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Structural-Metabolic Changes in Histaminergic Neurons of the Rat Hypothalamus in Conditions of Loss of Bile

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The aim of the present work was to evaluate structural and metabolic changes in histaminergic neurons in hypothalamic nucleus E2 in rats in conditions of complete external drainage of bile. Studies were performed on male Wistar rats ($n = 45$). Controls consisted of animals subjected to sham surgery with preservation of physiological bile flow throughout the experiment. Quantitative histological and histochemical methods were used. Serial frontal cryostat sections cut from the posterior hypothalamus were used for detection of the activity of the following enzymes: monoamine oxidase B, succinate dehydrogenase, NADH dehydrogenase, NADPH dehydrogenase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and acid phosphatase. Morphological studies of histaminergic neurons were performed on preparations stained with thionine. These studies showed that complete external drainage of bile led to transient size reductions and rounding of cell perikarya. Metabolic changes were seen within a day of bile loss and subsequently progressed. All energy metabolic pathways were suppressed and acid phosphatase activity was increased on day 5.

KEY WORDS: brain, histaminergic neurons, morphometry, histochemistry, bile.

The histaminergic neuron system of the brain is involved in regulating many of the body's functions, systems, and reactions: sleep and waking, movement activity, feeding and drinking behavior, memory and learning, pain and stress, the mechanisms of neurohumoral regulation, and cardiovascular control [12]. The bodies of histaminergic neurons in animals and humans are located exclusively in the posterior hypothalamus, where they form five groupings (nuclei), E1–E5 [11]. Nucleus E2 is the largest and functionally the most active; it contains more than half the histaminergic neurons of the brain [5]. There is as yet insufficient evidence to say that these five groups of neurons perform different functions, so they can be regarded as a unitary functional group [4]. The axons of histaminergic neurons are distributed to all parts of the brain, controlling other neurotransmitter and morphofunctional systems of the CNS. Histamine is synthesized in histaminergic neurons from the amino acid L-histidine, a process involving the enzyme histidine decarboxylase. Specific and effective histamine uptake systems in the CNS are not seen, so after release it is inactivated exclusively by metabolism, which involves the enzymes histamine-N-methyltransferase, monoamine oxidase B (MAO B), and aldehyde dehydrogenase [12].

Impaired enterohepatic circulation of bile in humans and animals, is associated with changes in mental function and behavior, in brain functions, in analytical and synthetic processes, in excitation and inhibition, in electrical spike activity in the cerebral cortex, and in corticovisceral and viscerocortical reflex interactions [7, 10]. However, there are very few histological studies of the brain in conditions of impaired bile outflow, and those which exist address only cholestasis.

Treatment of the world's most prevalent hepatobiliary system pathology – biliary stone disease – in clinical con-

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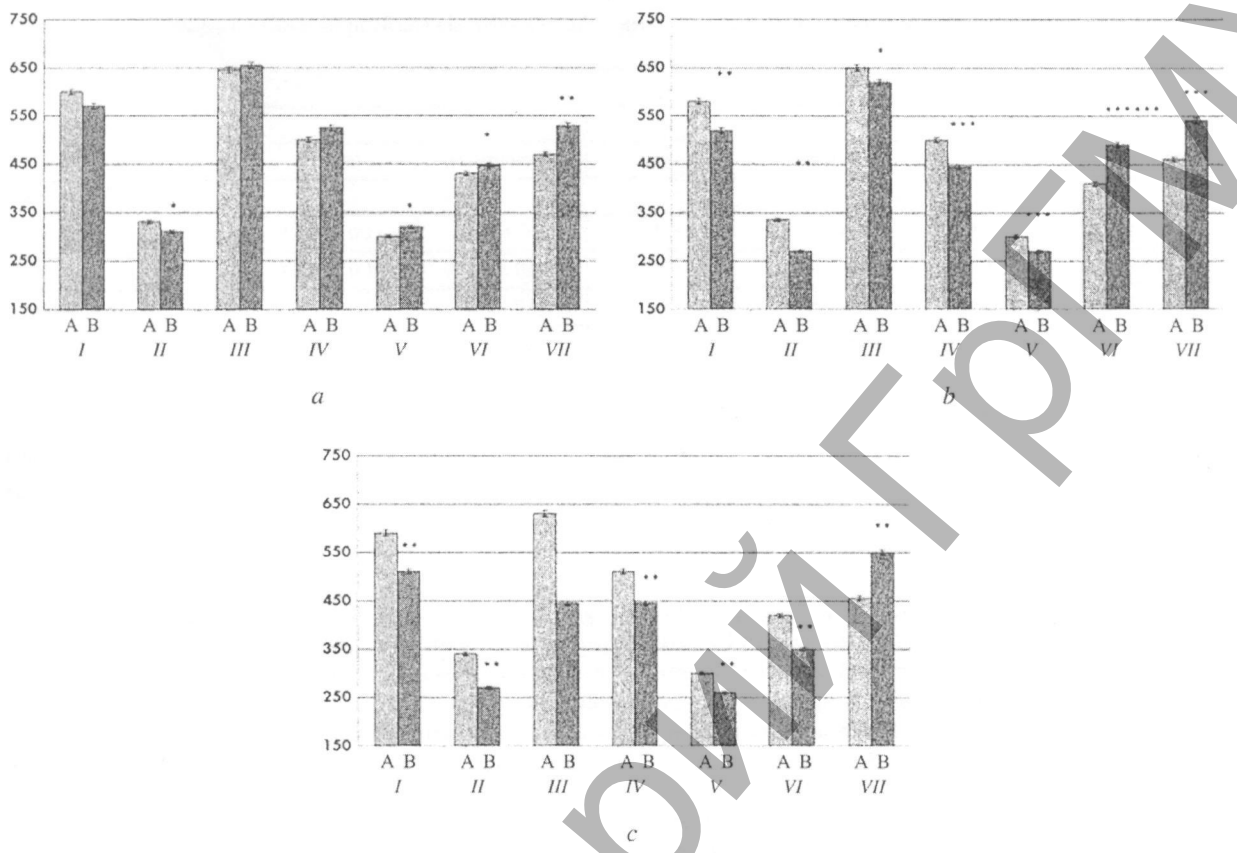

Fig. 1. Enzyme activities in the cytoplasm of histaminergic neurons in nucleus E2 of the rat hypothalamus during complete drainage of bile. *a*) Drainage of bile for one day; *b*) for three days; c) for five days. Horizontal axes: A = controls; B = bile drainage; study enzymes: *I*) monoamine oxidase B; *II*) succinate dehydrogenase; *III*) NADH dehydrogenase; *IV*) NADPH dehydrogenase; *V*) glucose-6-phosphate dehydrogenase; *VI*) lactate dehydrogenase; *VII*) acid phosphatase; the ordinate shows enzyme activity (OD units × 1000); $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ on comparison with controls; vertical bars show standard errors.

ditions requires surgical drainage of the bile pathways [7], i.e., external removal of bile with complete loss from the body. However, bile is an important liver secretion, which contains bile acids, phospholipids, cholesterol, bilirubin, and proteins [8]. Bile emulsifies fats and improves the absorption of fatty acids, cholesterol, and fat-soluble vitamins in the intestine, stimulates intestinal motor function, has bacteriostatic activity on the intestinal flora, and is involved in many metabolic processes. The importance of bile is apparent in structural and functional impairments arising in patients' bodies in conditions of chronic bile loss: disturbances to calcium metabolism, blood acid-base balance, the development of hemorrhagic diathesis, decreases in blood albumin levels, and impairments in liver and kidney, as well as nervous system, function [2]. However, the state of individual morphofunctional systems and brain cell types, particularly hypothalamic histaminergic neurons, has not been definitively studied.

The aim of the present work was to identify structural and metabolic changes in neurons in histaminergic hypothalamic nucleus E2 in conditions of complete external bile drainage.

MATERIALS AND METHODS

Studies were performed in accord with the "Regulations for Studies Using Experimental Animals" on 45 male Wistar rats weighing 250 ± 50 g. Complete external bile drainage was modeled by creating common bile duct fistulae as described by Vasilevskaya [1]. After 12 h starvation, rats were anesthetized with ether and underwent laparotomy; the common bile duct was exposed immediately beneath the confluence of the lobular hepatic ducts and a polyethylene catheter 4–5 cm long, connected to the inlet of a bile collector, was inserted into its proximal end to a distance of 3–4 mm. The

TABLE 1. Sizes and Shapes of Histaminergic Neurons in Nucleus E2 of the Rat Hypothalamus and in Animals at Different Durations of Complete External Bile Drainage ($\bar{x} \pm s_{\bar{x}}$)

Neuron parameter	Time after surgery, days					
	1		3		5	
	control	experimental	control	experimental	control	experimental
Diameter, μm:						
minimum	13.69 ± 0.21	13.47 ± 0.21	13.56 ± 0.24	13.81 ± 0.12	13.23 ± 0.16	13.44 ± 0.10
maximum	21.23 ± 0.16	20.41 ± 0.29*	20.80 ± 0.27	20.16 ± 0.21	20.75 ± 0.12	20.36 ± 0.20
Perimeter, μm	66.4 ± 0.3	64.2 ± 0.7*	65.1 ± 1.2	63.4 ± 0.6	63.8 ± 0.3	63.3 ± 0.4
Cross-sectional area, μm^2	217 ± 3	205 ± 4	210 ± 5	210.3 ± 2.8	204.7 ± 2.2	205.3 ± 2.3
Volume, μm^3	2424 ± 55	2225 ± 56	2310 ± 91	2313 ± 50	2218 ± 35	2227 ± 38
Shape factor	0.620 ± 0.010	0.630 ± 0.020	0.630 ± 0.020	0.660 ± 0.010*	0.640 ± 0.010	0.650 ± 0.003
Elongation factor	1.58 ± 0.04	1.55 ± 0.04	1.57 ± 0.04	1.490 ± 0.020	1.60 ± 0.03	1.54 ± 0.03

*$p < 0.05$ compared with control.

catheter was hermetically sealed into the duct using a silk ligature; a second ligature was placed just below the first, at the distal part of the duct, to prevent regurgitation of the contents of the common bile duct into the abdominal cavity. The bile collector was attached with three silk ligatures to the animal's skin, allowing free movement of the rats in their cages. Animals of the control group underwent sham surgery and physiological bile flow was maintained throughout the experiment. At 1, 3, and 5 days after surgery, animals were decapitated under ether anesthesia. The skull was opened quickly, the brain was removed, the hypothalamus was harvested and then frozen and stored in liquid nitrogen. Serial frontal sections of the posterior hypothalamus of thickness 20 μm were prepared in a cryostat at a temperature of –15°C, orientated using a stereotaxic atlas [14]. Histaminergic nucleus E2 was located from the known distribution of histaminergic nuclei of the hypothalamus [5, 11].

Cryostat sections were stained with thionine using the Nissl method and were used for reactions to detect the major histamine-catabolizing enzyme MAO B [6], which serves as a marker for histaminergic neurons in the hypothalamus [5]. The characteristics of the general metabolism of histaminergic neurons were studied by treating neighboring cryostat sections by standard histochemical methods for detection of oxidoreductases associated with the Krebs cycle (succinate dehydrogenase (SDH)), glycolysis (lactate dehydrogenase (LDH)), electron transport (HAD-DH and NADPH-DH), and the pentose phosphate shunt (glucose-6-phosphate dehydrogenase (G6PDH)), as well as acid phosphatase (AP, a lysosome marker enzyme) [9].

Quantitative determination of morphometric and cytophotometric parameters was performed using a Bioscan NT 2.0 (Belarus) computerized image analyzer with an objective magnification of ×40 and a video camera magnification of ×7. Microscope images were captured, digitized, and displayed on a computer monitor. Using preparations stained by the Nissl method, the outlines of the images of the perikarya of histaminergic neurons in the E2 nucleus of the hypothalamus were selected with the cursor. The computer program automatically yielded digital values for the sizes (minimum and maximum diameter, perimeter, area, volume) and shapes (the elongation factor, i.e., the ratio of the maximum and minimum diameters, and the form factor, i.e., 4 × the area/perimeter^2) of neuron perikayra. The activities of the enzymes of interest in the cytoplasm of histaminergic neurons were assessed by using the cursor to select the outlines of the cytoplasm of the perikarya on images of the corresponding histochemical preparations displayed on the computer monitor, with subsequent automatic determination of the mean optical density at the absorption peak of the stained reaction product. Thus, at least 30 neurons were assessed in each animal, with at least 150–200 neurons in each experimental group. The resulting primary numerical data were processed using non-parametric statistics run on Statistica 6.0 for Windows. Differences between the control and experimental groups were regarded as significant at $p < 0.05$ (Mann–Whitney U test).

RESULTS

Analysis of the data obtained in the present experiments demonstrated changes in the sizes and shapes of histaminergic neurons in the E2 nucleus on drainage of bile from the body. One day after the start of bile removal, there were reductions in the maximum diameter (by 3.9%) and perimeter (by 3.3%) of neuron bodies; there were tendencies to an increase in the shape factor and a reduction in the elongation factor (Table 1). On day 3 of bile loss, there was a statistically significant increase in the shape factor (by 4.8%)

and tendencies to reductions in the perimeter, maximum diameter, and elongation factor, i.e., histaminergic neurons become somewhat rounder and more spherical on days 1 and 3 of the experiment. On day 5 of bile drainage, there were no changes in the shapes and sizes of histaminergic neuron bodies (see Table 1).

On drainage of bile for one day, the cytoplasm of histaminergic neurons in the E2 nucleus showed statistically significant changes in the activities of several enzymes: AP activity increased by 10.7%, LDH increased by 5%, and G6PDH increased by 5.1%, while SDH activity decreased by 4.7% (Fig. 1).

Drainage of bile for three days led to further increases in LDH (by 17.8%) and AP activity (by 14.4%) and decreases in SDH (by 13.8%), NADPH-DH (by 12.8%), NADH-DH (by 4.2%), G6PDH (by 9.6%), and the key enzyme of histamine metabolism MAO B (by 12.1%) activities. Drainage of bile for five days led to further significant alterations in metabolism in histaminergic neurons. MAO B activity in experimental animals decreased by 14.6%, SDH by 16%, G6PDH by 14.5%, NADPH-DH by 14.6%, LDH by 16.9%, and NADH-DH by 13.5%. The only activity to increase was that of the key lysosomal enzyme AP, which showed further a increase (by 20.9%) (see Fig. 1).

DISCUSSION

The results obtained here provide evidence that loss of bile from the body due to complete drainage evoked significant structural-metabolic changes in the histaminergic neurons of the hypothalamus. Thus, at one day, the perikarya of neurons showed some reductions in size and increases in rounding; by three days, cells were more rounded, and spherical, and at five days, recovered their shapes and sizes. The transient changes in neuron size and shape may be associated with losses of cytoplasmic water due to impairment of the body's water-salt balance in acholia [2]. Unfortunately, the use of only cryostat sections in these experiments prevented measurement of neuron nucleus and nucleolus size and the ratio of their sizes. Recovery of parameters of histaminergic neurons at five days of bile drainage does not mean that their structural changes were reversed. The nature of the changes probably altered such that they were no longer detected by morphometry.

Impairments in enzyme activities in the cytoplasm of histaminergic neurons in the E2 nucleus were more significant and increased with increasing duration of bile drainage. Thus, bile drainage for one day led to increases in LDH, AP, and G6PDH activities, only the activity of the mitochondrial marker enzyme SDH decreasing. Bile drainage for three days produced further increases in the activities of the lysosome marker enzyme AP, along with increases in the activity of the anaerobic glycolysis enzyme LDH, though the activities of the remaining enzymes, including G6PDH, decreased. This suggests that during this period, histaminergic neurons showed reductions in synthetic processes and tissue respiration, though the cells tried to maintain viability using glycolysis and to eliminate intracellular damage by activating autophagic processes directed at removing damaged organelles. Loss of bile for five days suppressed the activities of all the enzymes studied except AP, which showed a further increase in activity, i.e., all energy metabolic pathways were suppressed and the impairments can be regarded as irreversible, leading to death of brain neurons and the entire body in conditions of further bile loss. In fact, continuation of bile drainage led to the death of all the animals on days 6–7 of the experiment [3].

Many bile components are known to be able to cross the blood-brain barrier and have direct effects on the brain. Data have been obtained showing increases in the receptor-dependent uptake of lipoproteins in the brains of animals during bile drainage [13]. The alterations detected here demonstrate the important role of bile in maintaining the structural-metabolic state of histaminergic neurons in the hypothalamus. It remains unclear whether these are the direct result of impairments in the enterohepatic circulation and the insufficiency of bile components in the blood or of severe abnormalities developing in the body. On the other hand, the changes may reflect the active participation of the histaminergic neurons of the hypothalamus in the adaptive reactions of the body in conditions of acholia. Overall, these data provide a more complete understanding of the role of bile in maintaining structural-metabolic homeostasis in brain neurons and in the CNS sequelae of impairments to the enterohepatic bile circulation.

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