STRUCTURAL DAMAGE TO THE MESENCEPHALIC RETICULAR FORMATION INDUCED BY IMMOBILIZATION STRESS

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It was shown previously that prolonged emotiogenic immobilization stress leads to increased permeability of the blood–brain barrier (BBB) and, in some cases, to injury to the vessel walls in the brain parenchyma, rupture of which is accompanied by the outflow of plasma and blood cells into the surrounding nerve tissue, and to the release of damaged brain structures and endothelial cells into the general circulation. Definite selectivity of damage to brain structures during emotional stress has been discovered. One region in which the earliest and most marked destructive changes in the intracerebral vessels were found is the mesencephalic reticular formation (MRF) [1, 5].

It was decided to investigate the duration of damage to the intracerebral vessels and nerve and glial cells connected with them, and induced by emotional stress. Accordingly, this paper gives data on the dynamics of changes in structures of MRF at different stages after stress.

EXPERIMENTAL METHOD

Experiments were carried out on 54 male Wistar rats weighing 250-300 g. The rats were kept under standard animal house conditions with free access to food and water. Stress was induced by immobilization on a wooden board [8] for 1.5 days, with interruptions. The total duration of immobilization was 13 h in a 37-h experiment. The state of the animals during and after immobilization was assessed by determining changes in rectal temperature, the picture of the gastric mucosa, and the presence or absence of blood-stained exudates from the nose and eyes. Immediately and 1, 2, 4, and 6 weeks after the end of immobilization the animals were decapitated, the brain was fixed in cold neutral formalin solution or in cold Carnoy's fluid, and serial paraffin and frozen sections were cut and stained by the methods of Klüver and Barrera, Einarson, Bielschowsky, and Nissl. Survey sections also were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Changes in the rectal temperature during the experiment were not significant: at the end of the experimental deviations from the initial level did not exceed 0.8°C. In 20% of animals, after stage I of immobilization, the rectal temperature fell by 1.5-2°C. A blood-stained exudate from the nose or eyes was observed in 60% of cases. The gastric mucosa of most animals was undamaged and two pinpoint ulcers were observed in the mucosa of only one rat and an erosion in that of another rat.

Immediately after immobilization, ruptured vessels were found in MRF of 75% of rats with blood cells excaping into the brain parenchyma (Fig. 1). No precise correlation was found between the presence and severity of the vascular lesions in the brain and peripheral organs (mucous membranes of the stomach, nose, and eyes) or with the time course of the rectal temperature during immobilization.

The number of damaged vessels was small and varied from 1-2 to 5-7 in each animal. Most frequently the damaged vessels were venules (50-60% of cases) or arteriovenular anastomoses

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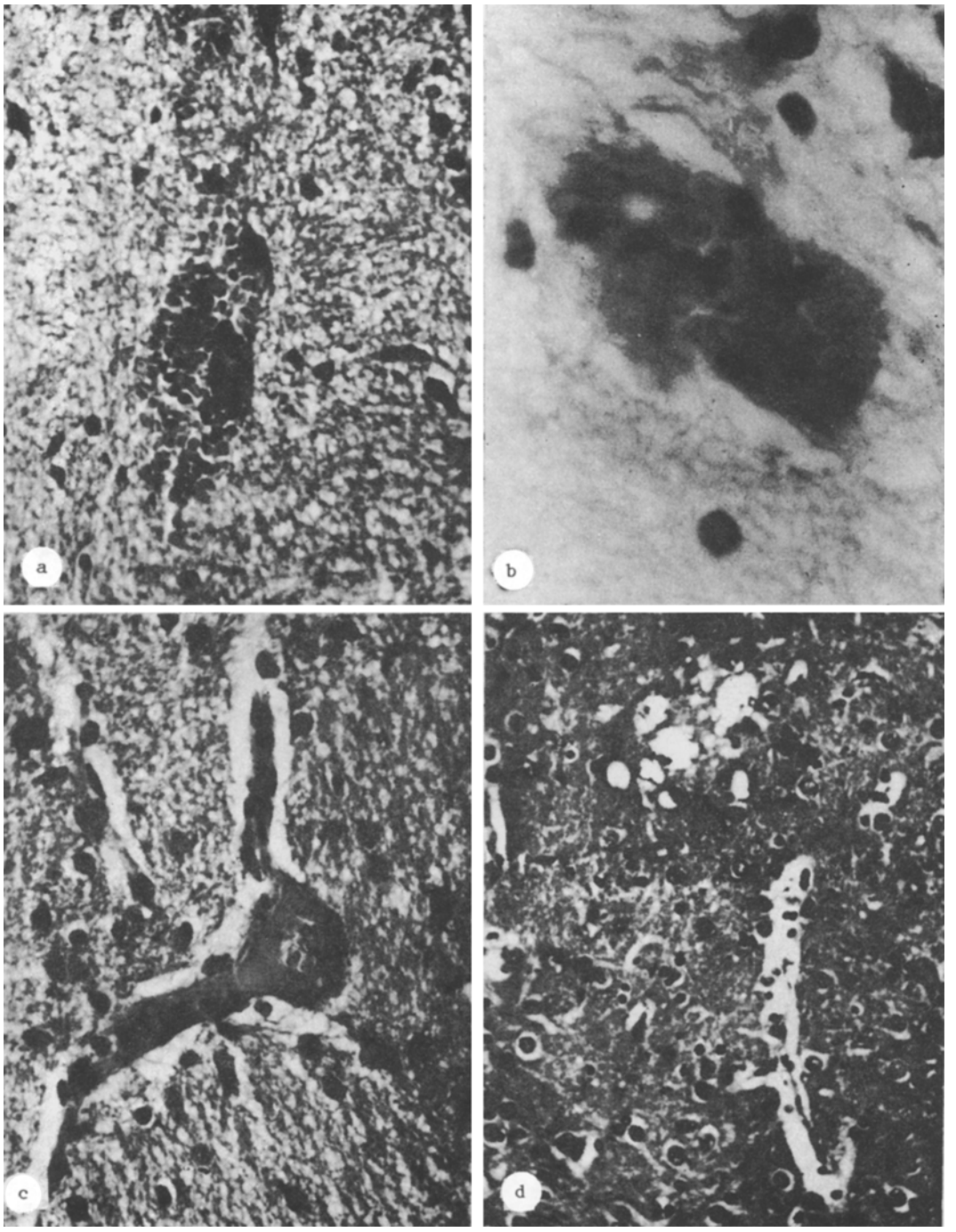

Fig. 1. Changes in intracerebral vessels in MRF after immobilization stress. a) Microhemorrhage; b) diapedesis of erythrocytes and lymphocytes (arrows) from venule, edema; c) edema around arteriolovenular anastomosis; thickening of blood plasma; aggregated erythrocytes in lumen of vessel; d) destruction of capillary (one arrow); softening of nerve tissue (two arrows). a, b, c) Immediately after immobilization, d) 4 weeks after immobilization. Magnification: a, c) 400; b) 1000; d) 200×.

and postcapillary venules (30-40%), less frequently arterioles and capillaries (10-20%). Most of the vessels in MRF were in a state of edema. Edema and porosity of the walls again were more marked in venules and less marked in arterioles.

Immediately after immobilization destructive changes were observed in nerve and glial cells, and these changes were even more marked 1 week after stress (Fig. 2). The severest changes in the neurons were noted in the oral part of MRF, at the level of the posterior

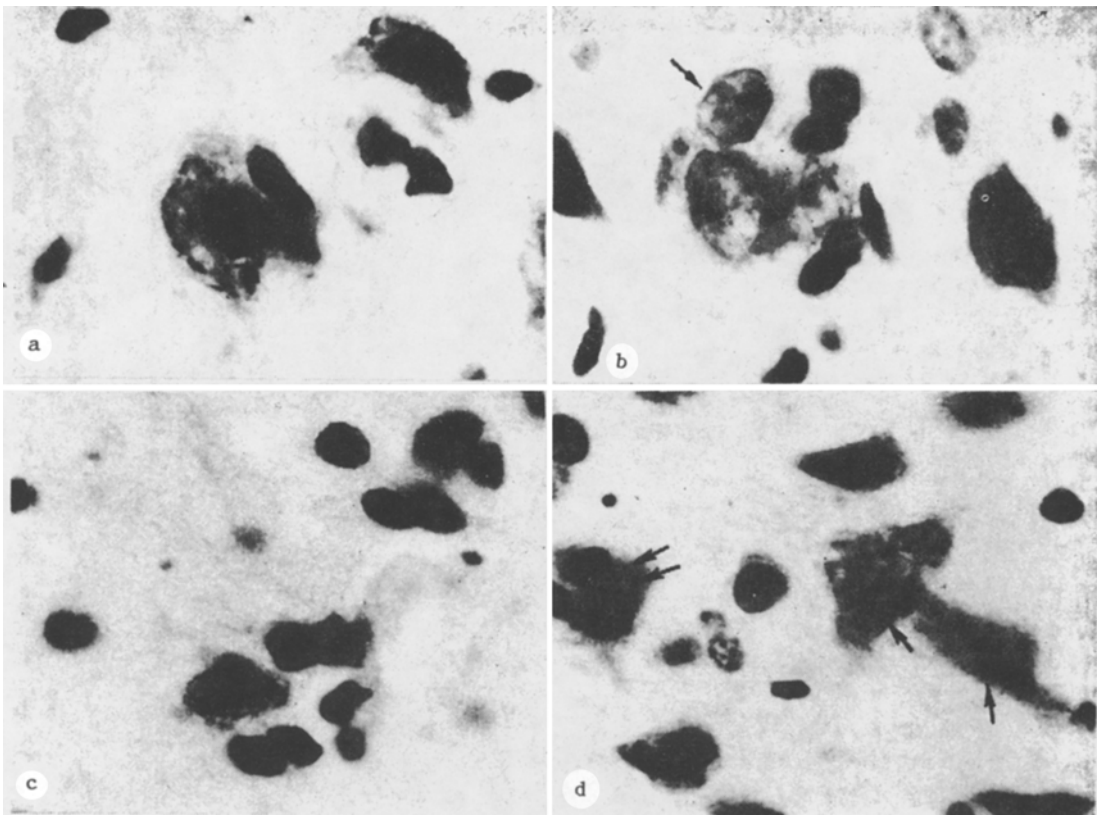

Fig. 2. Morphological changes in neurons after immobilization. a) Central chromatolysis, formation of large clumps of Nissl's substance along periphery of neuron, hyperchromic angular nucleus without any clearly defined nucleolus; b) hypochromic neurons surrounded by edematous macroglial cells; vacuolation of cytoplasm, liquifaction of Nissl's substance; hyperchromic angular nucleus (arrow); c) gliosis near hypochromic vacuolated neuron; liquefaction of Nissl's substance; dissected outline of nucleus; uneven staining of nuclear membrane; marked power of nucleolus; activation and edema of macroglial cells; d) ghost cells (one arrow), neuronophagy (two arrows), activation and edema of glia. a) 2 weeks after immobilization, b, c, d) 1 week. Einarson's stain. 1000×.

commissure — in the oral part of the deep mesencephalic nucleus and the magnocellular nucleus of the posterior commissure.

Many of the altered MRF neurons showed marked hypochromia (1 week after immobilization), liquefaction and partial disappearance of the Nissl's substance, and vacuolation, often affecting the greater part of the cytoplasm. The nucleus was irregular and angular in shape and characterized by intense staining or, conversely, by an indistinct nuclear membrane, often folded. The nucleolus in many cells had a complex mulberry-like shape and was displaced toward the periphery of the nucleus. A minority of neurons was characterized by hyperchromia, and the boundaries of the nucleus and cytoplasm were indistinct. Pictures of gliosis, neuronophagy and, sometimes, lysis of neurons with the formation of cell ghosts also were observed. Meanwhile some neurons of MRF were indistinguishable from the control.

Thus immediately after immobilization and 1 week later, neurons in MRF showed destructive changes which varied in severity. The reaction of the nucleolus, and also the presence of chromophilic cells suggest the possibility of a compensatory enhancement of functional activity and of repair processes even in this early stage.

Macroglial (astrocytes) and oligodendroglial cells were strongly activated immediately and 1 week after immobilization. Chromatin granules were large, numerous, and brightly stained. Proliferation of the oligodendroglia was observed. The clearly distinguishable pale rim around the nuclei of the glial cells was evidence of edema. Thus in the glia also, both regressive (edema) and progressive changes (proliferation, activation of nuclear chromatin) were found.

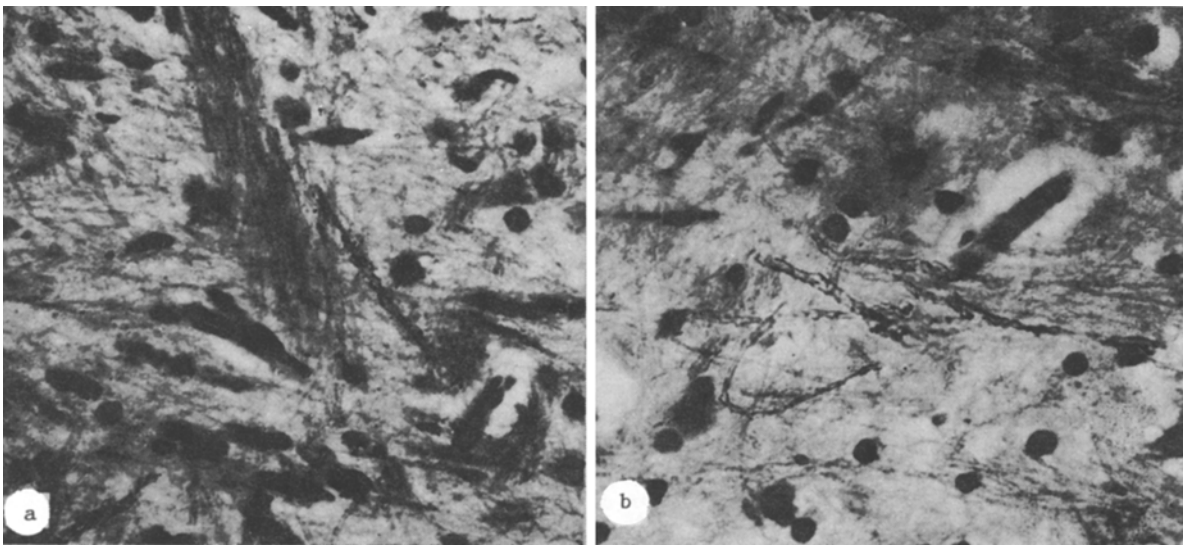

Fig. 3. Varicose swelling and fragmentation of myelin fibers in MRF (arrows). Method of Klüver and Barrera, counterstained with cresyl violet. 400×. a) Immediately after immobilization, b) 2 weeks after immobilization.

The changes described above in the glial cells 2 weeks after immobilization remained at the same level on the whole as 1 week after stress. However, after 2 weeks the degree of damage to the nerve cells of MRF was increased: their vacuolation was more severe, in some cases pycnosis of the nuclei and perinuclear edema were observed, and in many cases pericellular edema also. After 1 and, in particular, 1.5 months, even though tissue edema still persisted, the neurons as a whole differed only a little from the controls, although at this stage also edematous cells and pictures of gliosis and neuronophagy were still seen.

Deformed myelin fibers (swollen, vacuolated, fragmented) were found immediately after immobilization and at all times later in the investigation (Fig. 3). They were distributed in MRF and also in tracts connecting the brain stem with the diencephalon — the posterior commissure, posterior longitudinal bundle, medial lemniscus, tectospinal tract, and dorsal and ventral tegmental decussations. Both thin and thick myelinated fibers showed signs of destruction. It must be pointed out, however, that small numbers of deformed fibers could be seen in the fiber tracts mentioned above and in MRF of the control animals also, evidently due to the specific nature of functioning of MRF and of the fiber systems connected with it.

Substantial individual differences were found in the response of structures of MRF at all stages of the poststress period studied, probably largely depending on whether the particular animal developed hemorrhage or not during immobilization.

It can thus be concluded from the results described above that lesions of blood vessels of the brain parenchyma caused by severe emotional stress lead to long-term structural disturbances. All components of nerve tissue, whether neurons, glial cells, blood vessels, or fibers, show substantial pathological changes long after exposure to severe stress. Meanwhile signs of recovery also were observed. Consequently, the phase of repair of the structures of nerve tissue developed virtually simultaneously with the damage, as was observed previously [4].

As the results show, disturbed functions of the intracerebral vessels under conditions of stress due to immobilization is a long-acting initiating factor, which limits restoration of nerve and glial cells. If the possible causes of this phenomenon are examined, it can evidently be concluded that the main cause of damage to the brain vessels in emotional stress is stress-induced dysfunction of the noradrenergic system of the brain. Besides data in the literature on the role of the locus coeruleus in the regulation of BBB function [6, 7], the situation described above was confirmed by the present experiments. Pharmacologic blockade to the noradrenergic structures of the brain by the substance DSP-4 caused damage to intracerebral vessels in virtually all parts of the brain. Immobilization stress against the background of blockade of the locus coeruleus led to even more marked injuries to the vessels of the brain parenchyma [2].

The particular vulnerability of MRF, where brain vessels are damaged first and most severely in emotional stress, may be due to a certain specific feature of the energy metabolism of this part of the brain — relatively high activity of anaerobic and relatively low activity of aerobic energy metabolism [3].

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RESPONSE OF SINGLE CELL POPULATIONS OF THE PARAVENTRICULAR HYPOTHALAMIC NUCLEI TO CARBOHYDRATE LOADING AND STARVATION IN RATS

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With the discovery of direct nervous connections between the paraventricular nuclei (PVN) of the hypothalamus and the dorsal nuclei of the vagus (DNV) in the medulla [4, 8] a possible role for PVN in the regulation of the hormonal basis of carbohydrate homeostasis via the paraventriculovagal nerve pathway began to be postulated [1, 3]. Experimental confirmation of such a possibility could be given by observations on insulin-dependent food responses, which have been studied in animals with different procedures aimed at the PVN region [5, 6]. With an increase in interest in the study of the role of PVN in the regulation of homeostatis functions, it has become increasingly evident that in modern research changed ideas on the structure of these nuclei must be taken into account. At the present time about 10 cell populations are distinguished in them, differing in the character of their nervous connections and the composition of their neuropeptides and neurotransmitters [2, 7]. Hence it is clear that any attempt to study the role of PVN in the regulation of carbohydrate homeostasis requires clarification of the individual contribution of each separate subnucleus in the regulation of this homeostatic function.

In the investigation described below correlation was studied between structural changes in the cells in each subnucleus of PVN and experimental changes in the parameters of the blood sugar: lowering of their values by starvation and their elevation as a result of drinking 12% glucose solution instead of water.

EXPERIMENTAL METHOD

The test object consisted of 30 mature male Wistar albino rats weighing 180-200 g, of which 10 were deprived of food for 6 days but allowed free access to drinking water; 10 animals were kept on a standard diet but, instead of drinking water, they were given a 12% solution of glucose for 20 days; 10 animals served as the control and received a pellet diet and

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