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Brief Report

Glomerulonephritis and sodium retention: enhancement of Na^+/K^+-ATPase activity in the collecting duct is shared by rats with puromycin induced nephrotic syndrome and mice with spontaneous lupus-like glomerulonephritis

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Abstract

Background. In rats with puromycin aminoglucoside-induced (PAN) nephrotic syndrome, micropuncture studies have localized the site of sodium retention to the collecting duct. We have confirmed this finding by demonstrating a two-fold increase in Na^+/K^+-ATPase activity specifically limited to the cortical collecting duct in PAN rats. To further define whether this phenomenon was dependent on the chemical induction of the nephrotic syndrome or was a general phenomenon observed in glomerulonephritis, we measured Na^+/K^+-ATPase activity in nephron segments from mice with spontaneous lupus-like nephritis.

Methods. Hydrolytic activity of Na^+/K^+-ATPase was measured in three isolated nephron segments: proximal convoluted tubule, thick ascending limb and cortical collecting duct. The Na^+/K^+-ATPase activities were measured in PAN rats, sham-injected controls, and in (MRL × BXSB) F1 male mice which develop a well established spontaneous lupus-like glomerulonephritis by 4 months of age and their controls. Control mice have the same genetic background, but lack the Yaa mutant gene responsible for autoimmune acceleration and are free of glomerular lesions at 4 months of age.

Results. In (MRL × BXSB) F1 male mice, Na^+/K^+-ATPase was similar to control mice in the proximal convoluted tubule and the thick ascending limb. In contrast, cortical collecting duct Na^+/K^+-ATPase activity was two times higher in (MRL × BXSB) F1 mice than controls. These results were identical to those observed in PAN rats compared to their sham-injected controls studied 7 days after an intraperitoneal injection of puromycin or isotonic saline, respectively.

Conclusions. Enhancement of Na^+/K^+-ATPase activity localized to the cortical collecting duct is a general characteristic of glomerulonephritis independent of its mode of induction, i.e. chemical *versus* autoimmune. Therefore, the experimental model of PAN is suitable to study the underlying mechanisms leading to Na^+/K^+-ATPase dysfunction.

Key words: adenosine triphosphatase; nephron segments; proteinuria

Introduction

Glomerular diseases with or without nephrotic syndrome, are most often accompanied by sodium retention and/or a urinary concentrating defect. Puromycin aminoglycoside-induced (PAN) nephrotic syndrome is one of the most extensively studied models of glomerulonephritis in rats. This experimental model mimics the minimal change disease glomerulopathy found in human pathology. The characteristic lesion in both the experimental model and human disease consists of vacuolation and flattened foot processes of podocytes [1]. In addition to glomerular lesions, functional alterations of tubular transport have been demonstrated in puromycin-treated animals. A defect in osmotic equilibration across the collecting duct has been recognized along with an 87% decrease in aquaporin-2 expression and a 70% decrease in aquaporin-3 expression in normal-appearing cells of the inner medullary collecting duct as determined by transmission electron microscopy [2]. Micropuncture studies have shown that sodium reabsorption is specifically increased in the collecting duct while there is no change in the other segments of the nephron [3]. We have confirmed this finding by demonstrating an enhancement of Na^+/K^+-ATPase activity as expressed by an increase in the enzyme turnover limited to the collecting duct [4,5].

The aim of this study was to define whether the increase in Na^+/K^+-ATPase activity observed in the cortical collecting duct of rats with PAN nephrotic syndrome was specific to this experimental condition

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or a general phenomenon occurring in glomerular diseases associated with sodium retention. To answer this question we measured Na^+/K^+-ATPase activities in isolated nephron segments from mice with spontaneous lupus-like glomerulonephritis. The results obtained in mice confirm our previous observation in PAN nephrotic rats. That is, an isolated increase in Na^+/K^+-ATPase activity in the cortical collecting duct is present in these mice. Thus, enhancement of the cortical collecting duct capacity for sodium reabsorption demonstrated in experimental models appears to be independent of the model studied and may be also relevant for the pathophysiology of sodium retention in human glomerulonephritis.

Materials and methods

Puromycin aminoglycoside-induced nephrotic syndrome model

Male wistar rats weighing 100–130 g received a single intraperitoneal injection of 1 ml of a NaCl 0.9% solution with or without 15 mg/100 g body weight of puromycin aminoglycoside. Injection of puromycin aminoglycoside results in the developement of nephrotic syndrome (PAN) within 1 week. Animals were studied at day 7 following injection, when proteinuria and salt retention have reached their highest values [4,5].

Spontaneous lupus-like glomerulonephritis model

Lupus prone BXSB mice bearing a mutant gene, Yaa (Y-linked autoimmune acceleration), and MRL mice were purchased from Olac laboratory, Oxon, UK. Yaa − mice, lacking the Yaa gene, were developed in the laboratory of S. Izui by backcross procedure, and established at the twelfth backcross generation as previously described [6]. (MRL × BXSB) F1 and (MRL × BXSB. Yaa −) F1 hybrid mice were generated by local breeding. The development of a lethal form of lupus-like nephritis is markedly accelerated in (MRL × BXSB) F1 male mice bearing the Yaa mutation (50% mortality rate due to lupus nephritis at 8 months) as compared to (MRL × BXSB Yaa −) F1 male mice lacking the Yaa mutation (50% mortality rate at more than 24 months) [6]. At 4 months, mice possessing the Yaa mutant gene had developed a severe diffuse proliferative glomerulonephrits (Figure 1B) whereas mice lacking the Yaa mutant gene still exhibited normal glomerular structure (Figure 1A).

Isolation of single segments of the nephron

Under anaesthesia (pentobarbital 5 mg/100 g body weight, i.p.), the left kidney from both rats and mice were perfused through a catheter inserted in the aorta with a dissection solution containing: 137 mM NaCl, 5 mM KCl, 8 mM $MgSO_4$, 0.33 mM NaH_2PO_4, 1 mM $MgCl_2$, 10 mM Tris—HCl, 1 mM $CaCl_2$, pH 7.40 and 0.18% (wt/vol) collagenase (from *Clostridium histolyticum*, 0.87 U/mg, Serva Feinbiochemica). After perfusion, the kidney was immediately removed and sliced into small pyramids, which were incubated at 30°C for 20 min in aerated dissection solution containing 0.09% (wt/vol) collagenase. Pyramids were then thoroughly rinsed in ice cold dissection solution and stored

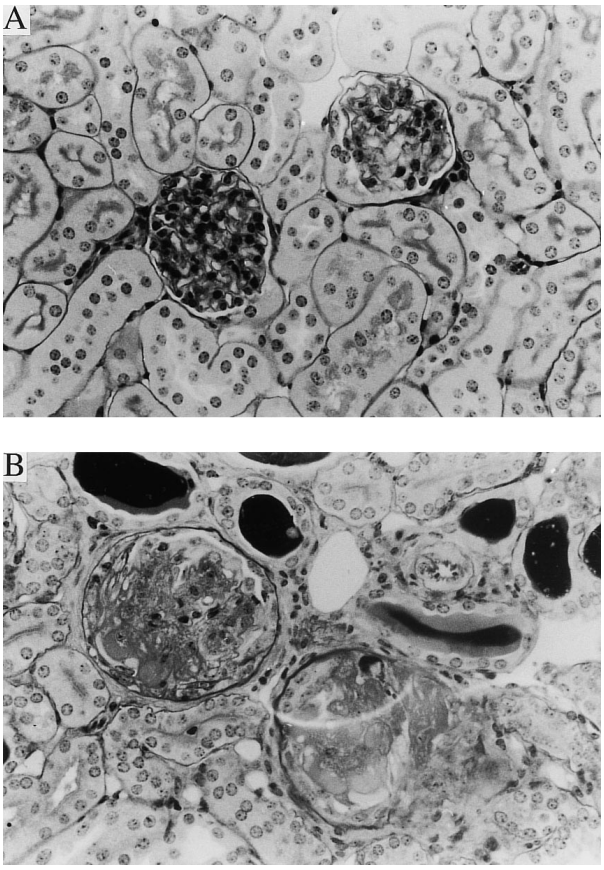

Fig. 1. (**A**) Normal glomeruli from a 4-month-old (MRL × BXSBYaa −) F1 hybrid mouse; PAS 200 ×. (**B**) Proliferative glomerulonephritis from a 4-month-old (MRL × BXSBYaa) F1 hybrid mouse; PAS 200 ×.

at 4°C until used. Tubule segments were microdissected in dissection solution at 4°C under stereomicroscopic observation. Proximal convoluted tubule, thick ascending limb and cortical collecting duct were isolated and identified by morphological and topographical criteria as previously described [7].

Na, K-ATPase activity measurement

The hydrolytic activity of Na^+/K^+-ATPase was determined in single segments of the nephron as previously described using a radiochemical assay based on the measurement of Pi released from [γ^{32}P]ATP [8]. Briefly, each tubule was individually transferred with 1 µl of incubation solution into the well of a bacteriological slide coated with dried BSA. The length of each tubule, which serves as reference for ATPase activity, was determined by photography. The tubules were then thoroughly rinsed with ice-cold distilled water and submitted to a freezing—thawing step in 0.2 µl of distilled water. This procedure permitted the removal of ions and permeabilized cell membranes, allowing the access of substrates into the cells. After addition of 1 µl of ATPase assay solution (see composition below), samples were incubated for 15 min at 37°C. Incubation was stopped by cooling and by addition of 5 µl of 5% (wt/vol) cold trichloracetic acid. Samples were then transferred into 2 ml of 10% (wt/vol) activated charcoal. After mixing and centrifugation, the radioactivity was measured by liquid scintillation of 500 µl

aliquots of supernatant which contain the Pi formed from ATP.

For measurement of total ATPase activity, the ATPase assay solution contained: 50 mM NaCl, 5 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 100 mM Tris—HCl, 10 mM Na_2ATP, and tracer amounts (5 nCi/μl) of [γ^{32}P]ATP (NEN, 2–10 Ci/mmol) for total ATPase activity. For basal Mg-ATPase activity measurements, Tris−HCl was 150 mM, NaCl and KCl were omitted and 1 mM ouabain was added. The pH of both solutions was adjusted to 7.4. Na^+/K^+-ATPase activity was calculated as the difference between the mean total and Mg-ATPase activities, respectively, and was expressed as pmol ATP/mm/h and data are expressed as mean ± SEM from 5–7 animals.

Statistics

Statistical comparisons between groups were performed by the unpaired Student's *t* test. Differences were considered significant for $P < 0.05$.

Results

Figure 2 summarizes our results. Rat and mouse Na^+/K^+-ATPase activities of the different nephron segments from control and nephrotic animals were compared. In controls *versus* PAN rats, Na^+/K^+-ATPase activities were (pmol ATP/mm/h): proximal convoluted tubule, 2954 ± 369 *vs* 2769 ± 230; thick ascending limb, 5352 ± 711 *vs* 5239 ± 803; and cortical collecting duct, 363 ± 96 *vs* 848 ± 194 ($P < 0.01$), respectively. In control *versus* (MRL × BXSBYaa) F1 mice, Na^+/K^+-ATPase activities were (pmol ATP/mm/h): proximal convoluted tubule, 979 ± 140 *vs* 1045 ± 148; thick ascending limb, 2213 ± 284 *vs* 2286 ± 246; cortical collecting duct, 311 ± 82 *vs* 755 ± 122 ($P < 0.01$), respectively. Na^+/K^+-ATPase activities measured in proximal convoluted tubule and thick ascending limb from control mice are lower than those found in rat. In contrast, Na^+/K^+-ATPase activities are similar in rat and mouse cortical collecting duct.

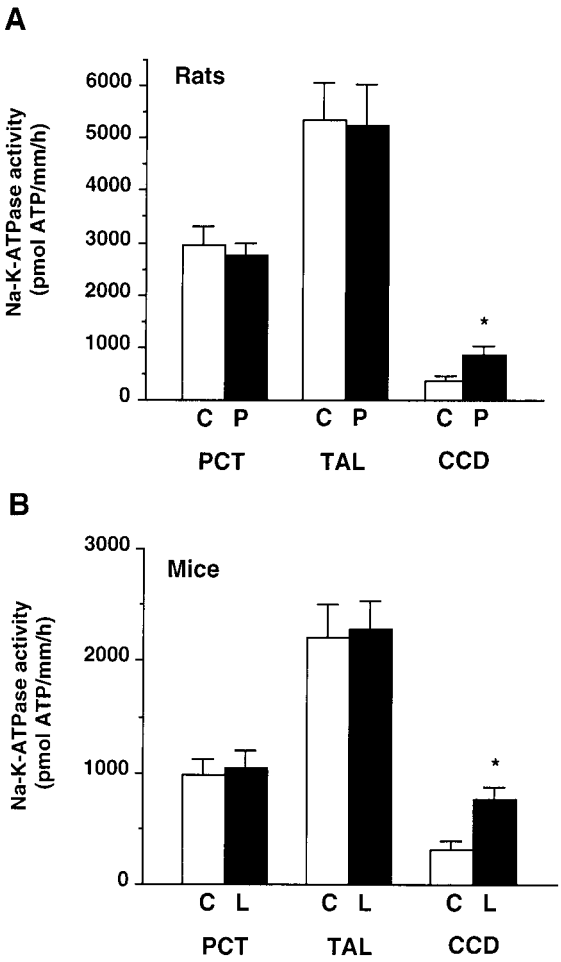

Fig. 2. Na^+/K^+-ATPase activity in proximal convoluted tubule, thick ascending limb and cortical collecting duct of rats with puromycin aminonucleoside-induced nephrotic syndrome and mice with spontaneous lupus-like glomerulonephritis (close bars) compared to controls (open bars). (**A**) PAN nephrotic syndrome rats 7 days after puromycin-aminoglycoside or sham injection. (**B**) Spontaneous lupus-like glomerulonephritis in 4-month-old (MRL × BXSBYaa) F1 hybrid mice and their controls, (MRL × BXSBYaa−) F1 hybrid mice. Each bar is mean ± SEM from 5–8 animals.

Discussion

In renal tubular epithelial cells, the level of Na^+/K^+-ATPase activity corresponds to the sodium reabsorption rate. Using the puromycin-induced nephrotic syndrome model in the rat, we have reported an enhancement of the Na^+/K^+-ATPase activity specifically limited to the cortical collecting duct [4,5]. This finding was in agreement with the increased sodium reabsorption described in PAN-nephrosis cortical collecting duct [3]. In PAN rats, the two-fold increase in Na^+/K^+-ATPase activity does not appear to be under hormonal control. Indeed, the enhanced Na^+/K^+-ATPase activity is independent of aldosterone [4]. Moreover, ouabain, an endogenous Na^+/K^+-ATPase inhibitor is elevated in the kidney tissue of PAN rats, serving as appropriate response to their increased extracellular fluid volume. However, ouabain does not blunt the Na^+/K^+-ATPase activity, further supporting a dysfunction of the regulation of this pump [4]. Finally, this enhanced Na^+/K^+-ATPase activity does not correlate with the severity of the proteinuria [4], as recently confirmed by another group [10].

To evaluate the relevance of this finding for glomerular diseases and to exclude a mechanism related to the chemical properties of the drug used in the PAN-nephrosis, we measured Na^+/K^+-ATPase activity in an autoimmune model of spontaneous glomerulonephritis in mouse. In these mice, the Yaa mutant gene is responsible for accelerating the development of a lupus-like glomerulonephritis and deletion of this Yaa gene dramatically slows down this process [6]. Four-month-old mice with the same genetic background with the exception of the Yaa mutant have not yet developed the glomerulonephritis (Yaa−) (Figure 1A) and serve as control to the experimental group of

animals (Yaa) which already exhibit a severe glomerulonephritis at this age (Figure 1B) [6]. This study demonstrates that Na^+/K^+-ATPase activities in the proximal convoluted tube and the thick ascending limb of mice with a lupus-like glomerulonephritis are identical to control animals. In contrast, the Na^+/K^+-ATPase activities measured in the cortical collecting duct are two times higher in the diseased mice than in their controls (Figure 2). In agreement with a previous report [9], Na^+/K^+-ATPase activities measured in proximal convoluted tubule and thick ascending limb were lower in mouse than in rat. However, in the present study, these differences are more apparent since Na^+/K^+-ATPase activities measured in mouse and rat were in the lower and higher range of normal values, respectively. This finding may be due to the specific strains of animals we used.

The results obtained in mice with glomerulonephritis are identical to those previously reported in PAN rats. They demonstrate that the enhanced Na^+/K^+-ATPase activity is independent of the mode of induction of the glomerulonephritis (chemical *versus* immunological) and species, thus supporting an association between the glomerular lesions and changes in the sodium transport rate by the cortical collecting duct. Therefore, the increase in cortical collecting duct Na^+/K^+-ATPase activity might be a general phenomenon shared by several types of glomerular diseases accompanied by sodium retention. Based on this observation, one may postulate that the podocyte and the principal epithelial cell of the cortical collecting duct are common targets for a yet unknown mediator which leads to alteration of these cells. The PAN rat model should be suitable for investigating the nature of the link between the glomerular lesions and the functional changes in tubular transports as well as for studying the molecular basis of the dysfunction of the Na^+/K^+-ATPase documented in cortical collecting duct of several types of glomerular diseases associated with sodium retention.

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