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HISTOCHEMICAL ANALYSIS OF GLYCOSAMINOGLYCAN CONTENT IN THE CHOROID PLEXUS DURING HYDRATION AND DEHYDRATION

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KEY WORDS: vasopressin; water and electrolyte metabolism; choroid plexus; cerebrospinal fluid.

Vasopressin is known to participate in the regulation of cerebrospinal fluid (CSF) production by inducing an iso-osmotic decrease in its volume [4]. It has been suggested that the hormone modifies the transport function of the epithelium of the choroid plexus through its effect on structures of glycosaminoglycan nature sensitive to it, and that this mechanism is similar to that known for its action on the collecting tubules of the kidneys.

This paper describes a histochemical analysis of changes in the choroid plexus during maximal depression of secretion of endogenous vasopressin and in response to injection of posterior pituitary extract. The results obtained in these extreme situations can shed light on the role of glycosaminoglycans (GAG) in the mechanism of the change in permeability of the choroid plexus under the influence of vasopressin and the role of this process in preservation of the volume and composition of the CSF.

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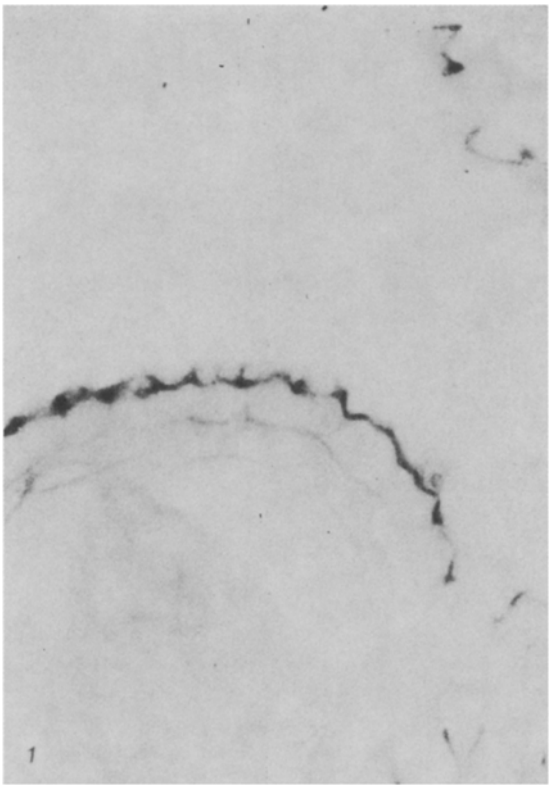

Fig. 1. Acid GAG in epithelium of choroid plexus of control dog. Here and in Figs. 2 and 3: staining by Hale's method, 160 ×.

EXPERIMENTAL METHOD

Chronic experiments were carried out on mongrel dogs weighing 6-8 kg, into the lateral ventricle of which a metal cannula 30 mm long and 1 mm in diameter had previously been implanted. The animals were divided into three groups. Animals of group 1 (control) received an injection of 0.5 ml of artificial CSF of the following composition (in mM) through the cannula: NaCl 136, KCl 2.9, $MgCl_2$ 4.4, $CaCl_2$ 4.6, $NaHCO_3$ 14.3 mM (pH 7.40). Dogs of group 2 were hydrated before the experiments by injecting water into the stomach in a dose of 5% of the body weight, after which they also received 0.5 ml of artificial CSF by injection through the cannula into the lateral ventricle. Animals of group 3 were given a solution of posterior pituitary extract into the lateral ventricle in a dose of 25 mU/kg body weight. The animals were killed by intravenous injection of a lethal dose of sodium thiopental 1.5 h after water loading and 30 min after injection of the hormone or CSF. The choroid plexus was removed, fixed with 10% neutral formalin, dehydrated in alcohols of increasing concentration, taken through benzene, and embedded in paraffin wax. Sections 5-6 μm thick were cut for histochemical analysis. High-polymer GAG were detected by Hale's method (with colloidal iron), and polyanionic GAG, irrespective of their degree of polymerization, were determined and differentiated by pH-dependent staining with alcian blue (from Serva, West Germany), with pH of the dye ranging from 1.0 to 2.8-3.0 [1, 6, 8], and with toluidine blue at pH values from 4.0 to 6.0 [7]. Differentiation of sulfate-containing from carboxylated GAG was carried out with the aid of chemical control: mild methylation and alkaline demethylation [8]. To identify hyaluronic acid and chondroitin-sulfuric acid C an enzyme control was set up with testicular hyaluronidase (from Reanal, Hungary) at 37°C for 2 h in a concentration of 25 mg %.

EXPERIMENTAL RESULTS

Histochemical analysis on sections of the choroid plexus revealed compounds in them which stained by Hale's method. This staining was completely abolished by treatment of the sections with testicular hyaluronidase. The alcianophilic staining of these compounds took place only with dye at pH 2.8, and not after mild methylation of the sections. Staining was restored after demethylation, possible evidence of the presence of active carboxyl groups in these compounds. Weak metachromatic staining with toluidine blue at pH 4.0 indicates the

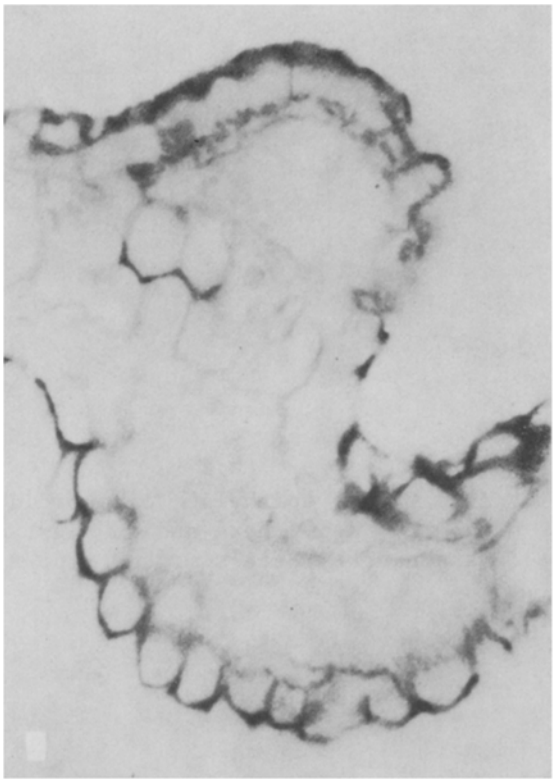

Fig. 2

Fig. 3

Fig. 2. Considerable increase in deposits of acid GAG on ventricular surface of epithelial cells of choroid plexus of dog receiving water equivalent to 5% of body weight.

Fig. 3. Decrease in content of acid GAG in epithelium of choroid plexus after injection of 25 mU/kg of posterior pituitary extract into lateral ventricle.

presence of a very small quantity of active sulfate groups. Intensive metachromatic staining with toluidine blue at pH 5.0-6.0 is evidence of the polyanionic properties of the compounds studied, due to the orderly arrangement of many active carboxyl groups in them. The character of the histochemical reactions of the substances analyzed and their hydrolysis by testicular hyaluronidase indicate that they belong to the class of acid GAG, containing chiefly hyaluronic acid, and only very small quantities of chondroitin-sulfuric acid C.

GAG were found in the form of thin-layer deposits on the ventricular surface of the choroid epithelium (Figs. 1-3). Some increase in the density of this material in the form of plugs was observed above regions of intercellular junctions of epithelial cells. The GAG content in different intact dogs could vary from moderate to high, as was manifested by differences in the degree of density and thickness of their deposits. Less GAG than in the epithelium was found in the epithelial basement membrane.

During hydration of the dogs a sharp increase in the GAG content in the epithelium and basement membrane (Fig. 2) was observed in the choroid plexus of the dogs. GAG were revealed in the form of a dense, fairly wide layer on the ventricular surface of the cells. Thickening of the GAG layer above the intercellular junctions in the form of plugs became more obvious in the hydrated dogs. Compaction of the GAG layer on the epithelium, more marked because of the increased intensity of staining, may perhaps be due to an increase in the degree of polymerization of the GAG.

Injection of posterior pituitary extract into the cerebral ventricle caused changes in the histochemical picture of the choroid plexus opposite to those observed in the hydrated dogs. Under the influence of pituitary extract the GAG content fell sharply compared with that in intact and hydrated animals (Fig. 3). On the ventricular surface of the cells GAG appeared in the form of extremely fine, palely stained, loosely packed and uneven deposits, or they were quite undetectable histochemically. GAG likewise were not found in the epithelial basement membrane. The sharp fall in the content and change in the state of GAG

were evidently connected with their depolymerization under the influence of the pituitary extract injected into the lateral ventricle.

Changes in GAG found in the choroid plexus resemble greatly those observed in the interstitial tissue of the renal medulla during hydration and under the influence of vasopressin. Interaction of GAG and mucolytic enzymes of hyaluronidase type in the kidneys is known to lie at the basis of the function of the concentration mechanism. Through these biochemical reactions vasopressin changes the permeability of the collecting tubules for water, thus enabling the concentration of the urine excreted to be varied [2, 3]. It can be tentatively suggested that the similarity we have found is not accidental. Very probably GAG play the same role in the choroid plexus as in the kidney, participating in the mechanism controlling permeability of the epithelium for water and determining the quantity of CSF formed.

It has been shown that vasopressin travels along axons of the supraoptic and paraventricular nuclei to reach not only the neurohypophysis, but also the CSF in the cerebral ventricles [5]. Inhibition of its secretion during hydration makes the epithelium impermeable for water, because of polymerization of GAG, and maintains the volume of CSF at a constant level despite the developing hydremia. The increase in vasopressin secretion, typical of dehydration and reduction of the blood volume, increases permeability through depolymerization of GAG and thus facilitates the entry of water into the CSF, to keep its volume constant.

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ULTRASTRUCTURAL STUDY OF LYMPH NODE CELLS IN EXPERIMENTAL BURN TRAUMA

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KEY WORDS: lymph nodes; nerve trauma.

Although the greatest importance is attached to the study of the immunology of burns [1, 7, 8], much still remains unexplained. In particular, there is much evidence of the presence, on the one hand, of hyperplasia of cells of the lymphoid and plasma series in lymph nodes [8, 10] and, on the other hand, of the presence of severe secondary immunologic deficiency in burns [5]. Howard et al. [11] concluded from data in the literature that the main role in the development of immunologic deficiency is played by disturbances predominantly of humoral immunity, but there is no information on the structural pathology of the immunocompetent cells and on their fine structure. This makes differentiation of intermediate forms distinguished purely by ultrastructural features and evaluation of the structural and functional dynamics of the blast transformation process impossible.

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