ROLE OF ACID MUCOPOLYSACCHARIDES OF THE KIDNEY IN THE MECHANISM OF REABSORPTION OF OSMOTICALLY FREE WATER

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Histochemical characteristics of acid mucopolysaccharides (MPS) of the renal medulla were compared with the indices of kidney function in dogs during water and osmotic diuresis and polyuria induced by administration of heparin. Changes in staining for MPS were found to correspond to the intensity of reabsorption of osmotically free water. The results suggest that changes in the physicochemical properties of MPS are one of the significant factors determining the permeability of structures of the renal medulla to movement of water from the lumen of the tubules into the interstices.

KEY WORDS: kidney; diuresis; reabsorption; mucopolysaccharides.

The basic point of A. G. Ginetsinskii's hypothesis regarding the mechanism of action of antidiuretic hormone on the mammalian kidney is the view that hyaluronidase and the system of acid mucopolysaccharides play a role in regulation of the permeability of the medullary structures for movement of water along the osmotic gradient [2, 4]. However, the problem of whether changes in the state of mucopolysacchrides are directly connected with the facultative reabsorption of water remains under discussion [9, 11, 18, 19, 21].

The object of this investigation was to ascertain the presence of correlation between the histochemical characteristics of acid mucopolysaccharides of the kidney tissue and the intensity of reabsorption of osmotically free water in polyuria stimulated by factors differing in their mechanism of action: administration of water, of an osmotic diuretic, and of heparin (a competitive inhibitor of hyaluronidase).

EXPERIMENTAL METHOD

Experiments were carried out on 24 dogs weighing 10-12 kg. At a preliminary operation on the animals the ureters were exteriorized on the abdominal wall, a gastric fistula was formed, and the jugular vein was catheterized. Water diuresis was induced by injecting water into the stomach in a volume of 7% of the body weight. Urea was used as the osmotic diuretic and also was injected into the stomach (1 g/kg). Heparin (Gedeon Richter, Hungary) was injected intravenously in a dose of 1250 units/kg.

The intensity of reabsorption of osmotically free water was determined by calculating the parameter $T^F_{H_2O}$ from the cryoscopic data [3]. The filtration level was determined from the endogenous creatinine clearance [8]. The water excretion was calculated as a percentage of the quantity filtered.

Acid mucopolysaccharides in the medulla of the kidney removed from the animal guillotined at the height of polyuria were detected by histochemical methods: staining by Hale's method [1] and toluidine blue at different pH values, with appropriate controls [17].

EXPERIMENTAL RESULTS

Administration of water to the animals led to a change in renal function typical of water diuresis: an

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TABLE 1. Changes in Indices of Renal Function and Histochemical Characteristics of Acid Mucopolysaccharides in Interstitial Tissue of Renal Papilla during Various Types of Polyuria (M ± m)

Indices of renal function and histochemical staining for mucopolysaccharides	Administration of water (n=9)		Administration of urea (n=9)		Injection of heparin (n=6)	
	initially	maximum of response	initially	maximum of response	initially	maximum of response
Diuresis (in ml/min · m^2)	0.5±0.2	11.2±3.7	0.8±0.5	4.3±0.5	0.3±0.2	3.7±1.2
Water excretion (in % of filtration)	0.95±0.24	14.0±0.8	1.1±0.4	7.1±1.3	0.9±0.2	5.5±1.1
Filtration (in ml/min · m^2)	61.0±4.0	58.2±5.0	51.1±6.0	50.4±6.2	61.0±3.0	61.8±4.2
Reabsorption of osmotically free water (in ml/min · m^2)	0.69±0.14	-5.6±0.6	0.49±0.14	4.5±1.0	1.4±0.2	-1.8±0.6
γ-Metachromasia	–	+	–	–	–	+
Staining by Hale's method	–	+	–	–	–	+

increase in the rate of urine production without any significant change in filtration (Table 1) which was entirely the result of depression of the reabsorption of osmotically free water in the distal segment of the nephron.

Microscopic investigation of the kidney in the region of the papilla showed the characteristic picture of hyperhydration – intense staining of acid mucopolysaccharides and of the interstitial tissue, and high epithelium of the collecting tubules (Fig. 1a).

The mechanism of diuresis induced by an osmotic diuretic differed in principle from that described above. The rate of diuresis after administration of an osmotically active agent increases as a result of inhibition of the proximal reabsorption of water. Reabsorption of osmotically free water occasionally is not inhibited but, by contrast, is increased [6, 15, 20]. In the writers' earlier investigation [7], osmotic diuresis, by contrast with water diuresis, was found to be accompanied by high hyaluronidase of the urine. As-

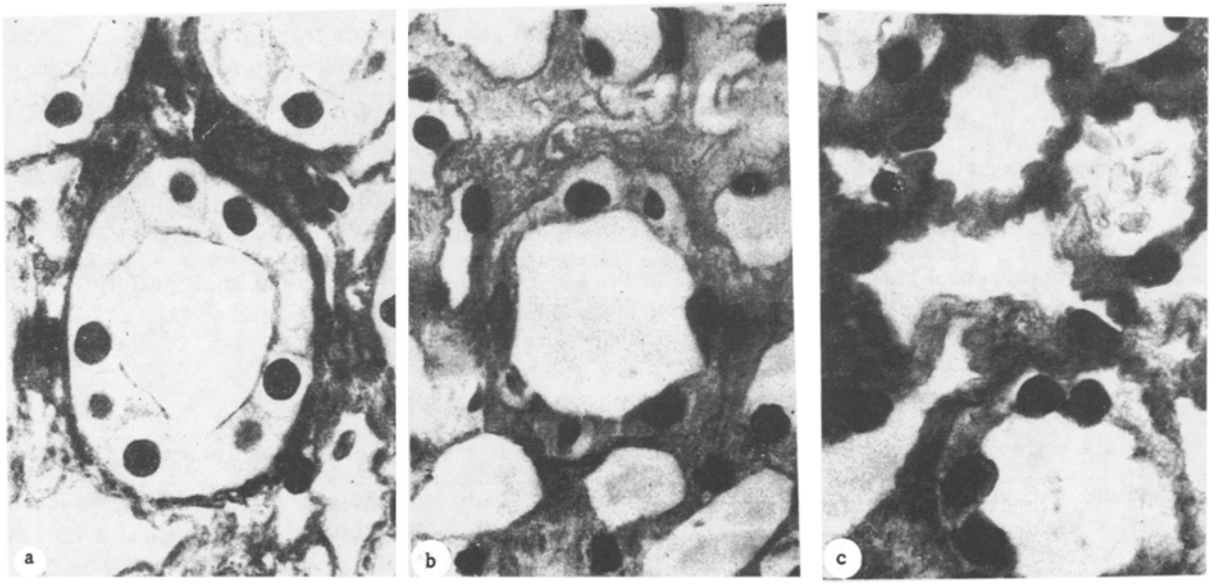

Fig. 1. Transverse section through dog kidney in the region of the middle third of the papilla: a) 40 min after administration of water; b) 30 min after administration of urea; c) 30 min after injection of heparin. Toluidine blue, pH 4.8, immersion, objective 90 ×.

suming that the acid mucopolysaccharides of the medulla are the substrate for action of renal hyaluronidase, and that a change in these substances is an essential stage in the distal reabsorption of water, it ought to be expected that the histochemical characteristics of the renal mucopolysaccharides in osmotic and water diuresis would differ significantly.

In the series of experiments with osmotic diuresis a rapid increase in the rate of urine production was observed in the dogs after intragastric injection of urea. The increase in diuresis was not accompanied by any significant change in filtration, and it took place against the background of gradually increasing reabsorption of osmotically free water (Table 1). The morphological picture of the kidney at the height of diuresis was as follows: the intensity of staining of the acid mucopolysaccharides in the region of the renal papilla was greatly reduced, the epithelium of the collecting tubules was flattened, and the capillary network surrounding the collecting tubules was open (Fig. 1b). Despite the development of polyuria, the cytological and histochemical characteristics of the medullary substance in osmotic diuresis were thus similar to those usually found in animals under the influence of antidiuretic hormone.

An increase in urine production can be stimulated by intravenous injection of heparin [5]. It was concluded from the marked decrease in concentration index during the period of development of polyuria that the action of heparin is due to inactivation of hyaluronidase and a decrease in the permeability of the collecting tubules for water. In the present experiments a marked polyuria also was observed after the intravenous injection of heparin. The increase in diuresis developed simultaneously with a sharp decrease in the resorption of osmotically free water whereas the filtration level remained unchanged (Table 1).

Investigation of the kidney obtained from an animal at the height of heparin-induced diuresis revealed distinctive changes in the medulla (Fig. 1c). Although the general histological picture was similar to that observed in the kidney of the dehydrated animal (flattened epithelium of the collecting tubules and open vascular network in the interstitial tissue, although the shape of the capillaries was a little modified, giving the impression of slightly shrunken and empty vessels), at the period of maximal diuresis after injection of heparin, a well-marked γ-metachromasia of the fibrous structures was observed in the interstitial tissue and basement membranes of the collecting tubules. The intensity of staining for acid mucopolysaccharides corresponded to that found in dogs during maximal water diuresis, when the mucopolysaccharides were in a high-polymer state.

When discussing the role of mucopolysaccharides in the reabsorption of water the concept, suggested by several workers [13, 14, 16], of the existence of protein–polysaccharide macromolecules in the solution in the form of diffuse molecules forming a special type of gel filter of interstitial tissue, which is capable of reversibly changing the resistance to the flow of fluid, is particularly important. The unique distribution of acid mucopolysaccharides and their fractions among the morphological structures of the kidney is not accidental and some definite relationship must exist between the concentration of mucopolysaccharides and the functional significance of topographical zones of the kidney. The concentration of hyaluronic acid and condroitin sulfates, determined in the region of the medulla, is fairly high – of the order of 2 mg/g [10]. Consequently, the content of mucopolysaccharides in the renal papilla is quite sufficient for the formation of a structural lattice, with the properties of a "molecular sieve," and the permeability of the interstitial tissue for water diffusion must be determined by the physicochemical state of the mucopolysaccharides.

The results of comparing the histochemical characteristics of the mucopolysaccharides of the medulla with the parameters of kidney function in the various types of polyuria indicate that the histochemical features of the mucopolysaccharides correspond to changes in the intensity of reabsorption of osmotically free water. In cases in which the diuresis is increased by inhibition of distal reabsorption of water (water of heparin diuresis), acid mucopolysaccharides stain intensely in the renal medulla and are probably in a high-polymer state. Conversely, when diuresis is increased through inhibition of water transport in the proximal segment of the nephron only, and distal water reabsorption continues to remain at a high level (osmotic diuresis), the intensity of staining of the acid mucopolysaccharides is sharply reduced, possibly as the result of a profound change in their physicochemical state (depolymerization or degradation to diffusible fractions).

The results concerning correlation between the state of mucopolysaccharides and the intensity of transport of osmotically free water form a basis for the conclusion that a change in the physicochemical properties of these compounds is probably one of the most important factors determining the permeability of medullary structures to the flow of water along the osmotic gradient from the lumen of the tubules into the interstices.

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