Regulation of Na\(^+/\)H\(^+\) exchanger-NHE3 by angiotensin-II in OKP cells

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Abstract

Previous studies have shown that circulating Angiotensin II (A-II) increases renal Na\(^+\) reabsorption via elevated Na\(^+\)/H\(^+\) exchanger isoform 3 (NHE3) activity. We hypothesized that prolonged exposure to A-II leads to an increased expression of renal NHE3 by a transcriptionally mediated mechanism. To test this hypothesis, we utilized the proximal tubule-like OKP cell line to evaluate the effects of 16-h treatment with A-II on NHE3 activity and gene expression. A-II significantly stimulated NHE3-mediated S-3226-sensitive Na\(^+\)/H\(^+\) exchange. Inhibition of transcription with actinomycin D abolished the stimulatory effect of A-II on NHE3-mediated pH recovery in acid-loaded OKP cells. This prolonged exposure to A-II was also found to elevate endogenous NHE3 mRNA (by 40%)—an effect also abolished by inhibition of gene transcription. To evaluate the molecular mechanism by which A-II regulates NHE3 expression, the activity of NHE3 promoter driven reporter gene was analyzed in transient transfection assays. In transfected OKP cells, rat NHE3 promoter activity was significantly stimulated by A-II treatment, and preliminary mapping indicated that the A-II responsive element(s) is present between 149 and 548 bp upstream of the transcription initiation site in the NHE3 gene promoter. We conclude that a transcriptional mechanism is at least partially responsible for the chronic effects of A-II treatment on renal NHE3 activity.

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1. Introduction

Angiotensin-II (A-II) is an essential hormone that exerts pleiotropic actions in the renal proximal tubule including modulation of transport [1,2], metabolism [3], and cell proliferation [4]. A-II is a powerful vasoconstrictor and strong mediator of intravascular volume regulation [5]. A-II also regulates NaCl reabsorption [5,6] through both direct effects on the proximal tubule, and indirectly via effects on aldosterone secretion. A-II stimulates Na\(^+\) uptake in isolated proximal tubule cells through elevations in the activity of an amiloride-sensitive Na\(^+\)/H\(^+\) exchanger (NHE) which could be inhibited by A-II receptor antagonist saralasin [7]. Subsequently, a wide range of evidence has been acquired showing that the specific NHE isoform NHE3 is acutely regulated by A-II [8,9]. The acute regulation of NHE activity on the renal proximal tubules is biphasic; low concentrations of A-II (10\(^{-11}\) M) have been shown to stimulate Na\(^+\)/H\(^+\) antiport activity, whereas high concentrations (10\(^{-7}\) M) inhibit NHE activity utilizing distinct signaling pathways [10–12]. A-II regulates NHE3 activity after binding to membrane AT1 receptors in the proximal tubules [5,13]. More recent studies have shown that the increase in NHE3 activity induced by acute A-II treatment involves stimulating protein kinase C [8], activating a non-receptor tyrosine kinase [11], or redistributing of NHE3 protein [14,15].

The effect of chronic A-II treatment on NHE3 expression and the regulation of salt and water balance is less clear. A recent in vivo study showed that long-term A-II treatment increased the abundance of NHE3 in the thick ascending limb and in the proximal tubule brush border, an effect which may contribute to the observed enhancement of renal Na\(^+\) and HCO\(_3\)\(^-\) reabsorption in response to A-II [16]. Our group has also
demonstrated that increased renal NHE3 activity coincides with significantly elevated NHE3 protein and mRNA abundance in rats chronically infused with A-II via implanted miniosmotic pump [17]. These findings suggest that prolonged exposure to elevated levels of A-II increases NHE3 activity which may result partially from a transcriptional response of NHE3 gene.

Previous work has demonstrated that the proximal tubule-like OKP cells provide an excellent model system to study in situ regulation of the NHE3 gene [18–20]. The OKP cell line, established from opossum kidney proximal tubule, expresses NHE3 in a similar fashion to that observed in the proximal tubule apical membrane [18]. For this reason, OKP cells have been used as a model for understanding hormonal regulation of NHE transporter expression. In OKP cells, NHE expression and activity are elevated by endothelin, acidsides, and glucocorticoids, and are inhibited by PTH and exogenous cAMP analogs [19,21–23]. The effects of A-II on NHE in these cells have not been investigated. In the present study, we utilized OKP cells to evaluate A-II mediated changes in NHE expression, and to determine the molecular mechanisms by which A-II may exert its effects on NHE3 promoter activity. In these studies, we demonstrate that long-term A-II treatment (16 h) induces transcriptionally mediated (actinomycin D-sensitive) increase in NHE3-driven pH recovery, paralleled by a significantly elevated NHE3 mRNA abundance and its gene promoter activity. These results suggest a role for A-II in transcriptional regulation of the NHE3 gene.

2. Methods

2.1. Cell culture

OKP cells, a gift from Dr. R. J. Alpern, are a clonal opossum kidney cell line, originally described by Cole et al. [24]. OKP cells (passages 21–30) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. The same batch of FBS was used in all experiments. Cells were cultured at 37 °C in a 95% air–5% CO2 atmosphere and passed every 48–72 h. For experimentation, OKP cells were grown to confluence, rendered quiescent by removing the serum for 48 h, then studied. In A-II treatment experiments, cells were incubated with 100 mM (10−7 M) A-II (Sigma; St. Louis, MO) or vehicle (saline) for 16 h. A-II is stable for 24 h–48 h in cell culture [25,26]. In all experiments, control and experimental cells were from the same passage and were assayed on the same day. Media and other reagents used for cell culture were purchased from Irvine Scientific (Irvine, CA).

2.2. Measurement of NHE activity

NHE activity was analyzed by measuring the rate of intracellular pH (pHi) recovery after induction of an acid load in the absence of NaHCO3. pHi was assessed by monitoring the fluorescence emission of the pH-sensitive dye SNARF-1 (Molecular Probes, Eugene, OR). For these experiments, cells were incubated with 100 nM (10−7 M) HOE-694, which inhibits both NHE1 and NHE2, and 20 μM S3226 was used to assess the specific activity of NHE3 [29,30]. In addition, recovery from the acid load was assessed during induction of H+-ATPase with 0.4 μM bafilomycin A1 [31].

2.3. RNA purification and Northern blot analyses of OKP cells

mRNA was isolated from three different passages of OKP cells, utilizing the Fast-Track mRNA purification kit (Invitrogen, Carlsbad, CA). A 5′-labeled OKP cell-specific NHE3 cDNA antisense probe was generated using strip-EZ PCR kit (Ambion, Austin, Texas), according to the manufacturer’s protocol. The opossum NHE3 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. L42522). The primer sequence was as follows: 5′-CTACATCATCGCAGTCTGGTA-3′ (sense) and 5′-TCAC-CAGAGACAGAAGGAAG-3′ (antisense), which produced an OKP cell cDNA fragment of +1453 bp to +1114 bp. The 662 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. 5 μg of mRNA was fractionated on 1% 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 NHE3 gene expression.

2.4. Semiquantitative RT-PCR analysis of NHE3 gene expression

mRNA was purified from OKP cells treated for 16 h with A-II (100 nM) or vehicle (saline). RT-PCR conditions were described with standard methods [32]. The primers used to detect NHE3 were designed from OKP NHE3 mRNA (GeneBank accession no. L42522). The forward primer was at 453 bp to +484 bp, the 662 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. 5 μg of mRNA was fractionated on 1% 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 NHE3 gene expression.
Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Construct</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>−548/+39</td>
<td>5′-GACTACCGGTCCCTTTCATCCCTATGTCC-3′ (forward)</td>
</tr>
<tr>
<td>2</td>
<td>−149/+39</td>
<td>5′-GACTACCGGTACAGTAGTGCGGCTGA-3′ (forward)</td>
</tr>
<tr>
<td>3</td>
<td>−95/+39</td>
<td>5′-GACTACCGGTGGTGGTAGTCCAAGTGCG-3′ (forward)</td>
</tr>
<tr>
<td>4</td>
<td>−548/+39</td>
<td>5′-TACACTCGAGTCAGCCCTCAGCGCTAT-3′ (reverse)</td>
</tr>
</tbody>
</table>

NHE3 mRNA expression levels were estimated by taking a ratio of NHE3 over β-actin amplicon optical densities.

2.5. Construction of reporter plasmids

Luciferase reporter plasmids used in this study were derived from pGL3 basic (pGL3b; Promega), which contains the firefly luciferase gene. A construct containing −1360/+58 bp of the rat NHE3 promoter was described previously [31]. The other constructs (−548/+39, −149/+39, −95/+39) were prepared by PCR amplification using the −1360/+58 bp construct as a template, primer 4 as a common reverse primer and primers 1, 2, and 3 as forward primers (Table 1). For these constructs, PCR products were digested with Mlu I and subcloned into pGL3-basic vector, at the Mlu I sites. All plasmid constructs were confirmed by sequencing on both strands.

2.6. Transient transfection and functional promoter analysis

OKP cells (at passages 21–30) were cultured in 24-well plates. When cells reached ~70–80% confluence, liposome-mediated transfection was performed as follows: 0.5 μg of promoter construct DNA (constructs mentioned above), 30 ng of pRL-CMV (Renilla luciferase reporter construct used as an internal standard; Promega), and 5 μl of Lipofectamine (GIBCO/BRL; Grand Island, NY) were mixed with 200 μl of Opti-MEM (GIBCO/BRL) for 30 min at room temperature. Then the mixture was added to the cells, the cells were incubated for 5 h, and then an equal volume of DMEM containing 20% FBS was added. On the next day, the medium was removed and replaced with standard medium with 10% FBS. Twenty-four hours later, cells were harvested for reporter gene assays. For A-II dose response studies, transiently transfected cells were treated with 0, 10\(^{-10}\), or 10\(^{-7}\) M A-II for 16 h before they were harvested. For transcriptional inhibition studies, transiently transfected cells were pretreated with actinomycin D (100 nM; Calbiochem-Novabiochem; San Diego, CA) for 2 h and then treated with 100 nM A-II for 16 h in the presence of actinomycin D before they were harvested. Promoter reporter assays were performed using the Dual Luciferase Assay Kit according to the manufacturer’s instructions (Promega). Luciferase activities were measured with a tube luminometer (FB12; Zylux; Maryville, TN). Each experiment was repeated a minimum of three times with different passages of cells.

2.7. Statistical analyses

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

3. Results

3.1. The effect of A-II concentration on NHE3 promoter activity in OKP cells

Previous studies on A-II mediated acute responses have shown that lower concentration of A-II (10\(^{-10}\) M) stimulates NHE3 activity, while higher A-II concentration (10\(^{-7}\) M) inhibits NHE3 activity. To test if these concentrations used for acute regulation have similar effect, NHE3 promoter constructs transfected OKP cells were treated with different concentrations of A-II for 16 h before analyzing the promoter activity. As shown in Fig. 1, lower concentration of A-II (10\(^{-10}\) M), which acutely stimulates NHE3 activity, had no effect on stimulating NHE3 promoter activity. High concentration of A-II (10\(^{-7}\) M), which has been shown to inhibit NHE3 activity in the acute setting, stimulated NHE3 gene promoter activity. As a control, pGL3 B vector showed no change in all A-II concentrations used in the experiments.

3.2. Effects of chronic A-II treatment on functional NHE activity in OKP cells

To measure the NHE activity in OKP cells, the initial rate of recovery of intracellular pH (pHi) following an acid load was measured in the presence of 0.4 μM bafilomycin A (to inhibit the V-type H\(^+\)-ATPase), and the absence of medium NaHCO\(_3\). NHE3-specific activity was assessed by further addition of 20 μM HOE-694 to inhibit NHE1 and 2, and S-3226 to inhibit NHE3 specifically [31]. Bafilomycin by itself had no significant effect on pHi recovery rate, whereas 20 μM HOE-694 reduced the pH recovery rate by about 37% compared to control (Fig. 2). The pH recovery rate measured in the presence of both bafilomycin and 20 μM HOE694 was not significantly different than recovery measured with 20 μM HOE694 alone. To specifically evaluate NHE3 activity, recovery rates were measured in the presence of both bafilomycin and 20 μM S-3226. 20 μM S-3226 reduced the pH recovery rate by about 60% compared to control (Fig. 2). No significant recovery was observed with all inhibitors
present (not shown). These data indicate that NHE transport (NHE1-3) is the only process for pHi recovery from an acid load in the absence of NaHCO3 and also suggest that the activity remaining in the presence of 20 μM HOE694 is due to NHE3. In a second series of experiments, the effect of 16 h of treatment with 10⁻⁷ M A-II on purported NHE3 mediated transport activity was evaluated by measuring pH recovery in the presence of bafilomycin and 20 μM HOE694. A-II treatment led to a doubling of NHE3 mediated transport activity (Fig. 3). To evaluate if transcription of new NHE3 was required for this A-II induced increase in activity, cells were preincubated with transcriptional inhibitor actinomycin D. Actinomycin D blocked the A-II mediated increase in HOE694-insensitive activity, indicating that A-II mediates changes in NHE3 activity through a transcriptional mechanism. The dependence of the A-II induced increase in HOE694-insensitive recovery on functional NHE3 activity was directly evaluated using S-3226. Treatment with S-3226 reversed the A-II induced rate of recovery (Fig. 3), suggesting that the A-II mediated increase was specifically dependent on an increase in NHE3 activity.

3.3. A-II increases NHE3 mRNA abundance in OKP cells

Since the observed increase in NHE3 activity was sensitive to a transcriptional inhibitor, actinomycin D, we next evaluated the effects of 16 h treatment with A-II on endogenous expression of NHE3 mRNA in OKP cells by Northern blots. Hybridization with opossum NHE3-specific cDNA probes showed that NHE3 mRNA abundance was increased by ~44% in A-II-treated OKP cells, as compared to controls (2.70 ±0.09 densitometric units in A-II treated cells, versus 1.88±0.05 in controls; n=3) (Fig. 4). To demonstrate that the effect of A-II on NHE3 gene expression was due to transcriptional regulation, OKP cells were first treated with actinomycin D for 2 h and then treated with A-II for 16 h in the presence of actinomycin D. Due to prolonged exposure (18 h) to transcriptional inhibitor actinomycin D, we were not able to obtain enough mRNA to perform Northern blot experiments. Therefore NHE3 mRNA abundance was determined by semiquantitative RT-PCR with OKP-specific NHE3 and β-actin primers (Fig. 5A). Preliminary experiments established standard curves that related amount of RT-reaction used for PCR to amount of amplified product, and all subsequent
experiments were carried out within the linear portions of these curves. Results showed that A-II treatment increased NHE3 mRNA abundance by ∼42%, and this increase was abolished by actinomycin D treatment (1.38±0.03 densitometric units, control; 1.96±0.1, A-II-treated, n=4; 0.42±0.13, actinomycin D alone; 0.38±0.13, A-II-treated in the presence of actinomycin D; n=3) (Fig. 5B).

3.4. Rat NHE3 gene promoter analysis in OKP cells

To determine the A-II responsive region in the rat NHE3 gene promoter, four reporter constructs (pGL3/−1360 bp, pGL3/−548 bp, pGL3/−149 bp, pGL3/−95 bp) and the promoterless pGL3-basic were transfected into OKP cells. Reporter gene assays were performed 40 h after transfection. The luciferase assay data showed that each of these promoter constructs was functional in OKP cells. Compared with pGL3/basic controls, these promoter constructs resulted in eighteen to twenty-six-fold stimulation of reporter gene activity (Fig. 6A; n=3–6).

To test the effect of A-II on rat NHE3 gene promoter activity, OKP cells were transiently transfected with promoter constructs and then treated with 10−7 M A-II or saline for 16 h before they were harvested. Activity of Renilla luciferase (from cotransfected pRL-CMV vector) was not altered by treatment with 10−7 M A-II, thus validating its use as an internal control (data not shown). Exposure to A-II for 16 h increased NHE3 promoter activity in cells transfected with the pGL3/−1360 bp (by 44%±5%) and the pGL3/−548 bp (by 74%±12%)

Fig. 3. Effect of Chronic A-II Treatment on Specific NHE3 Functional Activity. Bars show the mean rates of recovery (±S.E.) measured within the first minute after NH4Cl washout from 25 to 30 cells. All measurements were performed in the presence of Basilomycin A (0.4 μM) and 20 μM HOE-694. The remaining rate of recovery measured in the presence of both inhibitors is used as an estimate of the NHE3 functional activity (NHE-3 Specific). Act. D-100 nM actinomycin D. S-3226 at 20 μM. The drugs were added 5 min prior to initiation of the acid load. All-16 h pretreatment with 100 nM A-II. *Significantly different than recovery measured in the absence of A-II (leftmost bar). †Significantly different than recovery measured in the absence of A-II pretreatment alone (middle bar).

Fig. 4. Northern blot analysis of NHE3 mRNA expression in OKP cells. (A) A representative Northern blot experiment. Blots were probed with opossum NHE3-specific and β-actin-specific probes. The hybridization signal at 8 kilobase pairs (kb) represents NHE3, and hybridization signal at 2 kb represents β-actin. C—control, A-II—angiotensin-II (both panels). (B) Quantitative summary from NHE3 Northern blot experiments. Data are presented as a ratio of NHE3 to β-actin mRNA levels. Values are means±S.E.; n=3. *Indicates statistical significance compared to control.

Fig. 5. Effect of actinomycin D (Act.D) on NHE3 mRNA expression in A-II-treated OKP cells. (A) Representative results of semi-quantitative RT-PCR analysis of NHE3 mRNA. mRNA isolated from OKP cells treated under different conditions was used for first-strand cDNA synthesis. Subsequent PCR was performed with subsaturation levels of the RT reaction, and NHE3 or β-actin primers were used in separate reactions. Equal volumes of PCR reactions for NHE3 and β-actin were loaded on the same gel and visualized with ethidium bromide. (B) Quantitative summary from NHE3 semi quantitative RT-PCR experiments. Data are presented as a ratio of NHE3 mRNA to β-actin mRNA levels. Results are means±S.E. from 3 to 4 separate experiments. The results showed that the ∼42% increase in NHE3 mRNA abundance induced by A-II treatment was abolished by Act.D treatment (100 nM, for 18 h). *Indicates statistical significance compared to control.
constructs, as compared to saline treated cells. The pGL3/−95 bp and pGL3/−149 bp constructs showed no increase in promoter activity with $10^{-7}$ M A-II treatment (Fig. 6B).

To determine whether the A-II effect on NHE3 gene promoter activity was due to transcriptional stimulation and not altered reporter mRNA stability, OKP cells were first transfected with NHE3 promoter constructs (pGL3/−548 bp or pGL3/−95 bp) and then cells were treated with $10^{-7}$ M A-II or saline for 16 h. Fold induction is shown as the ratio of luciferase activity in A-II-treated cells to that in saline treated cells. Values are means±S.E.; number of replicates=3–5. *Statistically different for pGL3/−548 bp and pGL3/−1360 bp vs. all other constructs.

4. Discussion

Previous studies demonstrated that long-term A-II treatment increases NHE3-mediated Na$^+$ absorption, and NHE3 protein abundance in rat renal cortex [16,17]. Studies also showed that under acute A-II treatment conditions, $10^{-10}$ M A-II stimulates NHE3 activity, while $10^{-7}$ M A-II inhibits NHE3 activity. Here, we demonstrate that $10^{-10}$ M A-II has no effect on NHE3 gene expression, while $10^{-10}$ M A-II stimulates NHE3 gene expression in OKP cells under A-II chronic treatment (Fig. 1). These results suggest that the mechanism of A-II regulation on NHE3 activity may be different between acute regulation and chronic regulation. Our data also show an A-II-induced (16 h with 100 nM A-II) increase in NHE3-specific activity in OKP cells (Fig. 3). The rate of pH recovery from an acid load, measured in the presence of 20 μM HOE-694 plus baflomycin, and in the absence of HCO$_3^-$, was assumed to be a specific measure of NHE3 activity. This assumption is based on the fact that HOE-694 is more effective in inhibiting NHE1 and 2 (IC$_{50}$=5 μM in PS120 cells) than NHE3 (NHE3 is unaffected at HOE-694 concentrations up to 70–100 μM) [34]. Although it is conceivable that this increase in 20 μM HOE-694-insensitive Na$^+$/$H^+$ exchange activity could be partially related to the activity of another NHE not yet described in these cells, results obtained with S-3226, a specific NHE3 inhibitor, point to NHE3 as the major mediator of A-II-mediated pH recovery (Fig. 3). Additionally, the contribution of HCO$_3^-$ coupled transporters or V-type H$^+$-ATPase has been ruled out by our protocols. Thus, based on analysis of H$^+$ recovery rates, we concluded that A-II doubled NHE3-specific functional activity. Such an increase could be due to a direct activation of the transporter itself, insertion of stored transporters into the plasma membrane, or de novo synthesis of the transporter. Our data show that actinomycin D treatment inhibits NHE3 activity induced by A-II treatment, suggesting that transcriptional regulatory mechanisms might be involved.

To test our hypothesis about transcriptional regulation of NHE3 activity by A-II, we studied endogenous NHE3 mRNA
levels in OKP. We demonstrated a significant increase in NHE3 transcript abundance with chronic A-II treatment, an effect that was abolished by inhibition of gene transcription with actinomycin D (Fig. 5). These results suggested that chronic A-II treatment stimulates NHE3 activity at least partially through gene expression regulation. In addition, A-II clearly regulated NHE3 promoter activity in cells transiently transfected with reporter gene constructs, which further supports that A-II effect on NHE3 activity involves activating NHE3 promoter. Therefore, our data suggest that chronic A-II exposure increases NHE3 activity, primarily through a transcriptionally mediated mechanism. It is interesting to note that A-II treatment elicited a similar increase in NHE3 mRNA abundance in in vivo studies in rat kidney cortex where changes in mRNA expression paralleled changes in immunoreactive protein expression [17]. To evaluate the interactions of A-II with the NHE3 gene promoter, we developed a series of reporter constructs based on fusions of unique promoter fragments and firefly luciferase.

Four different rat NHE3 gene promoter constructs (pGL3/−1360 bp, pGL3/−548 bp, pGL3/−149 bp, and pGL3/−95 bp) transfected into OKP cells, demonstrated significant activity. Furthermore, the two longer promoter constructs (pGL3/−1360 bp and pGL3/−548 bp) were responsive to A-II treatment, whereas two shorter 5′-deletion constructs (pGL3/−149 bp and pGL3/−95 bp) were not. Interestingly, promoter construct pGL3/−360 bp showed a ~44% increase by A-II treatment, whereas the pGL3/−548 bp promoter construct showed a ~74% increase by A-II treatment. These observations suggest that the putative A-II responsive element(s) is located between 149 and 548 bp upstream of the transcription initiation site, with a modular cis-element(s) located further upstream, between −548 and −1360 nt.

A-II is known to exert its effects through two different A-II receptor subtypes, designated as type 1 receptor (AT1-R) and type 2 receptor (AT2-R) [35]. Many known biological actions of A-II are mediated by stimulation of the AT1-R, which is a member of the G protein-coupled seven-transmembrane-spanning receptor family [36]. Cano et al. demonstrated that acute treatment with A-II at physiological concentrations stimulates NHE activity in OKP cells via a cAMP-independent mechanism mediated by AT1-R and a pertussis toxin-sensitive G protein [20]. Therefore, it is possible that the effect of A-II on NHE3 gene expression in OKP cells is mediated by AT1-R in our experiments. Several A-II responsive elements mediated by AT1-R signal transduction have been identified within the interleukin-6 [35], the plasminogen-activator inhibitor type-1 (PAI-1) [37], and the cyclin D1 genes [26]. These A-II responsive elements include a cAMP response element (CRE)-binding sequence in the interleukin-6 gene [35], an Sp1 binding sequence in the PAI-1 gene [37], and an early growth response gene-1 (Egr-1) binding sequence in the cyclin D1 gene [26]. We searched the rat NHE3 gene promoter region (−548 bp to −149 bp), and found that one sequence (−395 bp-CCGGGGCGTGTGCA-382 bp) is identical to the Sp1 binding sequence which appears in the PAI-1 gene [37]. It remains to be determined whether binding of Sp1 transcription factor to this putative cis-element participates in the mechanism of A-II regulation of the rat NHE3 gene transcription.

In summary, we demonstrate that prolonged exposure of renal proximal tubule epithelial cells to A-II in a concentration previously demonstrated to acutely inhibit Na+/H+ exchange [11,12] results in transcriptionally mediated increase in NHE3. This finding adds a new dimension to the biphasic and concentration-dependent effects of angiotensin II on NHE3-mediated renal acidification, and epithelial Na+ and HCO3− reabsorption. Further studies focusing on identification of the A-II responsive element(s) and trans-factors involved in A-II regulation of the NHE3 gene are required to fully define the transcriptional mechanism underlying this phenomenon.

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