MORPHOMETRIC ULTRASTRUCTURAL STUDY OF ADRENAL ADRENOCYTES AND NORADRENOCYTES IN RATS EXPOSED TO IMMOBILIZATION STRESS OF VARIED DURATION

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The important role of catecholamines (CA) in the response of the organism to an extraordinary stimulus is not now disputed. The participation of cells of the adrenal medulla synthesizing most of the CA in the response to stress has become a topic for active biochemical study [2, 5, 6]. However, there have been only a few investigations of the ultrastructure of the medullary tissue under stress conditions, and as a rule only qualitative characteristics of changes taking place have been described [3]. Information on the quantitative study of CA-containing cells and, in particular, the dependence of these changes on the dynamics of the stress response, is virtually not to be found.

The aim of this investigation was to study the number of chromaffin granules, containing adrenalin (A) and noradrenalin (NA), in the tissues of the rat adrenal medulla, and also a morphometric analysis of the redistribution of these structures at different stages of development of immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180 ± 20 g. A stress situation was created by fixing the animals by the neck and lumbar region in special frames. The duration of exposure to stress (3, 24, and 48 h) was chosen in accordance with the stages of development of the general adaptation syndrome studied previously by the use of this model of immobilization [4]. The adrenal medulla was fixed by Tranzer's method in the writers' own modification [1]. By this method it was possible to differentiate between A- and NA-containing cells in the same specimen. The number of A- or NA-containing granules per conventional unit area of cytoplasm was counted in electron micrographs by morphometry and their relative percentages in the cell were determined. For the morphometric study of ultrastructural changes in the adrenocytes and noradrenocytes 30 electron micrographs were chosen at random. The principles of stereology were used for the analysis.

EXPERIMENTAL RESULTS

Three types of structures were conventionally distinguished in the electron-microscopic and cytochemical study of the rat adrenal medulla in the population of secretory granules: vesicles filled with the cytochemical reaction product over the whole area of section (full), partly filled vesicles (half-full), and vesicles containing no cytochemical reaction product (empty). These types of granules are illustrated in the electron micrograph (Fig. 1). The results of counting the number of these structures in intact animals and during stress are given in Table 1 and in the histogram in Fig. 2. It follows from the results of morphometric analysis that the distribution of the types of secretory granules thus distinguished in the adrenocytes and noradrenocytes of the rat adrenals was similar in character. For instance, most of the structures (50-60%) fall into the category of full vesicles (Table 1), about 30% of the vesicles are half full, and only about 15% of the secretory granules consist of empty

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TABLE 1. Distribution of A- and NA-Containing Secretory Granules (in %) in Chromaffin Cells of Rat Adrenal Gland during Immobilization Stress of Varied Duration (M ± m)

Response to stress	Duration of immobilization, h	Adrenocytes			Noradrenocytes		
		full	half-full	empty	full	half-full	empty
Normal	0	52,4±5,9 (100,0±11,3)	32,2±4,8 (100,0±14,9)	15,4±2,5 (100,0±16,2)	64,0±9,2 (100,0±14,4)	23,1±3,5 (100,0±15,1)	13,0±2,2 (100,0±16,9)
Alarm stage	3	40,0±4,8* (76,3±9,2)	46,1±5,1* (143,2±15,8)	13,9±1,5 (90,3±9,7)	59,1±6,6 (92,3±10,3)	25,2±2,8 (109,1±12,1)	15,7±2,2* (120,5±16,9)
Adaptation stage	24	27,7±3,2* (52,9±6,1)	46,6±5,5* (144,7±17,1)	25,7±3,0* (166,9±19,5)	24,8±2,9* (44,4±4,5)	25,8±3,1 (111,7±13,4)	49,4±7,4* (380,0±5[illegible],9)
Exhaustion stage	48	11,0±1,6* (21,0±3,0)	28,9±3,5 (89,7±10,9)	60,1±7,7 (390,2+50,8)	16,8±2,2* (26,3±3,4)	33,6±4,1* (145,5±17,7)	49,6±8,1* (381,5±62,3)

Legend. Changes (in %) in each of the three types of granules during stress shown in parentheses. *$p \leq 0.05$.

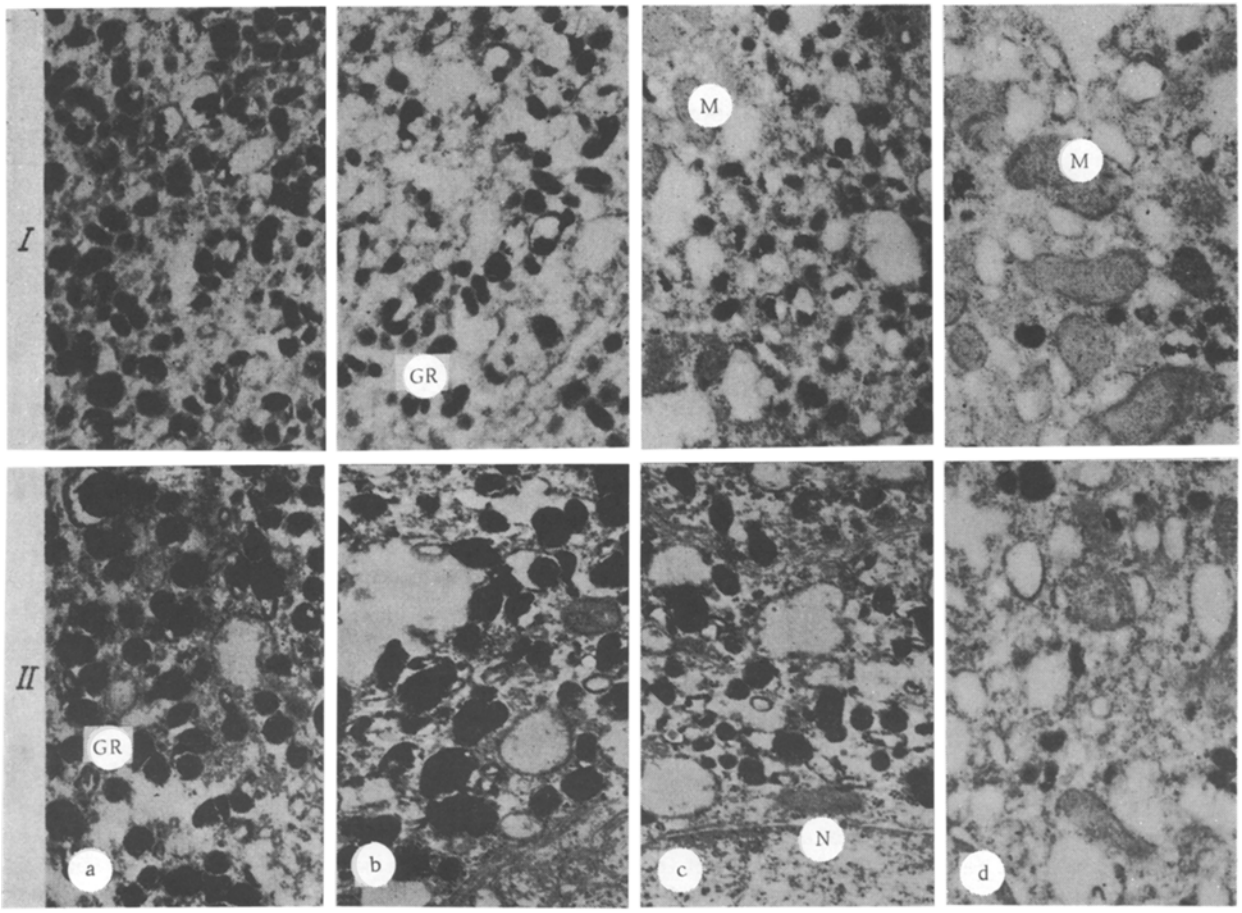

Fig. 1. Electron-microscopic determination of A-containing (I) and NA-containing (II) granules in chromaffin cells of the rat adrenals during immobilization stress of varied duration. a) Normal; b) after 3 h, c) after 24 h, d) after 48 h of stress. N) Nucleus, M) mitochondrion, GR) secretory granules. 18,000 ×.

vesicles. When the structures were counted per conventional unit of area of the cytoplasm of a medullary cell (Fig. 2) the largest number of vesicular structures consisted of full vesicles (3-4 in adrenocytes and 5-6 in noradrenocytes). The distribution of the number of half-full granules had a maximum at 1-2 structures, and that of empty granules was at 0-2 structures.

However, all the parameters studied showed considerable changes as a result of exposure to stress. For instance, even in the alarm stage, i.e., after 3 h of immobilization stress,

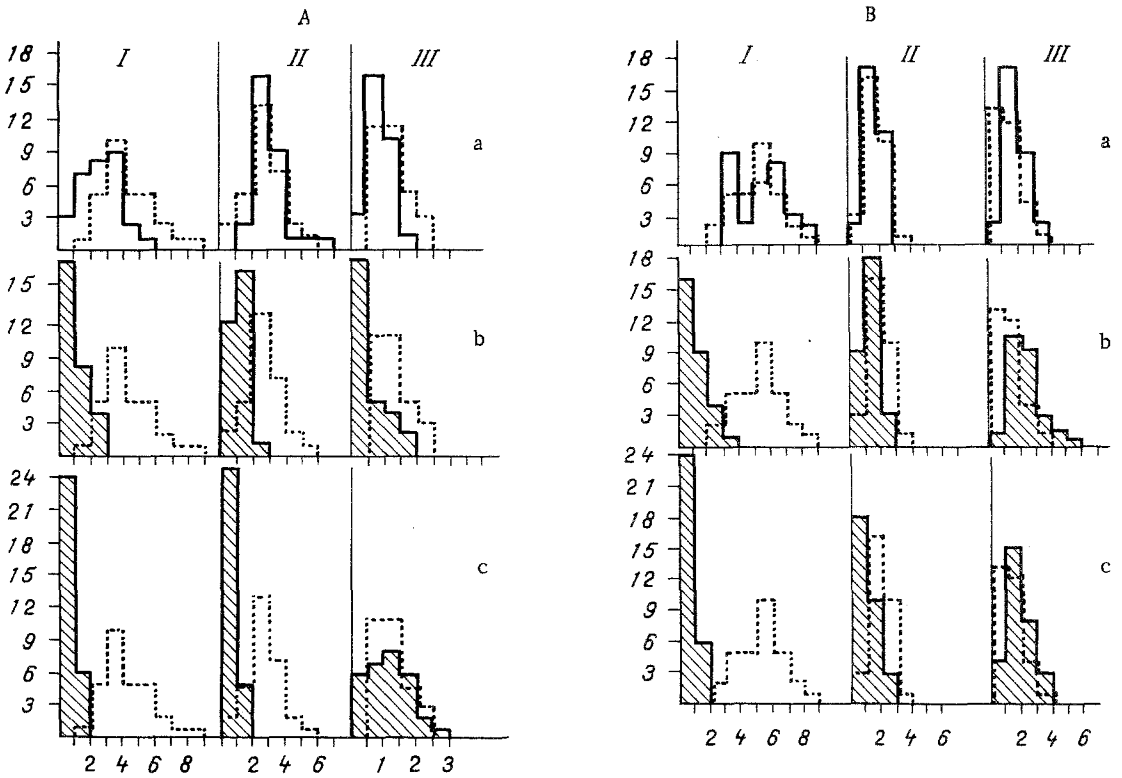

Fig. 2. Histogram of distribution of number of secretory granules in adrenocytes (A) and noradrenocytes (B) of adrenal glands of rats exposed to immobilization stress of varied duration. Abscissa, number of structures; ordinate, number of cells. Broken line — normal, continuous line — exposure to stress. A) Alarm stage (3 h of stress), B) adaptation stage (24 h of stress), C) exhaustion stage (48 h of stress). I, II, III) Full, half full, and empty granules, respectively.

morphometric analysis revealed a change in the distribution of the composition of the secretory granules. This process took the form of a shift of the histograms to the left for the full and half full structures. The character of distribution of the secretory granules changed in the following manner: the proportion of full structures fell to 76%, whereas that of the half full structures increased by 43% of the control level.

Much more marked changes in the distribution of the reserve granules was observed after 24 h of stress (Fig. 2, Table 1), i.e., at the resistance stage. In this period the changes affected both adrenocytes and noradrenocytes. At this stage the proportion of empty vesicles was about doubled. Incidentally, the content of half full granules in the adrenocytes remained increased (just as after 3 h of stress) by nearly 50%, whereas the proportion of vesicles of this type in the noradrenocytes was almost unchanged compared with the intact level.

An increase in the duration of immobilization to 48 h, corresponding to the exhaustion stage of the general adaptation syndrome, led to an even greater degree of emptying of the chromatin granules (Fig. 1d). For instance, the total number of full granules and their proportion in A- and NA-containing cells were appreciably reduced to only 21 and 26%, respectively. A sharp increase in the number of empty vesicles (Fig. 2) and also an almost fourfold increase in the proportion of these structures in the population of both A- and NA-storage granules were evidence of considerable CA release. At this period of the stress reaction changes in the distribution of the half full vesicles differed in degree: although their absolute number was reduced, the relative percentage of these structures was almost unchanged in the adrenocytes and increased in the noradrenocytes (Table 1, Fig. 1d).

Incidentally, there is no information in the literature on quantitative changes in the ultrastructure of the cells of the adrenal medulla during immobilization stress. The results of the present morphometric study agree with data in the literature on qualitative changes in the ultrastructure of the medullary cells under stress conditions. Many workers have shown [3] that the stress response leads to a reduction in the population of chromaffin granules, i.e., in the number of full vesicles. These changes correspond to biochemical data on a decrease in the content of A and NA in the adrenals during immobilization stress [7]. According to the principle enunciated by Viveros et al. [8], CA are released from the chromaffin granules of the adrenal medulla in accordance with the "all or nothing" rule. Consequently, the pool of half full vesicles studied in this investigation, can be regarded as a morphometric indicator of newly formed CA reserves, and not as the result of half-emptying of CA as a result of release of the transmitter from the vesicles or its diffusion during processing of the tissue.

The most important of the facts established must be discussed from these standpoints. For instance, during the first hours of development of the stress reaction (the alarm stage) an increase in the proportion of full and half full vesicles was found in the adrenocytes. These changes affected the noradrenocytes to a lesser degree. These facts can be taken as evidence not only of the release of CA, which is known from the work of other investigators, but also of a different process, namely an increase in their synthesis. The morphometric data are confirmed by biochemical investigations which showed that CA synthesis can be accelerated during the first hours of development of the stress reaction [2, 5]. During continued exposure to stress and the development of a more powerful response to the stressor (the onset of the adaptation stage), the relationship between CA release and synthesis changes somewhat. In this situation, however, despite massive release of CA, their synthesis is still evidently activated, although it does not prevail over the processes of release. This is shown by the following facts: 1) the marked decrease in the proportion of full vesicles in adrenocytes and noradrenocytes; 2) the relative increase in the number of half full vesicles in the adrenocytes and their unchanged level in noradrenocytes; 3) marked accumulation of empty vesicles in both kinds of cells in the adrenal medulla.

In the last stage of the response to stress (the exhaustion stage) the relationship between these processes is evidently shifted strongly toward continuing release of CA. On the basis of the available data it can be concluded that CA synthesis at this stage is inhibited, and that is why the accumulation of vesicles with CA is unable to make good the standard distribution of the vesicle population. It can also be postulated that CA synthesis remains the same as before. In that case the process of replenishment likewise cannot exceed release. At the same time, it follows from the results that during the development of the response to stress changes affect primarily the adrenocytes, followed by the noradrenocytes, and only after this has occurred do the processes of CA release referred to above proceed almost parallel to each other.

Thus immobilization of rats leads to considerable structural and functional changes in the adrenocytes and noradrenocytes of the rat adrenal medulla, expressed as a marked change in the redistribution of the types of structures in the population of CA-reserve granules. The degree of this structural change depends on the duration of immobilization stress and it exhibits characteristic differences in each stage of stress.

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