Ultrastructural Changes of the Microcirculatory Bed and Nerve Cells of the Reticular Formation of the Midbrain in Rats with a Hypertensive Response to Immobilization-Induced Emotional Stress

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Extensive protrusions of the nuclei of endothelial cells into the lumen of the vessel, separation of the plasma membranes of endotheliocytes between the neighboring gap junctions, and proliferation of the granular endoplasmic reticulum of endothelial cells of the microvessels are discovered in Wistar rats responding to immobilization stress by a rise of the arterial pressure. Signs of chromatolysis are frequently encountered in the nerve cells.

Key Words: *emotional stress; arterial hypertension; reticular formation of midbrain; microcirculatory bed; nerve cells*

The reticular formation of the brainstem plays a crucial role in the development of the stress responses caused by an emotional factor [1,2].

It has been previously demonstrated, by injecting Trypan Blue in the circulatory bed and subsequently preparing the brain after Falk-Hillarp, that the permeability of the blood-brain barrier markedly increases in emotional stress [3]. In this case the most pronounced changes have been noted in the reticular formation of the midbrain.

In the present study the state of the wall of the circulatory bed and of nerve cells of the midbrain reticular formation was investigated by electron microscopy in rats with a hypertensive response to emotional stress.

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MATERIALS AND METHODS

The experiments were carried out on 8 Wistar rats weighing 180-200 g. One day prior to the experiment a catheter was implanted in the caudal artery under sodium pentobarbital (50 mg/kg) anesthesia. On the day of the experiment the arterial pressure (AP) was measured in unrestrained animals. The rats were then immobilized by firmly fixing the head and the paws. AP was continuously recorded throughout the experiment. The experiment lasted 6.5 h, whereupon the animals, in which AP during the experiment was 40-50 mm Hg higher than the initial level (80+8 mm Hg), were perfused via the left ventricle with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 10-15 min under pentobarbital anesthesia. Tissue pieces were fixed in 1% osmic acid and then dehydrated and embedded in Epon-812. Ultrathin sections prepared on an LKB III ultratome (Swe-

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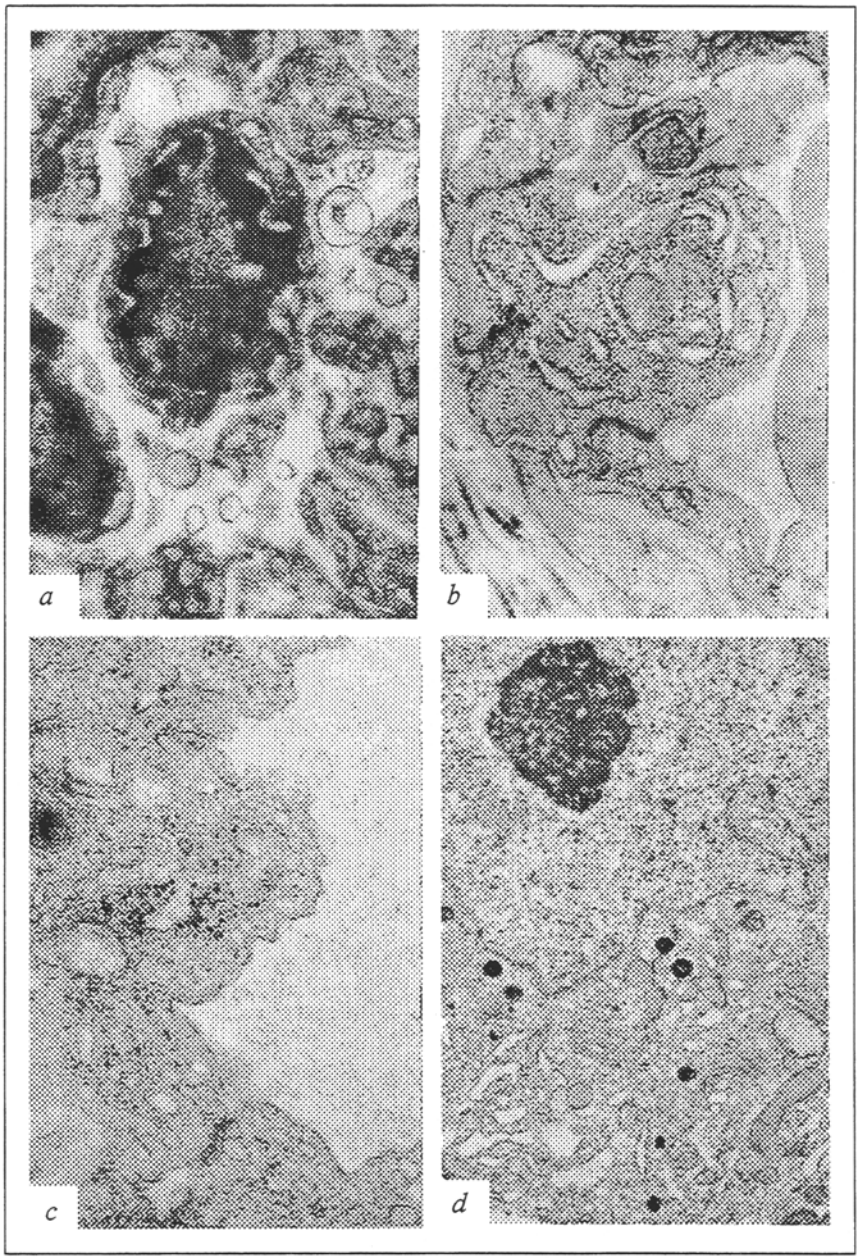

Fig. 1. Microcirculatory bed and neurons of midbrain reticular formation in rats after a 6.5–h immobilization. *a*) nuclei of endothelial cells surrounded by an area of cytoplasm in vessel lumen; ×8,000. *b*) enlarged cisternae of granular endoplasmic reticulum in an endotheliocyte; ×11,000. *c*) abundant pinosomes in endotheliocytes; ×11,000. d) nerve cell: displacement of nucleus to periphery of perikaryon, vacuolization of cisternae of granular endoplasmic reticulum, emergence of abundant polysomes; ×5000.

den) were contrasted in uranyl acetate and lead citrate. Electronograms were obtained on a Philips electron microscope (Holland).

RESULTS

As a rule, in the control animals the nuclei of endothelial cells of the capillaries, arterioles, and venules are oblong or oval. In the karyoplasm, granular bodies are evenly distributed or form clusters, in most cases adjacent to the nuclear membrane. Mitochondria, abundant ribosomes, elements of the rough and smooth endoplasmic reticulum, the Golgi apparatus, and solitary lysosomes and multivesicular bodies are observed in the cytoplasm of endothelial cells. Pinosomes and vacuoles, which are most often bound to the apical plasma membrane and more rarely to the basal membrane, are components characteristic of endotheliocytes. Pinocytosis is more pronounced in the arterioles and capillaries than in the venules; its magnitude also depends on the diameter of the vessel. In the majority of cases the gap junctions between endothelial cells are intact.

In rats exhibiting the hypertensive response to the stress load, the nuclei of endotheliocytes frequently protrude into the lumen of the vessel. In some sections such nuclei, surrounded by an area of cytoplasm, largely obscure the lumen of the vessel; notably, this is characteristic of arterioles (Fig. 1, *a*). It is worthy of note that endothelial cells show signs of markedly enhanced activity of the protein-synthesizing system: a sinuous shape of the nuclear membrane and enlarged cisternae of the smooth endoplasmic reticulum (Fig. 1, *b*) and of the Golgi apparatus. The number and size of mitochondria increase, especially near the gap junction, the mitochondrial matrix often being vacuolized. The plasma membranes of adjacent endotheliocytes are separated in the zone between neighboring gap junctions. The number and size of pinosomes increase, notably in arterioles (Fig. 1, *c*). Ruptures are encountered in the wall of the microvessel, and organelles (more often, swollen mitochondria) enter the vessel lumen. Sometimes clusters of collagen fibers can be observed in the zone of edema.

Intensified pinocytosis and separation of endothelial cells are known to be indicative of an increased permeability of the wall of the circulatory bed. The changes of the ultrastructure of the

protein-synthesizing apparatus in endothelial cells which were discovered in our experiments attest to a marked emotional stress-induced metabolic upheaval. In an analysis of these findings it must be taken into account that histochemical methods also testify to a change of metabolism in the vascular wall of the microcirculatory bed of animals with experimental hypertension [4]. In the opinion of Djurcic et al. [4], under these conditions reorganization of the metabolism also plays an important role in changing the permeability of the blood-brain barrier.

In our experiments many neurons of the reticular formation of the midbrain exhibited signs of partial or, sometimes, total chromatolysis, which manifested itself in an enlargement and vacuolization of the cisternae of the granular endoplasmic reticulum and emergence of free polysomes in great numbers. The nuclei of such neurons are frequently displaced to the periphery, and their membrane has a sinuous shape, sometimes with extended protrusions of the cytoplasm (Fig. 1, *c*). Such ultrastructural changes have been observed during long-term hyperfunction or damage to the nerve cell [6] and attest to the enhanced protein synthesis required in repair processes [5]. The presence of a large body of such neurons may testify to the disruption of a specific function which is performed by the neuronal network composed by these neurons.

Presynaptic terminals, packed with vesicles of diverse size and density, are rather frequently encountered near the vessels in the animals examined.

Analysis of our findings suggests that during emotional stress-induced arterial hypertension, the permeability of the microcirculatory bed is markedly changed, which results from both separation of endothelial cells and enhancement of pinocytosis in them, as well as from marked metabolic rearrangements in these cells. In addition, damage to some of the vessels may indicate to appreciable impairments of the blood-brain barrier. Many of the nerve cells are functionally overloaded and this, along with the damage to the blood-brain barrier, may play an important role in the development of cardiovascular disease.

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