Morphological Characteristics of the Inner Medullary Zone in the Kidneys of Brattleboro and Wistar Rats during Blockade of Prostaglandin Synthesis

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Morphological characteristics of the inner medullary zone in the kidneys in Brattleboro and Wistar rats were studied during blockade of prostaglandin synthesis. The absence of a diuretic effect of prostaglandins on the kidneys was accompanied by structural changes in the transepithelial and interstitial barriers for an osmotic flow of water.

Key Words: *hyaluronan; β-glucuronidase; diclofenac; collecting ducts*

The ability of mammalian kidney to concentrate the excreted fluid is determined by the presence of barriers for the flow of osmotically free water. They are presented by specialized dense epithelium and structures of the interstitial space, which mainly consists of hyaluronan. The neurohypophyseal hormone vasopressin (VP) is a major regulator of water-salt metabolism, which causes specific structural reconstruction of the epithelium in collecting ducts and typical changes in glycosaminoglycans (GAG) and interstitium of the renal medulla [4]. It contributes to reabsorption of osmotically free water. These changes are observed in the inner medullary zone of the kidney containing various structural elements for the concentrating mechanism. Previous studies showed that the hydroosmotic effect of VP is modulated by prostaglandin (PG) E_2 at various stages of hormonal signal transduction [10,12]. It was hypothesized that PG E_2 inhibits the response to VP due to modulation of cAMP level and intracellular Ca^{2+} concentration [5,6]. However, there is no general agreement on the reaction of structural elements of the osmotic concentration system under conditions of VP and PG E_2 interaction.

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Here we studied morphological characteristics of cells and composition of the interstitial space in the inner medullary zone (main barriers for the osmotic flow of water) during blockade of PG synthesis.

MATERIALS AND METHODS

Experiments were performed on adult Wistar rats and homozygous Brattleboro rats with hereditary hypothalamic diabetes insipidus [14]. Our study was conducted according to the requirements of the Helsinki declaration on animal's welfare. The rats of these strains were divided into 2 groups of 8 specimens each (control group; and experimental group, administration of sodium diclofenac in a dose of 0.1 mg/100 g twice a day for 2 days). The efficiency of osmotic concentration was evaluated from osmolality of the excreted urine (cryoscopic method; OMT-5-01 osmometer, Burevestnik).

Renal sections were examined under an Axioscop 40 light microscope (Carl Zeiss; ocular ×16, object lens ×100). The images were photographed using an AxioCam digital photographic attachment. A histochemical study was conducted at the level of the middle third of the renal papilla, which consists of structures for osmotic concentration. GAG were identified in paraffin sections of the kidney by the method of Hale

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with colloidal iron (blue staining of the Hale-positive material). β-Glucuronidase was identified by bright red staining in frozen sections of the kidney by the method of simultaneous azo coupling using naphthol-AS-BI-β-glucuronide (Sigma) as the substrate. The height of the epithelium and lumen of the collecting ducts were measured morphometrically using the samples stained by hematoxylin and eosin (AxioVision 4.5 software).

The significance of between-group differences was evaluated by Student's *t* test for independent samples and two-way analysis of variance (genotype and effect of the product as independent variables).

RESULTS

Homozygous Brattleboro rats with hypothalamic diabetes insipidus are characterized by polydipsia, polyuria [8], and excretion of hypotonic urine (123±11 mOsm/kg H_2O). In Wistar rats with normal function of the VP gene, low osmolality (comparable with that in Brattleboro rats, 195±29 mOsm/kg H_2O) was achieved due to dietary regimen. Blockade of PG synthesis in experimental animals led to an increase in osmotic concentration. These changes were more pronounced in VP-deficient animals: osmolality of excreted urine in these animals increased to 568±35 *vs*. 333±43 mOsm/kg H_2O in Wistar rats.

Diclofenac-induced activation of the osmotic concentration system was followed by structural reorganization of the inner medullary zone in the kidneys (Table 1). Blockade of PG synthesis in Brattleboro rats was followed by flattening of the epithelium; its height significantly decreased (compared to control animals) and the lumen of collecting ducts increased. On the contrary, in Wistar rats the lumen of collecting ducts significantly decreased, while the height of the epithelium increased. This reaction of the epithelium was probably related to changes in cytoskeletal components after blockade of the diuretic effect of PG E_2. Our results are consistent with published data that the transepithelial osmotic gradient is accompanied by changes in collecting duct cells of the inner medullary zone in the kidneys [3,11].

Osmotic flow of water in the kidney also depends on permeability of extracellular structures (glycocalyx and intercellular space around the elements of the concentrating mechanism). Hyaluronan plays an important role in the formation of this barrier for water diffusion. Hyaluronan serves as the major component of the extracellular matrix, which is produced by interstitial cells of the inner medullary zone and epitheliocytes of the collecting ducts. Histochemical analysis of hyaluronan in the kidney of control Brattleboro rats revealed no specific staining for GAG in the interstitium of the renal papilla (Fig. 1, *c*). These data are consistent with the results of previous experiments [1,9]. During blockade of PG synthesis, hyaluronan was identified in the glycocalyx and cytoplasm of interstitial cells (granules of the Hale-positive material; Fig. 1, *d*). Histochemical staining of GAG in the inner medullary zone of the kidney in control Wistar rats illustrates the presence of hyaluronan in the interstitial space (fiber-like structures; Fig. 1, *a*). Blockade of PG synthesis in rats with a normal function of the VP gene had no effect on staining of the Hale-positive material in the interstitium. Specific staining was revealed on the glycocalyx of collecting duct epitheliocytes (Fig. 1, *b*).

β-Glucuronidase serves a marker of the hyaluronate–hydrolase system, which plays a role in hydrolysis of extracellular GAG [13]. Histochemical analysis for β-glucuronidase revealed the presence of enzyme granules nearly in all structural elements of control Brattleboro rats. The number and size of

TABLE 1. Morphometric Characteristics of the Inner Medullary Zone in the Kidneys of Wistar and Brattleboro Rats (*M*±*m*)

Parameter	Wistar		Brattleboro	
	control	diclofenac	control	diclofenac
Height of the epithelium in the collecting ducts, μ	6.00±0.03	6.33±0.03***	6.53±0.05^{+++}	6.04±0.04***$^{+++}$
Lumen of the collecting ducts, μ	20.65±0.15	20.13±0.16*	13.53±0.14^{+++}	17.79±0.19***$^{+++}$
Number of β-glucuronidase granules in epitheliocytes, per cell	2.54±0.07	3.20±0.06**	6.82±0.29^{+++}	9.96±0.33***$^{+++}$
Number of β-glucuronidase granules in interstitial cells, per cell	5.88±0.27	5.38±0.27	10.35±0.81^{+++}	20.61±1.42***$^{+++}$

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to the control group from the same strain of rats; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 compared to the same group from another strain of rats.

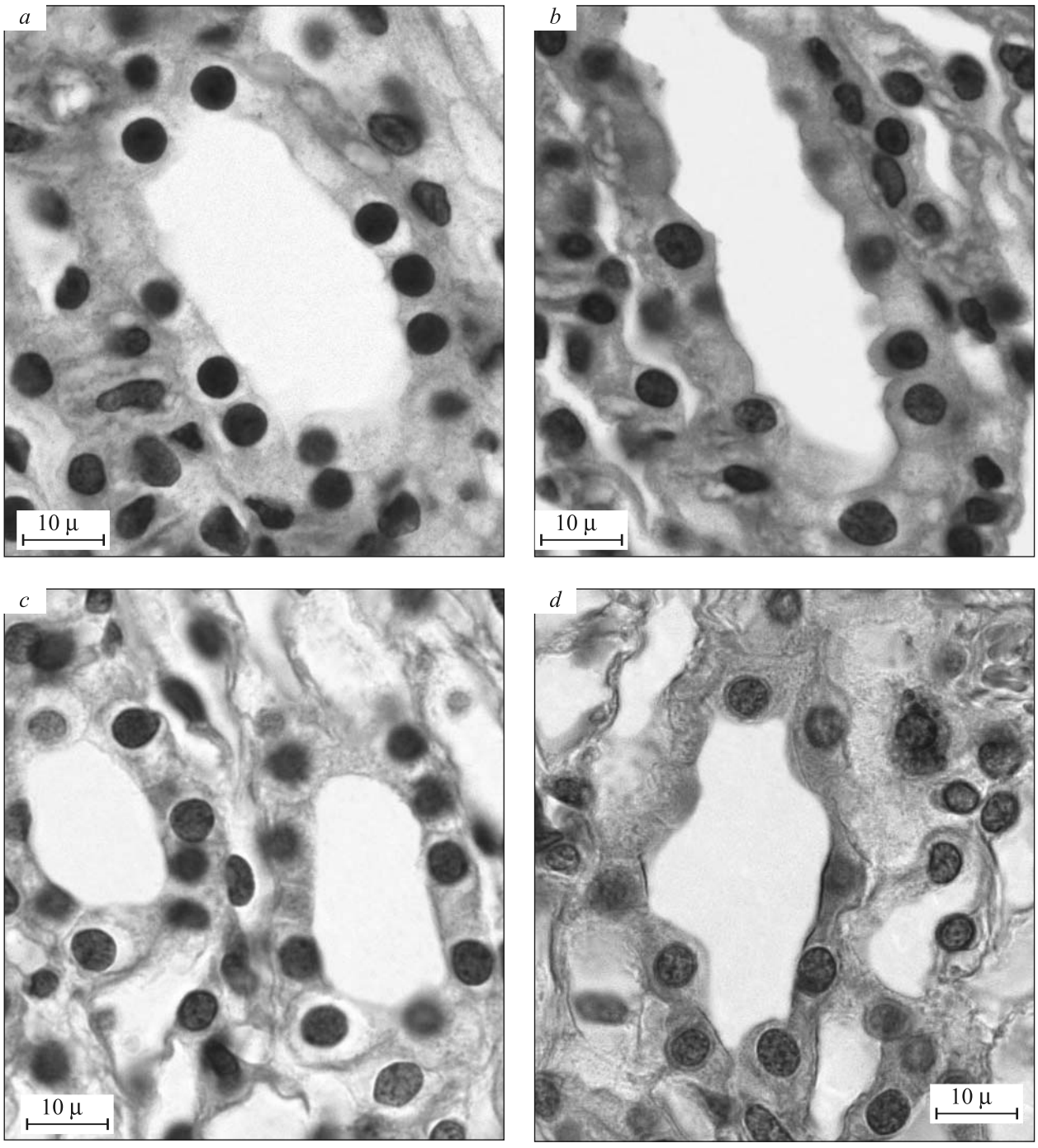

Fig. 1. Localization of hyaluronan in the middle third of the renal papilla in Wistar (*a*, *b*) and Brattleboro rats (*c*, *d*). Control (*a*, *c*) and treatment groups (sodium diclofenac; *b*, *d*). Staining by the Hale method; ×1600.

these granules varied significantly (Fig. 2, *c*; Table 1). According to the results of the histochemical study for β-glucuronidase, diclofenac administration was followed by an increase in the number of enzyme-containing granules in epitheliocytes of the collecting ducts and particularly in interstitial cells (Fig. 2, *d*; Table 1). Epitheliocytes of the collecting ducts and interstitial cells of the inner medullary zone in control Wistar rats contain a lower number of β-glucuronidase granules than those in control Brattleboro rats ($F_{(1,1799)}$ (epitheliocytes)=1375.13, $p<0.001$; $F_{(1,405)}$ (interstitial cells)=240.91, $p<0.001$; Fig. 2, *a*; Table 1). Blockade of PG synthesis in rats with normal neurohypophyseal function was followed by accumulation of enzyme granules in epitheliocytes. However, the amount of these granules remained unchanged in interstitial cells

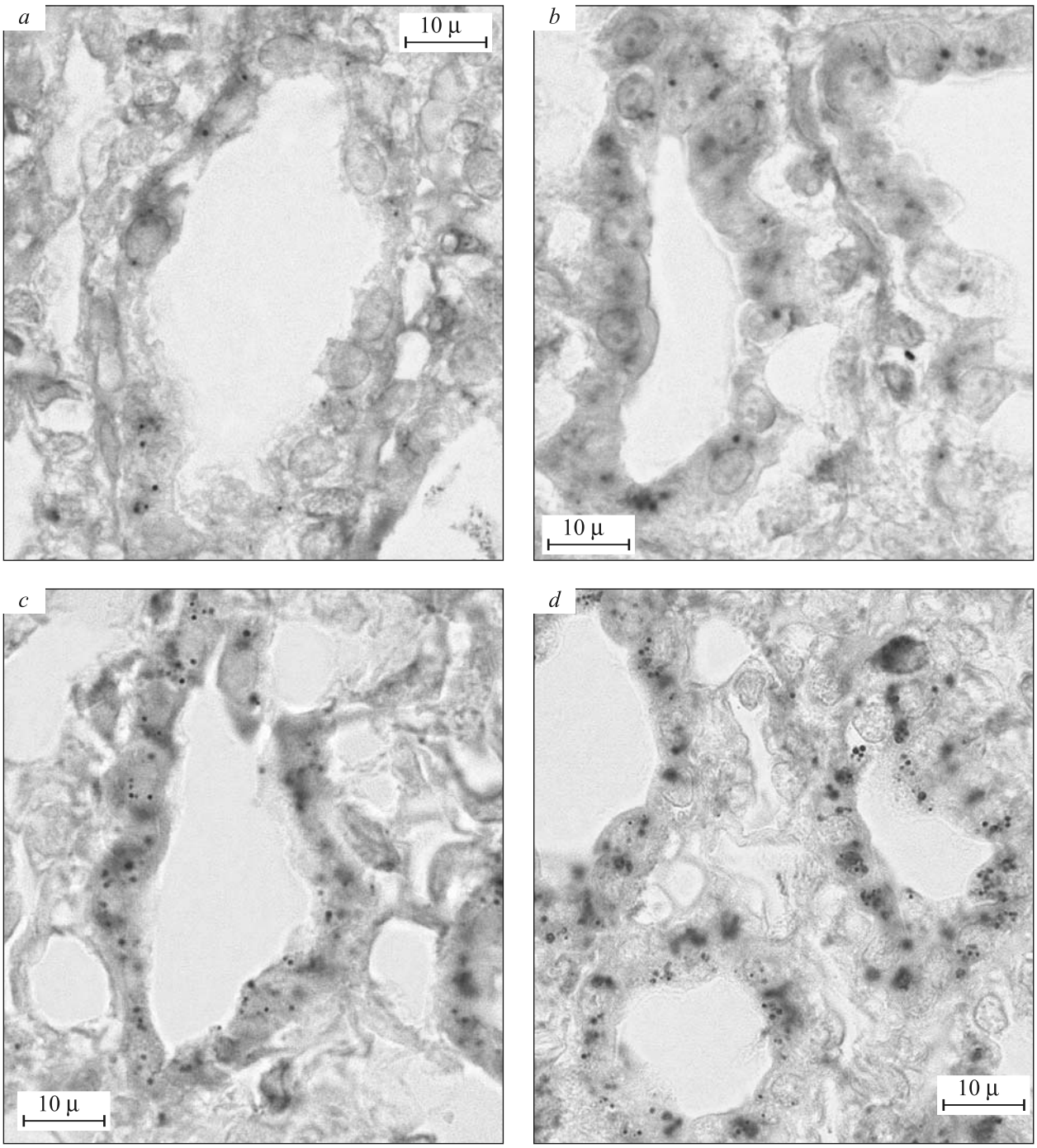

Fig. 2. Localization of β-glucuronidase on sections from the middle third of the renal papilla in Wistar (*a*, *b*) and Brattleboro rats (*c*, *d*). Control (*a*, *c*) and treatment groups (sodium diclofenac; *b*, *d*). Staining by the method of simultaneous azo coupling; ×1600.

(Fig. 2, *b*; Table 1). Comparison of β-glucuronidase distribution showed that this enzyme is accumulated in epitheliocytes of the collecting ducts in all experimental animals. The degree of these changes depended on neurohypophyseal function of animals ($F_{(1,1799)}$=69.31, p<0.001). Published data show that variations in the size of granules illustrate differences in the location of β-glucuronidase [2]. An increase in the number of large granules in various cells of the inner medullary zone of the kidney probably reflects a relatively high level of enzyme synthesis and its accumulation in the endoplasmic reticulum [7].

We conclude that the degree of structural changes in the inner medullary zone of the kidneys is different in rats with a normal function of the VP gene and VP-deficient animals after blockade of PG synthesis.

Hence, the absence of the diuretic effect of PG E_2 on the kidneys is accompanied by changes in the transepithelial and interstitial barriers for an osmotic flow of water.

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REFERENCES

1. L. N. Ivanova, V. A. Lavrinenko, A. V. Babina, and I. I. Khegai, *Byull. Eksp. Biol. Med*., **146**, No. 11, 580-584 (2008).
2. A. Abou-Haila, D. R. Tulsiani, M. D. Skudlarek, and M. C. Orgebin-Crist, *J. Androl*., **17**, No. 3, 194-207 (1996).
3. C. L. Chou, M. J. Yu, E. M. Kassai, *et al*., *Am. J. Physiol. Renal Physiol*., **295**, No. 1, F192-F201 (2008).
4. P. Hansell, V. Goransson, C. Odling, *et al*., *Kidney Int*., **58**, No. 5, 2061-2068 (2000).
5. N. Hatae, Y. Sugimoto, and A. Ichikawa, *J. Biochem*., **131**, No. 6, 781-784 (2002).
6. R. L. Hebert, H. R. Jacobson, and M. D. Breyer, *Am. J. Physiol*., **259**, No. 2, Pt. 2, F318-F325 (1990).
7. R. M. Lösel, E. Falkenstein, M. Feuring, *et al*., *Physiol. Rev*., **83**, No. 3, 965-1016 (2003).
8. M. Maghnie, *Horm. Res*., **59**, Suppl. 1, 42-54 (2003).
9. W. G. Mcauliffe, *Am. J. Anat*., **157**, No. 1, 17-26 (1980).
10. S. P. Nadler, J. A. Zimpelmann, and R. L. Hebert, *Am. J. Physiol*., **262**, No. 2, Pt. 2, F229-F235 (1992).
11. S. Nielsen, J. Muller, and M. A. Knepper, *Ibid*.., **265**, No. 2, Pt. 2, F225-F238 (1993).
12. Y. Sakairi, H. R. Jacobson, T. D. Noland, and M. D. Breyer, *Ibid*., **269**, No. 2, Pt. 2, F257-F265 (1995).
13. R. Stern, *Glycobiology*, **13**, No. 12, 105R-115R (2003).
14. H. Valtin, *Handbook of Physiology. Renal Physiology*, Vol. 11, Sect. 8, 1281-1316 (1992).