Ultrastructural Reorganization of Adrenal Cortex in Rats Exposed to Hypoxia and Treated with Nandrolone

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Single and repeated hypoxic exposures induced different ultrastructural changes of varying intensity in adrenocorticocytes of rat adrenal zona fasciculata and zona reticularis. Destructive changes in cells were caused by the antianabolic effect of hypoxia and inhibition of regeneration and plastic processes. Treatment with nandrolone (Nerobolil, Gedeon Richter) diminished this effect and stimulated secretory activity of adrenocorticocytes.

Key Words: *hypoxia; adrenal cortex; adrenocorticocytes; ultrastructure*

Hypoxic states (HS) are the most important problem in modern biology and medicine [6,8,12,14]. Multicomponent and multifactorial nature of HS is now well established [5]; however, triggers of functional and metabolic reactions induced by HS are difficult to identify.

Ultrastructural reorganization in the adrenal cortex in HS is of special interest, since the adrenal glands, on the one hand, mediate the development of nonspecific organism's response to stress (in particular, hypoxia) [1,4,7,9,10] and, on the other hand, similarly to other organs are subjected to hypoxia. Increasing severity and duration of hypoxic exposure (HE) induced considerable structural and functional changes in the adrenals leading to their secretory insufficiency.

Of particular importance is the problem of correction of HS, in particular, the use of anabolic steroids. These substances exhibit no specificity with respect to hypoxia, but can diminish its antianabolic effect.

The aim of the present study was to investigate adrenocorticocyte (ACC) reorganization in the zona fasciculata and zona reticularis of rat adrenal glands during HE and the effect of nandrolone of these processes.

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MATERIALS AND METHODS

Experiments were carried out on 104 Wistar rats. Single HE was modeled by "elevating" the animal in a pressure chamber to an altitude of 9000 m above the sea level for 1 h. Repeated HE were modeled in the same chamber by elevating the animals for 1 h to an altitude of 5000 m every day for 11 days and to 9000 m during day 12. The ascent rate was 50 m/sec. The animals were divided into 6 group: group 1 animals were subjected to single HE; group 2 — single HE+nandrolone; group 3 — repeated HE; group 4 — repeated HE+nandrolone; group 5 — intact animals; group 6 — nandrolone treatment.

Commercial nandrolone (Nerobolil, Gedeon Richter) heated to body temperature was injected intramuscularly in a dose of 5 mg/100 g body weight 24 and 12 h before decapitation.

For electron microscopy, adrenal gland tissue fixed in 4% paraformaldehyde and postfixed in 1% OsO_4 was processed using standard methods and embedded in Epon and Araldite. Ultrathin section were prepared on a LKB-III ultratome and examined under a JEM-1010 electron microscope at 40 kV.

RESULTS

No considerable changes in the body weight and the weight of the adrenal glands were observed in group

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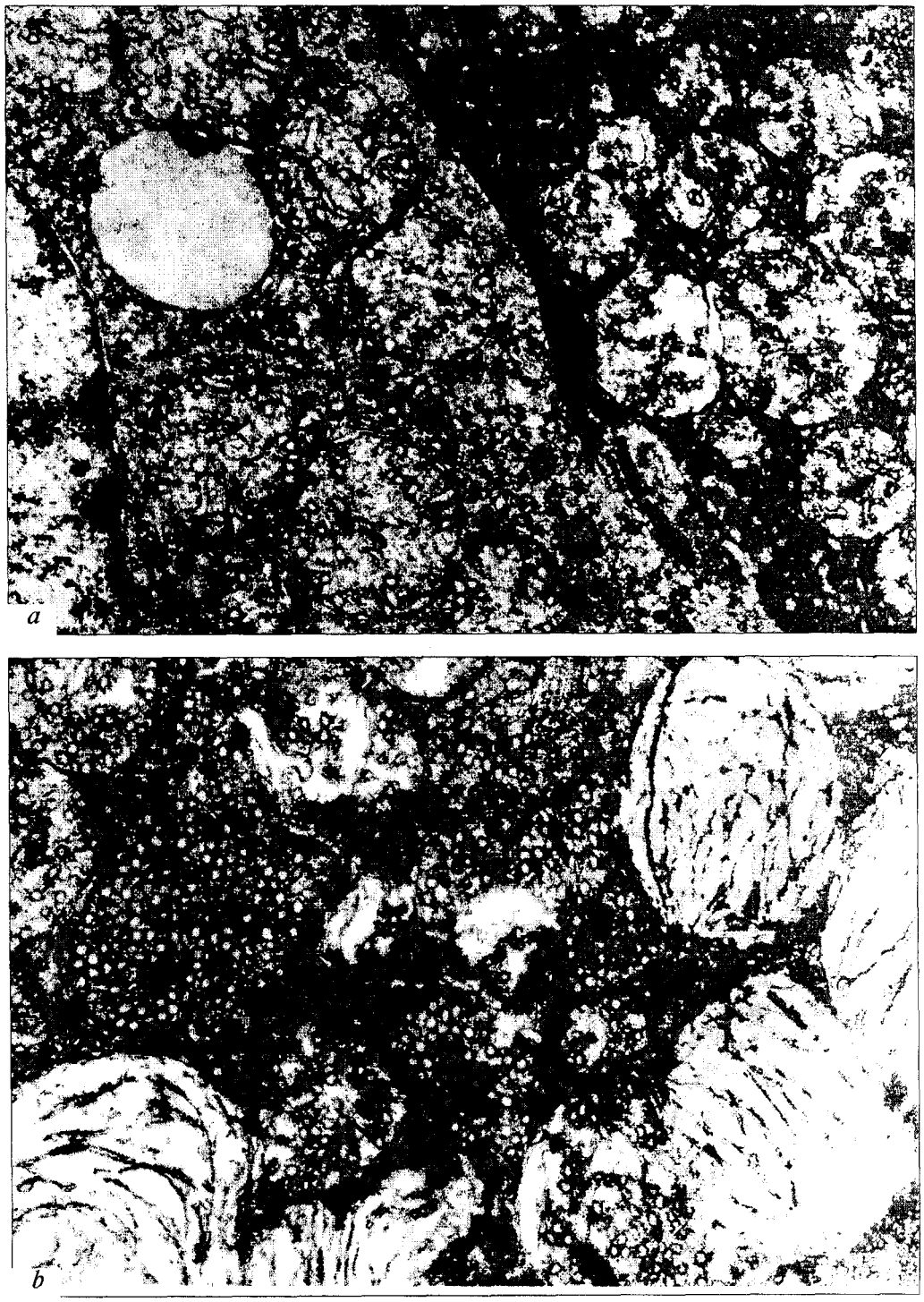

Fig. 1. Ultrastructural changes in adrenocorticocytes (ACC) after single hypoxic exposure. *a*) fragments of dark and light ACC, marked destruction of mitochondrial cristae in dark ACC, ×12,000; *b*) membrane transformation of lipid droplets, ×15,000.

1 animals (the weight of the left adrenal gland was 18.8±0.1 mg *vs.* 19.5±0.2 mg in the control), and therefore their relative weight remained unchanged. In group 2 rats, the relative weight of the adrenals decreased by 9% ($p<0.05$) due to the increase in the body weight by 8% ($p<0.05$) in comparison with the control. At the same time, single HE reduced the total width of the zona fasciculata and zona reticularis by 31% (from 820.3±10.3 to 562.5±16.5 μ, $p<0.05$) and the width of the zona glomerulosa by 29%, i.e. induced atrophy of the adrenal cortex. Pretreatment with nandrolone did not abolish this effect. The absence of

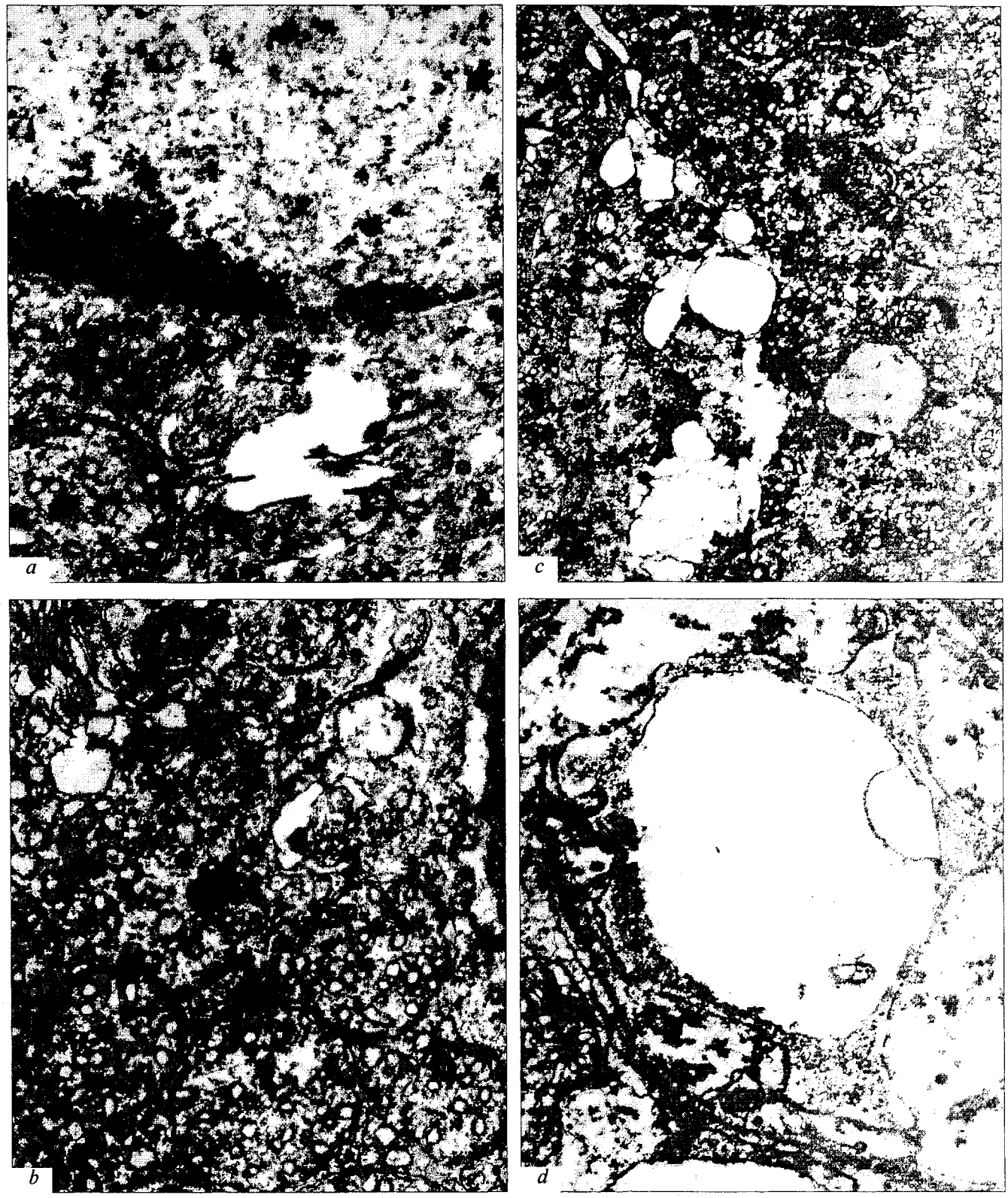

Fig. 2. Ultrastructural changes in adrenocorticocytes (ACC) after hypoxic exposures in groups 2 (*a*), 3 (*b*), and 4 (*c, d*) rats. *a*) unequal dilation of dictyosomes and partial membrane destruction in ACC of the zona fasciculata, ×20,000; *b*) developed Golgi complex in ACC of the zona reticularis, ×15,000; *c*) enlarged spaces between adjacent ACC in the zona fasciculata, ×10,000; *d*) necrobiotic changes in sinusoidal endotheliocytes, ×15,000.

changes in the weight of the adrenals was probably associated with plethora observed in all animals subjected to acute HE.

Atrophy of the adrenal cortex in acute hypoxia [11,13] probably reflects deep inhibition of anabolic processes under conditions of energy deficit and predominance of catabolic processes in ACC.

In group 3 animals, the body weight remained unchanged, while the weight of the adrenals significantly increased from 19.5±0.2 to 21.8±0.5 mg (by 12%, $p<0.05$) and their relative weight increased by 11% ($p<0.05$). In group 4 rats, the weight of the adrenals increased to 21.1±0.3 mg (by 8%, $p<0.05$); the body weight in this group also increased and, therefore, the relative weight of the adrenals remained unchanged.

The observed increase in the weight of the adrenal glands after repeated HE is probably associated with hyperplasia of ACC, especially in the zona fasciculata/reticularis, which is confirmed by the increase in their total width to 1088.3±17.3 μ (by 33%, $p<0.05$). Similar changes were noted in group 4 rats. However, in this group widening of the zona glomerulosa was more pronounced. Analogous changes in the weight of the adrenal glands and width of the zona fasciculata were previously observed in animals subjected to stress and after repeated injections of ACTH [4].

Marked functional heterogeneity of the zona fasciculata/reticularis was observed after single HE: cells with regular structure and light cytoplasm (moderate electron density, light cells) and cells with dense condensed cytoplasm (dark cells) were seen (Fig. 1, *a*).

In zona fasciculata cells, the number of liposomes greatly varied: some cells contained large agglomerates of liposomes, while others contained only solitary lipid inclusions. Most liposomes lacked osmiophilic substances and contained membrane structures (Fig. 1, *b*). Mitochondria in light cells underwent ultrastructural changes: focal lysis of the matrix and destruction of cristae; some mitochondria were replaced with residual bodies (myelin-like structures). Enhanced autophagocytosis in ACC was confirmed by increased number of secondary lysosomes.

In dark cells we observed extensive lysis of mitochondrial matrix and reduction of cristae (Fig. 1, *a*). All mitochondria contained electron-dense inclusions (crystalline bodies). No organelles can be distinguished in the cytoplasm of dark cells, while light ACC contained solitary vesicles of the smooth endoplasmic reticulum. The Golgi complex was reduced.

Ultrastructural changes of ACC in the zona reticularis were similar to those in the zona fasciculata. It should be noted that ACC of the zona reticularis contained practically no liposomes.

In group 2 rats, ultrastructural changes in ACC were less pronounced than in group 1. Structural and functional heterogeneity of ACC was preserved: light and dark cells were found in the zona reticularis, while zona fasciculata contained cells with electron-dense cytoplasm differing from dark cells. The number of liposomes decreased in all cells of the zona fasciculata.

Interestingly, large aggregates of polysomes and numerous vesicles of the smooth endoplasmic reticulum were observed in ACC (especially, at the cell periphery) under these conditions, which suggested activation of repair and secretory processes in cells. Golgi complex was present in all cells, but was characterized by unequal dilatation and partial destruction of dictyosomes (Fig. 2, *a*). Aggregates of many coated vesicles and small primary lysosomes were found nearby the Golgi complex.

After repeated HE, structural and functional heterogeneity of ACC disappeared, all cells had similar structure. ACC of the zona fasciculata contained a decreased number of liposomes, but mitochondria were more abundant and greatly varied in size. Some cells contained abnormal mitochondria. In practically all cells liposomes were depleted or underwent membrane transformation.

In the cytoplasm, vesicles of the smooth endoplasmic reticulum were seen between mitochondria, polysomes were noted in practically all cells. The Golgi complex and few coated vesicles were arranged nearby the nucleus (Fig. 2, *b*). In some cells secondary lysosomes with lipid inclusions were observed.

Analogous ultrastructural changes were noted in ACC of the zona reticularis. However, these cell contained practically no lipid droplets.

An important feature in the structural and functional rearrangement of ACC in both zones was dilation of intercellular spaces between membranes not lining the sinusoids (Fig. 2, *c*). These regions often contained membrane structures, vacuoles and residual bodies. The same structures were found in the sinusoid lumens. Sinusoidal endotheliocytes in the zona fasciculata/reticularis were markedly altered. Sometimes pronounced edema and almost total lysis of the cytoplasm and cell organelles were observed; only solitary pinocytotic vesicles were seen.

In group 4 rats, ultrastructure of ACC practically did not differ from the control. Cell cytoplasm was more condensed and contained multiple mitochondria with vesicular cristae. Fine structure of mitochondria generally corresponded to normal, but some cells contained abnormal mitochondria. The number of liposomes was reduced, some of them were replaced with concentric membrane structures. Vesicles of the smooth endoplasmic reticulum were seen in the cytoplasm. Necrobiotic changes in endotheliocytes were preserved in this group (Fig. 2, *d*).

The observed ultrastructural changes in ACC of the zona fasciculata/reticularis in acute hypoxia reflected not only stimulation of steroidogenesis (reduced number of liposomes) in response to stress [2,3], but also impairment of repair and plastic processes and predominance of catabolic processes leading to marked destructive changes in cells under these conditions. Nandrolone diminished the antianabolic effect of hypoxia, which manifested itself in an increased number of preserved cell organelles involved in biosynthetic processes and in a reduced number of dark cells. The latter represented (judging from their ultrastructure) necrobiotic cells, which were then resorbed by macrophages. After repeated HE, repair processes in ACC were reactivated and cell ultrastructure was to a great extent restored. Nandrolone promoted activation of anabolic processes in cells and reduced plastic deficiency, and consequently stimulated secretory activity of ACC.

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