INJURY TO THE BLOOD-BRAIN BARRIER FOLLOWING A SINGLE EXPOSURE TO IMMOBILIZATION STRESS

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UDC 612.824:616.45-001.1/.3

KEY WORDS: blood-brain barrier; emotional stress; arterial blood pressure; brain-stem reticular formation.

In recent years many investigations showing that the blood-brain barrier (BBB) in animals is permeable to some degree for macromolecules have been published: On injection of vital dyes into the blood stream they can be found in the parenchyma of the brain not only in zones known to be "outside the barrier" [3-5, 7], but also in other brain regions. It has been shown that BBB permeability depends on the functional state of the organism. Under pathological conditions, and also in experimental ischemia, hypercapnia, hypertension, and under the influence of ionizing radiation, burns, cerebral edema, and so on [6, 9, 14], permeability of the BBB is increased. The study of the problem whether permeability of the BBB varies during emotional stress is of considerable interest, including for medical practice.

The object of this investigation was a morphological study of BBB permeability (excluding zones known to be "outside the barrier") in control rats and after experimental emotional stress induced by immobilization.

EXPERIMENTAL METHOD

Male Sprague-Dawley rats weighing 150-250 g were immobilized by the method described previously [15]. During immobilization the blood pressure (BP) was recorded through a catheter inserted into the abdominal aorta 2 days before the experiment. After immobilization of the rats for 6.5 h, 1 cm^3 of a 2% solution of trypan blue in physiological saline was injected intraveneously. The rats were decapitated from 15 sec to 30 min after injection of the dye. The brain was quickly removed and treated by the Falk–Hillarp method [11, 13]. Intact male rats served as the control.

EXPERIMENTAL RESULTS

Vital dyes in the blood stream, conjugated with plasma proteins, form a complex which, after treatment of the brain by the Falk–Hillarp method, are revealed by their acid fluorescence. In control experiments red fluorescence was usually limited to blood vessel walls. In some cases foci of extravasation and penetration of the dye into the brain parenchyma are found. The intensity of extravasation was found to depend largely on the duration of the animals' survival after injection of trypan blue. Under the present experimental conditions the optimal time was 1 min. Permeability of BBB in control rats killed 1 min after injection of trypan blue was found to be present in the preoptic region, in the hypothalamus – in the arcuate, periventricular and, to a lesser degree, paraventricular nuclei, in the midbrain – in the ventral region of the tegmentum, in the medulla – in the reticular formation, chiefly in its ventral path, in the region of distribution of catecholamine-synthesizing groups A_1 and A_5 and between them, and in region A_2 and the nucleus of the tractus solitarius (nomenclature according to [12]).

In the case of a longer period of survival after injection of trypan blue, enlarged perivascular spaces could be seen, but they no longer contained any traces of the fluorescent dye.

Territorial variability of the density of the BBB was thus found in the control animals: Increased permeability of BBB was observed most often in structures located near the basal surface of the brain. The considerable individual variability of the density of BBB in the control must be noted.

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0007-4888/83/9607-0881$07.50

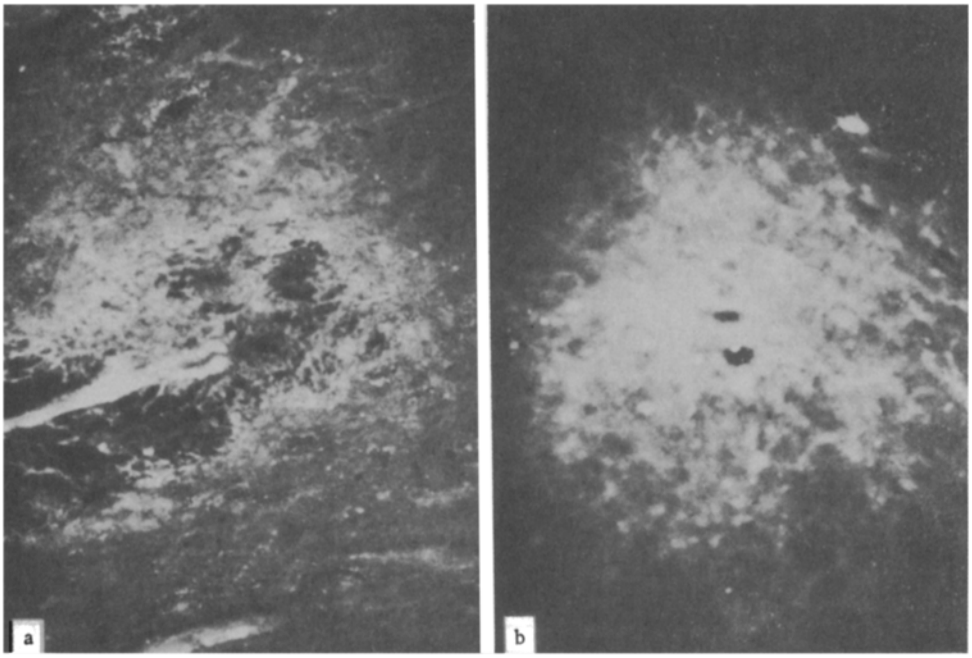

Fig. 1. Destruction of BBB in mesencephalic (a) and medullary (b) reticular formation after immobilization for 6.5 h. Injection of trypan blue, subsequent treatment by Falk–Hillarp method. Magnification 370.

After 6.5 h of immobilization the greatest changes in BBB were found in two regions of the brain-stem reticular formation – in the midbrain and medulla (Fig. 1). In the mesencephalic reticular formation rupture of blood vessels and an extensive zone of red fluorescence around them could be observed. In the medullary reticular formation the pattern of BBB permeability were different: The dye was distributed around the vessels in a circumscribed zone with more or less regular outlines.

Besides the two zones of the brain-stem reticular formation increased permeability after immobilization was a feature of the same BBB structures as in the control animals, although the increase was more clearly marked.

Strict dependence of the degree of disturbance of the BBB on the time course of BP during immobilization stress could not be detected although on the whole such a trend could be noted: The greatest disturbances of BBB were found in rats distinguished by a sharp rise (by 20-25 mm Hg) in BP during immobilization.

The results thus indicate significant injuries to BBB in two zones of the brain-stem reticular formation under conditions of a single long-term exposure to immobilization stress.

The decisive role of the medullary reticular formation in the mechanism of vitally important autonomic functions is well known. Investigations have demonstrated the leading role of the mesencephalic reticular formation in the mechanism of the various defensive responses, including immune, maintaining homeostasis [1, 2, 8, 10]. Destructive changes in neurons of the reticular formation in the oral and caudal zones of the brain stem, an inevitable consequence of damage to BBB, thus undoubtedly have a significant effect on the state of the physiological functions of the body and, consequently, they largely determine the severity of the consequences of emotional stress.

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ELECTROPHYSIOLOGICAL INVESTIGATION OF CONNECTIONS OF NUCLEI OF THE MEDIAL AND LATERAL ZONES OF THE RESPIRATORY CENTER

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UDC 612.282.014.421

KEY WORDS: respiratory neurons; connections of neurons; respiratory nuclei.

The medial and lateral zones of the respiratory center differ in their neuronal composition and functional role [1, 2, 4-9]. High-frequency stimulation of inspiratory and expiratory sites of the gigantocellular nucleus [5-7] changes the state of the overwhelming majority of inspiratory and expiratory neurons of the nucleus ambiguus and nucleus retroambiguus (ventral respiratory nucleus) and nucleus of the tractus solitarius (dorsal respiratory nucleus) and evokes spike activity in them [6-8]. The presence of various mutual functional connections has been established between neurons of the inspiratory and expiratory sites of the gigantocellular nucleus and the ventral and dorsal respiratory nuclei [5-8]. No information is available on direct connections between neurons of medial and lateral zones of the respiratory center, although there are indirect data suggesting their existence. One piece of such indirect evidence is the recovery of spike activity of respiratory neurons when it has ceased during stimulation of inspiratory sites [7, 8].

The object of this investigation was to study connections of neurons of inspiratory and expiratory sites of the gigantocellular nucleus with respiratory and reticular neurons of the ventral and dorsal respiratory nuclei by analysis of latent periods of electrical responses of the neurons to equivalent stimulation of these sites.

EXPERIMENTAL METHOD

Experiments were carried out on 12 spontaneously breathing cats anesthetized with pentobarbital (40 mg/kg, intraperitoneally). The preparatory operations and the technique of conducting the observations were described in [7, 8]. Bipolar (interelectrode distance 100 μ) or glass electrodes, filled with Wood's alloy, were used for stimulation. The tips of the glass electrodes were coated electrolytically with platinum. Electrical activity was recorded on the side of stimulation from the phrenic nerve at levels C4-C5. To record integrated activity of

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