MECHANISM OF THE EFFECT OF ANTIDIURETIC HORMONE ON EPITHELIUM OF THE COLLECTING TUBULES IN THE DOG KIDNEY

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Experiments on dogs shows that after administration of pituitrin P, containing antidiuretic hormone (ADH), an increase in osmotic concentration of the urine was accompanied by a decrease in height of the epithelium of the collecting tubules and an increase in the concentration of dry substance in the cells. A change in the height of the epithelial cells was produced by retrograde injection of solutions of mannitol into the collecting tubules or by incubation of slices of kidney tissue in solutions of different osmotic concentrations. It is postulated that the decrease in height of the epithelial cells in the collecting tubules after administration of ADH is the result of their dehydration because of the increasing osmolarity of the intratubular urine.

A morphological feature reflecting the action of pituitary antidiuretic hormone (ADH) on the mammalian kidney is flattening of the epithelium of the collecting tubules [1, 2, 5, 6]. It is not yet known whether the decrease in height of the cells is the result of apocrine secretion of the enzyme hyaluronidase [3], an accidental phenomenon [11], or a secondary event developing as the result of the increasing osmotic concentration of the intratubular urine.

The object of the investigation described below was to determine whether hypertonicity of the medium influences the epithelium of the collecting tubules in the dog kidney in vivo and in vitro.

TABLE 1. Height of Epithelium of Collecting Tubules and Concentration of Dry Substance in Epithelial Cells and Stroma of Dog Renal Papilla for Different Osmotic Concentrations of Urine

Osmotic concn. of urine (in mosm/liter)	Ht. of cells, in μ ($X \pm t \cdot m_x$ at P = 0.001)	Concn. of dry substance, in % ($X \pm t \cdot m_x$ at P = 0.001)	
		cytoplasm of epithelium of collecting tubules	collagen bundles of stroma
60	17,7±0,31	13,5±0,78	22,0±1,38
65	—	13,3±0,80	21,6±0,9
150	16,0±0,52	—	—
700	11,0±0,49	17,0±0,74	23,7±0,73
780	10,5±0,36	20,0±0,76	24,3±0,82
1120	9,5±0,44	28,7±0,96	25,5±0,93
1320	9,5±0,36	25,7±0,88	27,6±1,22
1500	9,5±0,35	32,5±0,41	25,6±0,96
1840	8,2±0,29	37,2±1,07	25,5±0,74

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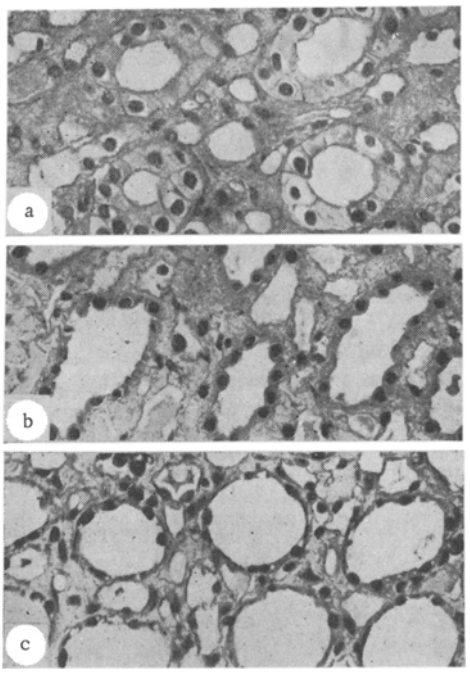

Fig. 1. Epithelium of collecting tubules in region of lower third of renal papilla in dogs: a) water diuresis, osmotic concentration of urine 60 mosm/liter; b) and c) after administration of pituitrin P, osmolar concentration of urine 1120 and 1840 mosm/liter respectively. Hematosylin-eosin, 320 ×.

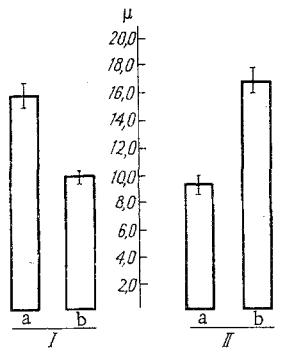

Fig. 2. Changes in height of epithelial cells of collecting tubules after retrograde injection of mannitol solutions with osmotic concentrations of 1500 (I) and 50 mosm/liter (II) into the kidney. A, B) Height of cells (in μ) in sections of control and experimental kidneys respectively.

EXPERIMENTAL METHOD

Mongrel dogs weighing 6-14 kg were used. The experiments were divided into three series: in series I the characteristics of the epithelium of the collecting tubules were studied in the kidney of dogs during water diuresis or the antidiuretic response (nine dogs); in series II the kidney tissue was investigated after retrograde injection of hyper- and hypotonic solutions of mannitol into the collecting tubules (14 dogs); in series III kidney slices were incubated in solutions of different osmotic concentrations (five dogs).

Hydration of the animals was produced by injecting water into the stomach in a dose of 7% of the body weight. The antidiuretic response was stimulated by intravenous injection of pituitrin P in a dose of 10 i.u./kg. At the height of the response the animals were decapitated and the kidney removed for morphological investigation.

The reflux method [8] was used to create artificial hyper- or hypotonicity of the medium in the lumen of the collecting tubules. Retrograde injection of hypotonic mannitol solution (1500 mosm*/liter) was carried out on the hydrated animal anesthetized with chloralose and with a rate of diuresis of 10-16 ml/min·m^2. The hypotonic solution (50 mosm/liter) was injected when the initial diuresis was 0.2-0.5 ml/min·m^2. The experimental and control kidneys were removed for morphological examination 30 min after occlusion of the ureter.

Kidney slices, 1 mm thick, were incubated in aerated Krebs-Ringer solution and in Krebs-Ringer solutions with the addition of mannitol to an osmotic concentration of 600, 1000, 1500, and 2000 mosm/liter for 20 min at 37°C. The osmotic concentration of the urine and solution was determined by a cryoscopic method [4].

Pieces of kidney tissue for morphological examination were fixed in formalin and embedded in paraffin wax. Sections were stained with hematoxylin-eosin. The height of the epithelial cells of the collecting tubules was measured with a type OM (15 ×) ocular micrometer in the region of the lower third of the renal papilla, where changes developing by the action of antidiuretic hormone (ADH) are most marked in dogs. The height of 100 cells in the section was measured, and 5-7 sections were used from each kidney.

Parallel sections from the same blocks of tissue were used to determine the concentration of dry matter in the epithelial cells of the collecting tubules and collagen bundles of the connective tissue of the renal papilla. The interference method in a uniform field with high image splitting [7, 9] was used. Measurements were made with a type MPI-5 (Poland) interference microscope. It must be remembered that measurements were made on paraffin sections of fixed material, and the results must therefore differ somewhat from the characteristic values measured in the living cell. However, since all the material was treated in the same way, the measurements were essentially of a systematic character and the results were comparable.

*Milliosmoles.

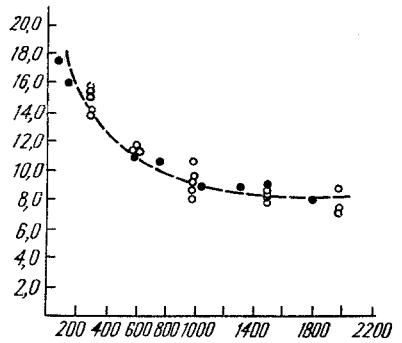

Fig. 3. Height of epithelial cells of collecting tubules as a function of osmotic concentration of urine (in vivo) and of incubation medium (in vitro). Filled circles show results of experiments in vivo; unfilled circles, results of experiments in vitro. Abscissa – osmotic concentration (in mosm/liter); ordinate – height of cells (in μ).

EXPERIMENTAL RESULTS

Significant differences were found between the epithelium lining the collecting tubules of the kidney in animals in states of hydration or of antidiuresis following administration of pituitrin (Fig. 1). In the kidney of the hydrated animal, excreting urine of low osmotic concentration, the epithelial cells were high and had abundant cytoplasm, a central nucleus, and distinct intercellular boundaries. After injection of pituitrin the epithelium of the collecting tubules was considerably flattened. The cells became elongated, their nucleus and cytoplasm were optically denser, their cytoplasm occupied a very small area around the nucleus, and the cell boundaries were practically indistinguishable.

Analysis of the height of the cells revealed a definite pattern: the higher the concentration of osmotically active substances in the urine, the more flattened the epithelium of the collecting tubules (Table 1). An increase in the osmotic pressure of the urine was also accompanied by changes in the concentration of dry substance in cytoplasm of the epithelial cells: during water diuresis, when the osmotic concentration of the urine was low and the epithelium was highest, the concentration of dry substance was 13%, but when the osmotic concentration of the urine was high and the cells were flattened the concentration of the dry substance rose sharply to 37%. This suggests that the decrease in height of the epithelium results from changes in the degree of hydration of its cells.

This hypothesis was confirmed in the experiments with retrograde injection of hyper- and hypotonic solutions of mannitol. In this way an osmotic concentration corresponding to a state of antidiuresis or of water diuresis could be created artificially in the lumen of the collecting tubules, and in this case both kidneys were under the influence of the same concentration of endogenous ADH circulating in the blood stream.

Injection of a hypertonic solution of mannitol created an osmotic environment in the collecting tubules which corresponded to the maximal ADH effect. Although preliminary hydration had caused a decrease in the ADH concentration in the animal's blood and the control kidney showed all the signs of water diuresis, in the experimental kidney the height of the epithelium was reduced by 41% compared with the control. Conversely, when the hypotonic solution was injected, the environment inside the tubules was typical of maximal water diuresis, and the height of the epithelium in the experimental kidney was virtually indistinguishable from the values obtained during natural high diuresis, with urine of an osmotic concentration of 50-60 mosm/liter.

The effect of a decrease in height of the epithelium of the collecting tubules (Fig. 2) was also observed when kidney slices obtained from a hydrated animal were incubated in solutions of increasing osmotic concentration. The height of the cells, with an increase in osmotic concentration of the incubating solution, corresponded to values observed under natural conditions in animals excreting urine of different osmotic concentrations (Fig. 3). Since the incubation fluid did not contain ADH, the changes in height of the epithelium could only have resulted from the dehydrating effect of the hypertonic solutions and could not have been the result of an active response to ADH.

By artifically changing the medium surrounding the epithelium, in experiments both in vivo or in vitro, changes could thus be produced in the epithelium such as are observed during antidiuresis or hyperhydration.

Flattening of the epithelial cells under the influence of ADH has been found only in the collecting tubules of mammalian kidneys. Experiments on isolated membranes of amphibians (skin, urinary bladder) have shown the opposite effect: swelling of the epithelial cells [13, 15]. Flattening of the epithelium of the collecting tubules in the mammalian kidney can be assumed to be due not to the direct effect of ADH on the cells, but to changes in the physicochemical conditions arising in the renal papilla as a result of action of the hormone.

The apical and basement membranes of epithelial cells are permeable to water. During water diuresis the apical surface of the cells in the collecting tubules is in contact with a strongly hypotonic solution, whereas the basal surface faces the interstitial papilla, in which the medium is kept hypertonic on account of the functioning of the concentration mechanism. Under these conditions the hypotonic effect is evidently stronger because the basal surface of the cells is relatively protected by the dense high-polymer structures of acid mucopolysaccharides, which create a considerable resistance to the current of fluid and of dissolved low-molecular-weight substances in this state [10, 12, 14]. As a result, the epithelial cells of the collecting tubules in an animal excreting hypotonic urine are strongly hydrated.

The main effects of ADH on the distal segment of the nephron are an increase in the permeability of the collecting tubules, an increase in the transport of osmotically free water from the lumen of the tubules into the interstitial tissues, and an increase in the osmotic concentration of the intratubular fluid. The mucopolysaccharide structures surrounding the collecting tubules undergo profound changes which led to an increase in their permeability. The epithelial cells of the collecting tubules are thus surrounded by a hypertonic medium, and when its osmotic concentration exceeds 660 mosm/liter, it evidently cannot withstand the dehydrating effect, so that the height of the cells is reduced and the concentration of solid matter in the cytoplasm rises. No marked changes in osmotic concentration take place in the interstitial tissues of the papilla such as are found in the lumen of the tubules, and it is interesting from this point of view to note that an increase in the osmotic concentration of urine is not accompanied by any significant increase in the concentration of solid matter in the collagen bundles of the papillary stroma.

It can be concluded from the analysis of these experimental results that changes in the epithelial cells under the influence of ATP are evidently unconnected with structural changes in the epithelium, but are the result of dehydration of its cells, a secondary reaction to an increase in the osmotic pressure of the medium.

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