BIOLOGICAL EFFECTS OF PULSED SHORT WAVE TREATMENT. AN EXPERIMENTAL STUDY

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Abstract

Pulsed short waves are short electromagnetic waves emitted as intermittent trains with a fixed duration, separated by free intervals of variable duration. The biological effects of pulsed short waves could be explained according to most of the authors by an activation of cellular enzymatic reactions, a stimulation of energy metabolism, a stimulation of liver function, of adrenal gland function and of the reticulocyte system, changes in cell permeability, by an increase of peripheral blood flow through the enhancement of local vascularization. This research aimed to investigate the biological effects of exposure to pulsed short waves at different doses on the adrenal glands of experimental animals, by structural and ultrastructural studies. The study included 35 animals assigned to 4 groups. Group I included 10 experimental animals exposed to radiation at a dose of 1/80 impulses/sec, group II, 10 animals exposed to a dose of 4/400 impulses/sec, group III, 10 animals exposed to a dose of 6/600 impulses/sec, for 10 min/day, and the control group consisted of 5 unexposed animals. Structural and ultrastructural changes of adrenal glands induced by the dose of 4/400 impulses/sec, compared to the unexposed control group and the dose of 1/80 impulses/sec, include an intensification of protein synthesis processes, an enhancement of energy metabolism in providing the energy required for an increased production of hormones, an intensification of collagen fiber synthesis processes in the capsule, necessary for healing. It was demonstrated that this dose induced an intensification of hormone synthesis and secretion, a stimulation of adrenal function. At the dose of 6/600 cycles/sec, a slight diminution of hormone synthesis and secretion activity was found, which was not below the limits existing in the unexposed control group, but was comparable to group II. This dose is probably too strong for experimental animals, inducing them a state of stress. The research demonstrated both the local and the general, systemic influence of pulsed short waves, the changes induced being for the most part strictly dependent on the dose used.

Key words: pulsed short waves, adrenal gland, Diapulse

Introduction

Pulsed short waves are short electromagnetic waves, emitted as intermittent trains with a fixed duration, separated by free intervals of variable duration.

Experimental research and the multitude of therapeutic effects observed in clinical studies suggest complex action mechanisms of pulsed short waves, up to the infrastructural level, with deep implications in cell metabolism [1].

If the favorable effects of continuous short waves are mostly due to tissue heat production (endothermia), a more complex action mechanism underlies pulsed short waves, which are athermal [2].

The biological effects of pulsed short waves could be explained according to most of the authors by an activation of cellular

enzymatic reactions, a stimulation of energy metabolism, a stimulation of liver function, of adrenal gland function and of the reticulocyte system, changes in cell permeability, by an increase of peripheral blood flow through the enhancement of local vascularization. However, all these processes require an increased energy consumption by cells and tissues [1].

Pulsed short waves are provided by the Diapulse device.

The work technique recommends that, regardless of the treated disorder, one of several regions, i.e. the liver, epigastrium or lumbar region, should be initially exposed (for action on the adrenal glands) [3].

There are few studies that evidence the cellular and subcellular changes of adrenal glands and the way in which the hormone synthesis and secretion function of these glands is affected after exposure to pulsed short waves at different doses.

The presence of favorable results in a number of disorders (acceleration of wound and burn healing, reduction of posttraumatic edema, antiinflammatory effects, accelerated regeneration of peripheral nerves and spinal cord) necessarily involves morphological and histological changes of the irradiated tissues.

The aims of this research were the following:

1. To study the biological effects of exposure to pulsed short waves at different doses on the adrenal glands of experimental animals, by structural and ultrastructural studies.
2. To evidence cellular and subcellular changes in the adrenal glands taken from rats exposed to pulsed short waves at different doses, compared to untreated control animals.

Material and method

Experimental animals. White Wistar female rats with a weight of 180-280 g were used for the experiment (Fig. 1).

Fig. 1. Groups of rats required for the experiment

Equipment. The Diapulse device, which provides high-frequency currents of 27.12 Mhz, with a wavelength of 11 m, was used [3].

Experimental model. 35 animals were assigned to 4 groups. Group I included 10 experimental animals exposed to radiation at a dose of 1/80 impulses/sec for 10 min/day, group II, 10 animals exposed to a dose of 4/400 impulses/sec for 10 min/day, group III, 10 animals exposed to a dose of 6/600 impulses/sec for 10 min/day, and the control group consisted of 5 unexposed animals, kept under the same living conditions as the animals of the three irradiated groups.

The duration of the experiment was 15 days. The entire body of the animals was exposed to pulsed short waves. There were no food or liquid restrictions during the experiment.

All procedures performed on the animals were in accordance with the EU ethical norms regarding the raising and sacrificing of experimental animals. Before starting the experiment, the approval of the Ethics Committee no. 39/23.04.2009 was obtained.

Right after the animals were sacrificed, the adrenal glands were rapidly collected and processed using electron microscopy methods and techniques.

The sections were examined with an Olympus BX 51 microscope, and the images were captured with a CCD Media Cybernetics camera, using the Image Pro Plus software. Subsequently, from the selected areas of the same blocks, ultrafine 40-60 nm thick sections were cut for electron microscopic studies. The sections

were cut with a Leica UC 6 ultramicrotome using DDK diamond knives, they were collected on electrolytic grids, were double contrasted with uranyl acetate and lead citrate, and finally were examined under a Jeol JEM 1010 transmission electron microscope (TEM). Images were captured with a Megaview III camera and were subsequently stored in the data base using the Soft Imaging Analysis software [4, 5, 6, 7, 8, 9, 10, 11].

Results

Macroscopic aspects

No changes were evidenced in any of the three groups exposed to pulsed short wave treatment (Fig. 2). No changes were found in the control group.

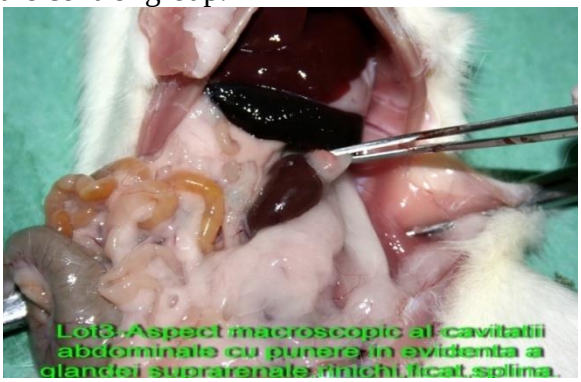

Fig. 2. Macroscopic appearance of the adrenal gland, without changes

Structural results obtained in the adrenal glands

The examination of the adrenal glands of the control rats by optical microscopy (100 X) evidenced no changes (Figs. 3, 4, 5).

The adrenal gland is enclosed in a connective capsule and the gland stroma is divided into three areas, zona glomerulosa, zona fasciculata and zona reticularis, while the medulla is found in the center.

The capsule is made up of collagen tissue, which extends to the cortical area through connective trabeculae that penetrate up to different depths. The support structure of adrenal gland cells consists of a reticular fiber network interposed between the capillaries and the cell cords of the entire gland, including in the medullary area. Along venules, relatively few collagen fibers are found.

The zona glomerulosa is situated right below the capsule, being relatively thin. It consists of cells arranged in bow-shaped clusters. In the rat, cells appear approximately spherical, with a central nucleus and the cytoplasm loaded with hormone lipid droplets.

Between the zonae glomerulosa and fasciculata, there is an intermediate area, formed by slightly elongated cells, which possess many mitochondria and only few lipid droplets in the cytoplasm.

The zona fasciculata is situated between the zonae intermedia and reticularis, without a clear delineation towards these areas. It contains polyhedral cells, which are slightly larger than those from the zona glomerulosa. The fact that cells are arranged in single-cell cords separated by sinusoidal blood capillaries is specific. The cords start from the proximity of the zona glomerulosa and extend to the zona reticularis. The cell nuclei are spherical, with one nucleolus or more rarely, two nucleoli. In the cytoplasm, there are many spherical lipid droplets and mitochondria.

The zona fasciculata is the most developed, extending over approximately 70% of the adrenal cortex. Its cells synthesize and secrete glucocorticoid hormones: cortisol, cortisone and corticosterone, hormones with a major role in carbohydrate, protein and lipid metabolism.

In the zona reticularis, cells are anastomosed, forming a network, and are separated by many sinusoidal blood capillaries. The cell nuclei are spherical. In the cytoplasm, there are mitochondria and relatively many lipid droplets. It is characteristic that the cells of this zone, particularly those found towards the medullary area, appear in two forms: the so-called ”dark” cells, which are more intensely colored, and the so-called ”bright” cells, less intensely colored. It has been proven that ”dark” cells are aged cells that contain many lipofuscin granules in the cytoplasm.

The cells of the zona reticularis synthesize and elaborate sexual steroid hormones: estrogen, androgen and progesterone.

Fig. 3. Control group. Adrenal gland, capsule, zona glomerulosa, loaded with hormone lipid droplets. 100x

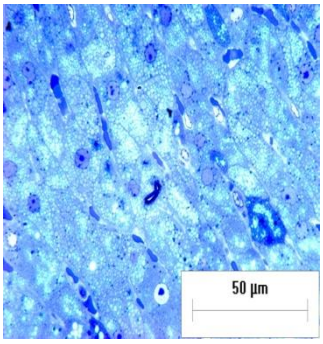

Fig. 4. Control group. Adrenal, gland, zona intermedia with fewer hormone lipid droplets. 100x

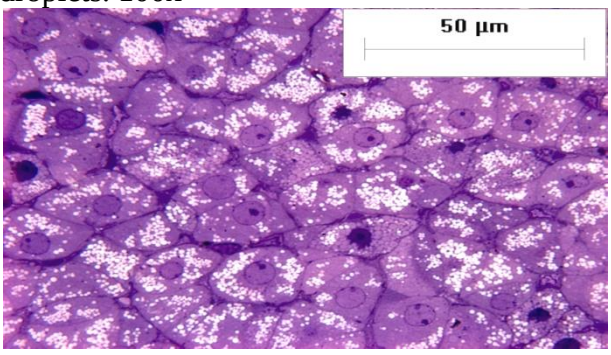

Fig. 5. Control group. Adrenal gland, zona fasciculata. Increased hormone secretion. 100x

Compared to the adrenal glands taken from the animals of the control group, the images obtained from the animals of group I, irradiated at a dose of 1/80 impulses/sec, indicate that irradiation induced a slight intensification of metabolic processes in the adrenocortical areas, but less in the zona glomerulosa. There were no changes in the capsule detectable with the optical microscope (Fig. 6).

The abundance of hormonal secretory granules in the cells of the zonae fasciculata and reticularis (Fig. 7) confirms the beneficial influence of irradiation, at a dose of 1/80 impulses sec, on hormone synthesis and secretion in rats.

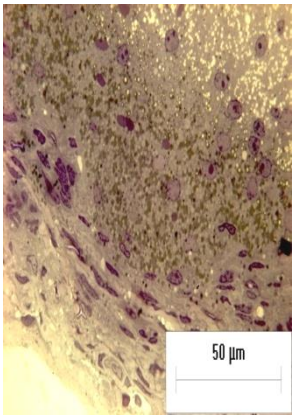

Fig. 6. Group I. Adrenal gland, capsule, zona glomerulosa. 100x

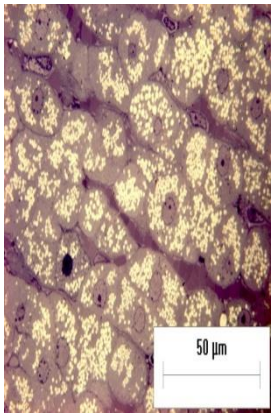

Fig. 7. Group I. Adrenal gland, zona fasciculata. Abundant hormonal secretory droplets. 100x

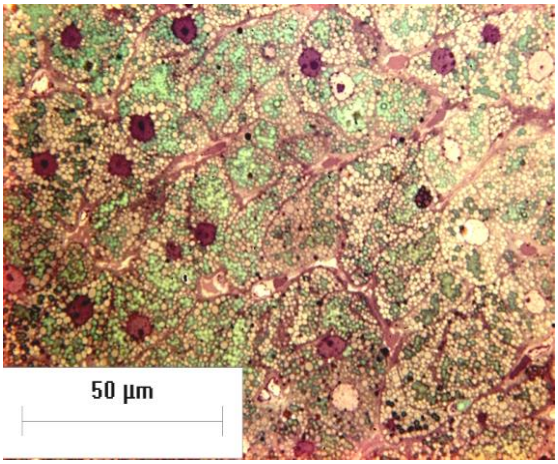

Fig. 8. Group I. Adrenal gland, zona reticularis. Abundant hormonal secretory droplets. 100x

In group II, irradiated at a dose of 4/400 cycles/sec, a greater abundance of collagen fiber bundles and a higher capillary density are seen, suggesting a higher blood supply, mainly in the zona glomerulosa.

Compared to images obtained from the adrenal glands of the control animals, as well as to those of the group irradiated at a dose of 1/80 impulses/sec, the effects in this group are particularly beneficial, greater than in the case of the previous group. In all images, in all three adrenal gland zones, there is an increase of hormone synthesis and secretion processes, evidenced by the particularly abundant hormonal secretory granules (Figs. 9-11) and by the absence of any degenerative morphostructural aspect. In

the zona reticularis, towards the medullary area, there are relatively few ”dark”, aged cells.

This picture showing an intense adrenal gland activity may suggest the fact that there is also an optimal metabolic activity in the whole organism.

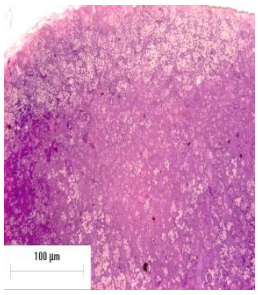

Fig. 9. Group II. Adrenal gland, capsule, zona glomerulosa. Abundant collagen fibers. 40x

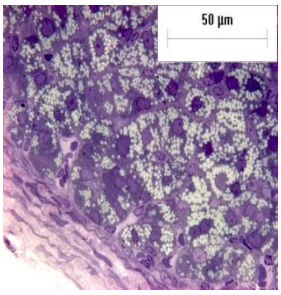

Fig. 10. Group II. Adrenal gland, zona fasciculata. Particularly abundant hormonal secretory granules. 100x

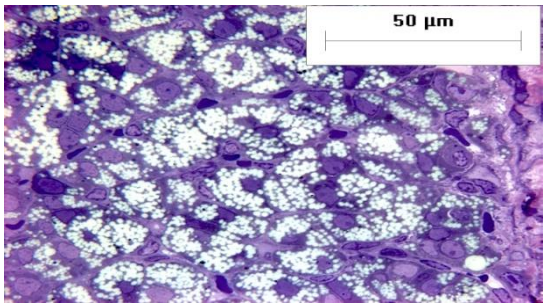

Fig. 11. Group II. Adrenal gland, zona reticularis. Particularly abundant hormonal secretory granules. 100x

In group III, irradiated at a dose of 6/600 impulses/sec, the general picture of the images obtained from all adrenal gland areas, compared to those of group I, and particularly of group II, indicates a disturbance of hormone synthesis and secretion activity, in the sense of a slight diminution, but not under the limits existing in the control gorup. In the zona glomerulosa (Fig. 12), a reduction in the number of lipid droplets is seen, which is not found in the preceding groups. In the zona fasciculata, there are portions with cells having a normal granular activity, and others with a slightly stimulated activity, in which isolated signs of storage of lipid droplets occur, which suggests their lower secretion outside the cells, while in some portions, in a small number of cells, hormone granules are fewer. In this zone, the presence of pyknotic nuclei (in the process of degeneration) is also seen, with a decreasing tendency of the metabolic activity of these cells (Fig. 13). In the zona reticularis, on the background of a rarefaction of hormone granules (Fig. 14), a great number of (dark) aged cells are present, which certainly have a lower metabolic activity that is also evidenced by the small number of lipid droplets present in their cytoplasm.

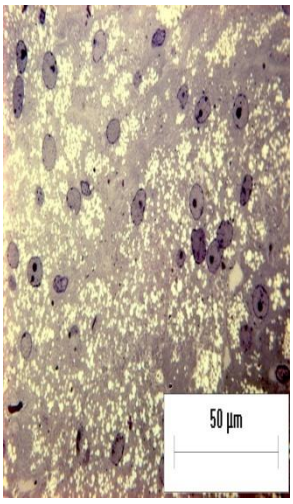

Fig. 12. Group III. Adrenal gland, zona glomerulosa. Reduction of lipid droplets. 100x

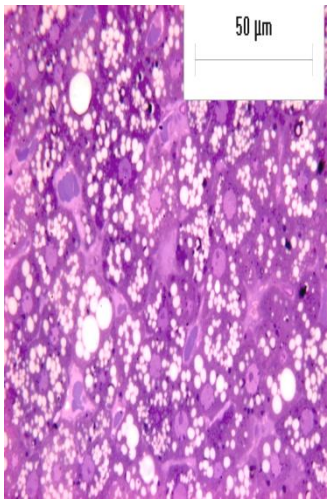

Fig. 13. Group III. Adrenal gland, zona fasciculata. Storage of lipid droplets. 100x

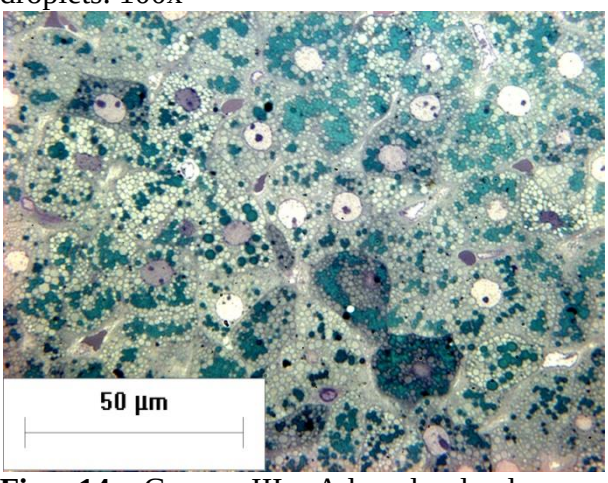

Fig. 14. Group III. Adrenal gland, zona reticularis.
Aged cells and fewer hormone granules are present. 100x

Utrastructural results obtained in the adrenal glands

Ultrastructural studies confirmed the results obtained by the analysis of semi-fine sections under the optical microscope, with the mention that the electron microscopic images obtained provided additional information and evidenced details at subcellular level.

In the control group, the zona glomerulosa is made up of cells that appear as groups of 3-5 cells in bundles delimited by connective trabeculae derived from the capsule, forming a not very thick continuous subcapsular layer. In each cell, the nucleus is usually central, oval-polyhedral, and the cytoplasm is loaded with hormone granules with a globular spherical lipid droplet appearance, having a diameter between 0.5-1 μm. Among the cell groups, blood capillaries are found (Fig. 15).

In the zona fasciculata, each cell has an approximately central nucleus, usually with a nucleolus and more rarely, two nucleoli. In the cytoplasm, there are numerous mitochondria and granules 0.5-1.2 μm in diameter in approximately equal proportions; their cristae are, characteristically for this area, tubular and appear as small vesicles in cross-section.

Among mitochondria, there are many small vesicles that form the smooth endoplasmic reticulum (SER), which is abundant in the adrenal gland cells, due to its participation in steroid synthesis processes. The granular endoplasmic reticulum (GER) or the rough endoplasmic reticulum (RER), involved in protein synthesis, is scarcely represented by short narrow profiles usually arranged along the mitochondrial membranes.

In each cell, a Golgi complex is situated in a cytoplasmic area immediately adjacent to the nucleus. Some lysosomes are also seen from place to place in the cytoplasm of each cell, as intensely electron-dense granules, 0.5 μm in diameter (Fig. 16).

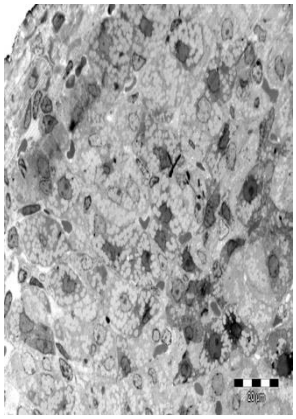

Fig. 15. Control group. Adrenal gland, zona glomerulosa

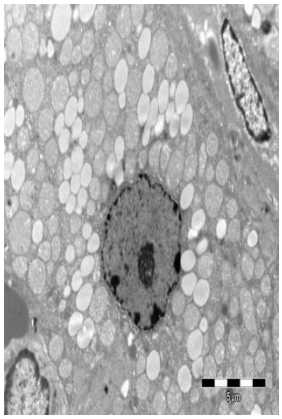

Fig. 16. Control group. Adrenal gland, zona fasciculata

In group I, irradiated at a dose of 1/80 impulses/sec, compared to images obtained in the control group, collagen bundles are more abundant in the capsule area, forming a denser layer (Fig. 18), which suggests a better protection of the gland.

In the zona glomerulosa, the cell cytoplasm contains many hormone granules, suggesting a sustained metabolic activity, slightly increased compared to the control group. The cell nuclei have different shapes, spherical, oval or polyhedral. Sinusoidal blood capillaries are found among the cells. Hormone granules have a slightly larger diameter, many of them reaching 1.5 μm in diameter compared to controls, 1 μm.

In the zona fasciculata, the cytoplasm is occupied by many spherical mitochondria and hormone granules in an approximately equal ratio, with small variations between the two components. Mitochondria are interspersed with many SER microvesicles and well-developed Golgi complexes in an intense activity state. Lysosomal formations are also present as intensely electron-dense granules, obviously more numerous than in the cells of the control group.

Mitochondria are normally structured, and through their high numbers, they have an optimal energy metabolic activity (Figs. 19, 20).

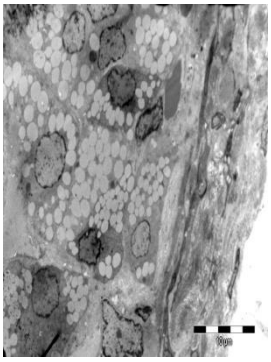

Fig. 18. Group I. Adrenal gland, capsule, zona glomerulosa

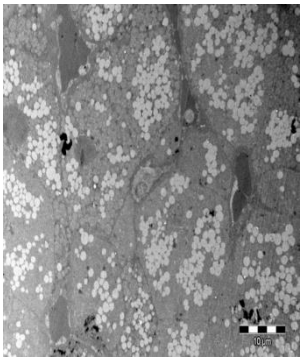

Fig. 19. Group I. Adrenal gland, zona fasciculata

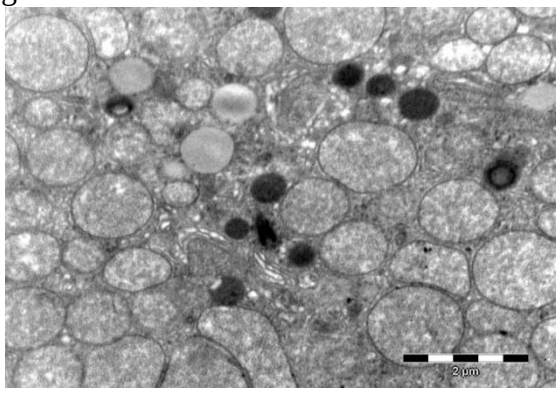

Fig. 20. Group I. Adrenal gland, zona fasciculata

In group II, irradiated at a dose of 4/400 impulses/sec, the analysis of the ultrastructural images obtained is in full accordance with the results presented following the analysis of semi-fine sections.
In the capsule, abundant normally structured collagen fibers are seen, with relatively many interspersed fibrocytes, which suggests an intensification of protein synthesis at this level. Subcapsular blood capillaries and venules are present (Fig. 21).
In the zona glomerulosa, cells have a particularly high metabolic activity. Their cytoplasm is overloaded with hormone granules, clearly indicating the fact that irradiation at a dose of 4/400 impluses/sec stimulates the metabolism of these cells for an increased hormone production (Fig. 21).
The zona intermedia has a greater extension compared to the control group, and its cells are overloaded with normally structured mitochondria, with many tubular cristae, suggesting an intense energy metabolic activity in providing the energy required for an increased hormone production, in all three adrenal gland zones. Mitochondria are interspersed with numerous SER vesicles, as well as Golgi complexes with an intense activity (Fig. 22).
Like in the case of the zona glomerulosa, in the zona fasciculata, cells are overloaded with hormone granules, suggesting their intense activity in the production of glucocorticoid hormones, due to the stimulation of this zone by irradiation at a dose of 4/400 impulses/sec (Fig. 23).

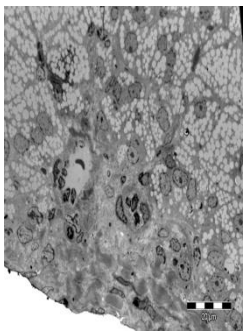

Fig. **21**. Group II. Adrenal gland, capsule, zona glomerulosa

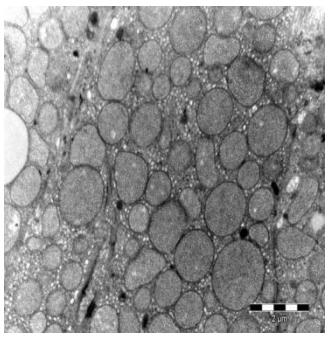

Fig. 22. Group II, adrenal gland, zona intermedia

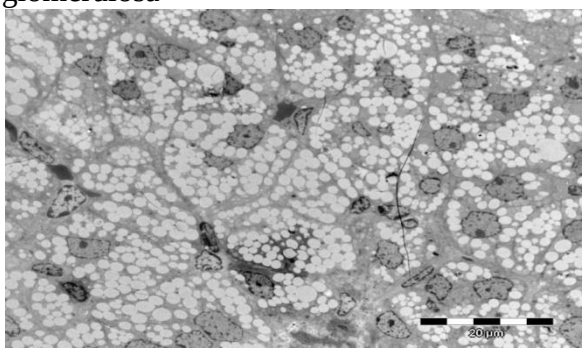

Fig. 23. Group II. Adrenal gland, zona fasciculata

In group III, irradiated at a dose of 6/600 impulses/sec, the capsule has a structure similar to that of the preceding group, so it has a good development, with the difference that more venules are present at this level.
In the zona glomerulosa, there is some disturbance of cell grouping in bow-shaped clusters, and cells and their nuclei have a polymorphic appearance and different shapes. The number of hormone granules in

the cytoplasm is diminished compared to group II and even group I.

It should be noted that small and dark aged cells, with very few hormone granules indicating their poor metabolic activity, appear in this area. In the capillaries, there is a mild erythrocyte stasis (Fig. 24).

The zona intermedia is formed by polyhedral cells, with many mitochondria in the cytoplasm and very few hormone granules. Some cell mitochondria have a slightly rarefied mitochondrial matrix, which indicates their decreased energy metabolic capacity. In these cells, nuclei are slightly hyperchromatic and their intermembrane space is dilated. In other cells, mitochondria have lost their spherical shape, being polymorphic, with a rarefied mitochondrial matrix (Fig. 25).

The zona fasciculata comprises cells with a diminished hormonal activity, but part of them have an activity similar to that of the control group. In other cells, nuclei have lost their spherical shape, becoming hyperchromatic, which are clear signs of pyknosis and reduced metabolic activity, evidenced by the decreased number of hormone granules. There are also cells with two nuclei, both of which have pyknotic tendencies, and the mitochondria of these cells have rarefied matrix and cristae. From all the mentioned above, it appears that in the zona fasciculata in particular, cells have a mosaic appearance.

In the zona reticularis, there are relatively high numbers of aged cells. All cells as well as their nuclei have a polymorphic appearance, and some of them have pyknotic tendencies. All cells contain a very small number of hormone granules. Small cell lysis and necrosis foci are seen among the cells (Fig. 26).

In the medullary area, there is a depletion of hormone granules and particularly of those containing epinephrine, a process achieved by the progressive degradation and vacuolization of the granules. All cells are affected, through an alteration-degeneration of the nuclei, which become hyperchromatic, with aberrant forms. Also, in the cytoplasm, almost all organelles, especially mitochondria, Golgi complexes and GER, undergo a dilation and vacuolization process.

It seems that this treatment dose represented a high stress for the organism, and the specific hormonal capacity of the adrenal medulla to respond to stress was overcome.

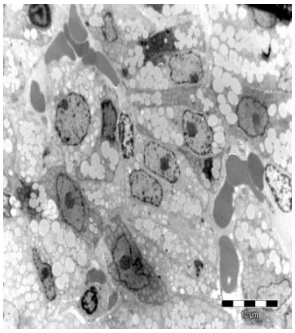

Fig. 24. Group III. Adrenal gland, zona glomerulosa

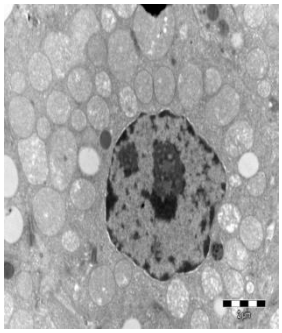

Fig. 25. Group III. Adrenal gland, zona intermedia

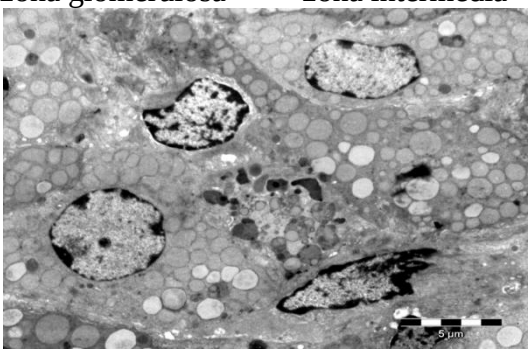

Fig. 26. Group III. Adrenal gland, zona reticularis

Discussions

The results obtained by optical and electron microscopy show that the application of treatment with pulsed short waves (Diapulse) to the experimental animals induced certain histological changes.

The most extensive changes were seen in groups II and III compared to group I.

The abundant hormonal secretory granules in the zonae fasciculata and reticularis confirm the beneficial influence of irradiation at a dose of 1/80 impulses/sec on the hormone synthesis and secretion process. However, we take into consideration the fact that this dose, which in humans is applicable to smaller areas and has a relatively low penetration power, was applied to the whole

body of the rats, and because of the smaller size of the animals, the penetration of radiation was probably higher.

At the dose of 4/400 impulses/sec, a greater abundance of hormonal secretory granules was seen in all three adrenal gland zones. There were no morphological or structural changes.

The structural and ultrastructural changes induced in the adrenal glands of group II (4/400 impulses/sec) compared to the control group and group I, i.e. an increase of protein synthesis, an intensification of energy metabolism in providing the energy required for an increased production of hormones, an enhancement of capsular collagen fiber synthesis required for healing, as well as the intensification of the activity of some mitochondrial enzymes observed by histoenzymological studies, support the presence of a stimulation of the adrenal gland function. Glucocorticoids have antiinflammatory effects, being widely used for the treatment of arthritis, of inflammatory diseases in general. Mineralocorticoid hormones, aldosterone and deoxycorticosterone, respectively, ensure the balance and the control of fluids and electrolytes [12, 13, 14].

Literature data show the presence of a correlation between the hormone secretion activity of the adrenal gland and the blood flow in the capillaries [19]; in our experiment, histological studies evidenced a higher capillary density in group II, suggesting a higher blood supply in the zona glomerulosa in the first place.

In animals of group III (6/600 impulses/sec), optical and electron microscopic studies revealed small cell lysis and necrosis areas, with a decrease in the number of hormone granules and a diminution of hormone synthesis and secretion activity.

In group III, some cell mitochondria had a slightly rarefied matrix, indicating a decreased energy metabolic capacity. We might say that for rats, this irradiation dose is too high, inducing stress.

However, the results obtained in these animals cannot be extrapolated as such to the human body.

The mitochondrion is an energy-converting organelle. Energy is converted to a form adequate for maintaining vital processes, i.e. ATP. In addition to its energy function, the mitochondrion has other metabolic functions. In mitochondria, ATP is produced through the oxidation of organic substrates in the presence of oxygen.

All cellular functions are dependent on a continuous energy supply. Energy is obtained by successive degradations of organic molecules during cellular respiration. The energy released by the breaking of chemical bonds that occurs during this process is finally stored in the form of ATP molecules. In cells with active respiration, ATP is an easy to obtain source of energy for all the metabolic functions of the cell [15, 16, 17, 18].

Conclusions

1. The optical and electron microscopic studies performed as part of this research evidenced cellular and subcellular changes in the adrenal glands taken from rats exposed to treatment with pulsed short waves at different doses, compared to the same organ of untreated control animals.

2. The structural and ultrastructural changes induced in the adrenal glands by the dose of 4/400 impulses/sec, compared to the unexposed control group and the dose of 1/80 impulses/sec, include an intensification of protein synthesis, an increase of energy metabolism in providing the energy required for an increased hormone production, an intensification of capsular collagen fiber synthesis, required for healing. At this dose, an enhancement of hormone synthesis and secretion, and a stimulation of adrenal gland function were demonstrated.

3. The general picture of the images obtained from all adrenal gland zones irradiated at a dose of 6/600 cycles/sec shows a slight diminution of hormone synthesis and secretion activity, which is not

below the limits existing in the untreated control group, but is comparable to group II. This dose is probably too strong for the experimental animals, inducing them a state of stress.

4. The studies demonstrated both the local and the general, systemic influence of pulsed short waves, the induced changes being for the most part strictly dependent on the dose used.

5. The favorable results obtained in a number of diseases following pulsed short wave treatment necessarily involve morphological and histological changes in the irradiated tissues.

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