Morphogenesis of Adaptation and Compensatory Reactions in Mouse Adrenals during Restitution after Thermal Exposure

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Single hyperthermia session causes an appreciable decrease in adrenocorticocyte count in the adrenal cortex in mice on day 3 of postthermal restitution. Morphological changes in the adrenals under conditions of developing stress reaction manifested in increased structural and functional heterogeneity of adrenocorticocytes in all cortical layers, exhaustion of lipid incorporations in the zona fasciculata cells, pronounced shrinkage of zona reticularis, developing against the background of hemodynamic disorders. Recovery of the total count of adrenocorticocyte population was associated with the appearance of small accumulations of immature cells in the subcapsular area of zona glomerulosa. Despite the absence of pronounced changes in the architectonics of all adrenocortical zones during postthermal restitution, recovery of zona reticularis was incomplete, which manifested in its shrinkage and persistence of degenerative changes in adrenocorticocytes.

Key Words: *whole-body hyperthermia; adrenocorticocytes; method of alkaline dissociation of tissues; morphometry*

The outcome of adaptive and compensatory processes after extreme exposure, including whole-body hyperthermia, largely depends on the reliability of the adrenal system function, specifically, on the intensity of changes and regeneratory processes in different layers of the adrenal cortex and medulla [2,5,6,11].

Studies of the mechanisms of physiological and reparative regeneration of the adrenal glands was for a long time focused on the search for location of proliferating adrenocorticocytes (ACC) and modes of newly formed cells transposition to sites of their damage and death [2,3,12,13]. Evaluation of the intensity of ACC death and elimination and the possibility of complete restitution of all morphofunctional compartments of the adrenal during and after exposure to extreme environmental factors is essential for evaluation of the adrenal regeneratory potential.

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Whole-body hyperthermia is a prevalent extreme ecological factors causing the development of the common adaptation syndrome and sometimes leading to death. The development of stress reaction and exposure of organs and tissues to numerous endotoxins forming during thermal stress is detrimental for all adrenal cell populations. On the other hand, the type and intensity of morphofunctional changes in the adrenals under these conditions are little studied.

We studied the morphogenesis of adaptation and compensatory reactions in the adrenals and evaluated the changes in ACC counts in CBA mice during different periods of postthermal restitution.

MATERIALS AND METHODS

Experiments were carried out on 36 adult male CBA mice exposed to single hyperthermia at 43°C. Control group consisted of 8 mice. All animals were kept under standard vivarium conditions with free access to water. The duration of thermal exposure was 35 min.

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Longer exposure led to overall deaths. Adrenals for morphological study were collected 30 min, on days 3, 7, and 14 after thermal exposure.

For photooptic examination the adrenals were fixed in 4% paraformaldehyde and processed for embedding in epoxy resins. Semithin sections were stained with Azur II.

The absolute count of ACC in the adrenal cortex of experimental animals was evaluated by the method of alkaline dissociation of fixed tissues [7]. The procedure was as follows: an adrenal released from fatty capsule was fixed in 10% neutral formalin for at least 10 days. Adrenal weight was measured, the medulla was removed, and the remaining fragments were put into 50% KOH for 15-18 h, after which they were treated in order to obtain dissociated ACC.

The cells were counted in Fuchs—Rosenthal cell chamber. Five chambers were filled simultaneously (two compartments of standard volume in each chamber).

Cell concentration was estimated by the formula: $c=A\times V\times 10^3/m\times 3.2$, where c is the number of cells per mg tissue, A is the mean number of cells in a chamber compartment, 3.2×10^3 volume of compartment (mm^3), m weight of the sample (mg), and V final volume of suspension (ml). The absolute number of cells in an organ was calculated from the organ weight. Cell concentration was determined repeatedly 10 times.

The width of the adrenal cortex was measured by the standard method using MOB-1-15^x ocular micrometer, 20 measurements per preparation.

The statistical processing included estimation of the means for the parameters and of dispersion and errors in the means using Student's t test [1].

RESULTS

Single whole-body hyperthermia did not cause appreciable changes in the adrenal weight in CBA mice during the entire period of postthermal restitution, but the relative weight of the gland decreased by day 7 after exposure (Table 1).

According to photooptic examination, the structure of zona glomerulosa and zona fasciculata of the adrenal cortex virtually did not change 30 min after hyperthermia. Pronounced changes were observed at the interface between zona reticularis and medulla, where connective tissue cords were loosened and thickened. Cell count decreased in this region, while cell-to-cell spaces were extended. Blood capillaries and sinusoids were moderately dilated and plethoric.

On days 3-7 after thermal exposure the architectonics of zona glomerulosa and zona fasciculata virtually did not change. The glomeruli in zona glomerulosa were notably smaller than in the control and were disposed irregularly. Structural and functional heterogeneity of ACC increased in all zones, being most pronounced in the zona reticularis (Fig. 1, *a*). The content of lipid incorporations decreased in the zona fasciculata ACC. Almost complete elimination of ACC was observed in the zona reticularis in the immediate vicinity of the medulla (Fig. 1, *b*). Diffuse infiltration of various adrenal layers with mononuclear cells was observed in some cases. An appreciable thickening and loosening of the adrenal connective tissue capsule was observed. Accumulations of small immature ACC were seen under the capsule (Fig. 1, *c*).

Hemodynamic changes involved all zones of the adrenal cortex and particularly the medulla: the sinusoids and capillaries were dilated and plethoric. Pronounced plethora of subcapsular capillaries, with frequently observed erythrocyte sludge and sometimes plasmorrhagia were seen.

By day 14 of postthermal restitution the connective tissue capsule remained moderately thickened and loose. Zona glomerulosa and zona fasciculata of the

TABLE 1. Weights, Morphometric Values, and Quantitative Evaluation of ACC Population in the Adrenal Cortex of CBA Mice after Single Whole-Body Hyperthermia ($M\pm m$)

Parameter	Control	Time after thermal exposure			
		30 min	3 days	7 days	14 days
Body weight, g	20.5±0.5	21.9±0.5	21.8±0.2	23.4±0.7	24.2±0.6
Adrenal weight, mg	2.3±0.2	2.20±0.17	2.20±0.17	2.00±0.01	2.00±0.01
Relative weight of the adrenal, mg/g	0.13±0.01	0.10±0.03	0.10±0.01	0.090±0.004*	0.08±0.004*
ACC concentration in 1 mg tissue, $\times 10^3$	199.1±3.1	143.1±7.4*	81.3±3.5**	191.4±8.5	235.6±14.8
ACC concentration in organ, $\times 10^3$	464.7±35.5	307.8±19.5*	175.4±9.9**	382.8±19.7	483.3±32.1
Cortical width, mm	0.46±0.03	0.41±0.02	0.54±0.01	0.39±0.01	0.41±0.09

Note. *$p<0.05$, **$p<0.01$ compared to the control.

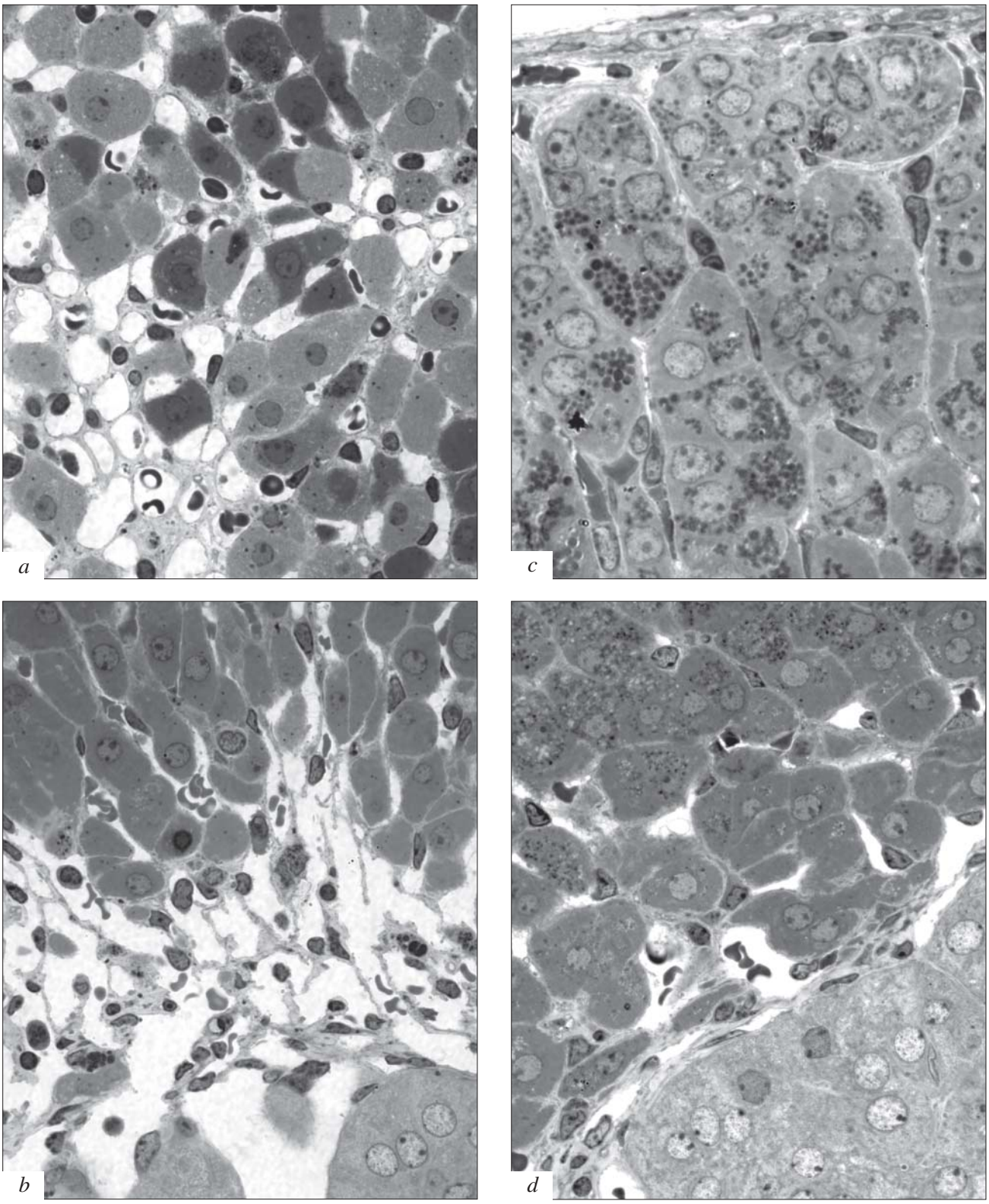

Fig. 1. Morphologic changes in mouse adrenals during restitution after thermal exposure. Semithin sections. Azur II staining, ×1000. *a*) intensification of structural functional heterogeneity in zona reticularis on day 3 of the experiment; *b*) appreciable elimination of adrenocorticocytes in zona reticularis 7 days after hyperthermia; *c*) thickening and loosening of connective tissue capsule, appearance of small accumulations of small adrenocorticocytes under it; *d*) decreased size of zona reticularis 14 days after hyperthermia.

cortical matter differed little from those in control animals. The width of zona reticularis greatly varied, but often remained decreased (Fig. 1, *d*). Massive elimination of ACC was observed in this zone, but it was less pronounced than during earlier period after hyperthermia. Connective tissue cords remained thickened and became rough. Hemodynamic disorders persisted.

Quantitative analysis of ACC population revealed notable fluctuations in their counts during the entire period of postthermal restitution (Table 1). ACC count decreased by 34% ($p<0.05$) 30 min after hyperthermia. By day 3 the count of ACC decreased most significantly (by 62%, $p<0.01$). By day 7 of postthermal restitution the total count of ACC increased, but remained 18% decreased compared to the control. By day 14 ACC count was 4% higher than in the control.

The decrease in ACC count 30 min and 3 days after hyperthermia was mainly caused by a significant decrease in cell concentration per mg tissue (by 28 and 59%, respectively, $p<0.05$ and $p<0.01$). Later (days 7 and 14) ACC concentration increased, which determined recovery of ACC count.

Morphometric analysis showed that the width of the adrenal cortex virtually did not change 30 min after hyperthermia. However, by day 3 of postthermal restitution this parameter increased by 17% and by day 7 of the experiment the width of the cortical layer was 15% lower than in the control. By the end of the experiment (day 14) this parameter approached the control (Table 1).

The detected diverse changes in the width of the adrenal cortex and total count of ACC indicate the importance of quantitative studies of parenchymatous cell population for evaluation of cellular and other possible mechanisms of adrenocortical hyper- or hypotrophy (degeneration), for which the width of the adrenal cortex is often regarded as the marker. Increased width of the adrenal cortex on day 3 of the experiment, paralleled by a decrease in ACC count, can be due to reorganization of the blood vessels caused by their plethora and changes in the connective tissue. Moderate hypertrophy of ACC during this period was observed only in the zona glomerulosa, but not in zona fasciculata and zona reticularis, where ACC decreased in size.

It was previously considered that any stress exposure led to adrenal hypertrophy [9], but later it was shown that ACC volume decreased [5,8]. These data are in line with our present findings.

Hence, the main regeneratory strategy of the adrenals during postthermal restitution is (at the cellular level) maintenance of ACC total count. The absence of necrotic changes in ACC in the adrenals during the entire period of postthermal restitution indicates apoptotic death of these cells and their elimination by macrophagal resorption. Reparative regeneration of the adrenals is realized mainly by proliferation of cells in the cambial layer (at the interface between zona glomerulosa and zona fasciculata) which migrate into the underlying zones [4]. We observed poorly differentiated cells in the subcapsular zone, which can be explained by high proliferative activity of the cambial reserve. According to some authors, ACC can proliferate in any adrenal zone under the effect of endogenous substances released by chromaffin cells, immune cells, and endotheliocytes [10,14].

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