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MICROSTRUCTURAL CHANGES IN THE THYROID GLAND OF THE PREWEANING AND WEANING RATS AFTER HETEROTYPIC STRESS

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Abstract:
Chronic stress modulates the activity of various neuroendocrine axes; while activation pattern of the hypothalamo-hypophyseo-adrenal axis (HHAA) is studied in details, the response of the hypothalamo-hypophyseo-thyroid axis (HHTA) is less understood, especially in terms of its response to different types of stressors by the follicular and parafollicular compartments of the thyroid gland. Recent papers presented data on the inhibitory effect of chronic stress on the HHTA, while exercise and low temperature were shown to be able to activate its function. Most studies were done on the adult experimental animals using genetic studies without consideration of the structural changes in the peripheral link of the HHTA in the growing body. Reports on the effect of the chronic variable stressors on the thyroid gland in early life are scarce, though during this period neuroendocrine axes are extremely sensitive to different adverse factors, such as stress, infection, inflammation and environmental changes. The objective of this research is to assess the microscopic changes in the thyroid gland of the preweaning and weaning rats exposed to chronic heterotypic stress compared to the homotypic one. Homo- or heterotypic stressors were chronically applied to the preweaning and weaning rat pups. After the end of the last stress session the animals were euthanized, thyroid gland was sampled, embedded in paraffin, sectioned and stained for thyroglobulin, calcitonin, proliferative cells nuclear antigen (PCNA) and caspase 3. The mucosa of the alimentary tract of the experimental animals was examined and the thymus and the adrenal glands were sampled and weighed to evaluate the depth of stress-induced changes in the body. Immunologically stained slides of the thyroid gland were assessed using Image Pro+ software. Our study showed that chronic stress resulted in the structural and immunohistochemical changes of the thyroid gland in the preweaning and weaning experimental animals which indicate an inhibition of its function in the type of stress-related pattern. Both homo- and heterotypic stressors caused microscopic alterations in the thyroid gland, the extent of which depended both on the initial age of the experimental animal and the type of the stressor applied. The number and the size of the thyroglobulin-positive cells significantly decreased, and the volume density of the apoptotic cells significantly increased in the heterotypically stressed rat pups of both age subgroups with higher level of significance in the preweaning age subgroup. The number of calcitoninocytes was significantly increased in the weaning rat pups exposed to the heterotypic stress. The number of PCNA-positive cells significantly decreased only in the heterotypic stress group of both ages with higher level of significance in the preweaning age subgroup. A positive correlation was found between the volume density of the thyroglobulin-positive cells and the severity of the accidental thymic involution. Thus, our research demonstrated that weaning period is very sensitive to chronic stress for the thyroid gland of the experimental animals which by this age becomes mature enough to differentially respond to the various types of stress (homotypic versus heterotypic) both by the thyroid follicular and parafollicular compartments, and that thymus plays an important role in the functional capacity of the thyroid gland during stress in early life.

Key words: *Thyroid Gland, Chronic Stress, Weaning Period, Immunohistochemistry, Image Analysis*

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INTRODUCTION:

It is well known that HHAA is activated during stress exposure, but the role of the HHTA under stress conditions is less understood [1-8]. Recent investigations demonstrated that chronic stress downregulates the HHTA; on the contrary, exercise and cold exposure activate it both at the level of its central and peripheral links [4,8,9]. It was shown that the expression of the TRH by the neurons in the hypothalamic paraventricular nucleus and the HHTA are activated by the energy consuming factors, such as low temperature and exercise, while situations with a negative energy balance, such as fasting and chronic stress, are more likely to inhibit them [4,9]. Earlier studies demonstrated that certain types of psychological stress, not involving energy consumption, like noise and water maze, activate thyroid function [10,11] while immobilization and inescapable shock cause its inhibition [12-15]. The reason for these controversies is a sensitivity of the thyroid gland to many body- and environment-related factors, such as circadian rhythm, temperature of the environment, nutrition, voluntary exercise and some others. If several factors are combined, the effect of any one of them may be blunted, for example: hypophysiotropic paraventricular nucleus TRH neurons are upregulated by the exercise, but their response may be modified by the suppressive action of chronic stress [1,9]. New information regarding the activity of the HHTA in stress was provided by the researchers who described the role of adenylate cyclase-activating polypeptide, deiodinases and tannicytes in alteration of the function of the thyroid gland [1,16,17, 8].

There are few papers describing the changes in the central and peripheral links of the HHTA in the growing body, including the research which studied the consequences of the early life stress for the adulthood [7], but information on the stress-related modulation of the HHTA in early life is not sufficient.

Most of the studies on the chronic stress-induced changes of the thyroid function were done using repeating rather than variable (heterotypic) stressors which become more and more common for the everyday life of a modern human. It is known that chronically applied heterotypic stressors cause their own pattern of modulation of the HHAA. Taking into consideration the crosstalk of the HHAA and HHTA in adaptation to stress, the response of the HHTA exposed to the heterotypic stress needs to be further investigated [18,19].

Earlier it was shown stress-related modulation of HHTA depends on the immune function of the body [20] which may control it by several mechanisms including secretion of the thyroid stimulating hormones by the lymphoid cells, but this aspect of the modulation of the thyroid function in the body needs further clarification.

The objective of this research was to compare the effect of homo- and heterotypic stressors on the thyroid gland of the preweaning and weaning rats.

MATERIAL AND METHODS:

Preweaning and weaning Sprague Dawley rat pups (14 and 21 days of age accordingly) were used in this research. The experimental design was approved by the ethical committee of the Faculty of Medicine, UiTM, Selangor, Malaysia, protocol ACUC 4-11, 14.04.11.

The animals were divided into 3 groups, with 16 species per group: control, experimental - exposed to the homotypic stressor and experimental - exposed to the heterotypic stressor using a modified model by Choudhary [21]. Control rat pups were fully isolated from the experimental animals. At the end of the experiment the rats were sacrifised by decapitation under anaesthesia. Their thymus and left adrenal gland were sampled and weighed. The thyroid gland was sampled together with the trachea, processed in the tissue processor and embedded in paraffin. Histological slides were sectioned and stained by the routine H & E staining. Immunohistochemical staining was done using the following chemicals: antibodies against thyroglobulin and calcitonin (DAKO, Denmark), PCNA and caspase-3 (AbDSerotec, US). After staining with primary and secondary antibodies, streptavidin-biotin-peroxydase complex was applied with subsequent DAB staining, as recommended by the manufacturer of the chemicals. After immunohistochemical staining the slides were assessed with a help of the Image Pro+ 8.0 software (Media Cybernetics, US).

All numeric data are shown as the mean ± S.E.M, with 8 animals per subgroup. The results were processed using one-way ANOVA with subsequent Student–Newman–Keuls multiple comparison test; the results with $p<0.05$ were considered statistically significant.

RESULTS:

All experimental animals revealed stress-related changes, such as hypoplasia of the thymus (Fig.1), hyperplasia of the adrenal glands (Fig.2) and haemorrhages in the mucosa of the oesophagus and stomach. As follows from the Fig.1 and 2, rats pups of the both stress groups and age subgroups demonstrated significantly increased relative mass of the left adrenal gland and decreased relative mass of the thymus with higher level of significance in the heterotypic stress for the adrenal glands in both age subgroups and in the homotypic stress for thymus in preweaning rats.

Fig 1. Relative mass of the thymus (%) of the experimental and control rat pups, M+/-m.

Group/age	preweaning	weaning
Control	**0,439 ± 0,035**	**0,569 ± 0,054**
Homotypic	**0,342 ± 0,030***	**0,431 ± 0,037***
Heterotypic	**0,308 ± 0,026****	**0,394 ± 0,031***

*** - p<0.05***
***** - p<0.01** compared* to the age-matched control

Fig 2. Relative mass of the left adrenal gland (%o) of the experimental and control rat pups, M+/-m

Group/age	preweaning	weaning
control	**0,213 ± 0,019**	**0,175 ± 0,014**
Homotypic stressor	**0,272 ± 0,018***	**0,237 ± 0,016***
Heterotypic stressor	**0,329 ± 0,027****	**0,286 ± 0,023*****

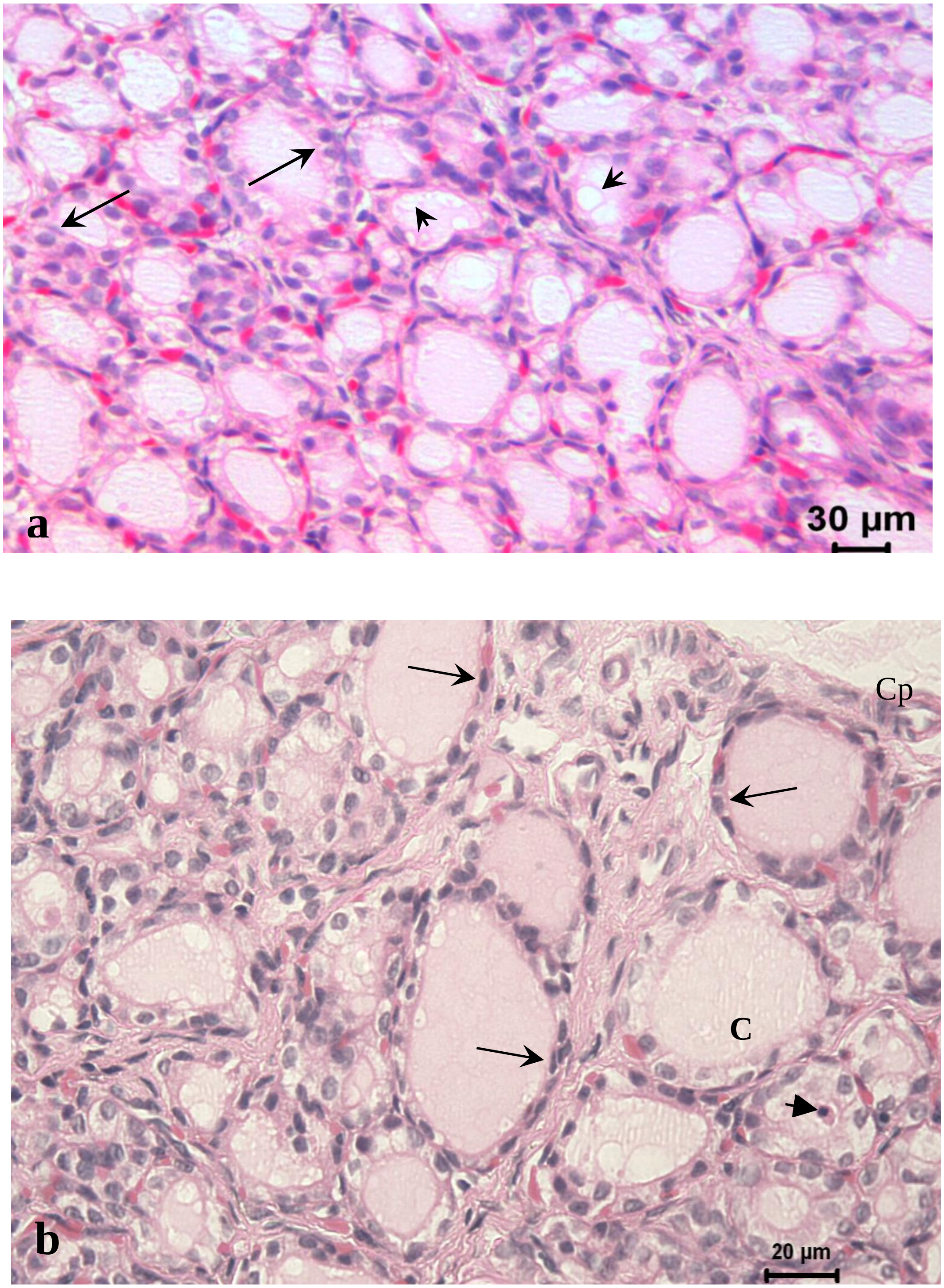
**** - p<0.05***

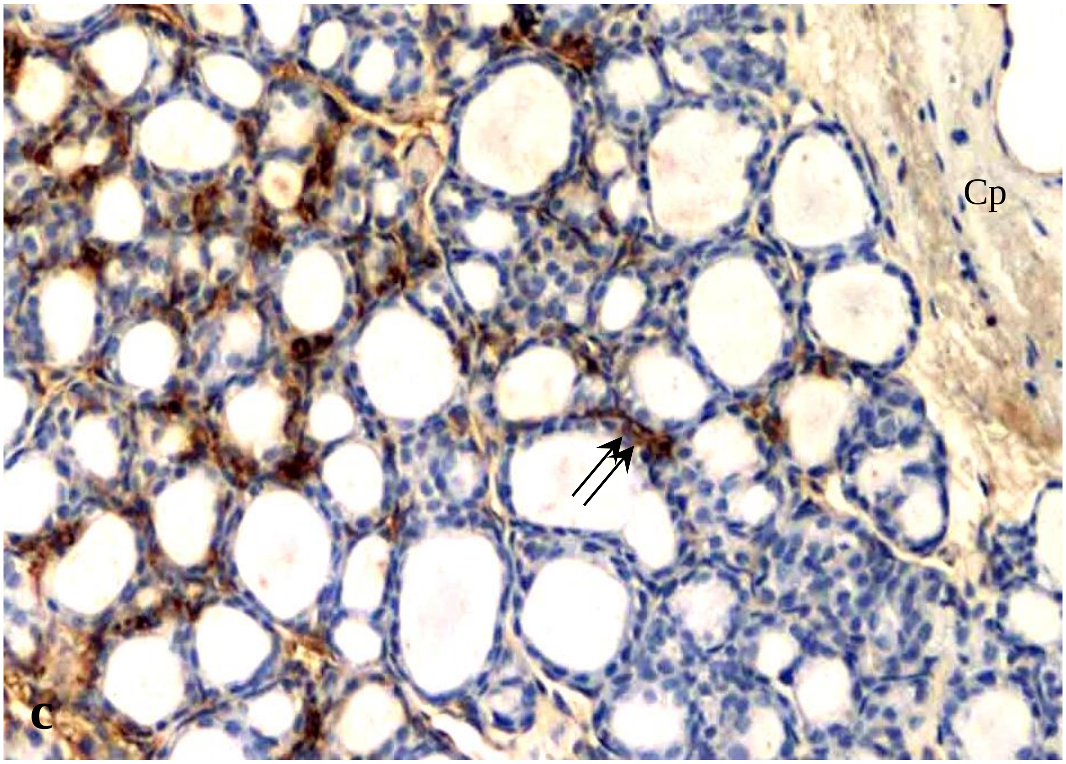
*****- p<0.01***

******- p<0.001*** compared to the age-matched control

Microscopic examination (Fig.3a-h) showed that in the control preweaning and weaning animals the thyroid gland was highly differentiated, contained mainly small and a few medium-sized follicles lined with predominantly cuboidal epithelium except the peripheral parts of the gland where the epithelium was often squamous. Colloid in the follicles was light basophilic with a few resorption vacuoles. Few larger lighter cells in the epithelial lining were identified as calcitoninocytes after immunohistochemical staining for calcitonin. Some calcitoninocytes were seen in the parafollicular space as well. Thyrocytes were highly positive for thyroglobulin, while the colloid was slightly positive. PCNA+ cells were evenly distributed in the parenchyma of the gland, while caspase-3-positive cells were scarce and they used to form conglomerates.

In stressed animals the microscopic picture depended on the type of stressor and the initial age of the animal. In homotypic stress group the changes in the preweaning rats were prominent, they involved the height of the epithelium, the number of the resorption vacuoles, the frequency of the sluffed off epithelial cells in the follicles and the frequency of apoptoses among the follicular cells. In heterotypic stress the changes were more severe, they involved the shape of the follicles, the density of the PCNA+ cells and calcitoninocytes, the intensity of the staining for thyroglobulin. Overall all these changes were more prominent in the preweaning rat pups compared to the weaning ones.

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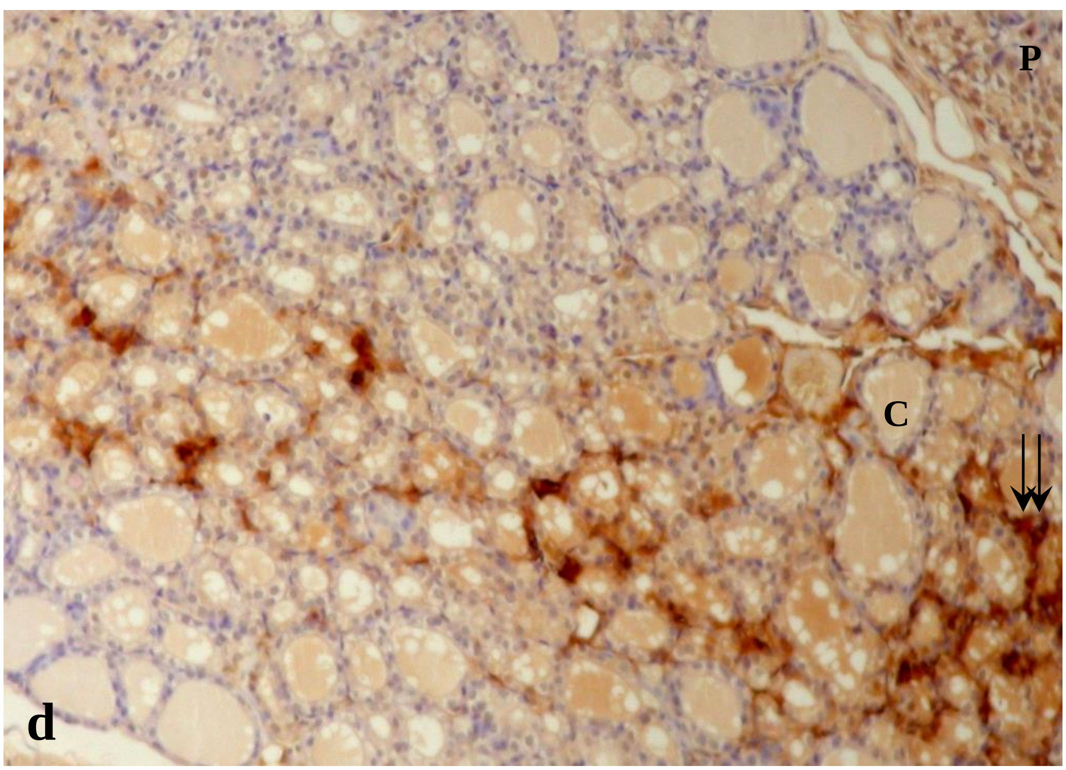
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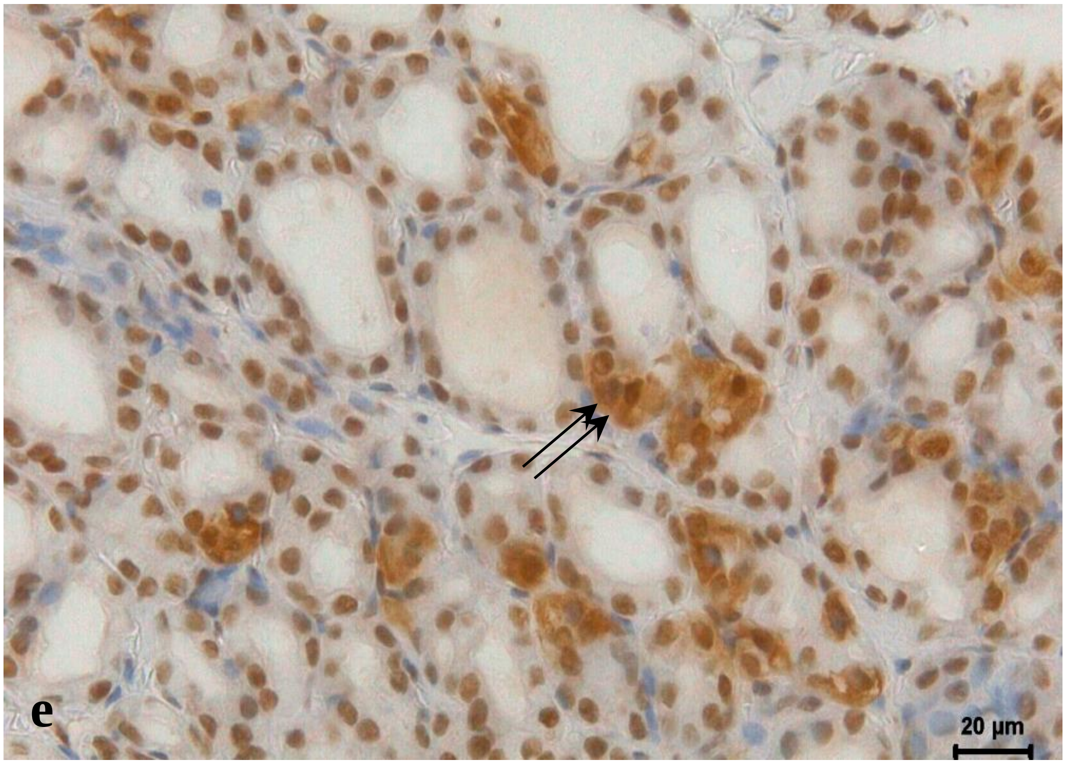
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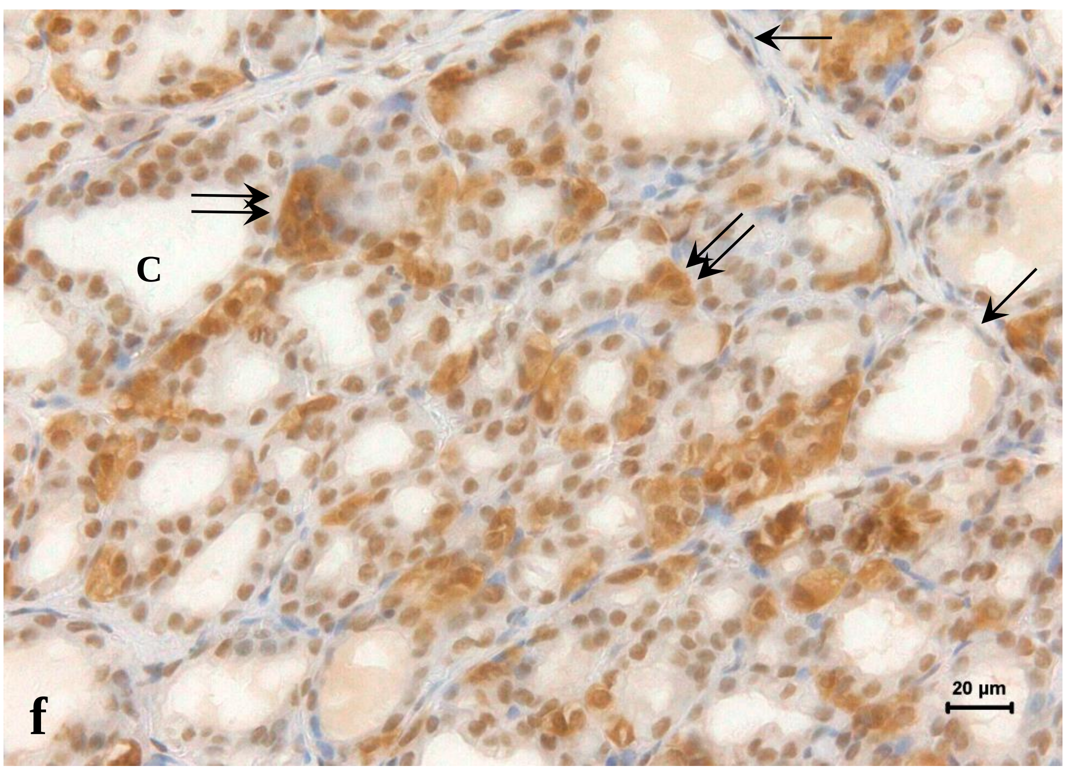
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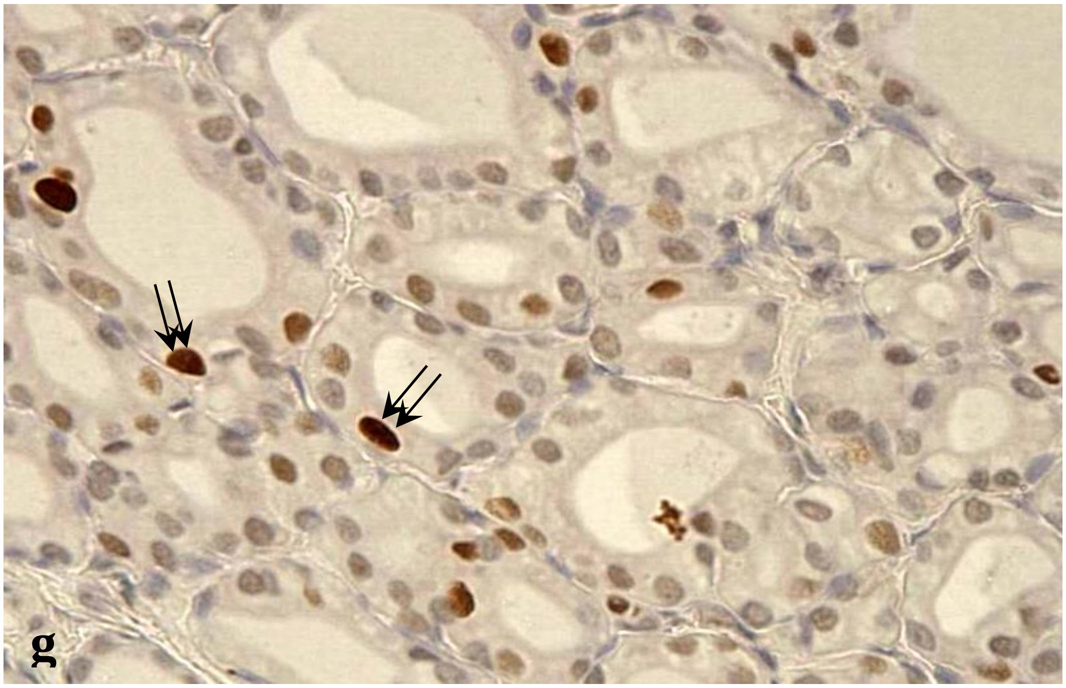

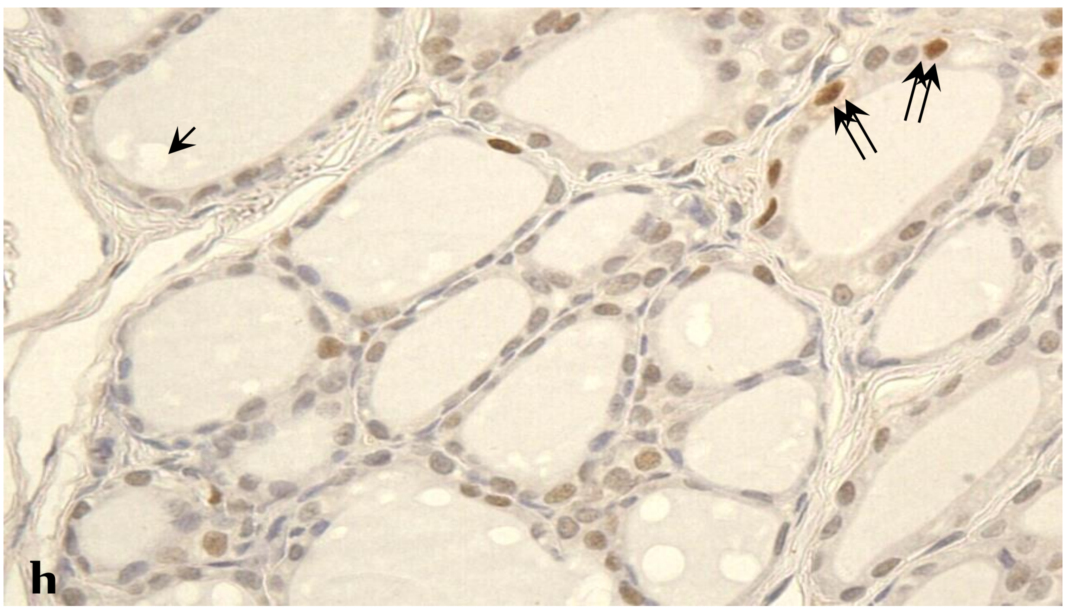

Fig.3. Microphotographs of the thyroid gland of the control (a,c,e,g) and experimental (repeating-d,f; variable-b,h) groups of rats; a,b,e,f,g,h – weaning rats, c,d - preweaning rats; a,b – H & E staining, c,d – staining for calcitonin, e,f – staining for caspase-3, g,h – staining for PCNA. Magnification x400 (b,g,h); x200 (a,e,f) and x100 (c,d).

Legends: long arrow – follicular epithelium, short arrow – resorption vacuoles, double arrow – immunopositive cells, arrowhead – sluffed off epithelim, C – colloid, Cp – capsule, P – parathyroid gland.

The results of the image analysis of the immunohistochemically stained sections of the thyroid gland are shown in the Fig.4-7.

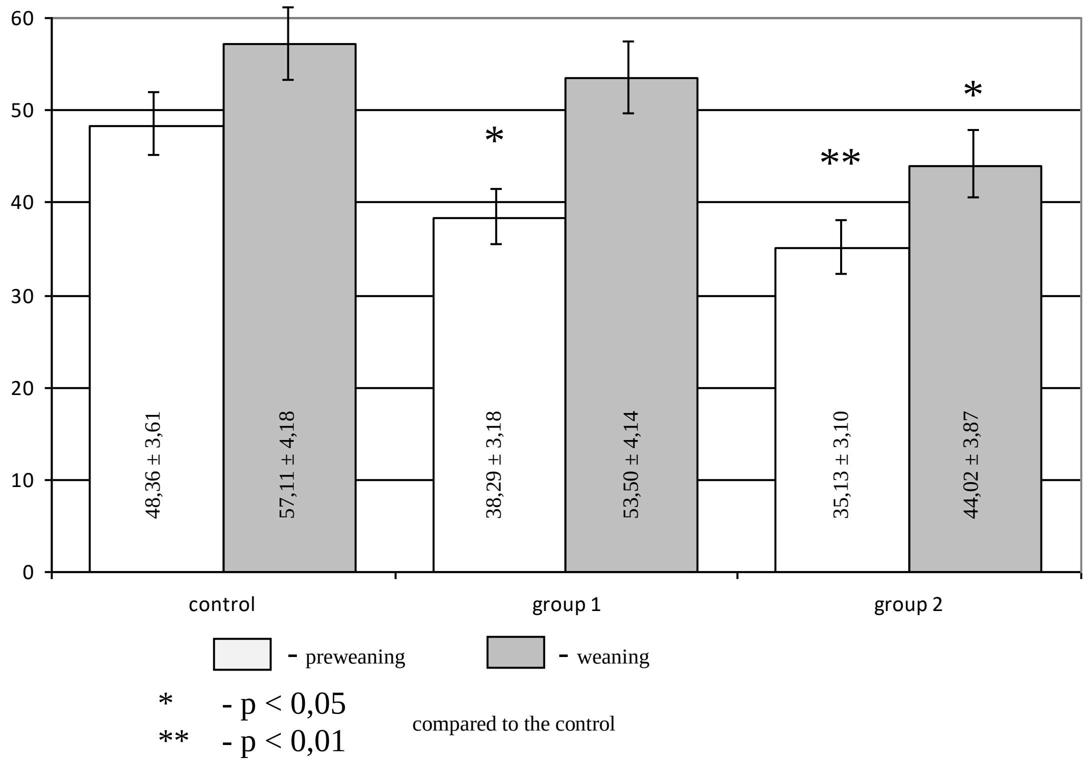

Fig. 4. Volume density of the thyroglobulin (%) in the thyroid gland of the stressed and control rat pups, M+/-m

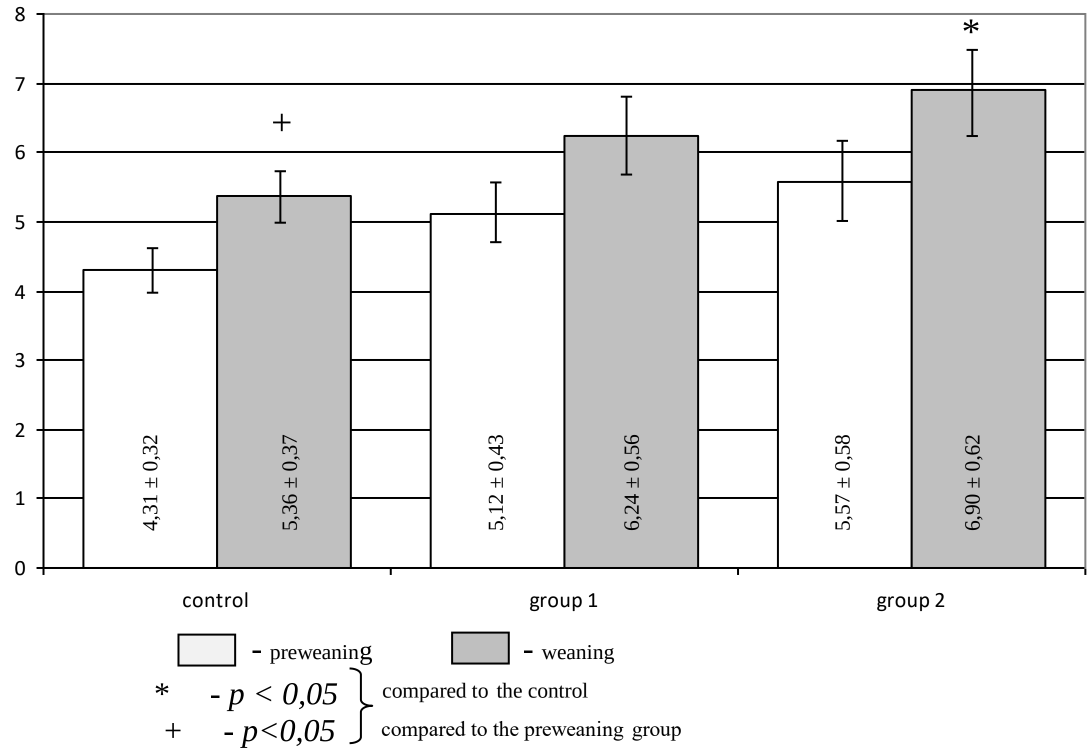

Fig.5. Volume density of the calcitonin (%) in the thyroid gland of the control and stressed rat pups (%), M+/-m

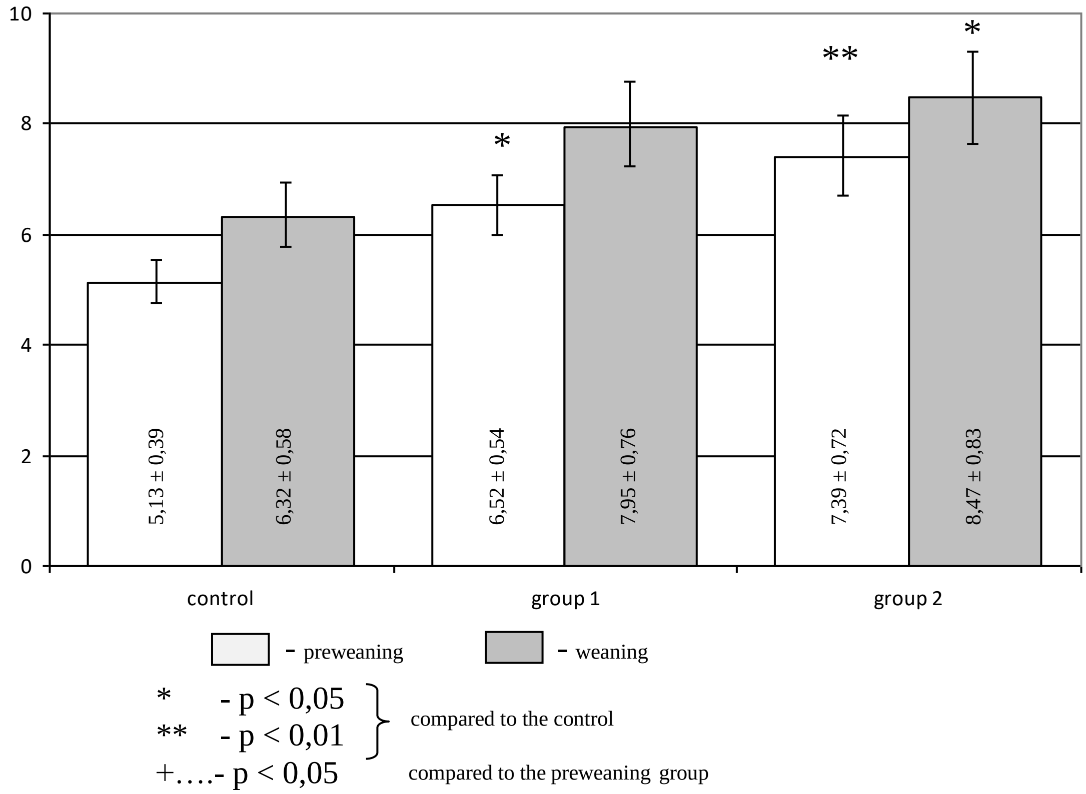

Fig.6. Volume density of the caspase-3 (%) in the thyroid gland of the control and stressed rat pups, M+/-m

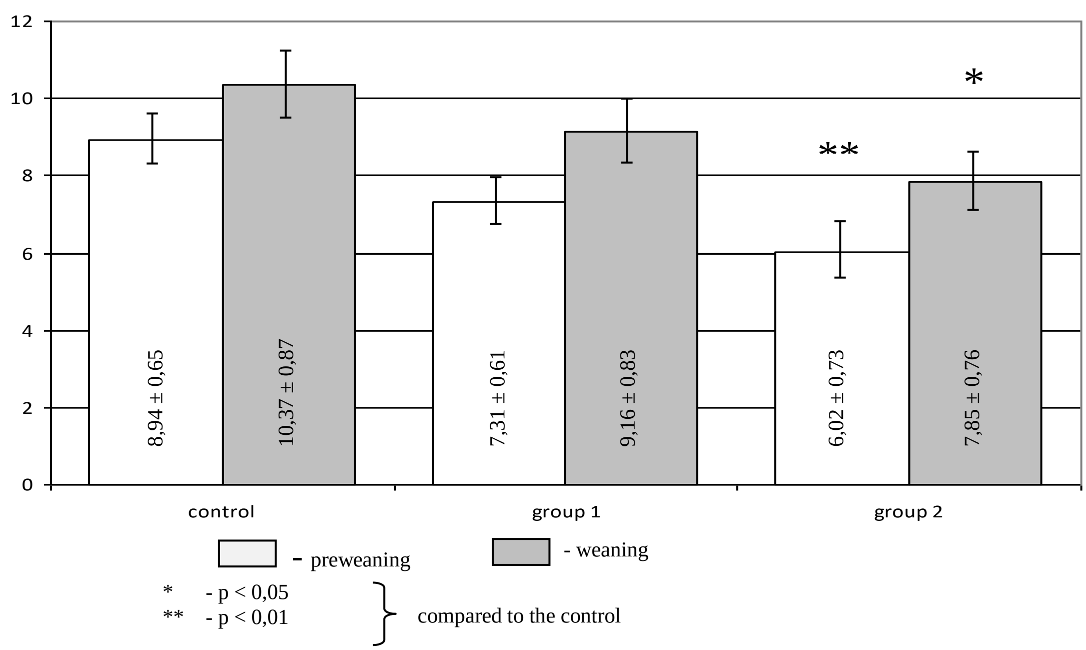

Fig.7. Volume density of the PCNA (%) in the thyroid gland of the control and stressed rat pups, M+/-m

DISCUSSION:

Our results concerning the suppressive effect of chronic stress on the thyroid gland as the peripheral link of the HHTA confirm the information of the other researchers on stress-related alteration of the HHTA in different types of chronic stress not involving the exposure to low temperatures and forced exercise [1-6] and expand it with the data regarding the microscopic stress-induced alterations in the thyroid gland at various ages of early life. On the contrary to most of the studies which were performed using genetic and molecular methods of evaluation of the thyroid function of the body [1-7], we focused on the structural stress-associated changes in the thyroid gland which demonstrate the severity of post-stress consequences in the follicular and parafollicular compartments of the gland. Most of the studies performed on the chronic stress-related modulation of the HHTA were done using repeating stressors [1-8], while in our research heterotypic stressors were applied which become more and more common in the modern life and which depict the range of the adaptational changes in both HHAA and HHTA and their stress-associated crosstalk.

Our results demonstrated the presence of the positive correlation between the functional activity of the thyroid gland assessed by the immunohistochemical staining for the thyroglobulin [22] and the severity of the accidental thymic involution, which indicates the importance of the thymus for the functional condition of the thyroid gland during early stages of postnatal ontogenesis. We also showed that heterotypic and not homotypic stress affects the proliferative activity of the follicular epithelium which is an important parameter of the functional activity of the thyroid gland [23]. Our data demonstrated the significant increase of the volume density of the calcitoninocytes with age and in heterotypic stress in the weaning rats. It was earlier shown [24] that the activity of calcitoninocytes in the thyroid gland does not depend on the level of the thyroid and thyrotropic hormones, which may be explained by the effect of the activated HHAA on the parafollicular compartment of the thyroid gland.

As a result, our study showed differential sensitivity of the thyroid gland to chronically applied homo- and heterotypic stressors which was also age-related within a short period of time from preweaning to weaning age and demonstrated that the dynamic developmental changes of the endocrine system in early life modulate its adaptational potential and predetermine its vulnerability to chronic stress.

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