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Correlated histological and morphometric study of kidney and adrenal gland from Guinea pig exposed to hyperbaric–hyperoxic environment

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Abstract
Our study revealed morphological changes in the two organs of Guinea pig kidney and suprarenal gland exposed to hyperbaric–hyperoxic environment. Proceeding from these data and knowing that in hyperbaric–hyperoxic environment the production of free oxygen radicals is increased, while the afferent arterioles undergo vasoconstriction, with direct implications on the cellular metabolism, our study puts forth the survey of the influence of hyperoxic environment on kidneys and adrenal gland – complex organs which are exposed to stress.

Keywords: hyperbaric–hyperoxic environment, free oxygen radicals, Guinea pig.

Introduction

Knowing that in hyperbaric–hyperoxic environment the production of free oxygen radicals [1] is increased with direct implication on the cellular metabolism our study puts forth survey of the influence of hyperbaric–hyperoxic environment [2, 3] on kidneys and adrenal gland.

The study of the complex answer and adaptation of human body to high depth is a major problem in hyperbaric medicine; hyperbaric conditions establish a long stimulation of sympathetic–adrenergic system. Stress is a biological phenomenon located somewhere between normal and pathological aspects and consists of two types of biological reactions: the first type represents the local and general response specific for each aggression, while the second type is related to the non-specific adaptative neuroendocrine reactions in order to enhance the resilience of human body [4, 5].

Our experimental study represents an original contribution by correlating data from the scientific literature with histometric data that confirm the involvement of suprarenal gland and kidney in the processes of eustress and distress and the implications of free oxygen radicals on the cellular metabolism.

Material and methods

In order to perform tests on animals we collaborated with the Department of Normal and Pathologic Physiology, Faculty of Medicine, „Ovidius" University of Constanţa, where is installed a hyperbaric chamber. The volume of barochamber is of 30.8 liters, with a total weight of 147 kg. The maximum admitted pressure was up to 20 ATA, but the work pressure that used during our experiment was 6 ATA. The laboratory animals were represented by Guinea Pigs (G.P.) with the weights 500–900 grams and body lengths about 250–300 mm. Ten G.P. comprised the group of animals with the weight at the beginning of experiment between 190–280 grams. According to the specific tables the animal's age was between 5–6 weeks.

This initial group was randomly split into two groups of five animals: control group and test group.

The test group was exposed for 50 consecutive days to ambient pressure of 6 ATA for a period of 30 minutes each, by using a pattern of compression and decompression commonly used in human diving. The respiratory mixture was compressed air [6].

After 50 days both groups were weighed and the animals were given a lethal dose of pentobarbital. We removed the kidneys and the histological pieces were processed using the routine histological techniques of paraffin embedding. Fixation lasted 48 hours at the laboratory temperature, and then the pieces were processed using paraffin embedding.

Serial cross sections were made at 4–5 microns, which were stained using Trichromic Goldner–Szeckelly, Masson and Hematoxylin–Eosin techniques in order to observe the general structure of kidney and adrenal gland. After staining the obtained specimens were examined under light microscope and captured by the computer using a videocamera. The obtained images were processed using image analysis software LUCIA G.4.10. A two-tailed dependent Student's t-test was used to compare the data of the two groups of animals. The Pt values under 0.05 were considered significant.

Results

The results of our study are shown in Figures 1–4 and Tables 1–3, revealing the morphological changes in the two organs of Guinea pig – kidney and suprarenal gland exposed to hyperbaric–hyperoxic environment. Our study puts forth the survey of the influence of hyperoxic environment on kidneys and adrenal gland – complex organs which are exposed to stress, because in hyperbaric–hyperoxic environment the production of free oxygen radicals is increased, while the afferent arterioles undergo vasoconstriction, with direct implications on the cellular metabolism.

Discussions

Stress is a biological phenomenon located between normal and pathologic and comprises two types of biologic reactions:

▪ first is the local and general response specific to each aggression

▪ the second category of biologic reactions is represented by the nonspecific responses of nervous and endocrine nature in order to increase the resilience of organism.

This neuroendocrine change during prolonged exposure of human body to stress was called by Selye "general adaptation syndrome" or "post-aggressive reaction" [1, 5].

The biological reactions from the general adaptative syndrome evolves after Selye's concept in three stages:

In the first stage, called "alarm reaction" appear acute phenomenon of shock (decreased blood pressure, increased capillary permeability, hemoconcentration, depression of central nervous system) followed by the counter shock reaction characterized by the hypertrophy and hypersecretion of suprarenal gland after the massive discharges of ACTH. This first stage induces the mobilization of the entire biologic potential increasing the resilience of organism to which participate neuro-endocrine factors such glucocorticoids, mineralo-corticoids and androgenic hormones.

▪ the second stages, resistance stage, is accomplished with the participation of renine–angiotensine system.

▪ the third stage, exhaustion stage, followed by sicknesss and death.

The studied histological specimens stained with staining techniques that give a general view of the renal corpuscules allowed us to make some interesting microscopic observations and morphometric study and at the suprarenal gland (both suprarenal cortex and suprarenal medulla) showed the structural modifications and in what degree these have an impact on metabolism and general status of the organism.

Comparing the control group and the test group, we observe at the control group various components of the nephron and the urinary space with a small volume, while the distal convoluted tubules are slightly dilated; in same time the renal corpuscles increase in size due to the fact that the urinary space is much larger than normal. To the test group image of the renal cortex [7, 8] showing renal corpuscles with increased urinary spaces and signs of protein dystrophy in the epithelium of proximal convoluted tubules. The renal corpuscles are relatively large with increased urinary spaces and are surrounded by convoluted tubules with dilated lumina (Figures 1 and 2).

The morphometric study performed by us was focused on the parameters of renal corpuscles, as follows: area of renal corpuscles – the principal size criterion, it expresses the real area, equivalent diameter of renal corpuscles, equivalent volume of renal corpuscles. Focusing on the urinary space we measured the following parameters: area of urinary space, equivalent diameter of urinary space, equivalent volume of urinary space [9, 10].

The renal fields examined from the specimens of Guinea Pig showed increased values of the surface occupied by renal corpuscles in the test group, which are statistically not significant (Pt>0.05).

The morphometric analysis showed that the equivalent diameter of renal corpuscles in the test group is slightly bigger than in the control group but statistically insignificant (Figure 3, Table 1).

Table 1 – ***Morphometric analysis of area of renal corpuscles*** **(μ^2)**

	Control group	Test group
1.	4750	5419.660
2.	5032.270	5430.600
3.	6725	5210.800
4.	4265.700	5322.370
5.	4115.560	5134.420
Mean	4977.706	5303.570
Pt	*0.205358*	

Stressing factors appear as stimuli that trigger efferences with hypothalamic starting point, that will modify concomitantly and differently the behavior or peripheral effectors, efferences that are either hormonal or nervous and witch by the action of mediators, increases concentration of STH, etc., will act on target organs determining a response with the significance of adaptative reaction, in the center of which stands the suprarenal gland.

In our experiment we surveyed in several successive stages the biodynamic changes that take place inside suprarenal gland.

All these structural aspects from suprarenal cortex are a reflection of adaptative capacity of suprarenal gland to stress factors (hyperbaric–hyperoxic environment), because initially an adaptative process take place, by morpho-functional changes at different levels in order to ensure the maintenance of life [11].

The results of adaptation are dependent of the ratio: intensity of stress and functional reserve of the organism. When the intensity of stress surpasses the organism's functional reserve and the maximum amplitude of adaptative resources, the status of diseases takes place and the animal may die [12–14].

The initial reactions is manifested by increased capillary permeability, as it is shown by our images, capillaries are dilated and filled with blood, with a retention of formatted elements of blood and erythrocytes.

We noticed the changes of the vascular bed as an adaptative reaction to the chemical stress; the increased intracellular spaces indicate an abundance of intestinal fluid that will finally lead to changes of ionic distribution and cellular metabolism.

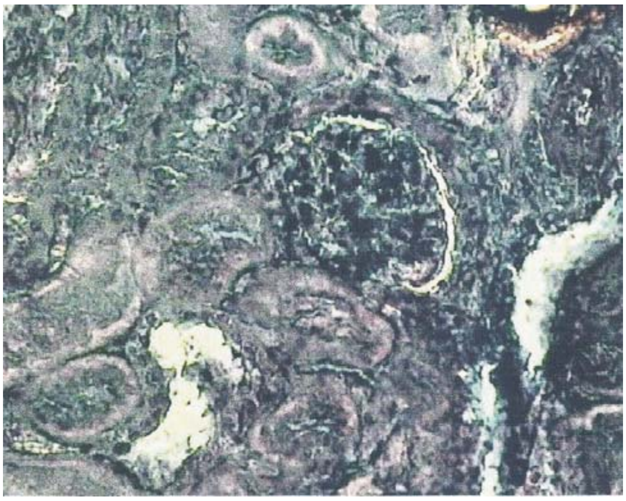

Figure 1 – ***Control group. Image of the renal cortex showing various components of the nephron. The urinary space has a small volume, while the distal convoluted tubules are slightly dilated (Masson's staining, ob. ×20)***

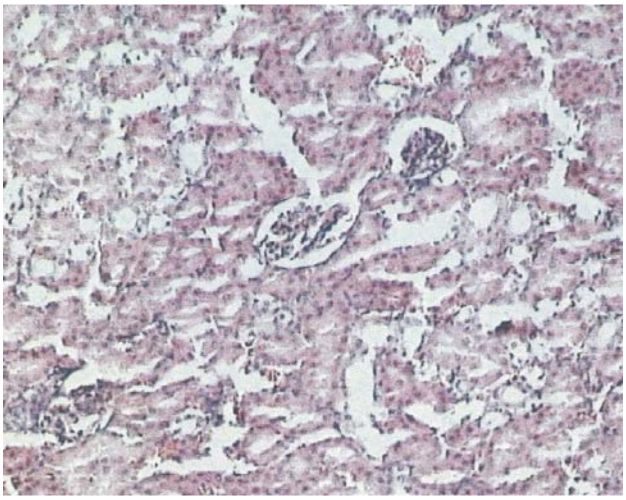

Figure 2 – ***Test group. Renal cortex with several renal corpuscles surrounded by convoluted tubules. The lumen of these tubules is slightly dilated (HE staining, ob. ×20)***

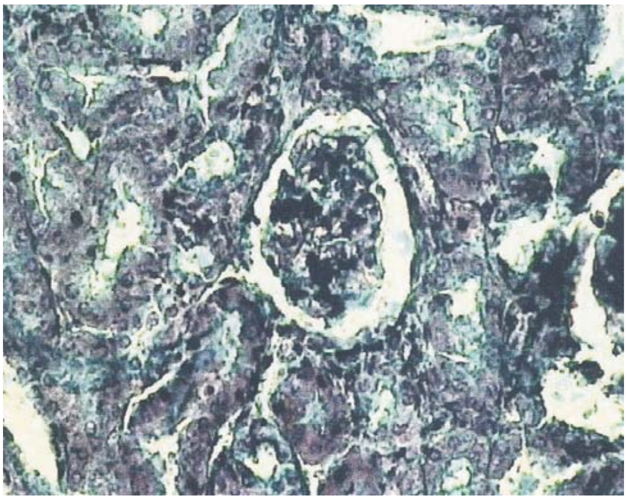

Figure 3 – ***Test group. Image of a renal corpuscles surrounded by convoluted tubules. The renal corpuscle has a large urinary space and the squamous epithelium of the parietal layer of Bowman's capsule is evident (Masson's staining, ob. ×20)***

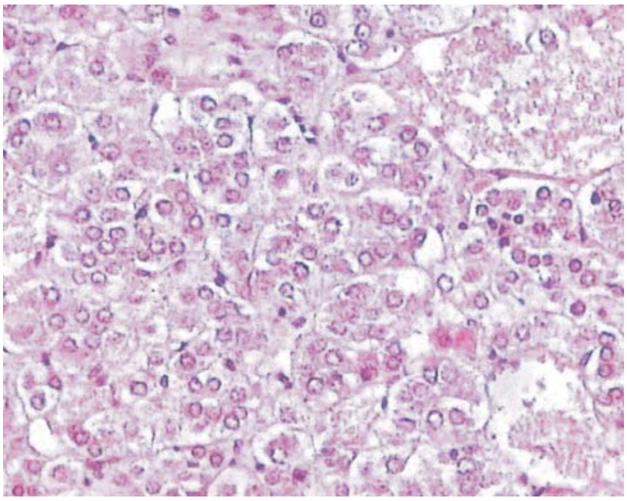

Figure 4 – ***Test group. Suprarenal medulla (HE staining, ob. ×20)***

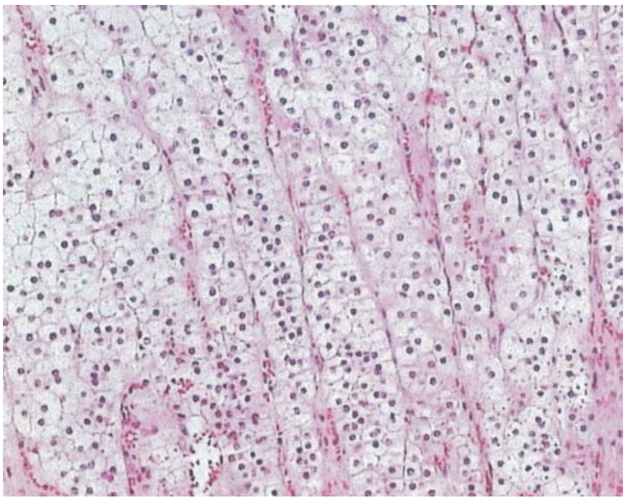

Figure 5 – ***Test group. Suprarenal gland, zona fasciculata (HE staining, ob. ×20)***

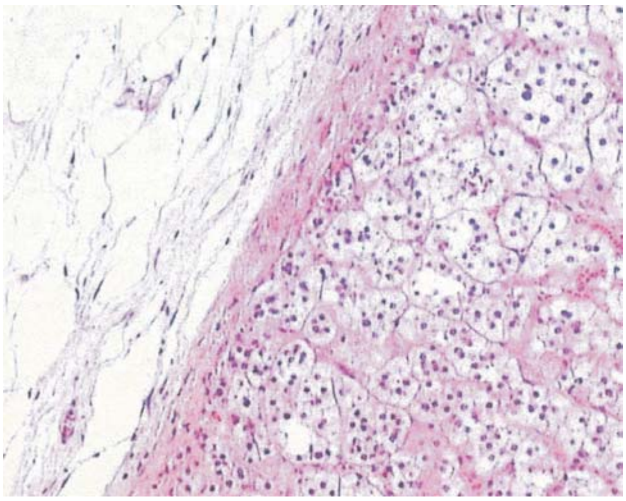

Figure 6 – ***Test group. Suprarenal gland, zona glomerulata (HE staining, ob. ×10)***

Our images show exactly these sequences in which the cellular structures, as well as those extracellular, appear to be modified.

This initial reaction manifests by increased capillary permeability as seen in our images with dilated capillaries, full of blood, with retention of formatted elements of blood, including erythrocytes.

We notice the changes of vascular bed as an adaptative reaction to the hyperbaric–hyperoxic stress, enlarged intracellular spaces that express an increased amount of interstitial fluid, that will lead to ionic distribution changes and finally changes in the cellular metabolism and enzymatic reaction.

These modifications in response to stress factors are explained by the compensating reactions involving always the axis hypophyse–thyroid–suprarenal glands.

Initially there is a strategic adaptative reaction, which if the stress factor continues to act will modify the homeostatic equilibrium and therefore leads to a status of shock. At the suprarenal gland we notice the highly dilated capillaries with retention of formed elements and erythrocytes. Following the dynamics of suprarenal medulla in these two groups shows the expression of neuro-vegetative and endocrine influence over suprarenal gland (Figure 4).

Our results represent the mean reactivity of studied animals because the triphase evolution of general adaptative syndrome depends not only on stress factor [15], but also on the individual reactivity according to general constitution, homeostatic equilibrium and resistance of the organism etc.

Following the dynamics of suprarenal cortex and suprarenal medulla modifications, and by calculating the ratio of these two surfaces with different embryonic origin, we notice that during hyperbaric–hyperoxic stress the cortex and medulla reflect distinct changes, as an adaptative eustress reaction on one hand, which comprises the biological responses that generate neuro-endocrine metabolic adequate reactions which tend to insure the reestablishment of homeostasis and then in the final phase – distress – inadequate reactions the generate the disease [16, 17].

The dynamic of suprarenal cortex surface and suprarenal medulla in the experiment shows that the suprarenal cortex slightly decreasing (Pt = 0.581076) compared to the control group. Instead, fasciculate area had an ascending dynamic.

The surface of suprarenal medulla increased significantly (Pt = 0.023527), compared to control group that involves a greater amount of catecholamines secreted by suprarenal medulla. From the graphic analysis of the dynamics of the three suprarenal cortex zones, *zona glomerulata* is less involved (Pt = 0.153828) in the regulation of homeostasis during stress.

In the test group there is a decreased area of *zona glomerulata* and corresponding increase of *zona fasciculata* (Figures 5 and 6).

From the graphic analysis of the dynamics of the three suprarenal cortex zones, *zona glomerulata* is less involved (Pt = 0.153828) in the regulation of homeostasis during stress.

In the test group there is a decreased area of *zona glomerulata* and corresponding increase of *zona fasciculata*. By our method we can not tell from *zona glomerulata* transform or not into cells of *zona fasciculata*, although at this level we noticed a great number of mitotic divisions (Tables 2 and 3).

Table 2 – *The mean surface of different zones of suprarenal gland (μ^2). Control group*

	Control group	Suprarenal medulla	Test group
1.	7698	1088	8786
2.	6719	1079	7798
3.	14417	1085	16584
4.	9101	991	10092
5.	8892	742	9634
Mean	*9365.4*	*997*	*19726*

Table 3 – *The mean surface of different zones of suprarenal gland (μ^2). Test group*

	Suprarenal cortex	Suprarenal medulla	Suprarenal gland
1.	9591	1968	11559
2.	8050	1085	9135
3.	17641	1384	19025
4.	10429	1489	11918
5.	7632	1862	9494
Mean	*10668.6*	*1557.6*	*12226.2*

In *zona fasciculata* there is a significantly grow of its surface (Pt = 0.021067).

Conclusions

After the action of hyperbaric–hyperoxic stress on the experimental material that is used the first segment that quantitatively react was proved to be the vascular corticosuprarenal system, the vessels appear flat, emptied of blood.

In the hyperbaric–hyperoxic stress actions we noticed that *zona glomerulata* is less involved, with negative dynamics, instead *zona fasciculata* is strongly involved.

The involvement of *zona fasciculata* is due to the intervention of endocrine factor on the hypothalamic hypophyse–suprarenal axis by the ACTH stimulation of this zone and increased levels of plasmatic cortisone.

Our study reveals that hyperbaric–hyperoxic environment induces dystrophic alterations of kidneys of Guinea pig quantified by the larger urinary spaces in the test group as well as a greater enfolding of glomerular capillaries showed by the greater perimeter of urinary spaces.

Focusing on the urinary space, we found significantly increased values of the area (Pt<0.002), equivalent diameter (Pt<0.001), volume equivalent to a sphere (Pt<0.005) and a perimeter (Pt<0.00003)

The biometrical study shows the total involvement of suprarenal gland in the process of stress. After hyperbaric–hyperoxic stress the first segment that quantitatively react was proved to be vascular cortico-suprarenal system, the vessels appear flat and emptied of blood; we noticed that *zona glomerulata* is less involved instead *zona fasciculata* is strongly involved.

By appreciating the dynamic cortex/medulla we observed that the cortex having a descending evolution compared to that of the medulla.

The hyperbaric–hyperoxic environment is a non-physiological, stressing factor to which the kidneys as well as other organs and systems find it hard to adjust.

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