Accumulation of Exogenous Histone in Rat Brain Parenchyma

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In vivo histoautoradiographic study of cerebral accumulation of exogenous histone after its intracarotid infusion to anesthetized rats with intact liver and animals with experimental cirrhosis showed that histone penetrates brain capillary endotheliocyte membranes and gets into the nervous tissue.

Key Words: *exogenous histone; blood-brain barrier; experimental hepatocirrhosis*

Exogenous histones are known to be antimicrobial and antiviral agents as well as factors improving nonspecific body resistance [3]. The spectrum of their positive properties makes it possible to extend the area of their practical application. Their ability to penetrate cell membranes increasing their permeability for other substances is of special interest. It allows to use histones for translocation of drugs with low ability to penetrate the brain. We found previously that exogenous histones get into the cerebral nervous tissue [1, 2,8] and developed the covalent histone conjugates with some drugs [4]. To clarify the morphological localization of exogenous histones in the cerebral tissue, it seems important to trace their transport through impaired (open) and intact (closed) blood-brain barrier (BBB). It is known that experimental liver cirrhosis is accompanied by structural changes in BBB impairing its function [5]. This makes it possible to monitor *in vivo* passage of infused protein through open vascular barrier.

In this study we investigated the cerebral accumulation and distribution of exogenous histone after its administration into the carotid artery of anesthetized rats with intact liver and rats with experimental liver cirrhosis.

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MATERIALS AND METHODS

Histone (histone sulfate from calf thymus, Reakhim, Biolar) and BSA (Sigma) were iodinated by a standard technique using $Na^{125}I$ and chloramine T [7]. Specific activity of labeled histone and BSA was 60 and 40 μCi/μg; respectively. The experiments were carried out on female albino rats weighing 175-200 g. The preparations (8-12 μg in 1 ml Ringer solution, 0.5-0.7 μCi) were slowly (0.2 ml/min) infused into the right common carotid artery to rats anesthetized with intraperitoneal hexenal (60 mg/kg). After 2-10 min the vascular system was perfused with 100 ml mixture of bovine serum and Ringer solution (2:1) containing 50 units/ml heparin. The mixture was introduced into the ascending branch of the aorta at a rate of 3 ml/min and flowed off through the opened right auricle. After perfusion the brain was removed and fixed in 10% neutral formalin. Samples of the motor cortex, hippocampus, hypothalamus, cerebellum, and olfactory bulbs for histoautoradiographic study were fixed in 10% neutral formalin for 12-24 h, embedded in paraffin and sectioned. The sections (7 μ thick) were covered with an Ilforld-L4 emulsion and exposed for 4 weeks at 4°C. Then they were developed, stained with hematoxylin and eosin, and studied under an Opton microscope to analyze the distribution of radioactive traces.

In vivo monitoring of diffusion movement through BBB allows to separate their real circulation-brain transport from the accompanying deposition (or bind-

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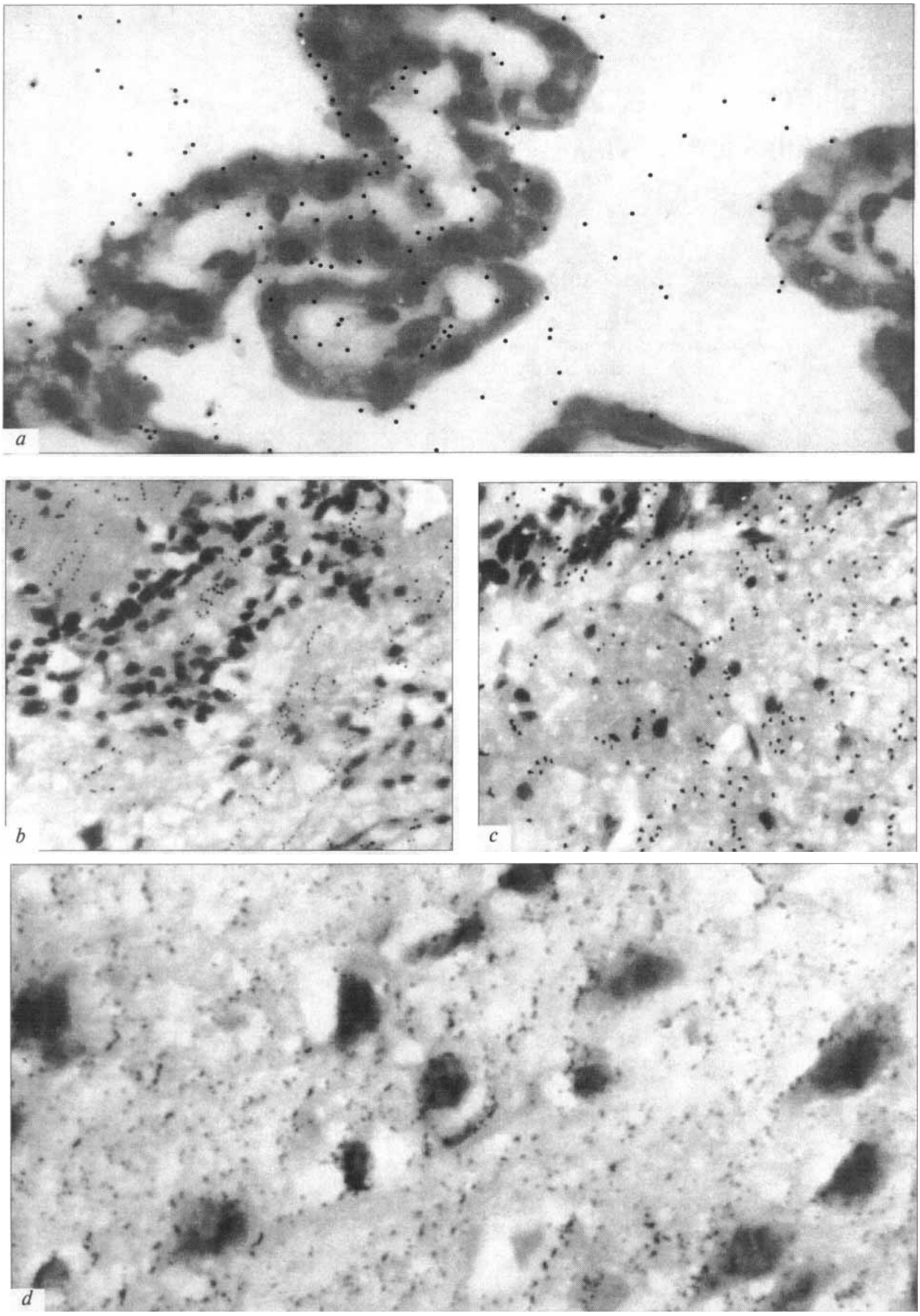
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ing) by cerebral capillaries. For this purpose we studied accumulation of ^{125}I-histone within different cerebral structures using a semiquantitative visual histoautoradiographic technique. ^{125}I-albumin that normally does not penetrate BBB [8] was used as the standard. The labeled protein was administered to control animals and its accumulation in the hippocampus, hypothalamus, sensorimotor cortex, chorioid plexus of the third ventricle, cerebellum, and olfactory bulbs was studied by histoautoradiography.

Dependence of protein transport on the state of vascular structures was analyzed under conditions of experimental impairment of BBB. Experimental liver cirrhosis was induced by intragastric CCl_4 in a single dose of 0.2 ml/100 g every other day for 6 months. As a result, we performed two types of control experiments providing the possibility to monitor the transport of histone through the intact (closed) and impaired (open) BBB.

RESULTS

Histone got into the brain tissue penetrating intact BBB (Fig. 1, *a, b*), but it was more intensely accumulated by brain tissue when BBB was impaired (Fig. 1, *c, d*). Increased BBB permeability in liver cirrhosis was evidenced by the presence of labeled albumin in the hypothalamus. In animals with intact liver, ^{125}I-albumin was not observed in the brain.

The analysis of brain labeling under conditions of intact and impaired BBB revealed intense accumulation of histone by the chorioid plexus of the third ventricle. The label was localized on the endothelial surface with traces in both plasma and basal membranes. This implies that histone could enter the brain tissue from the third ventricle.

Similar considerable labeling with nonhomogenous distribution of histone traces was found in the hippocampus and hypothalamus. In the hypothalamus, the neuropil contained a higher concentration of radioactive material compared to the area of cell bodies. Despite some differences in histone accumulation between the CA1, CA2, CA3, and CA4 areas of the hippocampus, the label was concentrated primarily around cell bodies in all these areas, while neuropil was labeled less intensely. Under conditions of intact BBB the sensorimotor cortex accumulated little, and the olfactory bulbs and cerebellum — only minor amount oflabeled histone. On the contrary, in rats with experimental liver cirrhosis histone was intensely accumulated within the sensorimotor cortex and cerebellum, where the label was distributed all layers — granular, ganglionic, and molecular.

Thus, the present study showed that exogenous histone after intravascular injection not only binds to endothelial membranes, but also penetrates the nervous tissue.

Relatively low intensity of this process under conditions of intact BBB suggests that there are some obstacles hampering the transport of histone though BBB. Some of histone molecules can be absorbed by membranes and bind to a negatively charged mucopolysaccharide layer, glycocalix, immediately adjoining the external membrane surface. For instance, extracellular histones probably originated from the nuclei of pericytes, endotheliocytes, neurons, and glia were found in isolated brain capillaries [9].

Histone transport through BBB seems to be determined by absorption-associated transcytosis. Endotheliocytes of brain capillaries are known to express specific receptors for circulating peptides and plasma proteins inparticular receptors for cationic proteins [11].

There is only indirect data on BBB permeability for lysine-rich histone (the study was carried out on tissue homogenates) [10]. The transport of cationic proteins such as albumin and IgG is mediated by absorptional transcytosis [14], while polycationic polymers (polylysine and protamine sulfate) directly affect biological membranes by changing their structure and thus increasing the permeability of BBB [12,13]. It is likely that histone transport is also provided by impaired of dense contacts between the endothelial cells of cerebral capillaries with the formation of channels between them.

There is an active search for BBB-penetrating protein carriers for drugs [6,11], however only a few transport polymers meet the necessary requirements. Insulin and transferrin as well as cationized albumin and IgG can find only limited use. Polylysine and protamine are characterized by pronounced toxicity. Histones have several advantages over these substances: they possess no specific biological activity, they are physiological compounds with low toxicity and minor antigenic properties, they open BBB for other substances in much lower concentrations. All this distinguishes histones from other substances making them very suitable for the role of potential carriers for haptens which usually do not penetrate BBB and other membrane structures.

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Fig. 1. Histoautoradiographs of the third ventricle chorioid plexus (*a*), the hippocampus (*b*), cerebellum (*c*), and the sensorymotor cortical area (*d*) 10 min after ^{125}I-histone infusion into the carotid artery of anesthetized rats with the intact liver, ×3500. *a*) labeling along the endothelial layer, the labels are seen in both plasmatic and basal membranes; *b*) labels are localized in neuropil; *c*) labels are located in the molecular layer and around Purkinje cells; *d*) intense labeling around neuronal bodies.

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