MECHANISM OF THE EFFECT OF THE HYPOTHALAMUS ON BILE FORMATION

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Experiments on dogs showed that stimulation of the anterior hypothalamus inhibits choleresis and reduces the synthetic capacity of the hepatocytes. Stimulation of the posterior hypothalamic nuclei increases chloresis and the synthetic capacity of the hepatocytes. The response of the liquid fraction of the bile is determined by the permeability of the connective-tissue stroma and of the intercellular spaces of the liver.

KEY WORDS: hypothalamic stimulation; bile formation; histochemistry of hepatocytes.

Stimulation of various hypothalamic structures modifies the bile-forming function of the liver [7, 8]. However, the available data are not sufficient to identify the localization of hypothalamic influences on bile formation.

This problem was examined in the present investigation.

EXPERIMENTAL METHOD

Acute experiments were carried out on 20 male dogs weighing 6.5-11 kg, anesthetized with chloralose, and with platinum electrodes implanted into the hypothalamus [3, 6]. The hypothalamus was stimulated with square pulses (40 Hz, 7.5 V, 0.2 mA, 0.5 msec) for 10 min from a type UÉS-1 electronic stimulator. The bile was collected through a polyethylene catheter introduced into the common bile duct, for a period of 6 h. To assess the state of permeability of the connective-tissue stroma and the cell membranes of the liver, the potassium and sodium clearance with the bile was determined by flame photometry [5], alkaline phosphatase activity in liver homogenates and serum was estimated [4, 9], and acid mucopolysaccharides were determined histochemically by the reaction with toluidine blue at different pH values (2.8-4.6). Their differential analysis was carried out after methylation, demethylation, sulfatation, and incubation in a solution of testicular hyaluronidase [2]. Some control sections were treated before the reaction with 0.2 N NaOH solution. The results of investigation of the qualitative composition of the bile (cholates by the Shire–Cooney method, bilirubin by the Van den Bergh method, and cholesterol by the Levchenko method), determination of cholinesterase activity in liver homogenates and blood serum [4, 8], and histochemical detection of glycogen [10] in the liver, and of RNA by Brachet's method and by luminescence microscopy gave evidence of the functional activity of the hepatocytes. Pieces of liver for histochemical investigation were taken during and after the end of the experiments. The numerical data were subjected to statistical analysis. The significance of differences was determined by Student's criterion. The histological preparations were assessed visually. Ten fields of vision were examined in the sections and 200 hepatocytes were counted in identical zones; the mean content of the particular substrate was then deduced with the aid of a five-point system of gradations.

EXPERIMENTAL RESULTS AND DISCUSSION

Before stimulation of the hypothalamus a high glycogen level was detected in the central and peripheral zones of the lobule in the liver of the intact animals. Cytoplasmic RNA was distributed as separate granules in the perinuclear region and close to the cell membranes. Nucleolar RNA was clearly defined. The RNA

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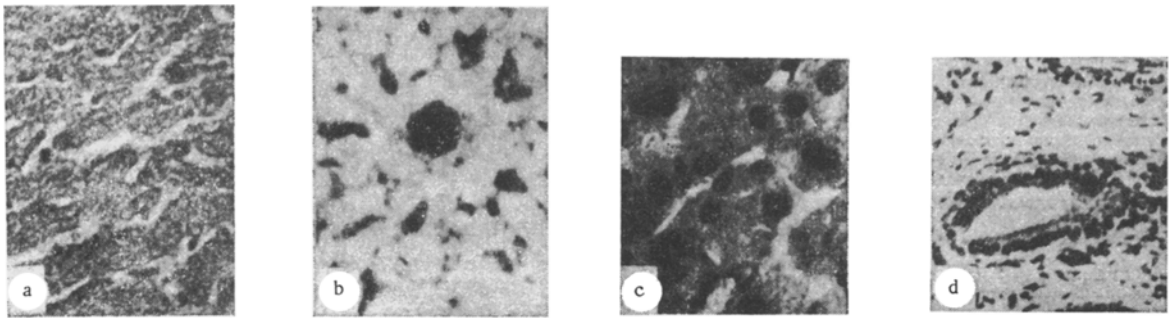

Fig. 1. Topochemistry of glycogen–RNA and nonsulfated mucopolysaccharides in liver after stimulation of anterior hypothalamic nuclei: a) glycogen utilization in hepatocytes. PAS reaction by McManus' method, 630 ×; b) distribution of RNA in hepatocytes. Acridine orange (1:10,000) in Ringer's solution pH = 5.0. Luminescence microscopy, 420 ×. Excitation filter 2-SS-4; suppression filter T-1 N; c) Depolymerization of nonsulfated mucopolysaccharides in intercellular spaces of liver. Toluidine blue, pH 4.0, 420 ×; d) depolymerization of nonsulfated mucopolysaccharides in connective-tissue stroma of bile ducts. Staining and magnification the same.

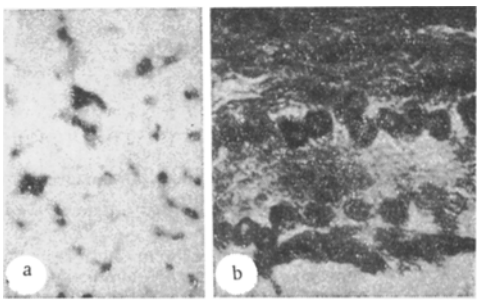

Fig. 2. Topochemistry of nonsulfated mucopolysaccharides in liver after stimulation of posterior hypothalamic nuclei: a) distribution of RNA in hepatocytes. Staining and magnification as in Fig. 1b; b) nonsulfated mucopolysaccharides in connective-tissue stroma and lumen of bile duct. Toluidine blue, pH 4.0, 630 ×.

content was inversely proportional to the glycogen content. γ-Metachromasia of the connective-tissue stroma of the liver was most marked at pH 4.0. The γ-metachromatic substrate in the hepatocyte cytoplasm formed single granules and small clumps. The intercellular spaces were closed and filled with γ-metachromatic substrate. In the connective-tissue surrounding the ducts γ-metachromasia increased toward the basement membrane. Acid mucopolysaccharides were present in small quantities in the bile ducts in the composition of the secretion and in the apical parts of the epithelium. Histochemical analysis of the γ-metachromatic substrate showed that it consisted of nonsulfated forms of acid mucopolysaccharides of the hyaluronate type.

After stimulation of the nuclei (supraoptic, anterior and lateral hypothalamic) of the anterior hypothalamus the glycogen content in the liver tissue fell or disappeared completely (Fig. 1a) and the intensity of the PAS reaction of the collagen fibers in the connective-tissue structures increased. Meanwhile a high content of homogeneous cytoplasmic RNA was clearly seen in the hepatocytes and Kupffer cells (Fig. 1b). The metachromatic substrate of the connective-tissue stroma and intercellular spaces showed generalized depolymerization (Fig. 1c, d). The content of acid groups of mucopolysaccharides in the connective-tissue structures was reduced. These changes took place in the direction from the periphery toward the center of the lobule and were connected with the adventitia of the blood vessels. Parallel with depolymerization of the acid mucopolysaccharides, the choleresis was reduced by 50% ($P < 0.01$) compared with the control, the concentration of potassium ions in the bile was reduced by 30.8% ($P < 0.01$), and the alkaline phosphatase activity in the blood serum was increased by 43.4% ($P < 0.05$). The associated changes between all these indices reflect increased permeability of the bile ducts, the possible cause of the increased reabsorption of water and salts, leading to concentration of the bile and a decrease in choleresis. As a result of the decrease in the liquid fraction, the concentration of cholates was considerably increased (by 58.5%; $P < 0.01$).

After stimulation of the nuclei of the posterior hypothalamus (lateral and medial mammillary, posterior hypothalamic) not only was the glycogen content reduced but the intensity of the PAS reaction of the hepatocytes also was weakened. The RNA content in the hepatocytes, Kupffer cells, and epithelium of the bile ducts was much higher than in the liver of the animals after stimulation of the anterior hypothalamic nuclei (Fig. 2a). The chief histochemical difference between the liver tissue during stimulation of the posterior hypothalamic nuclei compared with stimulation of the anterior was the different reaction of the γ-metachromatic substance. After stimulation of the posterior hypothalamus the degree of γ-metachromasia increased sharply (Fig. 2b). Meanwhile the choleresis was increased by 17.6% ($P < 0.01$) and the concen-

tration of potassium ions in the bile was increased by 24.1% ($P < 0.01$).

The permeability gradient of the connective-tissue stroma and intercellular spaces of the liver, the potassium and sodium clearance with the bile, and the alkaline phosphatase activity evidently determine the character of the reaction of the liquid fraction of the bile to stimulation of the anterior or posterior hypothalamus.

Differences between the results described above and data in the literature [1] can be explained by differences in the scope of the methods used.

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