the key mechanisms of thyrocyte regulation and shows a permissive (coordinating) role of glucocorticoids in respect to the given effects.

The state of chronic stress may be a cause of impaired iodine metabolism in thyroid cells, which can induce development of hypothyroiditis and autoimmune thyroid pathology. Deficiency of the pituitary-adrenal system enhances the probability of development of hyperthyroiditis.

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