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Effects of angiotensin II on NaPi-IIa co-transporter expression and activity in rat renal cortex

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Abstract

The kidney plays a major role in reabsorption of phosphate with the majority occurring in the proximal tubule (PT). The type IIa sodium-phosphate co-transporter (NaPi-IIa) is the main player in PT. The purpose of current study was to determine the effect of angiotensin II (A-II) on membrane expression of NaPi-IIa in the rat renal cortex. A-II (500 ng/kg/min) was chronically infused into the Sprague–Dawley rats by miniosmotic pump for 7 days. The arterial pressure and circulating plasma A-II level along with urine output were markedly increased in A-II rats. There was diuresis but no natriuresis. The phosphate excretion increased sevenfold on day 4 and 5.7-fold on day 7. There was no change in Na-dependent Pi uptake in brush-border membrane (BBM) vesicles between A-II-treated group and control on day 4, however, there was a 43% increase on day 7. Western blot analysis of NaPi-IIa protein abundance showed a parallel pattern: no change after 4 days of treatment and a 48% increase after 7 days of treatment. However, Northern blot analysis of cortical RNA showed no change in NaPi-IIa mRNA abundance on day 7. A-II stimulation of Na/Pi co-transport activity is a result of increases in the expression of BBM NaPi-IIa protein level and that stimulation is most likely mediated by posttranscriptional mechanisms.

Keywords: SLC34A1; Kidney; Brush-border membrane; Phosphate transport; Phosphaturia

1. Introduction

The kidney plays a major role in the maintenance of inorganic phosphate (Pi) homeostasis. Physiologic studies have revealed that the bulk of filtered Pi is reabsorbed in the proximal tubules, with a higher reabsorption rate in early segments of the proximal tubules [1]. Pi reabsorption in the proximal tubule is mediated by Na^+-dependent, secondary-active Na/Pi co-transporter(s) that represents the rate-limiting step of proximal tubular Pi reabsorption. Three distinct and unrelated families of mammalian Na/Pi co-transporters have been identified: type I, type II, and type III [2]. The type II Na/Pi co-transporter family contains the isoforms IIa [3,4], IIb [4,5], and IIc [6]. NaPi-IIa co-transporter is more abundant in kidneys of adult animals while NaPi-IIc is mainly present in kidneys of young animals [7]. NaPi-IIb is not expressed in kidney [5]. NaPi-IIa knockout mice [8] and NaPi-IIa localization [9] studies demonstrated that the Na/Pi co-transporter NaPi-IIa is the major player in renal Pi reabsorption and regulation. The NaPi-IIa co-transporter is regulated by several physiological effectors, including parathyroid hormone (PTH) [10–12], glucocorticoids [13], epidermal growth factor (EGF) [14], thyroid hormone [15,16], vitamin D_3 [17], and dietary phosphate [11,18–22].

Angiotensin II (A-II) is a powerful vasoconstrictor and strong mediator of intravascular volume regulation [23]. A-II has multiple effects on renal ion transport. It has been demonstrated that systemic infusion of A-II increased αENaC protein abundance in rat kidney cortex [24]. A-II

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stimulates Na^+/H^+ exchanger 3 (NHE3) activity in the thick ascending limb cells as well as in the proximal tubule brush border [25]. However, the chronic effect of A-II on the proximal tubular Pi reabsorption in the kidney remains unknown. The renal excretion of phosphate is renal perfusion pressure-dependent; the higher the renal perfusion pressure, the greater the excretion of phosphate, and this effect is independent of systemic pressure [26]. Enalapril, which blocks conversion of A-I to A-II, improves not only microalbuminuria but also abnormal calcium and phosphate excretion in microalbuminuric children with diabetes [27].

The purpose of the current study was to investigate molecular mechanisms involved in the effects of chronic A-II infusion on the renal Pi reabsorption and on NaPi-IIa expression. Our study reveals that long-term A-II infusion stimulates NaPi-IIa most likely by posttranscriptional mechanisms.

2. Materials and methods

2.1. Preparation of animals and tissues

Experiments were performed using adult male Sprague–Dawley rats (280 to 300 g, Harlan, Madison, WI), which were maintained in a temperature-, light- and humidity-controlled room. Throughout the experiments, animals had free access to standard rat chow (Ralson Purina, St. Louis, MO) and tap water before and during the study. All procedures were approved by the University of Arizona Institutional Animal Care and Use Committee.

Control rats and A-II-treated rats were chosen randomly to two experimental groups. One group of 16 rats received A-II infusion (Sigma, St. Louis, MO), at a rate of 130 ng/min (approximately 500 ng/kg/min), for a period of 7 days, while another group of 12 rats received equal volumes of saline administered in the same fashion and served as controls. The rats were anesthetized with sodium pentobarbital (50 mg/kg body wt; administered intraperitoneally), and an osmotic minipump (model 2001; Alza Corp., Palo Alto, CA) was implanted subcutaneously at the dorsum of the neck.

Several rats were maintained in metabolic cages, and 24-h urine and water intake were monitored 1 day prior to implantation of minipumps to establish the baseline value and on days 4 and 7 of infusion. Following collection, urine was stored at −20 °C prior to assays. The urine samples were measured by the University of Arizona Animal Care Diagnostic Laboratory.

Systolic arterial BP was measured in conscious rats on day 6 of infusion, using tail-cuff plethysmography (IITC INC/Life Science Instruments; Model 179; Woodland Hills, CA). Rats were killed by CO_2 anesthesia followed by cervical dislocation. The serum samples were measured by the University of Arizona Animal Care Diagnostic Laboratory. The Molybdate reaction, modified Jaffe and ion selective electrode methods were used for the measurement of phosphorous, creatinine and sodium concentrations, respectively. Plasma A-II concentration assay was performed with an enzyme immunoassay kit (Spibio, France). One renal cortex was excised, snap-frozen in liquid nitrogen, and stored at −80 °C until processing for mRNA purification. The other renal cortex was used for brush-border membrane vesicle (BBMV) preparation on the day of BBMV uptake or for Western blot analyses and was not frozen.

2.2. BBMV preparation of rat renal cortex

BBMVs were prepared from the renal cortex by the $MgCl_2$ precipitation technique as previously described [28,29].

2.3. Uptake analysis of rat renal BBMV

Uptake of phosphate was measured by a rapid filtration technique as described previously [13,18]. Pi transport was initiated by incubating 20 μl of the vesicular suspension with 80 μl of 100 mM NaCl or KCl, 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 0.1 mM KH_2PO_4, and tracer amounts of $KH_2^{32}PO_4$. The reaction was terminated at 10 s, which is within the linear rate phase [28,29], by addition of 2 ml of ice-cold stop solution (100 mM NaCl, 10 mM mannitol, 10 mM HEPES-Tris, pH 7.4, and 10 mM KH_2PO_4). The vesicles were then collected on 0.45-μm nitrocellulose filters and washed several times with 2–3 ml of ice-cold stop solution. The filters were dissolved in Ready-Protein scintillant (Beckman, Fullerton, CA), and radioactivity was measured by a scintillation counter. Radioactivity bound to filters in the absence of vesicles was used as background and was subtracted from the counts. The Na-dependent component of Pi uptake was obtained by subtracting uptake values in the presence of KCl from uptake values in the presence of NaCl. Values are mean±S.E. for each group and represent the results of three separate uptake reactions with samples isolated from different groups of animals. Three repetitions were performed on membrane vesicle preparations from three different groups of animals from both A-II-infused and saline-infused rats.

2.4. Western blot analysis of rat renal BBMV

BBMVs were purified from control rats and A-II-treated rats. Protein was quantitated by a Bradford protein assay. The BBMVs isolated from the renal cortex were diluted in at least an equal volume of Laemmli solubilization buffer (2% SDS, 10% glycerol, 1 mM EDTA, and 2 mM β-mercaptoethanol, pH 6.8) and placed on ice for 30 min. Protein samples were fractionated by 7.5% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked overnight in PBS with 0.05% Tween 20 (PBST) and 5%

nonfat dry milk. On the next day, the membranes were rinsed with PBST-0.1% milk and incubated for 40 min at room temperature with the primary antibody rabbit polyclonal antibody raised against mouse Na-P_i transporter-specific COOH-terminal peptide [at 1:4000 dilution] [30]. This antibody has been shown previously to cross-react with high specificity with the rat NaPi-IIa [13,31]. An anti-mouse/anti-rabbit secondary antibody (40 mU/ml; Boehringer Mannheim GmbH, Germany) was used with the reagent for chemiluminescent detection (Boehringer Mannheim). Membranes were stripped and subsequently reacted with β-actin antiserum (Sigma) at 1:5000 dilution. NaPi-IIa specific band intensities were determined by densitometric analysis utilizing GS-700 Imaging Densitometer and Quantity One software (Bio-Rad, Hercules, CA) and were normalized for β-actin band intensities on the same blot. Experiments were repeated three times with protein samples isolated from different groups of animals from both A-II-infused and saline-infused rats.

2.5. Northern blot analysis of rat renal cortex

Kidney cortex was removed and snap-frozen in liquid nitrogen. mRNA was isolated from the renal cortex, utilizing the Fast-Track mRNA purification kit (Invitrogen, Carlsbad, CA). A ^{32}P-labeled rat specific NaPi-IIa cDNA antisense probe was generated using strip-EZ PCR kit (Ambion, Austin, TX), according to the manufacturer's protocol. The rat NaPi-IIa cDNA, which was used as a template for making cDNA antisense probe, was produced by RT-PCR with primer pairs designed and synthesized based on GenBank (accession no. L13257). The primer sequence was as follows: 5′ -CCGTTGATGCTAGGCTTCCTT-3′ (sense) and 5′ -CACGGCAATGCTGGTAATCA-3′ (antisense), which produced rat NaPi-IIa cDNA fragment from +366 to +926 bp. The 560-bp PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and excised and purified from the gel. This PCR-generated product was confirmed by DNA sequencing. Five micrograms of mRNA was fractionated on 1.0% formaldehyde-agarose gels, transferred onto nylon membranes (Pierce), and cross-linked to the membrane by ultraviolet irradiation. The filter was hybridized with the probe overnight at 42 °C in 50% formamide containing hybridization buffer and washed under high stringency conditions (0.1× SSC–0.1% SDS at 42 °C). β-Actin-specific cDNA antisense probes were used as internal standards for quantitating NaPi-IIa gene expression.

2.6. Calculations and statistical analyses

Glomerular filtration rate (GFR) was estimated by calculating the renal clearance of creatinine (Cc_r) using the following formula:

$$Cc_r = [U/P]cr \times (V/bw)$$

where [U/P]cr denotes the urine to plasma concentration ratio of creatinine, and (V/bw) is the rate of urine flow divided by the body weight.

Fractional excretion of sodium (FE_{Na}) was calculated by using the following formula:

$$FE_{Na}\ (\%) = [U/P]_{Na} \times [P/U]_{Cr} \times 100$$

where $[U/P]_{Na}$ denotes the urine to plasma concentration ratio of sodium, and $[P/U]_{Cr}$ denotes the plasma to urine concentration ratio of creatinine.

Fractional excretion of phosphate (FE_{Pi}) was calculated by using the following formula:

$$FE_{Pi}\ (\%) = [U/P]_{Pi} \times [P/U]_{Cr} \times 100$$

where $[U/P]_{Pi}$ denotes the urine to plasma concentration ratio of phosphate, and $[P/U]_{Cr}$ denotes the plasma to urine concentration ratio of creatinine.

The renal tubular reabsorption of phosphate (TRP) was calculated by using the following formula:

$$TRP\ (\%) = (1 - C_{Pi}/C_{Cr}) \times 100$$

in which C_{Pi}/C_{Cr} denotes the phosphate to creatinine ratio of clearance.

The experimental data are expressed as mean±S.E. They were analyzed by ANOVA (StatView 5.0.1 version; SAS Institute, Cary, NC). P values of <0.05 indicate statistical significance.

3. Results

3.1. BP, plasma A-II levels and serum phosphorus levels

On day 6 of infusion, systolic BP was significantly elevated in the A-II-treated rats, compared to controls (190±4 mm Hg, A-II group, versus 120±3, controls; n=6 rats, P<0.001).

On day 7 of infusion, circulating plasma A-II levels were markedly increased in the A-II group, compared to controls (53.0±7.7 fmol/ml plasma, A-II group, versus 8.1±1.5, control; n=5 rats, P=0.02).

The serum phosphorus levels for the two groups were identical at the initiation of the study. The serum phosphorus was 6.1±0.6 and 8.6±0.3 mg/dL on days 4 and 7, respectively, in the control group, and 6.1±0.5 and 7.6±0.5 mg/dL on days 4 and 7, respectively, in the A-II group. There was no significant difference between A-II-treated and control rats in the phosphorus level during the study period (n=6 rats).

3.2. Glomerular filtration rate (GFR)

GFR was determined by 24-h urinary creatinine clearance. GFR for the two groups were nearly identical at the initiation of the study (A-II, 10.1±0.7 ml/min/kg bw, n=6 rats; control, 9.4±1.4, n=5 rats; P=0.3). On days 4 and 7 of

infusion, the GFR showed no significant difference between A-II-treated and control rats (on day 4 of infusion, 8.3±0.4 ml/min/kg bw, A-II group, versus 7.8±0.3, controls, P=0.1; on day 7 of infusion, 6.7±0.8 ml/min/kg bw, A-II group, versus 7.3±0.6, controls, P=0.07) (Fig. 1).

3.3. Water intake, urine output, Na^+ excretion and phosphate excretion

Water intakes for the two groups were identical at the initiation of the study (A-II, 122.4±7.7 ml/day/kg bw, n=6 rats; control, 139.4±21.9, n=5 rats). On day 4 of infusion, the average water intake for the A-II-treated rats was significantly higher than that for the saline-treated rats (385.8±27.6 ml/day/kg bw, A-II group, versus 133.1±4.9, controls). On day 7 of infusion, the average water intake for the A-II-treated rats was significantly higher than that for saline-treated ones (325.4±19.8 ml/day/kg bw, A-II group, versus 112.8±5.8, controls).

Urine outputs for the two groups were nearly identical at the initial study (A-II, 42.0±5.6 ml/day/kg bw, n=6 rats; control, 38.3±3.5, n=5 rats, P=0.6). On day 4 of infusion, the average urine output for the A-II-treated rats was significantly higher than that for saline-treated rats (287.4±34.3 ml/day/kg bw, A-II group, versus 58.4±4.2, controls, P=0.0003). On day 7 of infusion, the average urine output for the A-II-treated rats was decreasing but still significantly higher than that of saline-treated ones (226.2±20.6 ml/day/kg bw, A-II group, versus 54.9±4.0, controls, P=0.0001).

FE_{Na} for the two groups were nearly identical at the initiation of the study (A-II, 0.81±0.06, n=3 rats; control, 0.67±0.1, n=3 rats, P=0.3). FE_{Na} was not significantly changed between the A-II-treated rats and control, on days 4 and 7 of infusion (on day 4, A-II, 0.75±0.17, n=3 rats; control, 0.63±0.03, n=3 rats, P=0.5; on day 7, A-II, 0.69±0.11, n=3 rats; control, 0.56±0.03, n=3 rats, P=0.3).

FE_{Pi} significantly increased on days 4 and 7 of infusion. FE_{Pi} for the two groups was nearly identical at the initiation of the study (A-II group, 0.17±0.02, n=6 rats; control, 0.16±0.04, n=5 rats, P=0.8). On day 4 of infusion, the FE_{Pi} for the A-II-treated rats was significantly increased seven-fold, compared to control rats (1.55±0.22, A-II group, versus 0.22±0.04, controls, P=0.003). On day 7 of infusion,

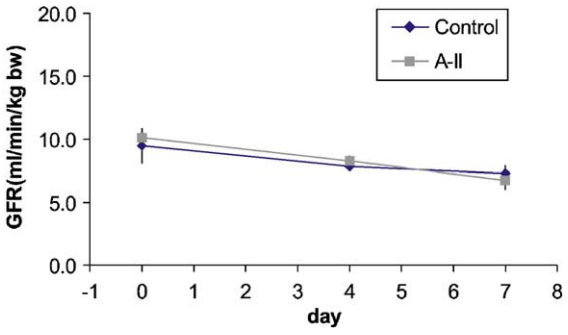

Fig. 1. Glomerular filtration rate. GFR was determined by creatinine clearance 1 day before pump implantation, at 4 and 7 days of infusion. Data are expressed as mean±S.E.

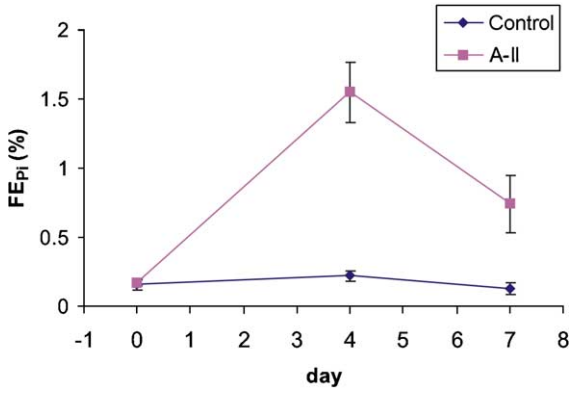

Fig. 2. Effect of A-II infusion on urinary phosphate excretion. Urine phosphate excretion was expressed as the renal fractional excretion of phosphate (FE_{Pi}, %). Data are expressed as mean±S.E. *P<0.05.

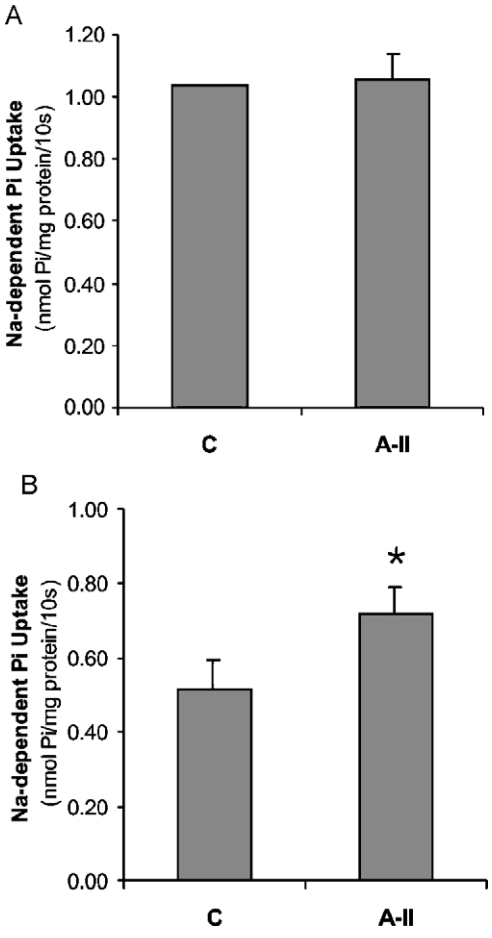

Fig. 3. Na-dependent phosphate uptake analysis in rat renal cortical BBMV. BBM vesicles were prepared from groups of rats treated with A-II or saline. Uptake of radioactive phosphate was measured by a rapid filtration technique with or without sodium at pH 7.4. Values are mean±S.E.; number of replicates=3. *P<0.05. Panel A shows Na-dependent phosphate uptake on day 4 of A-II infusion and panel B shows Na-dependent phosphate uptake on day 7.

the FE_{Pi} for the A-II-treated rats was significantly elevated 5.7-fold (0.74±0.21, A-II group, versus 0.13±0.04, controls, P=0.02) (Fig. 2).

TRP in the A-II-treated rats was significantly decreased by 1.3% on day 4 of infusion, and by 0.6% on the day 7 of infusion (day 4, 98.45±0.04, A-II group, versus 99.78±0.04, controls, P=0.002; day 7, 99.26±0.21, A-II group, versus 99.87±0.04, controls, P=0.02).

3.4. Uptake analysis of renal cortex BBMV

BBMV were purified from rats treated with A-II or saline. Uptake of radioactive phosphate was measured by a rapid filtration technique with or without sodium at pH 7.4, as described above. On day 4 of A-II infusion, there was no significant change in Na-dependent Pi uptake (in) between A-II-treated and control rats (1.06±0.08 versus 1.04±0.003 nmol/mg protein/10 s; Fig. 3A). However, on day 7 of infusion, there was a significant increase in Pi uptake after A-II infusion (0.72±0.08 versus 0.51±0.08 nmol/mg protein/10 s, P=0.03; Fig. 3B). Thus, 7-day A-II administration increased renal Na-Pi uptake by 43%.

3.5. Western blot analysis of BBM proteins with NaPi-IIa-specific antiserum

Renal BBMV protein was isolated from A-II-treated and control rats. Protein was fractionated by SDS-polyacrylamide gel electrophoresis (30 μg/lane) and transblotted onto nitrocellulose. Blots were reacted with antiserum specific for the NaPi-IIa co-transporter and β-actin. Western blot analyses with NaPi-IIa antiserum showed specific recognition of a 73-kDa protein and there was no change in NaPi-IIa protein level on day 4 of A-II infusion between A-II-treated group and control (1.37±0.08, A-II group, versus 1.46±0.07 densitometric units, controls; n=3;) (Fig. 4). However, A-II infusion increased this immunoreactive NaPi-IIa protein band by 48% on day 7, compared with controls (3.50±0.26, A-II group, versus 2.40±0.03 densitometric units, controls; n=3; P=0.01) (Fig. 5).

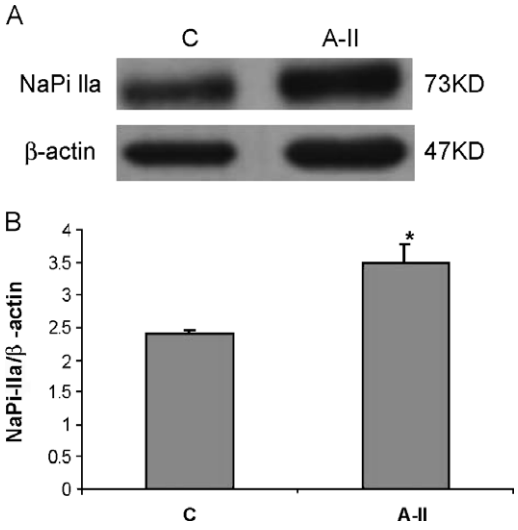

Fig. 5. Western blot analysis of NaPi-IIa protein in rat renal cortical BBMV on day 7 of A-II infusion. Panel A shows one typical Western blot experiment. Western blots were reacted with NaPi-IIa-specific and β-actin-specific antiserum. The band at 73 kDa is NaPi-IIa and the band at 47 kDa is β-actin. Panel B shows quantitative data from NaPi-IIa Western blot experiments. Data are presented as ratio of NaPi-IIa to β-actin protein levels. Values are mean±S.E.; number of replicates=3; *P<0.05.

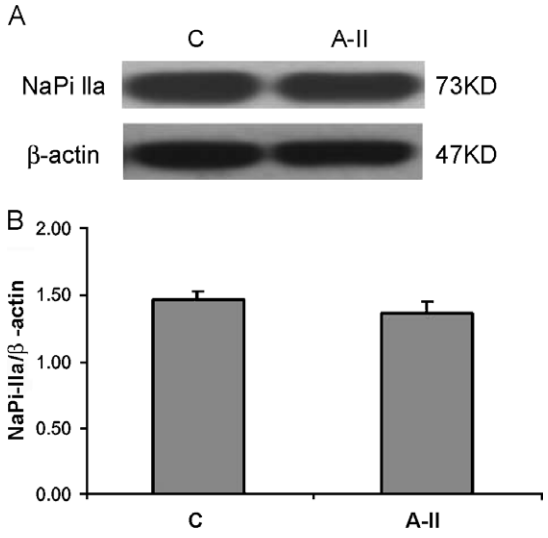

Fig. 4. Western blot analysis of NaPi-IIa protein in rat renal cortical BBMV on day 4 of A-II infusion. Panel A shows one typical Western blot experiment. Western blots were reacted with NaPi-IIa-specific and β-actin-specific antiserum. The band at 73 kDa is NaPi-IIa and the band at 47 kDa is β-actin. Panel B shows quantitative data from NaPi-IIa Western blot experiments. Data are presented as ratio of NaPi-IIa to β-actin protein levels. Values are mean±S.E.; number of replicates=3.

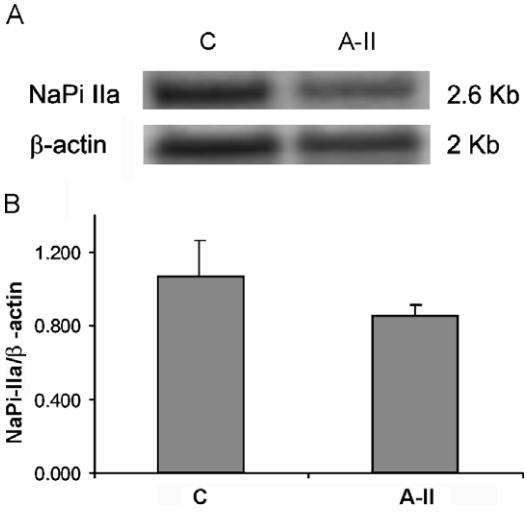

Fig. 6. Northern blot analysis of NaPi-IIa mRNA expression in rat renal cortex on day 7 of infusion. Panel A shows one typical Northern blot experiment. Northern blots were probed with NaPi-IIa-specific and β-actin-specific probes. The hybridization signal at 2.6 kb represents rat NaPi-IIa, and hybridization signal at 2.0 kb represents β-actin. Panel B shows quantitative data from NaPi-IIa Northern blot experiments. Data are presented as ratio of NaPi-IIa to β-actin mRNA levels. Values are mean±S.E.; number of replicates=3.

3.6. Northern blot analysis in the rat renal cortex

Northern blots analysis of mRNA isolated from kidney cortex was performed on A-II-treated and control rats. Hybridization of the membrane with rat NaPi-IIa cDNA specific probes demonstrated a 2.6-kilobase (kb) transcript in both groups (Fig. 6). Signal intensity was expressed as a ratio of NaPi-IIa to β-actin hybridization signal as determined by phosphorimage analysis. Statistical analysis of hybridization intensities showed no significantly differences in the 2.6-kb bands between A-II-treated and control groups on day 7 (0.85±0.05, A-II group, versus 1.07±0.19 densitometric units, controls; n=3; P=0.3) (Fig. 6).

4. Discussion

Among the sodium-phosphate co-transporter systems identified at the molecular level, a brush-border membrane type IIa Na/Pi co-transporter is the key player in proximal tubular Pi reabsorption [1,6]. Although this transporter is regulated by multiple physiological and pathophysiological factors, its regulation by chronic infusion of A-II has never been tested.

The present study utilized a previously established model of chronic A-II infusion [32–37] to study the long-term effects on renal Na/Pi co-transporter activity. In this study A-II infusion resulted in significant increase of systolic BP and plasma A-II levels, which was in agreement with previous studies [38]. The infusion dose of 500 ng min^{-1} kg^{-1} results in A-II plasma levels very similar to those found in the two-kidney one-clip Goldblatt model, which is widely used for renovascular hypertension studies [33]. In our study, we also noticed that A-II infusion did not alter the GFR on days 4 and 7 of infusion.

In our study, A-II infusion significantly increased water intake above control levels, which is consistent with previous reports that circulating A-II can act on specific brain receptors to stimulate thirst and A-II is essential for the drinking response [39]. We observed a large increase in the urinary excretion of water, but no change in Na^+ excretion. Thus, unlike in acute A-II infusion, there was no natriuresis but only diuresis.

In the present study, the phosphaturia observed on days 4 and 7 of A-II infusion is consistent with other researchers who have noted exaggerated phosphaturia in the hypertensive patients. The higher the renal perfusion pressure, the greater the excretion of phosphate [26,40]. Interestingly, Pi excretion on day 4 was increased by sevenfold with A-II infusion. On day 7 of A-II infusion, Pi excretion was coming down in A-II-treated rats, but was still higher than controls (5.7-fold). The fact that enhanced Pi excretion at day 4 showed a downward trend by day 7 (~50% decrease) without changing GFR suggested a compensatory increase of Pi reabsorption by day 7. To test this hypothesis, BBMV Na-dependent phosphate uptake was performed on days 4 and 7.

Na/Pi co-transporter activity was estimated by initial rate ^{32}P uptake studies with renal BBMVs. Na^+-dependent phosphate uptake did not show any change with 4 days of A-II treatment, while uptake showed a 43% increase with 7 days of A-II treatment. It is well known that at least three Na/Pi co-transporters are located on the apical membrane of proximal tubules, such as type I Na/Pi co-transporter and type IIc co-transporter. Murer et al. [1,6] and others have revealed that type I Na/Pi co-transporter is unlikely to be an important player in proximal tubular Pi flux. Electrophysiological studies suggest other roles for the type I Na/Pi co-transporter, such as chloride channel activity and transport of anionic organic compounds [41,42]. In addition, type IIc may play an important role in young animals, but not in adult animals [1,6]. Therefore, the Na^+-dependent Pi uptake measured in the current study is representative of NaPi-IIa activity. To further determine if the increase of Pi uptake is due to increased NaPi-IIa co-transporter protein, immunoreactive protein levels were assessed. Western blotting experiments showed a 48% increase in NaPi-IIa immunoreactive protein with 7 days of A-II treatment, which paralleled the changes observed in Na/Pi co-transporter. NaPi-IIa protein levels and Pi uptake were unaffected by 4 days of A-II treatment. Northern blotting did not detect changes in NaPi-IIa mRNA levels on day 7. These results indicated that the increase in BBM Na/Pi co-transporter activity with 7-day A-II treatment is associated with a similar increase in BBM NaPi-IIa protein abundance, but not with NaPi-IIa mRNA abundance. These findings suggest that regulation of NaPi-IIa co-transporter by A-II possibly occurs at the posttranscriptional level.

It is possible that A-II altered the amount of apical membrane-associated NaPi-IIa via a hormone-initiated cell-signaling cascade, leading to either decreased internalization from the apical BBM or increased delivery to the apical BBM. This possibility remains to be further established by evaluating whether the increased apical NaPi-IIa protein is due to changes in trafficking or in protein–protein interaction.

Other researchers have hypothesized that the exaggerated phosphaturia in hypertensive patients is at least partially due to a more profound suppression of proximal tubular reabsorption [26,40]. Our present data also revealed that the renal tubular reabsorption of phosphate (TRP) in the A-II-treated rats was significantly decreased by 1.3% on day 4, and by 0.6% on day 7, compared to control rats. There was tendency towards decrease in phosphate wasting from day 4 to day 7 as well as increased activity and expression of the NaPi-IIa transporter at day 7, suggesting that NaPi-IIa is involved in the compensation for phosphate lost in the urine.

In our study, it was observed that A-II-treated rats were able to maintain normal serum phosphate levels in the face of a large increase in urinary phosphate excretion. Although the renal phosphate sensing mechanism that is involved in

initiating the adaptive response to phosphate deprivation has not yet been defined, our data suggest that kidneys of A-II-treated rats are able to react appropriately. By increasing the renal absorption on day 7, it is able to reverse the trend of phosphate loss. Presumably, in most healthy animals serum phosphorous does not change unless the phosphoruria continues. Majority of the phosphorous in the body is stored in the bones and a minor shift into the intravascular pool should maintain normal blood levels for a period.

Renal tubular effects of A-II are believed to be mediated by A-II type 1 (AT1)-receptor. In our study this was not assessed, but enalapril which blocks conversion of A-I to A-II improves not only microalbuminuria but also abnormal calcium and phosphate excretion in microalbuminuric children with diabetes [27].

In view of our current data, we conclude that chronic A-II infusion stimulates NaPi-IIa co-transport activity by increasing the apical BBM expression of the type II Na/Pi co-transport protein. This stimulation of Na/Pi co-transporter activity by A-II is likely mediated by posttranscriptional mechanisms.

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References

[1] H. Murer, N. Hernando, I. Forster, J. Biber, Proximal tubular phosphate reabsorption: molecular mechanisms, Physiol. Rev. 80 (2000) 1373–1409.
[2] H.S. Tenenhouse, H. Murer, Disorders of renal tubular phosphate transport, J. Am. Soc. Nephrol. 14 (2003) 240–248.
[3] S. Magagnin, A. Werner, D. Markovich, V. Sorribas, G. Stange, J. Biber, H. Murer, Expression cloning of human and rat renal cortex Na/Pi cotransport, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 5979–5983.
[4] A. Werner, L. Dehmelt, P. Nalbant, Na+-dependent phosphate cotransporters: the NaPi protein families, J. Exp. Biol. 201 (1998) 3135–3142.
[5] H. Hilfiker, O. Hattenhauer, M. Traebert, I. Forster, H. Murer, J. Biber, Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 14564–14569.
[6] H. Murer, N. Hernando, I. Forster, J. Biber, Regulation of Na/Pi transporter in the proximal tubule, Annu. Rev. Physiol. 65 (2003) 531–542.
[7] H. Segawa, I. Kaneko, A. Takahashi, M. Kuwahata, M. Ito, I. Ohkido, S. Tatsumi, K. Miyamoto, Growth-related renal type II Na/Pi cotransporter, J. Biol. Chem. 277 (2002) 19665–19672.
[8] L. Beck, A.C. Karaplis, N. Amizuka, A.S. Hewson, H. Ozawa, H.S. Tenenhouse, Targeted inactivation of Npt2 in mice leads to severe renal phosphate wasting, hypercalciuria, and skeletal abnormalities, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 5372–5377.
[9] M. Custer, M. Lotscher, J. Biber, H. Murer, B. Kaissling, Expression of Na-P(i) cotransport in rat kidney: localization by RT-PCR and immunohistochemistry, Am. J. Physiol. 266 (1994) F767–F774.
[10] M. Jankowski, J. Biber, H. Murer, PTH-induced internalization of a type IIa Na/Pi cotransporter in OK-cells, Pflugers Arch. 438 (1999) 689–693.
[11] H. Murer, I. Forster, N. Hernando, G. Lambert, M. Traebert, J. Biber, Posttranscriptional regulation of the proximal tubule NaPi-II transporter in response to PTH and dietary P(i), Am. J. Physiol. 277 (1999) F676–F684.
[12] M.F. Pfister, J. Forgo, U. Ziegler, J. Biber, H. Murer, cAMP-dependent and -independent downregulation of type II Na-Pi cotransporters by PTH, Am. J. Physiol. 276 (1999) F720–F725.
[13] Y.S. Guner, P.R. Kiela, H. Xu, J.F. Collins, F.K. Ghishan, Differential regulation of renal sodium-phosphate transporter by glucocorticoids during rat ontogeny, Am. J. Physiol. 277 (1999) C884–C890.
[14] M. Arar, H.K. Zajicek, I. Elshihabi, M. Levi, Epidermal growth factor inhibits Na-Pi cotransport in weaned and suckling rats, Am. J. Physiol. 276 (1999) F72–F78.
[15] A.I. Alcalde, M. Sarasa, D. Raldua, J. Aramayona, R. Morales, J. Biber, H. Murer, M. Levi, V. Sorribas, Role of thyroid hormone in regulation of renal phosphate transport in young and aged rats, Endocrinology 140 (1999) 1544–1551.
[16] V. Sorribas, D. Markovich, T. Verri, J. Biber, H. Murer, Thyroid hormone stimulation of Na/Pi-cotransport in opossum kidney cells, Pflugers Arch. 431 (1995) 266–271.
[17] Y. Taketani, H. Segawa, M. Chikamori, K. Morita, K. Tanaka, S. Kido, H. Yamamoto, Y. Iemori, S. Tatsumi, N. Tsugawa, T. Okano, T. Kobayashi, K. Miyamoto, E. Takeda, Regulation of type II renal Na+-dependent inorganic phosphate transporters by 1,25-dihydroxyvitamin D3. Identification of a vitamin D-responsive element in the human NAPi-3 gene, J. Biol. Chem. 273 (1998) 14575–14581.
[18] J.F. Collins, N. Bulus, F.K. Ghishan, Sodium-phosphate transporter adaptation to dietary phosphate deprivation in normal and hypophosphatemic mice, Am. J. Physiol. 268 (1995) G917–G924.
[19] H.M. Hoag, J. Martel, C. Gauthier, H.S. Tenenhouse, Effects of Npt2 gene ablation and low-phosphate diet on renal Na(+)/phosphate cotransport and cotransporter gene expression, J. Clin. Invest. 104 (1999) 679–686.
[20] S. Kido, K. Miyamoto, H. Mizobuchi, Y. Taketani, I. Ohkido, N. Ogawa, Y. Kaneko, S. Harashima, E. Takeda, Identification of regulatory sequences and binding proteins in the type II sodium/phosphate cotransporter NPT2 gene responsive to dietary phosphate, J. Biol. Chem. 274 (1999) 28256–28263.
[21] S. Taufiq, J.F. Collins, J. Meaney, F.K. Ghishan, Dietary regulation of the renal sodium-phosphate (Na+/Pi) transporter during early ontogeny in the rat, Proc. Soc. Exp. Biol. Med. 215 (1997) 281–289.
[22] A. Werner, S.A. Kempson, J. Biber, H. Murer, Increase of Na/Pi-cotransport encoding mRNA in response to low Pi diet in rat kidney cortex, J. Biol. Chem. 269 (1994) 6637–6639.
[23] R. Gomez, S. El-Dahr, R. Chevalier, Vasoactive Hormones, Pediatric Nephrology, 4th ed., Lippincott Williams & Wilkins Press, 1999, pp. 83–99.
[24] K.T. Beutler, S. Masilamani, S. Turban, J. Nielsen, H.L. Brooks, S. Ageloff, R.A. Fenton, R.K. Packer, M.A. Knepper, Long-term regulation of ENaC expression in kidney by angiotensin II, Hypertension 41 (2003) 1143–1150.
[25] T.H. Kwon, J. Nielsen, Y.H. Kim, M.A. Knepper, J. Frokiaer, S. Nielsen, Regulation of sodium transporters in the thick ascending limb of rat kidney: response to angiotensin II, Am. J. Physiol., Renal. Physiol. 285 (2003) F152–F165.
[26] X. Wu, H. Sonnenberg, Effect of renal perfusion pressure on excretion of calcium, magnesium, and phosphate in the rat, Clin. Exp. Hypertens. 17 (1995) 1269–1285.
[27] H. Yuksel, S. Darcan, C. Kabasakal, A. Cura, S. Mir, E. Mavi, Effect of enalapril on proteinuria, phosphaturia, and calciuria in insulin-dependent diabetes, Pediatr. Nephrol. 12 (1998) 648–650.
[28] N. Nakagawa, N. Arab, F.K. Ghishan, Characterization of the defect in the Na(+)-phosphate transporter in vitamin D-resistant hypophosphatemic mice, J. Biol. Chem. 266 (1991) 13616–13620.

[29] N. Nakagawa, F.K. Ghishan, Transport of phosphate by plasma membranes of the jejunum and kidney of the mouse model of hypophosphatemic vitamin D-resistant rickets, Proc. Soc. Exp. Biol. Med. 203 (1993) 328–335.
[30] J.F. Collins, L.A. Scheving, F.K. Ghishan, Decreased transcription of the sodium-phosphate transporter gene in the hypophosphatemic mouse, Am. J. Physiol. 269 (1995) F439–F448.
[31] S. Taufiq, J.F. Collins, F.K. Ghishan, Posttranscriptional mechanisms regulate ontogenic changes in rat renal sodium-phosphate transporter, Am. J. Physiol. 272 (1997) R134–R141.
[32] L.X. Zou, A. Hymel, J.D. Imig, L.G. Navar, Renal accumulation of circulating angiotensin II in angiotensin II-infused rats, Hypertension 27 (1996) 658–662.
[33] A.M. Von Thun, R.C. Vari, S.S. el-Dahr, L.G. Navar, Augmentation of intrarenal angiotensin II levels by chronic angiotensin II infusion, Am. J. Physiol. 266 (1994) F120–F128.
[34] S. Rajagopalan, S. Kurz, T. Munzel, M. Tarpey, B.A. Freeman, K.K. Griendling, D.G. Harrison, Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone, J. Clin. Invest. 97 (1996) 1916–1923.
[35] D.I. Diz, P.G. Baer, A. Nasjletti, Angiotensin II-induced hypertension in the rat. Effects on the plasma concentration, renal excretion, and tissue release of prostaglandins, J. Clin. Invest. 72 (1983) 466–477.
[36] H. Kobori, L.M. Harrison-Bernard, L.G. Navar, Expression of angiotensinogen mRNA and protein in angiotensin II-dependent hypertension, J. Am. Soc. Nephrol. 12 (2001) 431–439.
[37] M.P. Dixit, L. Xu, H. Xu, L. Bai, J.F. Collins, F.K. Ghishan, Effect of angiotensin-II on renal Na+/H+ exchanger—NHE3 and NHE2, Biochim. Biophys. Acta Biomembranes 1664 (2004) 38–44.
[38] L.A. Cassis, D.E. Marshall, M.J. Fettinger, B. Rosenbluth, R.A. Lodder, Mechanisms contributing to angiotensin II regulation of body weight, Am. J. Physiol. 274 (1998) E867–E876.
[39] M.D. Evered, M.M. Robinson, P.A. Rose, Effect of arterial pressure on drinking and urinary responses to angiotensin II, Am. J. Physiol. 254 (1988) R69–R74.
[40] C. Chaimovitz, A. Spierer, H. Leibowitz, S. Tuma, O.S. Better, Exaggerated phosphaturic response to volume expansion in patients with essential hypertension, Clin. Sci. Mol. Med. 49 (1975) 207–211.
[41] H. Yabuuchi, I. Tamai, K. Morita, T. Kouda, K. Miyamoto, E. Takeda, A. Tsuji, Hepatic sinusoidal membrane transport of anionic drugs mediated by anion transporter Npt1, J. Pharmacol. Exp. Ther. 286 (1998) 1391–1396.
[42] A.E. Busch, A. Schuster, S. Waldegger, C.A. Wagner, G. Zempel, S. Broer, J. Biber, H. Murer, F. Lang, Expression of a renal type I sodium/phosphate transporter (NaPi-1) induces a conductance in *Xenopus* oocytes permeable for organic and inorganic anions, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5347–5351.