MORPHOLOGY AND PATHOMORPHOLOGY

Structural and Functional Changes in Epitheliocytes of Collecting Tubes in Renal Papilla of Brattleboro Rats Treated with Vasopressin

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The functional response of the kidney to desmopressin and morphological changes in epitheliocytes of collecting tubes were studied on homozygotic Brattleboro rats. Redistribution of β-glucuronidase fractions and increase in the number of osmiophilic granules reflecting increased production of vasopressin-dependent proteins and hyaluronate hydrolase exocytosis were typical structural correlates of the effect of vasopressin.

Key Words: *Brattleboro rats; desmopressin; β-glucuronidase; osmiophilic granules*

Vasopressin (VP), a neurohypophysial hormone, plays the main role in regulation of the renal concentrating function in mammals. The antidiuretic effect of VP manifests in restructuring of the collecting tube epithelium and characteristic changes in the interstitial glycosaminoglycans of the renal medulla [2,6]. Brattleboro rats with hereditary defect of VP synthesis have no endogenous hormone in the homozygotic state [13]. This model is widely used for evaluation of the molecular mechanisms of VP effect [14]. However, published data on the structural changes of the kidney in Brattleboro rats under the effect of VP are scanty. One of the most important morphological features of the kidneys in homozygotic Brattleboro rats, in contrast to heterozygotic and normal rats, is virtually complete absence of glycosaminoglycan-specific staining in the papillary interstitium [8], which corresponds to a sharp reduction in the content of hyaluronic acid and sulfated glycosaminoglycans in the medullary tissue [4], but activity of hyaluronate hydrolases (HH) metabolizing glycosaminoglycans is high in this zone. Extracellular hyaluronic acid is hydrolyzed by type 2 hyaluronidase fixed on the cell plasma membrane to large fragments, which are then internalized via receptor-mediated endocytosis and further hydrolyzed by type 1 hyaluronidase and exohydrolases (β-glucuronidase and β-N-acetate glucosaminidase) in lysosomes to di- and trisaccharides [11]. Exohydrolase β-glucuronidase participating in the final stages of glucosaminoglycane hydrolysis can serve as an indicator of the state of intracellular hyaluronate hydrolase system. Since this enzyme is well detected by histochemical methods, we compared functional response of the kidneys to VP in homozygotic Brattleboro rats with histochemical and ultrastructural changes in the principal cells of collecting tubes in the renal medullary zone.

MATERIALS AND METHODS

Experiments were carried out on adult homozygotic Brattleboro rats from Institute of Cytology and

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Genetics. The rats were divided into 2 groups: 1) controls and 2) animals intraperitoneally injected with desmopressin (VP; synthetic V_2 receptor agonist) in a dose of 20 μg/100 g for 4 days.

Urinary samples were collected from all animals before the experiment and before decapitation and the concentrations of osmotically active substances were measured. After experiment the control and experimental animals were decapitated, morphological studies were carried out at the level of the middle third of the renal papilla. Renal tissue for histochemical detection of β-glucuronidase was fixed in 10% formalin with calcium chloride for 24 h on cold and treated with gum sucrose at 4°C. Frozen sections were stained (at 37°C) by the method of simultaneous azo coupling using naphthol-AS-BI-β-glucuronide as the substrate. The reaction was evaluated by bright-red staining developing as a result of precipitation of the diazo dye at sites of enzyme accumulation. For ultrastructural studies the tissues were fixed in 3% glutaraldehyde in PBS (pH 7.4, fixative osmolarity corresponded to that of animal urine) and postfixed in 1% OsO_4. Tissue fragments were then dehydrated in ascending alcohols, acetone, and embedded in epon and araldite (6:1) mixture. The sections were made on a Tesla-100A ultramicrotome. Ultrathin sections were stained by the double contrast method with uranyl acetate and lead citrate after Reynolds.

RESULTS

Control homozygotic Brattleboro rats lacking endogenous VP were characterized by polydipsia and polyuria (the volume of drunk and excreted fluid reached 50-60% body weight; Fig. 1, *a*). Excreted urine was hypotonic because of low level of reabsorption of osmotically free water. Light microscopy and ultrastructural analysis of the principal cells of the collecting tube epithelium in the inner medullary zone of the kidney in Brattleboro rats showed morphological features (Fig. 1, *a-c*) characteristic of kidneys in hydrated normal rats and perfused fragments of collecting tubes in hyposmotic medium [3]. The epithelium of collecting tubes was presented by cubical cells with rare microvilli on the apical surface. Numerous contacts of lateral epitheliocyte membranes with multiple interdigitations were seen; intercellular spaces were absent. Cytoskeleton elements (microtubules and microfilaments) were found in the subapical zone. The presence of the terminal network in the perimembrane zone of principal cells prevents incorporation of water channel-carrying vesicles (aquaporines) into the apical membrane [10]. Numerous vesicular and vacuolar structures with coated and smooth-wall membranes were detected in the cytoplasm. Clathrin vesicles contained aquaporines [12]. The status of the nucleus, mitochondria with hydrated matrix, few organelles, and rarely seen osmiophilic granules confirm inhibition of synthetic processes. Significantly extended profiles of granular endoplasmic reticulum with light floccular contents were often seen, indicating accumulation of HH, which was confirmed by histochemical findings.

β-Glucuronidase was detected in virtually all principal cells of collecting tubes in the form of numerous stained small and large granules, their number and size varying in different cells (Fig. 3, *a*). Variations in the size of the granules can indicate different location of the enzyme. Presumably, small granules correspond to lysosomal location of the enzyme, while large granules are connected to the endoplasmic reticulum [7]. This distribution of β-glucuronidase can be explained by a variety of its functions. Enzyme heterogeneity of lysosomes,

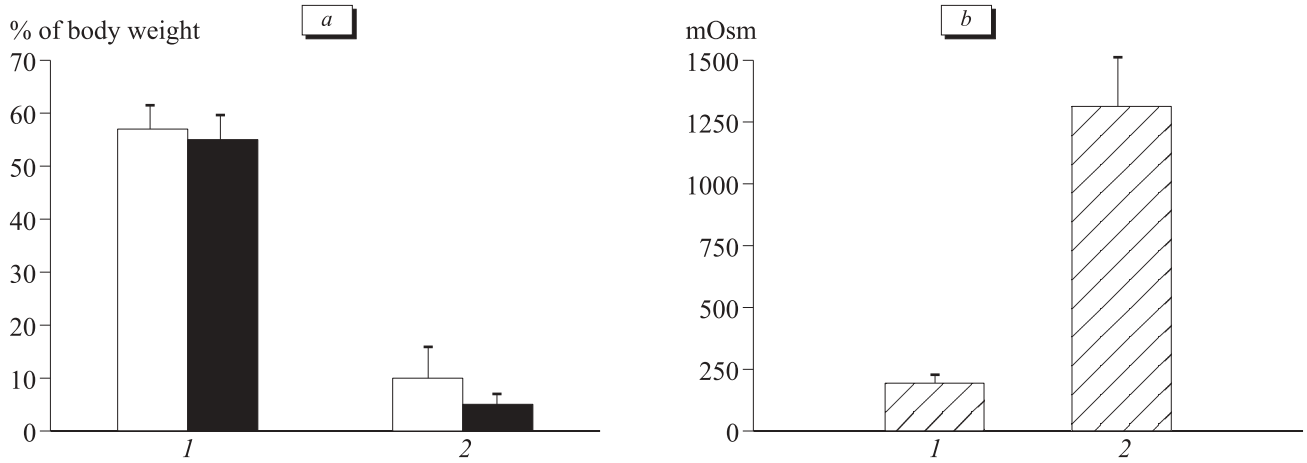

Fig. 1. Effect of DP on the content of consumed (light bars) and excreted (dark bars) fluid (*a*) and urine osmolarity (*b*). *1*) control: *2*) DP treatment.

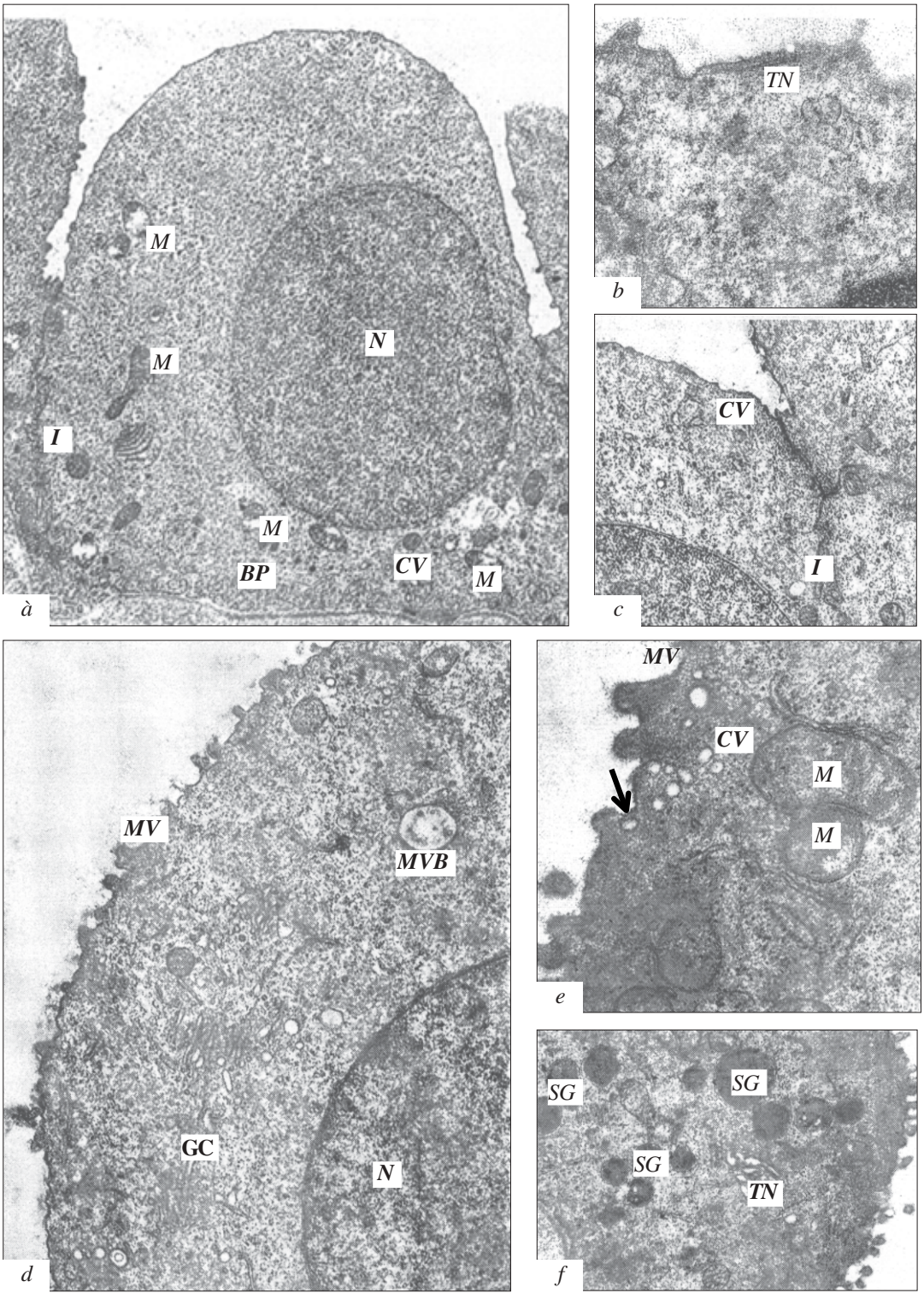

Fig. 2. Principal cells of collecting tubes in renal papilla of Brattleboro rats. *a-c*) intact animals; *d-f*) DP treatment. *N*: nucleus; *GC*: Golgi complex; *M*: mitochondria; *I*: interdigitations; *BP*: basal plication; *CV*: clathrin vesicles; *MV*: microvilli; *MVB*: multivesicular body; *SG*: secretory granules; *TN*: terminal network; arrow shows aquaporine incorporation. Staining by Reynolds' double contrasting. Electronogram. *a*) ×6700; *b*) ×18,700; *c*) ×33,600; *d*) ×21,000; *e*) ×28,300; *f*) ×10,000.

related to the time course of their transition from primary to secondary lysosomes, is also possible. The appreciable number of granules containing β-glucuronidase probably attests to high activity of this enzyme, which is in line with previous data according to which activity of β-glucuronidase in

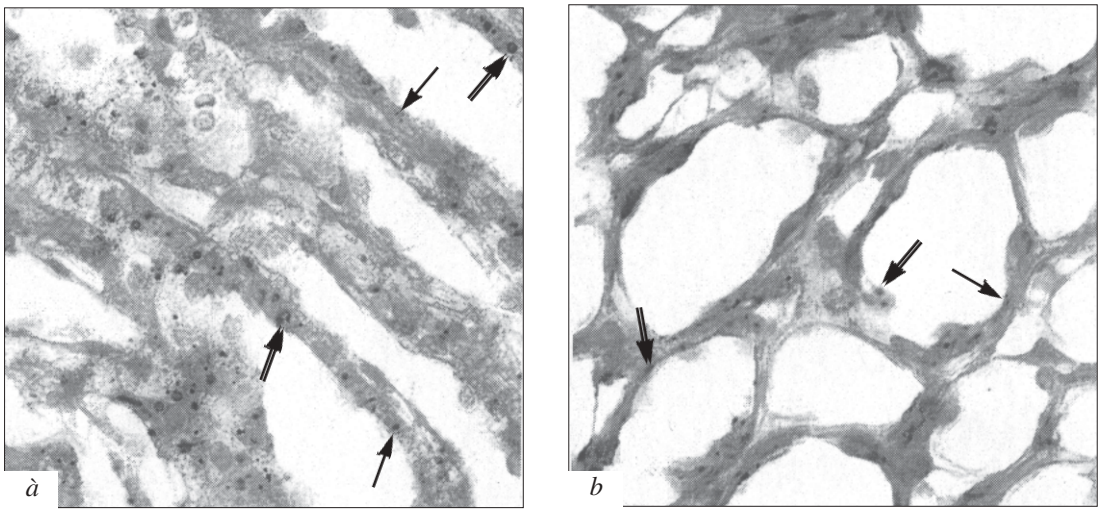

Fig. 3. Location of β-glucuronidase in collecting tubes of the middle third of Brattleboro rat renal papilla. *a*) intact animals; *b*) DP treatment. Arrow shows the lysosomal fraction, double arrow the membrane fraction. Simultaneous azo coupling method, ×1250.

the medulla of intact homozygotic Brattleboro rats is high and similar to that in heterozygotic Brattleboro and normal Wistar rats [1].

Treatment with DP intensively stimulated reabsorption of osmotically free water and, hence, induced antidiuresis and increased urine osmolarity to a maximum level; polydipsia disappeared (Fig. 1, *b*). These changes were associated with characteristic morphological restructuring of collecting tube principal epithelial cells: the height of these cells decreased from 0.270±0.002 to 0.24±0.05 mm ($p<0.05$), the diameter of the collecting tube increased from 0.70±0.02 to 0.94±0.03 mm ($p<0.05$; Fig. 2, *d-f*). Changes in the principal cells, typical of antidiuretic reaction and described for dehydrated animals, were detected at the ultrastructural level: flattening of epithelial cells, condensation of intracellular matrix due to appearance of numerous organelles, extension of cell-cell spaces. The apical membrane was covered with long thickened microvilli, there were numerous plasmalemma invaginations, covered with clathrin proteins on the cytoplasmic side. The terminal network structures were poorly seen or not detected, which presumably indicated depolarization of the terminal network F-actin [10] due to inactivation of GTP-bound Rho protein [12]. Disorganization of the terminal network facilitates translocation of clathrin vesicles to the apical membrane [9]. This process is supported not only by the cytoskeleton interactions with vesicles, but also by the release of intracellular Ca^{2+} [15]. The nucleus contained active nucleolus and parietal heterochromatin. Active state of Golgi complex and numerous vesicular smooth and protein-coated structures detected in the Golgi complex and apical part of the cell, presumably indicate increased protein production, because VP stimulates expression of genes encoding not only aquaporines, but also many other proteins [14].

A special feature of the reaction of renal collecting tube principal cells in homozygotic Brattleboro rats was the appearance of numerous granular structures in the cytoplasm. Large and small osmiophilic granules, heterogeneous by their contents, primary lysosomes, lipid incorporations, and multivesicular structures were seen.

In animals receiving DP, staining of sections for β-glucuronidase revealed decreased granularity and disappearance of the greater part of large granules (Fig. 3, *b*). Comparison with ultrastructural findings showed that granularity detected by histochemical methods was not identical to osmiophilic granules appearing under the effect of DP. It seems that the increase in the number of osmiophilic granules in principal cells is associated with stimulation of synthesis of aquaporines and other VP-dependent proteins, while disappearance of large granules containing β-glucuronidase reflects activation of this enzyme and its release from the cells into the interstitium. We previously showed that activation of hyaluronane hydrolysis enzymes is directly regulated by VP and detected a strict correlation between endo- and exohydrolase (including β-glucuronidase) activities and increase in urine osmolarity reflecting the antidiuretic effect of VP. Experiments on suspension of Wistar rat renal papilla and isolated amphibian urinary bladders showed that VP treatment led to not only activation of HH, but also to their exocytosis [5].

Hence, the data indicate the basic similarity of structural changes in the principal cells of collecting tubes under the effect of VP in homozygotic Brat-

tleboro rats with genetic defect of VP synthesis and the findings in rats with normal VP level. On the other hand, the absence of endogenous VP in Brattleboro rats helps to detect the morphological correlates of the effect of the hormone on protein synthesis and redistribution of lysosomal enzyme β-glucuronidase. The signs of β-glucuronidase activation and exocytosis seem to indicate the involvement of glycano-hydrolases in the metabolism of renal papillary interstitial hyaluronane and their role in the transport of water induced by VP.

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