Involvement of Interstitial Structures of the Kidney into Hydrosmotic Effect of Vasopressin (Morphofunctional Study)

L. V. Shestopalova, V. A. Lavrinenko, V. A. Shkurupiy*, and L. N. Ivanova

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Administration of desmopressin for 4 days to homozygous Brattleboro rats lacking endogenous vasopressin induced an increase in osmotic concentration and was accompanied by typical changes in the morphofunctional state of interstitial cells of the renal papilla. These changes increase the permeability of extracellular matrix, which attested to the involvement of interstitial cells into hydrosmotic reaction to vasopressin.

Key Words: *vasopressin; Brattleboro rats; kidney; interstitial cells; ultrastructure*

Neurohypophyseal hormone vasopressin (VP) is the major factor regulating water reabsorption in mammalian kidney. The sequence of intracellular molecular events induced by VP in the osmoregulating epithelium is now studied in detail (from interaction of the hormone with the receptor to incorporation of water channels aquaporins into the cell membrane) [11]. The final stage related to waster diffusion through structures of the interstitial space formed by cell elements and high-molecular-weight glycosaminoglycans (GAG) is the least studied step in the mechanism of VP action. An important role in the formation of a barrier for water diffusion in the interstitium is presumably played by hyaluronic acid (hyaluronan), the major component of extracellular matrix changing its permeability depending on polymerization degree. It was found that the content of hyaluronan in the renal papilla positively correlates with the intensity of diuresis and negatively correlates with urine osmolality [6]. Depolymerization and degradation of hyaluronan is catalyzed by lysosomal endo- and exohydrolases; hyaluronidase is the key enzyme in these processes [12]. Enhanced expression of genes encoding different types of hyaluronidases and their increased activity in the renal medulla is stimulated by VP [1,9]. Interstitial cells (IC) are the main structural elements responsible for production of the components of renal medulla interstitium [6]. Previous studies showed that VP induces specific structural changes in the epithelium of collecting tubules and typical modifications of the interstitium of the renal medulla [2,8]. However, VP-induced changes in structural organization of IC are little studied.

Here we studied morphofunctional peculiarities of IC in the renal papilla of Brattleboro rats lacking endogenous vasopressin due to genetic defect in the synthesis of this hormone and treated with V_2 VP receptor agonist desmopressin, a synthetic VP analogue.

MATERIALS AND METHODS

Experiments were carried out on 35 mature homozygous Brattleboro rats weighing 200-250 g. The animals were grown in a vivarium of Research Center of Clinical and Experimental Medicine, Siberian

Department of Physiology, Novosibirsk State University; *Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Russia. ***Address for correspondence:*** phisiol@fen.nsu.ru. V. A. Lavrinenko

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Division of the Russian Academy of Medical Sciences (Novosibirsk) under conditions of free access to food and water. Intact group consisted of 10 animals, 25 animals received intraperitoneal injections of desmopressin (20 μ/100 g body weight, 2 times per day for 4 days).

For evaluation of the efficiency of osmotic concentration, the concentration of osmotically active substances in spontaneous urine samples obtained before and after the experiment was measured by cryoscopic method using a MT-2 milliocmometer (Research-and Production Complex Burevestnik). After the experiment, the animals were decapitated.

Histochemical analysis of GAG was performed by the method of Hale on routinely prepared paraffin sections of the renal medulla. A segment of the right kidney was used for measurement of intrarenal sodium and urea gradients.

For ultrastructural study, the renal papilla from the left kidney was isolated. The tissue was fixed in 3% glutaraldehyde on phosphate buffer (pH 7.4) with 7% NaCl. Osmolarity of the fixative corresponded to osmolarity of the urine. The samples were postfixed in 1% osmium acid. Then, the tissue specimens were dehydrated in ascending alcohols and acetone and embedded in epon-araldite (1:6) mixture. Semithin and thin sections were prepared on a Tesla-100A ultratome. Ultrathin sections were stained by the method of double contrasting with uranyl acetate and lead citrate after Reynolds. Cell ultrastructure was analyzed under a JEM 100CX electron microscope at accelerating voltage of 80 kV.

Morphometry of cell structures was carried out using an open test system using a 0.26-μ grid spacing at a final magnification of ×38.500.

Reliability of the differences between morphometric parameters of IC was evaluated using Fisher *F* test.

RESULTS

Homozygous rats are characterized by polyuria, polydipsia, and excrete urine of low osmolality (Fig. 1). Analysis of intrarenal sodium and urea gradients confirmed low concentration capacity of the kidney in these animals (Fig. 2). Low content of GAG in the renal papilla is also typical of homozygous Brattleboro rats. Histochemical staining for hyaluronan was reduced in the interstitium; Hale-positive material was found only on the apical surface of epithelial cells.

It is known that the composition of the extracellular matrix is determined by IC, functionally active cells of the interstitium. The extracellular matrix of the interstitium is presented by a hydrate gel with fine fibrillar network consisting of proteoglycans, glycoproteins, and extracellular fluid [7]. Hyaluronan is the major GAG of the renal papilla matrix; in the middle third of the papilla, hyaluronan can be synthesized both in interstitial cells and beyond these cells [5,12] and its synthesis is not associated by the Golgi complex, but involves the granular endoplasmic reticulum [4]. Intracellular hyaluronan can be presented by hyaluronosomes; the formation of these membrane structures requires activation of the entire synthetic apparatus of the cell. Degradation of hyaluronan and sulfated GAG (also synthesized by IC) is catalyzed by lysosomal hyaluronidase and exohydrolases.

Ultrastructural study of the renal papilla revealed two types of IC (Fig. 3, *a*, *b*) Type 1 IC (IC1) are large cells with oval nucleus and low number of organelles presented by vacuolar structures, few osmiophilic granules and lipid droplets, numerous vesicular structures with coated and uncoated membranes. Profiles of the endoplasmic reticulum in IC1 are widened. Type 2 IC (IC2) are

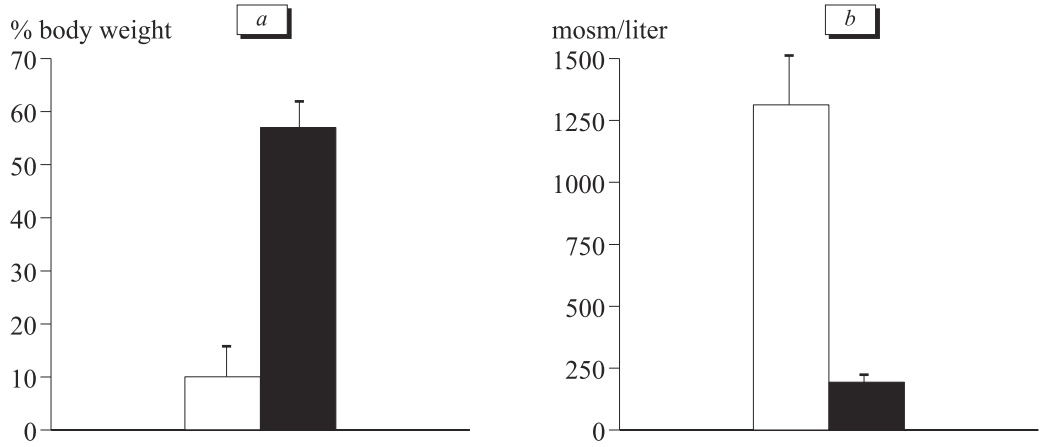

Fig. 1. Volume of excreted fluid (*a*) and urine osmolarity (*b*). Here and on Fig. 2: open bars: control; dark bars: desmopressin treatment.

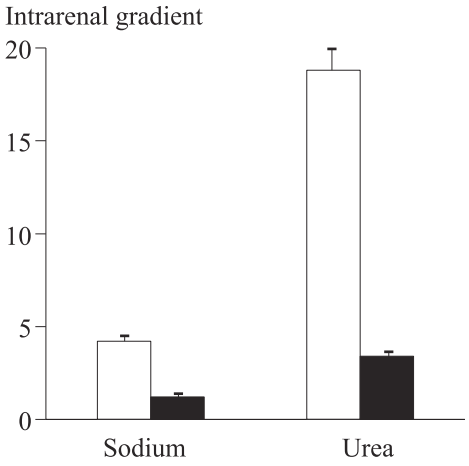

Fig. 2. Concentration gradients of sodium and urea.

small heteromorphic cells with dense cytoplasm and round nucleus. Profiles of the endoplasmic reticulum look like large vacuolar structures with electron-dense content. The cytoplasm of IC2 cells contain numerous large and small granules with osmiophilic and fine granular content. Both types of IC had similar localization; they are located in immediate proximity to epithelial cells of the collecting tubules, blood vessels, and loops of Henle.

The observed peculiarities of the ultrastructure and morphometric parameters (Table 1) attest to differential activity of IC1 and IC2 cells in intact animals. The state of the Golgi complex and endoplasmic reticulum and the presence of heterogeneous large and small osmiophilic granules in IC2 suggest that these cells more actively synthesize GAG and enzymes for their catabolism. The presence of primary and secondary lysosomes with heterogeneous matrix I the cytoplasm of these cells attested to the process of hyaluronan degradation.

Administration of exogenous desmopressin to Brattleboro rats for 4 days reduced fluid intake and excretion, and significantly increased osmolality of the urine (Fig. 1). Desmopressin considerably increased concentration gradient of the urine. Changes in functional parameters characterize marked increase in reabsorption of osmotically free water in the collecting tubules. An increase in histochemically detected GAG on preparations of the kidney from stimulated animals was visually noted.

Electron microscopy revealed hydratation of the interstitial space of the renal medulla; floccular and granular structures of different electron density were seen, which probably reflected depolymerization of hyaluronan. Similarly to the control group, IC of two types were present in stimulated animals. The state of synthetic apparatus in IC1 attests to activation of synthetic processes. The amount of disintegrated granules decreased, the content of lipid structures often forming membrane-coated groups considerably increased. This confirmed the assumption that lipid-containing cells are sensitive to VP [3] and their state directly depends on blood concentration of this hormone. This relationship is determined by prostaglandin-producing capacity of IC; the content of prostaglandins increases after hormone stimulation [13]. It remain unclear, whether the appearance of lipid inclusions in cells is related to enhanced synthesis of prostaglandin E2 involved in the regulation of water permeability [15] or it results from accumulation of arachidonic acid due to inhibition of prostaglandin synthesis.

In experimental animals, intracellular organization of IC2 was changed (Fig. 3, *c*). The Golgi complex was more pronounced than in the control and presented by 4-5 stacks of vacuolated cisterns; a tendency to an increase in its surface density was

TABLE 1. Morphometric Parameters of Renal Papilla IC in Intact and Stimulated Brattlboro Rats (*M*±*m*)

Morphological parameter	Control		Desmopressin	
	IC1	IC2	IC1	IC2
Surface density of Golgi complex, per 1 μ	0.52±0.07	0.80±0.19	1.04±0.17*	1.14±0.30
Surface density of endoplasmic reticulum, per 1 μ	0.57±0.27	3.17±0.63	1.39±0.50	1.20±0.26*
Numerical density of free ribosomes, per 1 μ^3	38.66±12.85	26.96±2.48	37.83±6.02	111.74±4.32*
Numerical density of polysomes, per 1 μ^3	64.78±24.01	37.18±8.30	65.07±17.21	79.54±17.82*
Content of electron dense granules per cell, %	26	56	31	24
Content of lipid droplets per cell, %	27	20	58	52
Content of disintegrating granules per cell, %	47	24	11	24
Content of uncoated vesicles per cell, %	28	37	53	44
Content of coated vesicles per cell, %	72	63	47	56

Note. *$p<0.05$ compared to the control.

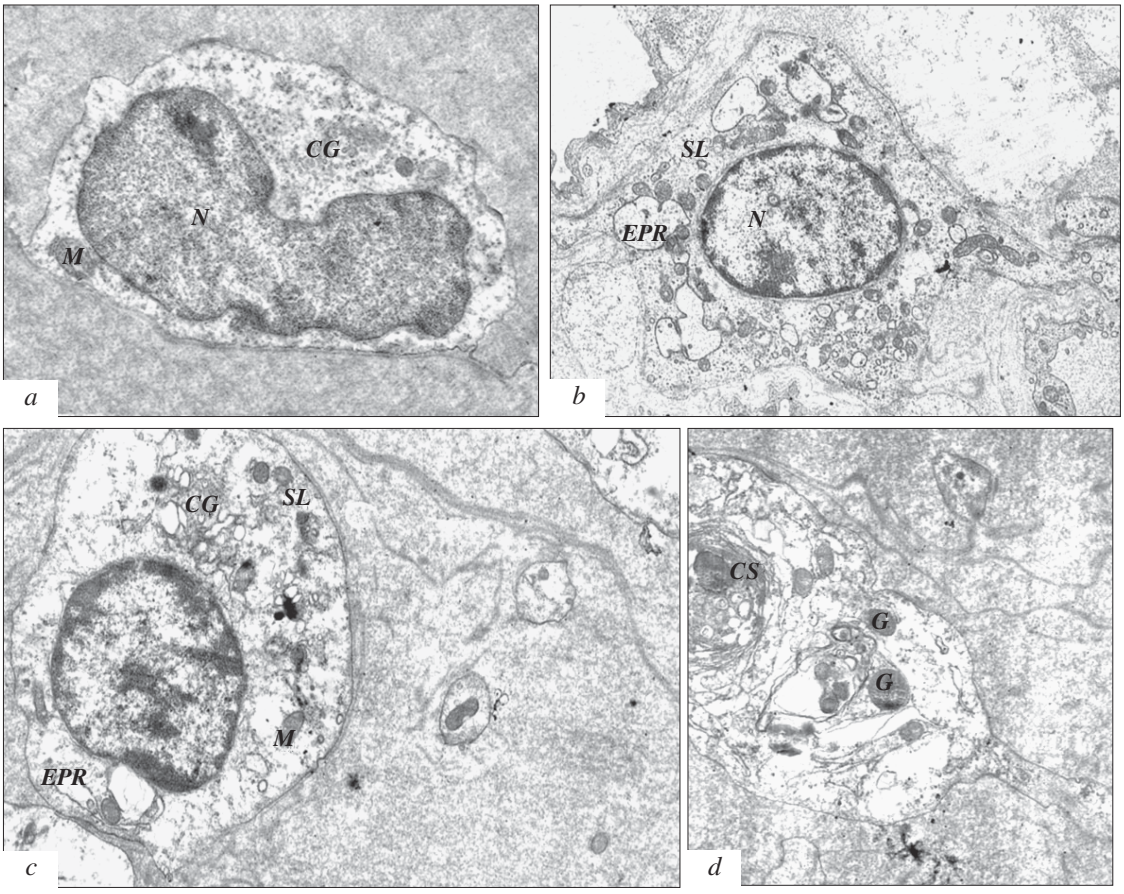

Fig. 3. IC in renal papilla of Brattlboro rats. *a*, *b*) control animals (IC1 and IC2, respectively); *c*, *d*) fragments of IC2 after desmopressin treatment. SL: secondary lysosomes; G: granules; CS: concentric structure; M: mitochondria; GC: Golgi complex; EPR: endoplasmic reticulum; N: nucleus. Double contrasting after Reynolds. Electronogram, magnification: *a*) ×4200; *b*) ×3500; *c*) ×4200; *d*) ×7000.

noted. The numerical density of free ribosomes and polysomes increased. Numerous vacuolar structures, non-coated and coated vesicles, were seen near the Golgi complex, which probably attested to enhanced synthesis of some VP-dependent proteins participating in modulation of water transport [14]. The surface density of the endoplasmic reticulum significantly decreased (Table 1), its profiles often looked like widened cisterns with light floccular content. The number of heteromorphic electron-dense large and small granules considerably decreased. There were cells with large osmiophilic granules, probably, secondary lysosomes, with signs of degradation. These cells often contained concentric structures including electron-dense granules, vacuoles with small dense inclusions, dark felt-like masses surrounded by several layers of endoplasmic reticulum profiles. The formation of these complexes can be a result of excessive transport of GAG from the extracellular matrix into the cell and their enhanced catabolism [10]. All hormone-induced changes in the structure of IC2 are most likely a reflection of changes in cell function. These changes include activation of protein synthesis (primarily, glycanohydrolases) involved in the increase in epithelium permeability, on the one hand, and inhibition of production of hyaluronan, an important component of the barrier reducing water reabsorption.

Thus, long-term administration of desmopressin to homozygous Brattleboro rats with genetic defect of vasopressin synthesis modulates the function of IC cells in the renal papilla, which leads to changes in the composition of the extracellular matrix increasing the permeability of the renal medulla interstitium in the zone of active water reabsorption.

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