Tissue Reorganization of Rat Adrenal Cortex Induced by Hypoxia and Its Correction with Nandrolone

O. P. Molodykh, E. L. Lushnikova, E. V. Koldysheva, and I. S. Proskuryakova

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Acute hypoxia has a pronounced catabolic effect, whereas repeated hypoxic episodes promote hypertrophy of the adrenal cortex. Steroid hyperproduction caused by stress reaction after single hypoxic episode persists after repeated hypoxic episodes. The relations between vessels and parenchyma play the key role in spatial reorganization of the adrenal cortex. Increased volume and surface-volume ratios between sinusoids and adrenocorticocytes point to the enhancement of transcapillary exchange and can be regarded as an adaptive compensatory reaction on the tissue level. Nandrolone decreases antianabolic and stress effects of hypoxia, thus potentiating compensatory adaptive reactions both after single and repeated hypoxic episodes.

Key Words: *adrenal cortex; hypoxia; nandrolone; morphology; stereology*

There are ample data concerning hypoxia and changes in tissues and organs caused by inadequate oxygen supply [1,5,7]. From biological point of view, hypoxia can be regarded as a stress factor, therefore oxygen deficiency induces unspecific stress reaction in the organism [8,10]. An important component of this reaction is activation of the adrenals and enhanced hormone synthesis and secretion. This strain combined with negative effect of oxygen deficiency on energy supply of biosynthetic processes in adrenocorticocytes (ACC) results in significant structural and functional changes in this organ and secretory insufficiency.

Recently, the possibitity of correcting hypoxic states with antihypoxants of different origin attracted attention of many authors [6,7,11]. However, hypoxia-induced morphological changes in the adrenal glands, particularly, spatial reorganization of the main tissue components, and the possibility of correcting these changes are not completely elucidated.

Laboratory of Histocytometry and Stereology, Laboratory of Pathophysiology and Functional Morphology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Science, Novosibirsk

MATERIALS AND METHODS

Adrenal glands of 104 male Wistar rats served as an object of complex morphological examination. Twelve rats served as the control. Hypoxia was modeled in a pressure chamber: the animals (n=33) were "lifted" to 9000 m at a rate of 50 m/sec once during 1 h. Repeated hypoxia was modeled by "lifting" the animals to 5000 m for 1 h during 11 days and to 9000 m for 1 h on day 12 of the experiment. Oil solution of nandrolone (NL, 19-norandrotestosterone phenylpropionate, synthetic androgen with anabolic activity) prewarmed to body temperature was injected twice into thigh muscles in a dose of 5 mg per 100 g body weight 24 and 12 h before decapitation (control rats, n=8), or before single (n=22) and repeated (n=7) hypoxic episodes.

For light microscopy, the adrenals were fixed in 10% formaldehyde and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin followed by Perls reaction. In addition, PAS reaction was performed. Ascorbic acid was revealed with acid solution of silver nitrate [12].

Semithin sections of epon-embedded tissue for stereological analysis were processed routinely. The

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sections were stained with 1% azure II and sudan black B. PAS reaction with azure II counterstaining was performed.

To evaluate the main stereological characteristics of the zona reticularis and zona fasciculata the following parameters were estimated: volume and surface density of ACC, their nuclei, sinusoids and connective tissue cells, volume density of connective tissue fibres and matrix. Secondary stereological parameters, e. g. stroma/parenchyma surface-volume ratio, nucleus/cytoplasm ratio and volume and surface-volume ratios between sinusoids and ACC were calculated from primary characteristics.

RESULTS

Single hypoxic exposure did not affect the relative weight of adrenals (Table 1), whereas NL decreased it by 9% due to an increase in body weight (Table 2). Acute hypoxia significantly decreased the total width of the zona fasciculata-reticularis, and this parameter did not return to the control level after NL injection. Repeated hypobaric hypoxia increased the relative adrenal weight (by 11%) and the width of all adrenal zones, especially zona fasciculata-reticularis (by 33%). After combined action of repeated hypoxia and NL, the relative adrenal weight remained on the control level, while alterations of the zone width showed the same tendency as under the influence of repeated hypoxia alone.

Single and repeated hypoxic episodes induced different morphofunctional changes in the adrenal glands.

Single exposure caused plethora of sinusoid capillaries, most pronounced in the zona fasciculata-reticularis, which was characterized by high structural and functional heterogeneity of ACC due to the presence of dark and light cells (Fig. 1, *a*). Sudanophilic lipid inclusions were found only in the external zona fasciculata. No ascorbic acid was detected after single hypoxia.

Repeated hypoxia caused enlargement of the sudan-negative layer (Fig. 1, *b*), probably due to cambial cell proliferation in response to adrenal hyperfunction. The number of sudanophilic inclusions in the external zona fasciculata was far below the control. Sinusoidal capillaries in the zona fasciculata-relicularis were equally plethoric, in the zona reticularis they were markedly dilated.

In intact animals, NL did not change the structure of the adrenal cortex except minor enlargement of the sudan-negative layer. After single hypoxia in combination with NL the structure of the adrenal cortex did not differ significantly from the control: pronounced sinusoidal plethora was observed only in the zona reticularis (Fig. 1, *c*). This zone was also characterized

TABLE 1. Morphometric and Stereological Parameters of Rat Adrenal Glands after Hypoxic Exposure ($M \pm m$)

Parameter		Control	Single hypoxia	Repeated hypoxia
Body weight, g		184.2±3.2	181.7±2.6	186.3±4.5
Adrenal weight, mg		19.5±0.2	18.8±0.2	21.8±0.5*
Relative adrenal weight, mg/g		10.56±0.12	10.35±0.07	11.73±0.06*
Width of zona glomeruloza, μ		145.1±8.1	103.2±11.2	175.1±13.9
Width of zona fasciculata-reticularis, μ		820.3±10.3	562.5±16.5*	1088.3±17.3*
Volume density, mm^3/cm^3				
Zona fasciculata	ACC	881.2±11.0	803.4±21.3*	862.3±11.6
	ACC nuclei	57.7±5.5	78.2±3.5*	77.6±2.6*
	sinusoids	49.9±4.3	101.6±5.2**	45.2±4.1
Zona reticularis	ACC	793.9±34.5	671.7±28.3*	794.1±14.9
	ACC nuclei	94.1±12.0	92.6±4.7	94.1±7.3
	sinusoids	93.6±15.3	200.5±17.2**	85.9±4.3
Surface density, m^2/cm^3				
Zona fasciculata	ACC	0.373±0.005	0.431±0.009*	0.429±0.057
	ACC nuclei	0.075±0.008	0.103±0.011	0.096±0.018
	sinusoids	0.087±0.014	0.114±0.036	0.073±0.017
Zona reticularis	ACC	0.415±0.006	0.370±0.030	0.474±0.015*
	ACC nuclei	0.128±0.004	0.101±0.007*	0.118±0.003
	sinusoids	0.095±0.011	0.148±0.006*	0.128±0.001

Note. Here and in Table 2: *$p<0.05$ and **$p<0.01$ compared to the control.

TABLE 2. Morphometric and Stereologic Parameters of Rat Adrenal Glands after Hypoxic Exposure against the Background of Nandrolone (NL) Pretreatment (*M±m*)

Parameter		Control	NL injection	Single hypoxia+NL	Repeated hypoxia+NL
Body weight, g		184.2±3.2	188.7±20.2	199.0±2.4*	196.4±2.4*
Adrenal weight, mg		19.5±0.2	20.2±0.2	19.8±0.1	21.1±0.3*
Relative adrenal weight, mg/g		10.56±0.12	10.68±0.10	9.60±0.05*+	10.77±0.11
Width of zona glomeruloza, μ		145.1±8.1	154.1±16.5	127.2±3.9	232.1±3.9*
Width of zona fasciculata-reticularis, μ		820.3±10.3	917.3±18.8*	596.5±8.9*	975.3±8.1*+
Volume density, mm^3/cm^3					
Zona fasciculata	ACC	881.2±11.0	897.8±0.6	835.0±12.2*+	818.3±14.5*+
	ACC nuclei	57.7±5.5	55.8±3.1	53.3±1.7	81.4±0.8*+
	sinusoids	49.9±4.3	34.5±4.5	95.6±11.1*+	91.7±7.8*+
Zona reticularis	ACC	793.9±34.5	805.8±25.8	677.6±28.2*+	790.4±20.9
	ACC nuclei	94.1±12.0	61.5±12.9	46.5±2.9*	75.2±3.0
	sinusoids	93.6±15.3	109.9±10.1	239.5±21.7*+	118.9±36.7
Surface density, m^2/cm^3					
Zona fasciculata	ACC	0.373±0.005	0.384±0.012	0.419±0.032	0.409±0.014
	ACC nuclei	0.075±0.008	0.064±0.004	0.068±0.004	0.092±0.010
	sinusoids	0.087±0.014	0.038±0.005	0.087±0.007	0.116±0.038
Zona reticularis	ACC	0.415±0.006	0.404±0.004	0.381±0.010	0.462±0.018+
	ACC nuclei	0.128±0.004	0.075±0.023	0.053±0.002**	0.097±0.004*
	sinusoids	0.095±0.011	0.091±0.006	0.138±0.038	0.135±0.033

Note. *$p<0.05$ compared to NL injection.

by structural and functional heterogeneity of ACC, which was less pronounced than after single hypoxia alone.

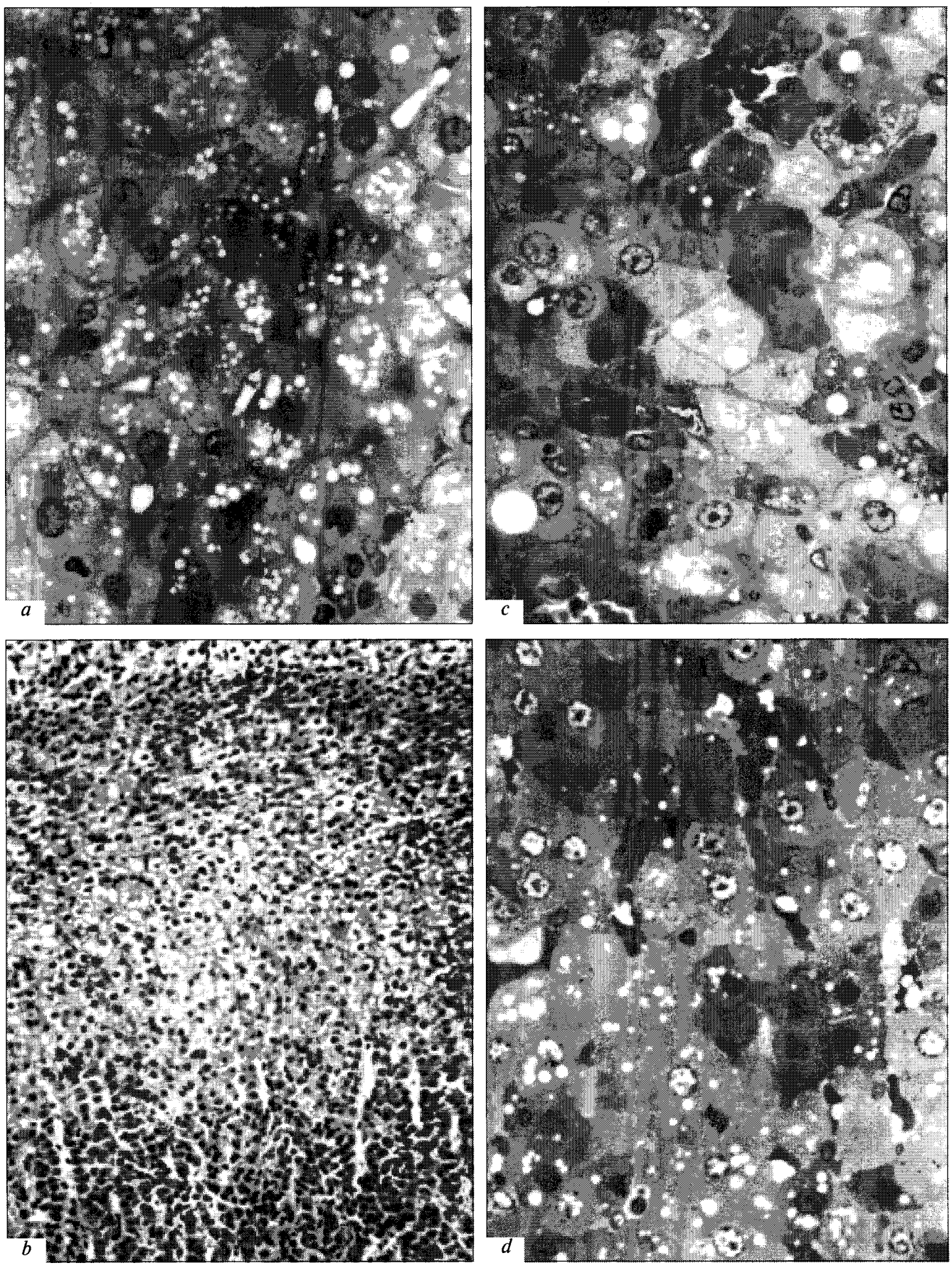
Repeated hypoxia after NL injection induced slight enlargement of the sudanophilic layer, ACC polymorphism in the zona fasciculata, and highly cell delipidization. In the zona reticularis, few dark cells and sinusoidal plethora were observed (Fig. 1, *d*).

Stereologic analysis showed that NL induced no significant changes in the surface and volume characteristics of the main tissue component of the adrenal cortex.

Single hypoxic episode decreased ACC volume density in the zona fasciculata-reticularis by 9 and 15%, respectively; this tendency persisted under the effect of NL treatment (Tables 1 and 2). The volume and surface density of sinusoids increased in both zona fasciculata (by 104 and 31%) and zona reticularis (by 114 and 56%). NL pretreatment also increased volume density of sinusoids by 84 and 56% in the zona fasciculata and zona relicularis, respectively, as compared to the control. In these zones sinusoid/ACC volume ratio also increased by 119 and 149% after single hypoxic episode (Fig. 2). NL pretreatment did not attenuate the effect of hypoxia and these parameters were increased by 96 and 214% in the zona fasciculata and zona reticularis, respectively (Fig. 3). Single hypoxic episode also induced a significant increase in the stroma/parenchyma volume ratio in the zona fasciculata and zona reticularis (by 119 and 145% respectively; Fig. 2). After NL pretreatment this parameter also increased by 74 and 202%, respectively (Fig. 3).

After repeated hypoxic exposure the volume density of ACC and sinusoids did not differ from the control, but their surface density tended to increase (Table 1). NL pretreatment decreased ACC volume density in the zona fasciculata by 7% and increased sinusoid volume density by 91% in both zones. After repeated hypoxia the sinusoid/ACC volume ratio tended to decrease, whereas NL increased this parameter by 104% (in the zona fasciculata; Fig. 3). Stroma/parenchyma volume ratio remained unchanged in both

Fig. 1. Hypoxia-induced morphological changes in the adrenal cortex of Wistar rats. *a*) dark and light cells in the zona reticularis of the adrenal cortex after acute hypoxia, ×800; *b*) enlargement of sudanophobic layer induced by repeated hypoxia; polymorphism of adrenocorticocytes in the zona fasciculata, ×200; *c*) pronounced sinusoidal plethora in the zona reticularis after nandrolone injection and single hypoxic exposure, ×800; *d*) accumulation of azure-positive cells in the zona reticularis of the adrenal cortex after nandrolone injection and repeated hypoxia, ×800. *a, c, d*) semithin sections stained with azure II; *b*) hematoxylin and eosin staining.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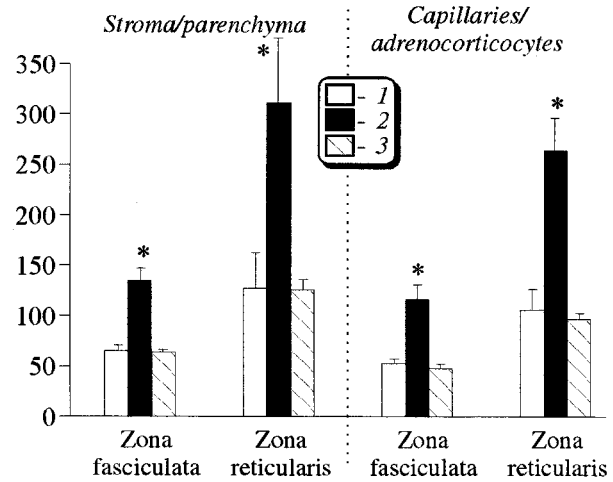

Fig. 2. Changes in volume ratio between the main parenchyma and stroma components of the adrenal cortex induced by hypoxia. *1*) control, 2) single, and 3) repeated hypoxia. *$p<0.05$ compared to the control.

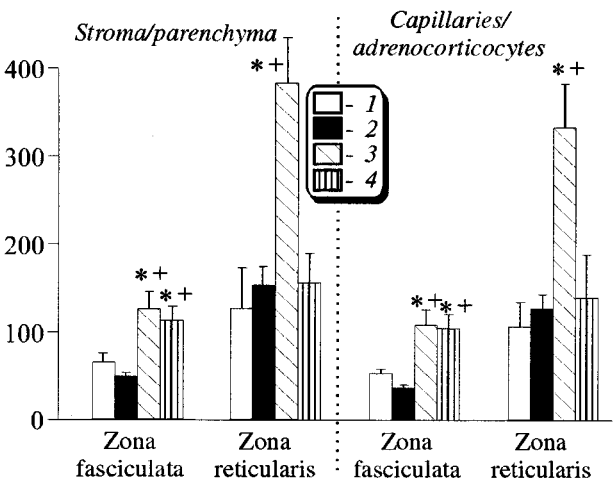

Fig. 3. Changes in volume ratio between the main parenchymal and stromal components of the adrenal cortex induced by nandrolone pretreatment and hypoxia. *1*) control, 2) nandrolone, 3) nandrolone single hypoxia, 4) nondrolone+repeated hypoxia. $p<0.05$ compared to the control (*) or nandrolone injection (+).

zones after repeated hypoxia, while NL pretreatment significantly (by 94%) increased it in the zona fasciculata (Fig. 3).

Thus, single hypoxic episode induced significant morphofunctional changes in the adrenal cortex involving all levels of its structural organization. At the organ level, this reorganization was manifested in a significant decrease in the width of the zona fasciculata-reticularis, while on the tissue level it appeared as an increased structural and functional heterogeneity of ACC against the background of pronounced hyperemia. Tissue spatial reorganization of the adrenal cortex induced by single hypoxic exposure is characterized by significant increase in the sinusoid/ACC volume and surface-volume ratios, which reflects adaptive compensatory changes at the tissue level. These changes are most pronounced in the zona reticularis. NL pretreatment attenuates the negative effect of single hypoxic exposure but does not prevent it completely. The protective properties of NL are most pronounced in the zona reticularis as increased sinusoid/ACC volume ratio at the tissue level and as less pronounced decrease in the width of adrenal cortex at the organ level.

The pattern of these changes in the zona fasciculata-reticularis suggests the key role of vascular-parenchymal interrelations in the reorganization of the adrenal cortex. The increase in the sinusoid/ACC volume and surface-volume ratios reflecting intensification of transcapillary exchange can be regarded as a compensatory reaction at the tissue level.

Repeated hypoxia increases the weight of the adrenals due to enlargement of the zona fasciculata-reticularis as a result of their hyperfunction in response to repeated stress. The increase in the adrenal weight depends on ACC hyperplasia, which is confirmed by their enlargement. Repeated hypoxia did not change significantly quantitative parameters of most tissue components in the zona fasciculata-reticularis.

Atrophic changes in the adrenal cortex induced by acute hypoxia can reflect sharp inhibition of anabolic processes under conditions of energy deficit and predominance of catabolic reactions in ACC [13]. The changes revealed in the adrenal cortex after repeated hypoxia are primarily associated with the development of general adaptation syndrome which is characterized by enhanced corticosteroid production, ACC hyperplasia, and pronounced hyperemia of the adrenal gland [2-4,9,14].

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