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EFFECT OF CHRONIC STRESS ON ULTRASTRUCTURE OF THE MYOCARDIUM AND HYPOTHALAMUS IN EMOTIONAL AND UNEMOTIONAL RATS

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Emotional-stress states often lie at the basis of development of many cardiovascular, nervous, and mental diseases. The severity of the pathological processes depends on individual characteristics and on differences in the type of response to stress. Different species and lines of experimental animals differ considerably in their response to a stress situation and in their sensitivity to psychotropic drugs [2, 3]. The study of the pathological manifestations of emotional stress in vitally important organs (myocardium, nervous system) and their dependence on individual characteristics is of great importance for the oriented pharmacological correction of these states.

In the investigation described below ultrastructural changes in the myocardium and hypothalamus of rats with different levels of emotional-behavioral reactivity, induced by chronic stress, were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male rats weighing 200 ± 26 g. A state of chronic emotional stress was produced in the animals by prolonged (7 days) selective deprivation of their rapid phase of sleep by Jouvet's "small platform" method [13]. Somatic responses to stress were assessed by counting the number of ulcers in the gastric mucosa and the change in body weight and in weight of the adrenals and thymus. Emotional-behavioral responses after the end of stress were studied in versions of the "open field" method, a dark chamber with holes, and the response to a moving object, and were estimated quantitatively and on a scale of points. Tissue from the left ventricle and anterior lobe of the hypothalamus (the region of the supraoptic and paraventricular nuclei) for electron-microscopy were fixed in 1% OsO_4 solution, dehydrated, and embedded in Araldite. Electron micrographs were obtained with the IEM-100B microscope. The number of glycogen granules was

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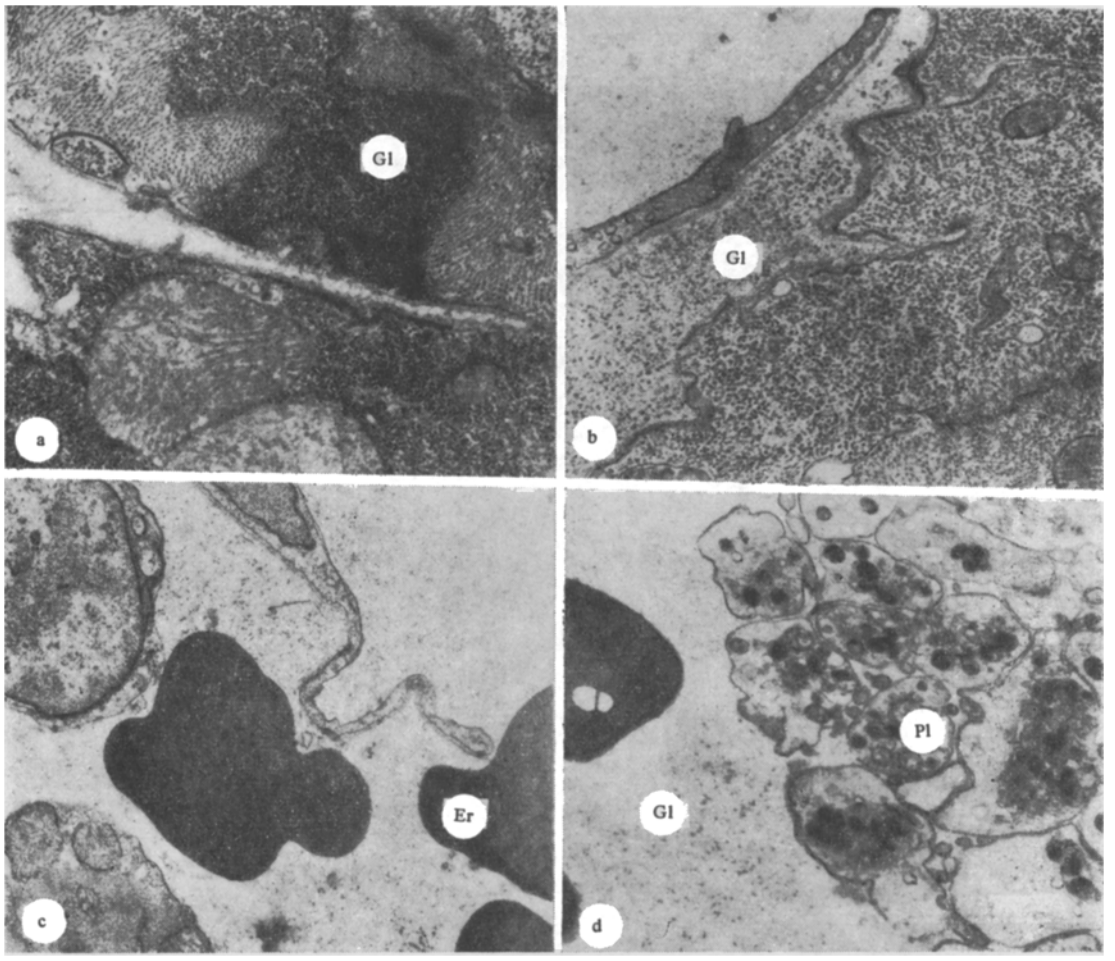

Fig. 1. Changes in myocardial ultrastructure in emotional rats: a) accumulation of glycogen granules (Gl) in cytoplasm of cardiomyocyte, 42,000×; b) glycogen granules in intercellular space, 42,000×; c) migration of erythrocytes (Er) from capillary lumen into intercellular space, 15,000×; d) aggregated platelets (Pl) and glycogen granules in capillary lumen, 20,000×.

counted in the cardiomyocytes by morphometry. Besides the ultrastructural investigation a microscopic study of the glycogen content in the myocardium also was undertaken by the PAS reaction.

EXPERIMENTAL RESULTS

Depending on changes in the animals' behavioral state after 7 days of stress two groups of rats were distinguished: "emotional" and "unemotional"; the animals also differed in the intensity of their somatic disturbances.

Emotional rats exhibited greater reactivity to stress. In the animals of this group, many erosions were observed in the glandular part of the stomach after stress, accompanied by severe edema and hyperemia of the mucosa. In the aglandular part of the stomach from two to four clearly distinguishable defects were found, namely ulcers 1-2.5 mm^2 in area. The weight of the adrenals of these rats was doubled (increase 100%), whereas the weight of the thymus decreased by 70%. In rats of the unemotional group, the physical disturbances were less marked. They had no visible ulcers of the stomach or edema and hyperemia of its glandular part. The weight of the thymus was reduced by only 39%, whereas the weight of the adrenals was increased by 21%. Considerable accumulation of glycogen granules was observed in the muscle cells in the myocardium of the emotional rats. The number of these granules reached 17.4 ± 3.0 (per conventional unit of area of myocyte), compared with only 1.22 ± 0.2 in the intact animals. Glycogen granules formed continuous zones beneath the sarcolemma (Fig. 1a) or accumulations between mitochondria and myofibrils, often forming "glycogenosomes" 6-8 μ in diameter. The increase in the quantity of glycogen in the

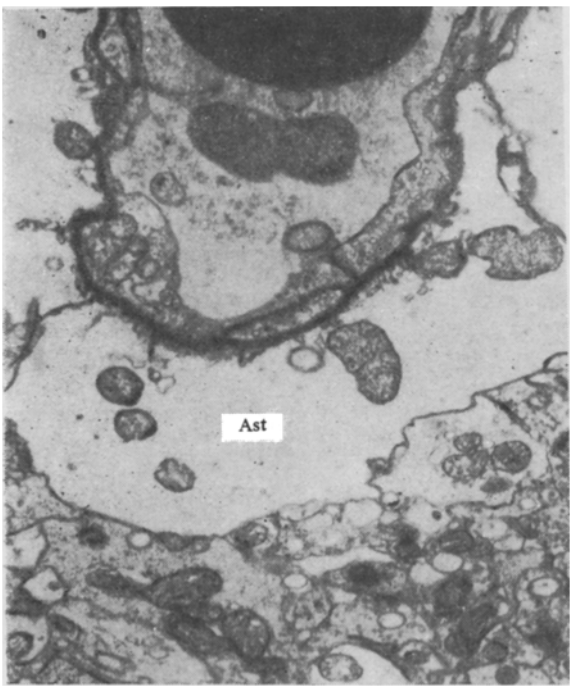

Fig. 2. Swelling pedicles of astrocycles (Ast) in supraoptic lobe of hypothalamus of emotional rat, 20,000×.

myocardium also was confirmed histochemically. Marked changes also were observed in the sarcolemma. The cell membrane became highly tortuous, forming numerous invaginations and evaginations. Sometimes the sarcolemma was ruptured, with release of mitochondria and glycogen granules into the intercellular space. Mitochondria in the myocytes were indistinguishable in structure from those in intact animals, but mitochondria outside the muscle cells underwent partial destruction. Myofibrils as a rule had their usual appearance although in some myocytes some segments of them were overcontracted. Often both separate collagen fibers and coarse bundles of such fibers were found in the intercellular space, together with numerous glycogen granules (Fig. 1b).

Marked changes were observed in the microcirculation of the emotional rats also. The cytoplasm of the endothelial cells of the myocardial capillaries contained many pinocytotic vesicles and became foamy in appearance. Sometimes the interendothelial junctions were separated and erythrocytes escaped into the intercellular space (Fig. 1c). The appearance of glycogen granules and of numerous conglomerates of aggregated platelets in the lumen of the vessels also was noted (Fig. 1d).

Marked swelling of the pedicles of the astrocytes adjacent to the capillaries (Fig. 2) and of the cytoplasm of the glial cells was observed in the hypothalamus of this group of animals. Just as in the myocardium, escape of erythrocytes from the capillary lumen also was observed in the hypothalamus of the emotional rats.

Electron-microscopic investigation of the cardiomyocytes of the unemotional rats revealed disappearance of glycogen granules by contrast with the intact and emotional rats. The results of the histochemical study confirmed this observation. A characteristic feature of the myocardium of these animals was the presence of segments of overcontraction of myofibrils and an increase in the number of lipid droplets in the muscle cells. Myofibrils often formed segments of contracture, in which it was virtually impossible to distinguish the structure of the sarcomeres (Fig. 3). The cisterns of the sarcoplasmic reticulum were greatly dilated. The structure of the mitochondria was normal. No changes could be found in the structure of the capillaries. The ultrastructure of the hypothalamus was virtually indistinguishable from that in the intact rats.

The most characteristic feature of the myocardium of the emotional rats was thus an increase in the number of glycogen granules in the myocytes, the intercellular space, and the capillary lumen and the presence of glycogenosomes and bands of overcontraction of the

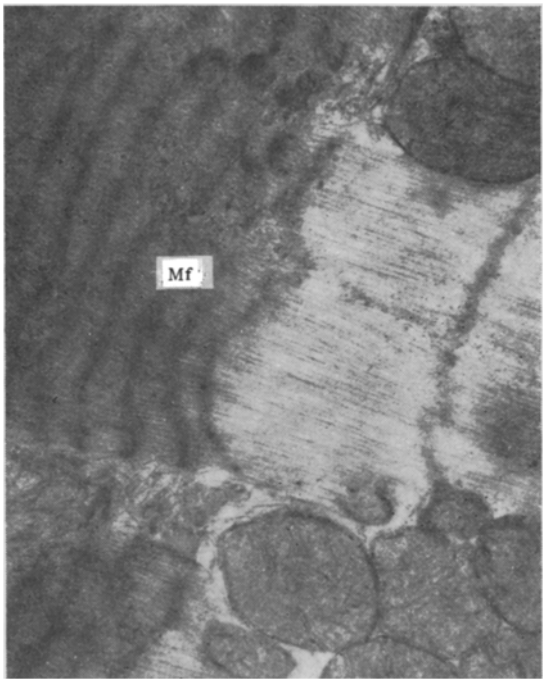

Fig. 3. Areas of overcontraction of myofibrils (Mf) in cardiomyocytes of an unemotional rat, 30,000×.

myofibrils. Similar changes also have been observed by other workers [11, 12, 14] in the myocardium after injection of exogenous noradrenalin into animals. It may be that the ultrastructural changes found in the present investigation in the myocardium are also due to an increase in the noradrenalin concentration in the circulating blood and, in particular, in the heart. Increased release of catecholamines into the blood during stress has often been reported [10]. The more marked activation of the adrenergic system in emotional than unemotional animals was observed by Bondarenko et al. [1]. Phasic changes in the glycogen content in the myocardium in stress have been described by Savina et al. [8]. Changes in the capillaries of the myocardium and hypothalamus of emotional animals are evidence of appreciable hemodynamic disorders. Psychoemotional stress is known to create the conditions for an increase in the thrombogenic properties of the blood [9] and also to increase the aggregating activity of the platelets [5]. The results of the present investigation confirm these conclusions. Escape of erythrocytes from the lumen of blood vessels may be the result of the hypertensive syndrome often observed during stress [6]. Swelling of the pedicles of the astrocytes and of the cytoplasm of the glial cells in the hypothalamus of emotional rats reflects their active participation in metabolism on the CNS and may be observed when the permeability of the blood–brain barrier is increased. This correlates with data obtained previously showing an increase in tyrosine hydroxylase activity in the brain of these animals [1]. High functional activity of the perivascular macroglia in immobilization stress has been described by Zurnadzhi et al. [3].

Ultrastructural changes in the myocardium of rats of the unemotional group were less marked (the presence of overcontraction bands in the myofibrils, enlargement of the lipid drops, dilatation of cisternae of the sarcoplasmic reticulum). Similar changes have been observed by many workers under the influence of exogenous adrenalin [11, 12, 15].

It can be concluded from a comparison of the results of the ultrastructural study of the myocardium and hypothalamus of emotional and unemotional animals in stress, and also differences in the severity of their physical disturbances, that the changes found depend on many causes, one of which is undoubtedly connected with a change in catecholamine metabolism. Increased reactivity of the emotional rats may perhaps be linked with a phase of long-term activation of the sympathicoadrenal system [1], i.e., the second phase of the change in catecholamine metabolism during stress [4], when marked release of noradrenalin from depots in nerve endings is observed. Changes in the ultrastructure of the myocardium and hypothalamus of the unemotional rats probably correspond to the first stage of the change in

catecholamine metabolism during stress [4], characterized by a rapid rise in the blood level of adrenalin and its more intensive supply to the heart.

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