Structure and Concentration Capacity of the Kidneys in Brattleboro Rats under Conditions of Long-Term Vasopressin Treatment

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The function and histochemistry of the kidney were studied in homozygous Brattleboro rats after 28-day treatment with exogenous arginine-vasopressin. The long-lasting effect of the hormone includes normalization of osmotic concentration, decrease in the number of β-glucuronidase granules in the medulla, and increasing content of hyaluronan in the interstitium. These changes promote physiological optimization of the antidiuretic reaction to vasopressin.

Key Words: *Brattleboro rats; arginine-vasopressin; β-glucuronidase; hyaluronan*

Genetically determined defect of vasopressin (VP) synthesis in homozygous Brattleboro rats manifests in polydipsia, polyuria, and essential changes in the morphology of the renal medulla [10]. Polyuria and hypotonia of the excreted urine are caused by impaired reabsorption of osmotically free water in the renal tubules and directly result from the absence of endogenous VP regulating cell membrane permeability in epithelium of collecting tubules. It was hypothesized that hyaluronic acid (HA), the main nonstructural component of the extracellular matrix, is involved in the regulation of water transport between the tubular structures and vessels of the concentration mechanism [5]. Homozygous Brattleboro rats are characterized by significantly reduced content of HA in the medullary zone of the kidney. The cause of this phenomenon remains unclear. It was shown that treatment with VP (desmopressin), a V_2 receptor agonist, for 4 days increased osmolarity of the excreted urine and induced VP-specific changes in epitheliocytes of collecting tubules, but had virtually no effect on staining of interstitial HA [1]. Interstitial cells are HA producers in the renal medulla [6]. Microautoradiography of the renal tissue sections revealed the presence of V_1, but not V_2 receptors of VP on renomedullary interstitial cells [9]. Later this observation was confirmed for interstitial cell culture [6]. It remained unclear whether low content of glycosaminoglycans (GAG) in the interstitium of the renal medulla in homozygous Brattleboro rats was directly caused by the absence of endogenous VP.

We studied the process of osmotic concentration, activity of β-glucuronidase (exohydrolase involved in final stages of HA metabolism), and interstitial HA status of the renal medulla under conditions of long (28 days) treatment with arginine-VP realizing its effect through V_2 and V_1 receptors in VP-deficient Brattleboro rats.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old male Brattleboro rats lacking endogenous VP due to deletion in the gene encoding VP [10]. The animals were divided into 3 groups, 5 per group. A 2ML4

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osmotic mini-pump (ALZET) releasing 125 pmol synthetic arginine-VP (Sigma) per hour was implanted subcutaneously in the scapular area to experimental animals narcotized with ether. This mini-pump is intended for 28 days of continuous work [3]. The presence of the hormone in the blood was evaluated by osmolarity of the released urine, which was measured on an MT-2 osmometer (Burevestnik Firm), and by the volume of daily consumed water [2]. Empty plastic capsules were implanted to control rats. Urinary specimens were collected throughout the entire experiment.

Histochemical studies were carried out at the level of the middle third of the renal papilla containing the structures responsible for osmotic concentration. In order to detect β-glucuronidase, the tissue specimens were fixed in calcium-formol for 24 h at 4°C and impregnated with cold gum sucrose solution. Frozen sections were stained at 37°C by the method of simultaneous azocoupling. Naphthol-AS-BI-β-glucuronide served as the substrate. The reaction was evaluated by bright red staining developing as a result of diazo stain precipitation in areas of enzyme concentration. For histochemical analysis of GAG, the papillary tissue specimens were fixed in 10% neutral formalin for 48 h, dehydrated, and embedded in paraffin. Identification of GAG in paraffin sections was carried out with colloid iron after Hale.

The significance of differences in the functional and morphological parameters between different groups was evaluated using Student's *t* test.

RESULTS

Intact homozygous Brattleboro rats lacking endogenous VP were characterized by low osmolarity of the excreted urine; daily water consumption in this group of rats surpassed 70% body weight (Table 1). Control rats did not differ from intact rats by this parameter. In experimental group, the osmolarity of excreted urine increased significantly and water consumption decreased 28 days after implantation of VP mini-pump. These values corresponded to those in rats with normal VP gene and in Brattleboro rats under conditions of long-term hormone treatment [10].

Structural reorganization of epitheliocytes corresponded to the observed functional changes (Table 1). The epithelium was flattened and its height significantly decreased in experimental rats in comparison with intact and control rats; the lumen of collecting tubules also decreased. One of the most important distinctive morphological features of the kidneys in Brattleboro rats in comparison with heterozygous and normal animals is virtually complete absence of GAG-specific staining in the renal papilla interstitium, which corresponded to decreased content of HA and sulfated GAG in the medullary tissue. The results of histochemical studies of HA in the kidneys of intact and control rats were similar to our previous data, indicating the absence of Hale-positive structures in the renal papillary interstitium (Fig. 1, *a*, *b*); rare large granules were seen in just few interstitial cells. Long-term treatment with arginine-VP revealed a clear-cut increase of histochemically detected GAG in the renal medulla in all examined animals of this group (Fig. 1, *c*). Homogenous Hale-positive material was detected in the interstitium and in the form of numerous granules in interstitial cells (Fig. 1, *d*). No fibrous structures and compact accumulations characteristic of heterozygous Brattleboro and normal Wistar rats were detected in the interstitium, which presumably reflected low polymeric status of HA. Histochemical staining for β-glucuronidase in intact and control rats revealed similar distribution of azo stain deposition in sections of the middle zone of the renal medulla (Fig. 2, *a*, *b*). Granules, varying by the number and size, depending on the cell type, were seen in virtually all structural elements. It was hypothesized that small peripheral granules corresponded to the lysosomal location of the enzyme, while large ones indicate the presence of the substratum in the endoplasmic reticulum [8]. Long treatment with arginine-VP was associated with a reduction of the total number of granules and redistribution of β-glucuronidase fractions: the granules

TABLE 1. Functional and Morphometric Characteristics of the Kidney in Brattleboro Rats (*M*±*m*)

Parameter	Group		
	intact	control	experimental
Daily water consumption, % body weight	74±10	68±9	11±2**
Urine osmolarity, mOsm/liter	144±8	165.2±9.4	1660.1±81.8**
Height of collecting tubular epithelium, μ	6.4±0.1	6.5±0.1	5.90±0.04*
Collecting tubule lumen, μ	13.8±0.2	14.2±0.1	16.8±0.2**

Note. *$p<0.01$, **$p<0.001$ compared to intact group.

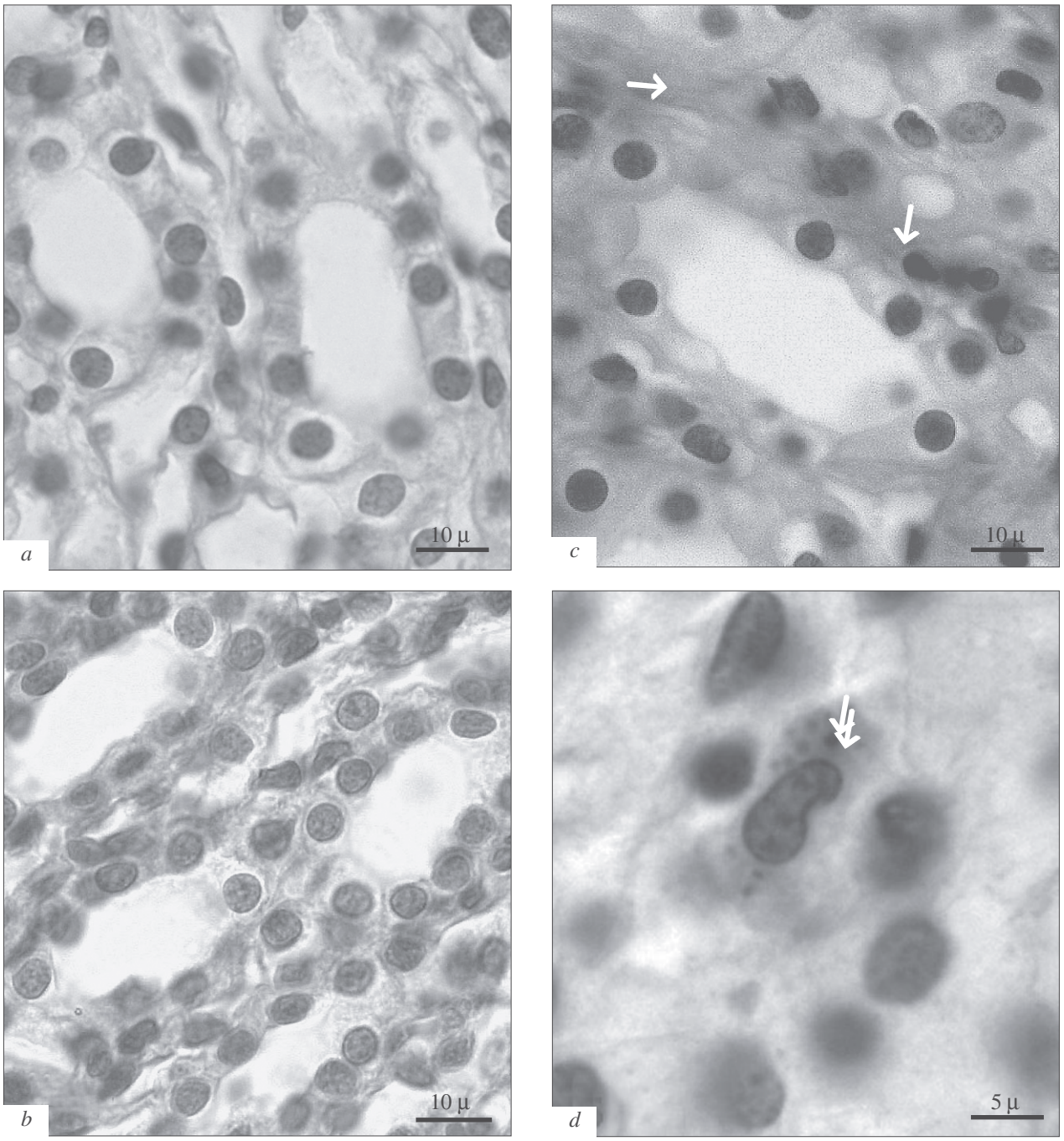

Fig. 1. Localization of HA in the middle third of the kidney in Brattleboro rats. *a*) intact; *b*) control; *c*) experimental group; *d*) GAG in an interstitial cell in the middle third of the renal papilla in experimental animals. Arrow: GAG in interstitium; double arrow: GAG granules in interstitial cell. Hale staining, ×1600.

in epitheliocytes migrated mainly into the lateral zone, while compactization and aggregation of granules around the nucleus was seen in few interstitial cells (Fig. 2, *c*). Changes in HA localization and staining intensity of β-glucuronidase can be regarded as a reflection of activation of lysosomal glycanohydrolases and exocytosis into the interstitium. Similar changes in β-glucuronidase localization were observed in the kidney of homozygous Brattleboro rats during 4-day treatment with desmopressin (VP V_2 receptor agonist) [1]. Activity of HA hydrolysis enzymes in homozygous Brattleboro rats is directly regulated by VP, and activities of endo- and exohydrolases (including β-glucuronidase) closely correlate with urine osmolarity reflecting the antidiuretic effect of VP. Experiments on Brattleboro rat renal

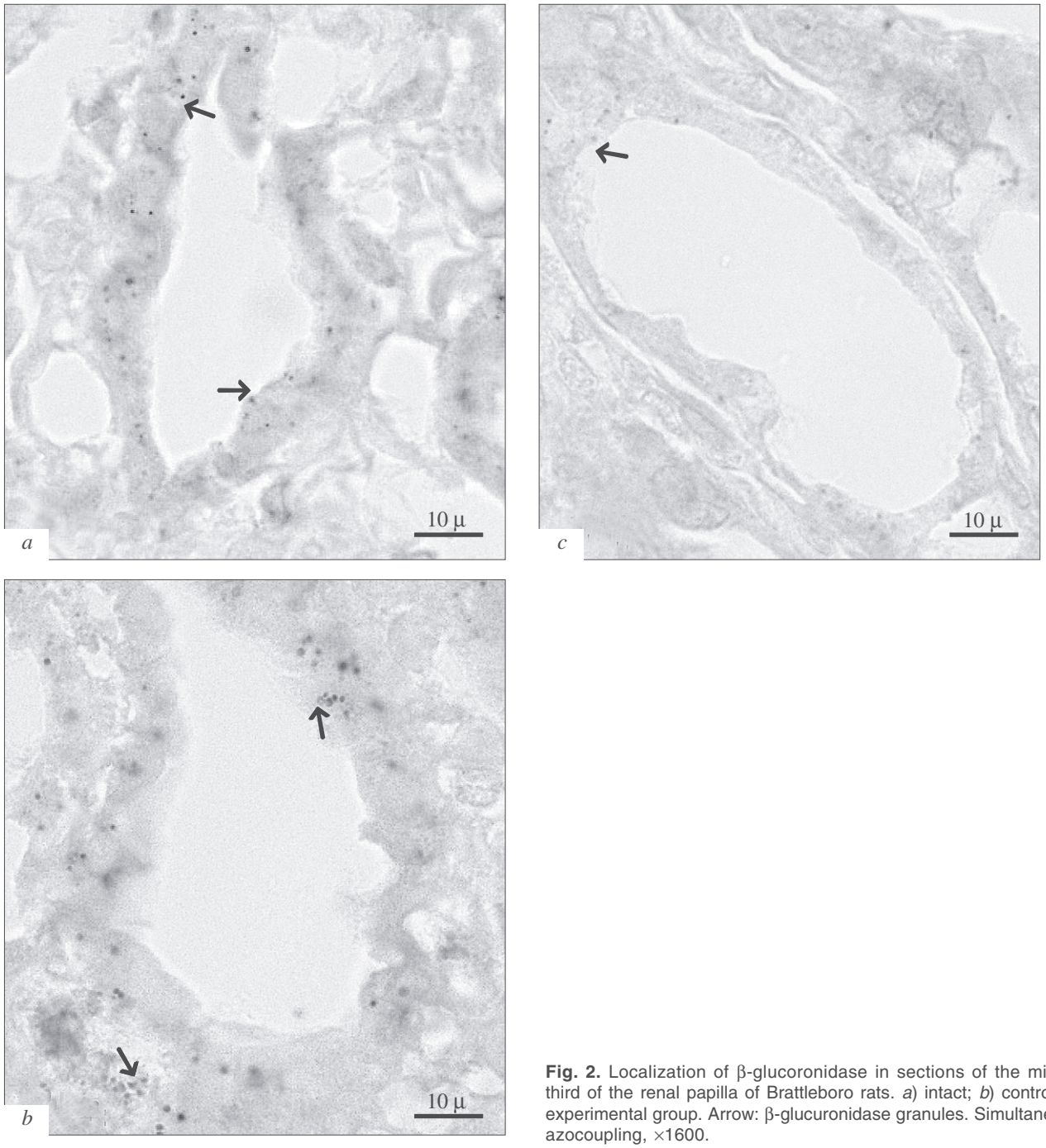

Fig. 2. Localization of β-glucoronidase in sections of the middle third of the renal papilla of Brattleboro rats. *a*) intact; *b*) control; *c*) experimental group. Arrow: β-glucuronidase granules. Simultaneous azocoupling, ×1600.

papillary cell suspension and isolated amphibian urinary bladders showed [7] that VP treatment was associated with not only activation of hyaluronate hydrolases, but also with exocytosis of enzymes into the incubation medium. Hence, our new study confirmed the hypothesis on the involvement of lysosomal hyaluronate hydrolases in the VP hydroosmotic effect.

Long-term treatment of Brattleboro rats with arginine-VP was associated with not only activation of lysosomal hyaluronate hydrolases (β-glucuronidase served as an indicator of their status), but also with accumulation of HA in the renal medullary interstitium. The content of HA in the renal interstitium depends on activity of its biosynthesis in the interstitial cells, hyperosmolarity served as a factor stimulating this process [4]. In the present study, the significant increase in osmolarity of released urine under the effect of VP (Table 1) was presumably directly related to the increase in osmotic gradient in the medulla. This factor probably stimulated HA biosynthesis. However, we cannot rule out activation of hyaluronate synthetase, the main en-

zyme of HA biosynthesis, by VP through V_1 receptors. The pattern of HA staining seems to indicate its low polymeric status, presumably because of simultaneous activation of hyaluronate hydrolase system. Detection of the key factor of HA biosynthesis necessitates further studies, while the decrease in its content in the renal medulla of VP-deficient homozygous Brattleboro rats seems to be caused by low level of HA biosynthesis under conditions of high activities of enzymes of its catabolism.

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