ULTRASTRUCTURAL MORPHOMETRY OF ADRENALIN- AND NORADRENALIN-STORING CELLS OF RAT ADRENALS AFTER PHYSICAL AND PSYCHOEMOTIONAL STRESS

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An importanr role in the responses of the body to stress is played by activation of the sympathicoadrenal system and, in particular, of the adrenal medulla, which has become the subject of active biochemical study [5]. However, only isolated accounts can be found in the literature of the study of the ultrastructure of adrenalin- and noradrenalin-storing cells of the adrenal medulla, and as a rule these give only a qualitative description [3].

With the aim of studying the ultrastructural organization of cells of the adrenal medulla, noradrenalin (NA)- and adrenalin (A)-containing cells were differently stained and a morphometric analysis was carried out of the distribution of A- and NA-storing granules and the state of the mitochondria and endoplasmic reticulum.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180 $\pm$ 30 g. Stress situations with a predominantly psychoemotional component were created by immobilizing the animals in special frames for 48 h, and also by depriving the animals for 7 days of the rapid phase of sleep (Jouvet's method [6], the model of stress was developed by N. A. Bondarenko), or by compelling them to run for 48 h in a drum revolving at a speed of 2 rpm (predominantly physical component). After decapitation of the animals the adrenal medulla was fixed by Tranzer's method [7] in the writers' modification: the tissue was treated in acetate buffer for 18 h, but the concentration of potassium bichromate was reduced to 0.065 M. In this way A- and NA-containing cells in the same specimen could be differentiated. The tissue was embedded in Araldite and sections were examined in the JEM-100B microscope. The number of granules containing A or NA and the number and relative volume of the mitochondria and cisternae of the endoplasmic reticulum per conventional unit area of the cell were counted on electron micrographs and calculated.

EXPERIMENTAL RESULTS

In the study of the above-mentioned models of stress the duration and strength of the stress stimulus were chosen so that the animals were at the same stage of the stress response — the stage of exhaustion (according to Selye). This was judged from changes in the following physical parameters: a decrease in weight of the thymus and spleen by 50-60%, an increase in weight of the adrenals by about 100%, a considerable decrease in the thickness of the lipid layer in the adrenal cortex, and the presence of ulcers in the gastric mucosa.

When the electron micrographs were analyzed three types of structures were conventionally distinguished in the population of A- and NA-containing granules (Fig. 1): 1) granules filled with the cytochemical reaction product throughout their area of section ("full"); 2) partially filled granules ("half empty"); 3) granules not containing any cytochemical reaction product ("empty"). The results of counting these structures in intact animals and under conditions of stress are given in Table 1.

It will be clear from Table 1 that a similar distribution of granules storing catecholamines (CA) was observed in the adrenalin- and noradrenalin-containing cells. For

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TABLE 1. Distribution of A- and NA-Containing Granules* in Chromaffin Cells of Rat Adrenals in Various Conditions of Stress

Experimental conditions	Adrenalin-containing cells			Noradrenalin-containing cells		
	full	half empty	empty	full	half empty	empty
Intact rats	52,4±6,9** (100±13%)	32,2±4,8 (100±15%)	15,4±2,5 (100±16%)	64,0±9,2 (100±14%)	23,0±3,5 (100±15%)	13,0±2,2 (100±17%)
Immobilization	24,8±2,9 (47±5%)	45,2±5,7 (132±18%)	30,1±4,2 (195±27%)	21,5±3,9 (34±8%)	42,6±5,3 (185±23%)	35,9±5,0 (276±38%)
Sleep deprivation	31,6±4,8 (60±9%)	43,5±6,0 (135±19%)	24,9±4,7 (162±29%)	26,8±4,1 (42±16%)	37,7±6,8 (164±30%)	35,5±7,5 (273±58%)
Running in a drum	17,3±2,6 (33±5%)	48,6±7,1 (150±22%)	34,1±7,4 (223±48%)	8,9±1,2 (14±2%)	67,9±13,1 (295±57%)	23,2±4,4 (178±34%)

*Conventional fraction of 100 structures.
**Confidence intervals given at the P = 0.05 level.

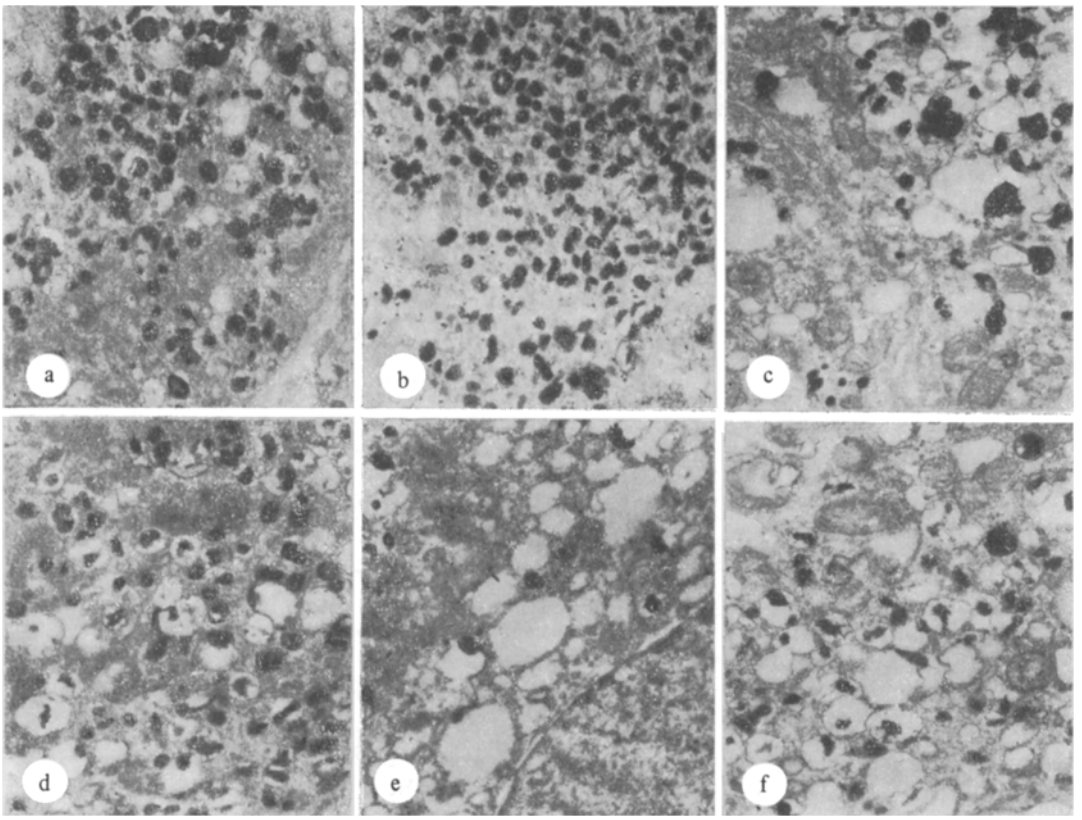

Fig. 1. Electron-microscopic demonstration of A- and NA-containing cells in rat adrenal medulla: a) noradrenalin-containing, b) adrenalin-containing cells in intact rat; c) noradrenalin-containing, d) adrenalin-containing cells in a rat after sleep deprivation for 7 days; e) noradrenalin-, f) adrenalin-containing cells in a rat after running for 48 h in the drum. Magnification 18,000.

instance, the largest number of structures corresponded to the fraction of "full" granules, the "half empty" granules accounted for about one-third, and the "empty" for about one-sixth of the total number of structures.

In a stress situation considerable changes took place in this distribution, with a decrease in the fraction of "full" and an increase in the number of "half empty" and "empty" granules. However, these changes varied in degree depending on the type of stress. The fraction of "full" granules usually decreased by a particularly marked degree, in both A- and NA-containing cells, during stress induced by running inside the drum and by a lesser degree during immobilization or sleep deprivation. The number of "half empty" granules

TABLE 2. Number and Relative Volume of Mitochondria and Cisternae of Endoplasmic Reticulum in Chromaffin Cells of Rat Adrenals in Different Stress Situations

Experimental conditions	Adrenalin-containing cells				Noradrenalin-containing cells			
	mitochondria		cisternae of reticulum		mitochondria		cisternae of reticulum	
	number*	relative volume	number	relative volume	number	relative volume	number	relative volume
Intact rats	0,50±0,06**	0,47±0,06	1,05±0,13	0,53±0,08	0,37±0,05	0,30±0,04	0,49±0,06	0,24±0,03
Immobilization	0,69±0,08	0,79±0,09	0,85±0,12	0,92±0,11	0,45±0,08	0,32±0,05	0,48±0,07	0,15±0,02
Sleep deprivation	0,63±0-08	0,83±0,10	0,86±0,10	1,24±0,14	0,42±0,07	0,33±0,04	0,45±0,07	0,13±0,02
Running in a drum	0,73±0,09	0,84±0,10	0,40±0,07	0,21±0,03	0,58±0,10	0,36±0,06	0,53±0,10	0,17±0,03

*Per conventional unit of area of cell.
**Confidence intervals of means given at the P = 0.05 level.

showed different changes in A- and NA-containing cells. The increase in the number of "half empty" granules in the NA-containing cells depended on the type of stress and was most marked during running in the drum. In the A-containing cells the number of "half empty" granules also increased, but was almost independent of the type of stress. The fraction of "empty" granules increased in all stress situations as a rule more than the fraction of "half empty" granules.

The decrease in the number of "full" granules and the increase in the number of "half empty" and "empty" granules found under stress reaction conditions are evidence of release of adrenalin and noradrenalin from the storage depots. These changes agree with biochemical data showing a decrease in the CA content in the adrenals during stress [5]. The greater release of CA obtained in the model involving running in a drum can evidently be explained by the more rigorous conditions of stress: the animals were completely deprived of all phases of sleep and pyschoemotional stress was aggravated by considerable physical work.

According to the results of ultrastructural analysis, a more marked decrease in the NA reserves was observed in stress caused by running in a drum than in the other stress situations, and also then the decrease in the A content. This may be evidence of the dominant role of the noradrenergic factor in the development of physical stress. The biochemical data on the participation of adrenergic and noradrenergic components during the performance of physical work are contradictory [1].

The results of the morphometric ultrastructural analysis of the state of the mitochondria and endoplasmic reticulum are given in Table 2. They show that during stress the number of mitochondria and cisterns of the endoplasmic reticulum and also their relative volumes changed by a lesser degree in the noradrenalin-containing cells than in those containing adrenalin. In the A-containing cells under these particular stress situations the number of mitochondria per conventional unit area of the cell had a tendency to increase compared with intact animals. An increase also was found in the relative volume of these organelles. The results and qualitative analysis of the electron micrographs indicate a tendency toward hyperplasia and some degree of swelling of the mitochondria in the adrenalin-containing cells. The number of cisternae of the endoplasmic reticulum under conditions of psychoemotional stress in these same cells showed a tendency to fall, but during running in the drum it fell to 0.40 ± 0.07. The relative volume of the reticulum increased during sleep deprivation, but during running the drum it decreased appreciably; this may perhaps be explained in cases of psychoemotional stress by swelling (dilatation) of the cisternae of the reticulum, but under conditions of physical stress by destruction of the elements of this organelle also. The results suggest that the depth of the ultrastructural changes in A-containing cells was greater in physical stress than in psychoemotional stress.

The results of the morphometric analysis agree with data in the literature [2, 4], according to which the functional strain on organs and tissues during stress may be accompanied by the following ultrastructural changes in the cells: swelling of the mitochondria, cleaning of their matrix, dilatation of structures of the Golgi complex and endoplasmic reticulum, a decrease in the number of ribosomes, and so on.

A stress situation thus leads to considerable changes in the structural and functional apparatus of chromaffin cells, which are expressed as marked emptying of the CA-storage granules, swelling of the mitochondria and endoplasmic reticulum, and partial destruction of the reticulum. The depth of these structural changes depends on the type of stress and is more marked in a situation dominated by physical stress.

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EFFECT OF MONOCHROMATIC RED LIGHT OF A HELIUM-NEON LASER ON THE MORPHOLOGY OF ZYMOSAN ARTHRITIS IN RATS

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Considerable experience has been gained in recent years in the treatment of rheumatoid arthritis by low-intensity laser radiation. The pathological basis for such treatment has not yet advanced beyond the stage of a working hypothesis. Judging from preliminary changes in the morphology of synovitis toward the end of treatment, laser radiation reduces tissue edema and the amount of fibrin on the synovial surface and between the synovial lining cells, reduces infiltration of the subjacent layer with lymphocytes and macrophages, and activates fibroplasia [2]. However, since laser therapy is usually given after preliminary anti-inflammatory therapy, and also because of the very varied picture of synovitis at the beginning of treatment and the incomparability of biopsy specimens obtained before and after treatment, it is difficult to assess the action of the laser beam on the inflamed synovial membrane.

The object of this investigation was to study the effect of laser radiation on the dynamics of the inflammatory process in experimental arthritis, in which the negative factors mentioned above were reduced to a minimum.

MATERIAL AND METHOD

Zymosan arthritis was chosen as the experimental model. Keystone et al. [3] induced arthritis in mice aged 6-8 weeks by injecting zymosan (a mixture of insoluble glycans from the yeast cell wall) into the knee joints in a dose of 0.02 ml of a sterile 1.5% suspension in physiological NaCl solution. In the present experiments, noninbred rats weighing 100-175 g were used as experimental animals. A 1.5% suspension of zymosan was injected into both

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