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Original Article

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The role of SGK-1 in angiotensin II-mediated sodium reabsorption in human proximal tubular cells

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Abstract

Background. The role of angiotensin II (Ang II) in mediating excessive sodium reabsorption in diabetic nephropathy is recognized. Serine-glucocorticoid kinase-1 (SGK-1) increases sodium–hydrogen exchanger-3 (NHE3) expression and is known to be upregulated in *in vitro* and *in vivo* models of diabetic nephropathy. However, a link between Ang II and SGK-1 in diabetic nephropathy has not been established.

Methods. Ang II production in cultured human proximal tubular cells was measured under normal (5 mM) and high (25 mM) glucose conditions. The Ang II type 1 receptor was identified by RT-PCR. SGK-1 and NHE3 mRNA and protein expression was measured in proximal tubule cells (PTCs) exposed to Ang II. EIPA inhibitable changes in cell sodium uptake were undertaken to confirm that alterations in NHE3 mRNA and protein were reflected in transport activity. SGK-1 was silenced in the PTCs using small interfering RNA to determine the role of SGK-1 in mediating Ang II-induced increases in NHE3-mediated sodium uptake.

Results. Ang II production by PTCs was significantly increased by exposure to high glucose ($P < 0.02$). Ang II increased NHE3 and SGK-1 mRNA expression to 275 ± 30% ($P < 0.02$) and 130 ± 10% ($P < 0.05$) respectively. Silencing of SGK-1 reduced Ang II-stimulated NHE3 protein expression to 49.8 ± 6.1% ($P < 0.05$) of control levels. SGK-1 silencing abolished increases in $^{22}Na^+$ uptake seen in Ang II-treated cells to 86.7 ± 1.6% of control values.

Conclusion. These data suggest that increased sodium reabsorption in renal proximal tubular cells considered to be due to Ang II in diabetes mellitus is mediated through SGK-1 expression.

Keywords: angiotensin II; diabetes; human proximal tubule; Na^+–H^+ exchange; SGK-1

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Introduction

The importance of the renin–angiotensin system in the pathogenesis of diabetic nephropathy has been demonstrated clinically by the reduction in proteinuria and slowing of progression of nephropathy achieved by the administration of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers to patients with diabetes mellitus [16,17,21]. The role of autocrine Ang II has not been directly demonstrated in the human proximal tubule. Analyses of human biopsies in established diabetic nephropathy have shown reduced glomerular angiotensin type 1 (AT_1) receptor expression, which is postulated to reflect a regulatory response to high intrarenal Ang II concentrations [24]. However, the autocrine effects of angiotensin II (Ang II) on tubular function, including transport mechanisms likely to be involved in both hypertension [4] and albumin reabsorption [10], and the downstream signalling pathways have not been demonstrated in a human model.

In animal models Ang II stimulates an increase in the activity of NHE3 in the proximal tubule, which is mediated through the AT_1 receptor [6]. Therefore, local increases in intrarenal Ang II are likely to contribute to enhanced tubular Na^+ reabsorption and hypertension observed early in the course of diabetic nephropathy. In Na^+–H^+ exchanger isoform 3 (NHE3) knockout mice, systolic and arterial blood pressures are reduced, suggesting a key role for NHE3 in maintaining these parameters [18]. Prior studies from our laboratory in an OK model of proximal tubular cells have demonstrated that tubular albumin reabsorption is stimulated in the presence of high glucose, which is dependent on the intact NHE3 activity [5].

Hypertension and microalbuminuria are clearly early signs of diabetic nephropathy. NHE3 regulates both Na transport and tubular protein uptake and catabolism in the proximal tubule by regulating intracellular and endosomal pH [10]. Hence, in this study we investigated the effects of high glucose on the production of Ang II and its autocrine effects on transport in the human proximal tubule.

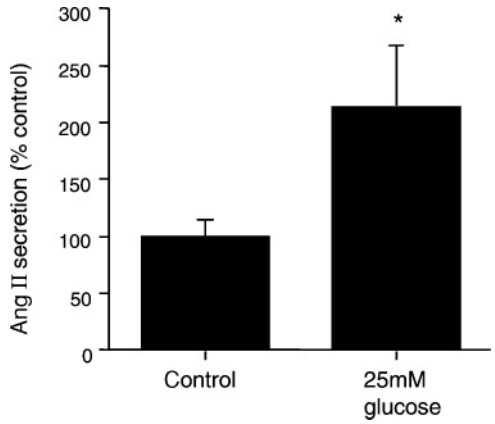

Fig. 1. Effect of high glucose on Ang II production in PTC. Results are expressed as a percentage of standardized control values and represent the mean ± SEM of six experiments. $^*P < 0.02$ versus control.

We have demonstrated that SGK-1 protein expression is upregulated in the proximal tubule of human kidney by high glucose and epidermal growth factor (EGF) [23]. We and others have shown SGK-1 to be involved in regulating NHE3 expression and its phosphorylation [25]. Furthermore, the activity of the prime transporter in the proximal tubule involved in the apical reabsorption of sodium, i.e. NHE3, has recently been shown to be increased by SGK-1 [28] interacting via the Na^+–H^+ regulatory factor isoform 2 (NHERF-2). The current study was therefore undertaken to investigate the interactions between high glucose, Ang II, NHE3 and SGK-1 in the proximal tubule. More specifically, we assessed the effect of high glucose on Ang II secretion in PTCs and changes in SGK-1 and NHE3 mRNA and protein expression due to Ang II. We also examined a possible role for SGK-1 in mediating changes in NHE3 expression due to Ang II by silencing SGK-1 using small interfering (si) RNA technology.

Methods

Patients

Segments of macroscopically normal renal cortex were obtained under aseptic conditions from patients undergoing nephrectomy for small (<6 cm) tumours. Patients were accepted for inclusion into the study if there was no history of renal or systemic disease known to be associated with tubulointerstitial pathology. In addition, the presence of a histologically normal cortical tubulointerstitium at sites removed from the tumour was confirmed by independent pathological examination. Written informed consent was obtained from each patient prior to surgery and ethical approval for the study was obtained from the Royal North Shore Hospital Human Research Ethics Committee.

Cell culture

The methods for primary culture of the human proximal tubule cell (PTC) are described elsewhere [13]. In brief, tubular fragments were derived from renal cortex by collagenase digestion and the resultant digest resuspended in 45% Percoll (Pharmacia, Uppsala, Sweden) in saline and separated by isopyknic ultracentrifugation. The tubular fragments were resuspended in serum-free hormonally defined media (HDM) consisting of a 1:1 (vol:vol) mixture of Dulbecco's modified Eagle's and Hams F-12 media (DMEM/F-12, ICN Pharmaceuticals Inc. Costa Meas, CA, USA), supplemented with 10 ng/ml (1.64 nM) EGF (Collaborative Research Inc., Bedford, MA, USA), 5 μg/ml human transferrin, 5 μg/ml (0.87 μM) bovine insulin, 0.05 μM hydrocortisone, 50 μM prostaglandin E1, 50 μM selenium and 5 pM tri-iodothyronine (all from Sigma Chemical Co., St Louis, MO, USA). The cells were plated into tissue culture flasks and incubated in humidified 95% air/5% CO_2 at 37°C, and the media were changed every 48 h until the cells reached confluence. The cells were harvested using CR-Dispase (Becton Dickinson, Bedford, MA, USA) and stored in liquid nitrogen. When required, the cells were thawed, and, after reaching confluence, were subcultured into the experimental chambers. These cells were designated as passage 2. The ultrastructure, growth and immunohistochemistry of PTC have been well characterized in our laboratory and shown to reflect reproducibly the biology and physiology of their *in vivo* counterparts [13].

Experimental protocol

All experiments were carried out on quiescent, near confluent, passage 2 PTC. Cells were made quiescent by incubation for 24 h in 5 mM glucose basic media (DMEM/F-12 containing 5 μg/ml human transferrin) without growth factors. We have previously used flow cytometry to demonstrate that cells are arrested in the G-0 phase of the cell cycle following this treatment (unpublished observations). Following quiescence, cells were grown in basic media containing 5 mM ('normal' glucose) or 25 mM glucose ('high' glucose) ± Ang II as per the protocols detailed below, for the observation period.

Angiotensin II assay

Ang II production by PTC under normal and high glucose conditions was determined in cells cultured from six kidney specimens. PTCs were grown to confluence in 25 cm^2 flasks, made quiescent and treated for 72 h with a medium containing either normal glucose or high glucose. Supernatants were harvested and stored at −80°C, and cell protein was measured by BioRad protein assay. Ang II in supernatants was quantified using the Ang II Enzyme Immunoassay (EIA) Kit (SPI-BIO, Massy Cedex, France). Briefly, Ang II was extracted from supernatants using Phenyl 10 μm columns (J.T. Baker, Phillipsburg, USA) and dried under vacuum. Ang II eluates were resuspended in 250 μl of EIA buffer and then the ELISA performed as per manufacturer's instructions. Results were expressed as pg of Ang II secreted per mg of cellular protein.

Western blotting

PTCs were exposed for 48 h to 5 mM glucose (control), high glucose (25 mM) or Ang II (10^{-10}). Briefly, to extract

Table 1. Competitive PCR primer sequences

cDNA	Primer sense	Primer sequence 5′ to 3′ size	Target size	Competitor size
NHE3	Sense	GTTCTTCACCGTCATCTTCCA	444	309
	Antisense	AGCGCTGACATTTTCTCTCAG		
	LAS	ACGCGTGACATTTTCTTCTCTCAGGGCCACGTAGCTGATGGC		
SGK-1	Sense	GCCAATGGAGTTGGGGACAG	574	465
	Antisense	TGCCTTGGGCTACCTGCATT		
	LAS	TGCCTTGGGCTACCTGCATTGCACAACATCCTTC TGTC		

Table 2. RT-PCR primer sequences

cDNA	Primer sense	Primer sequence 5′ to 3′ Size	Target size
AT-1 receptor	Sense	TTAGCACTGGCTGACTTATGC	643 bp
	Antisense	GCCGTGTCCACAATATCTGC	
SGK-1	Sense	GACTGTGGACTGGTGGTG	353 bp
	Antisense	CAGGCTCTTCGGTAAACT	
Actin	Sense	CATGTACGTTGCTATCCAG	757 bp
	Antisense	CGCAACTAAGTCATAGTCC	

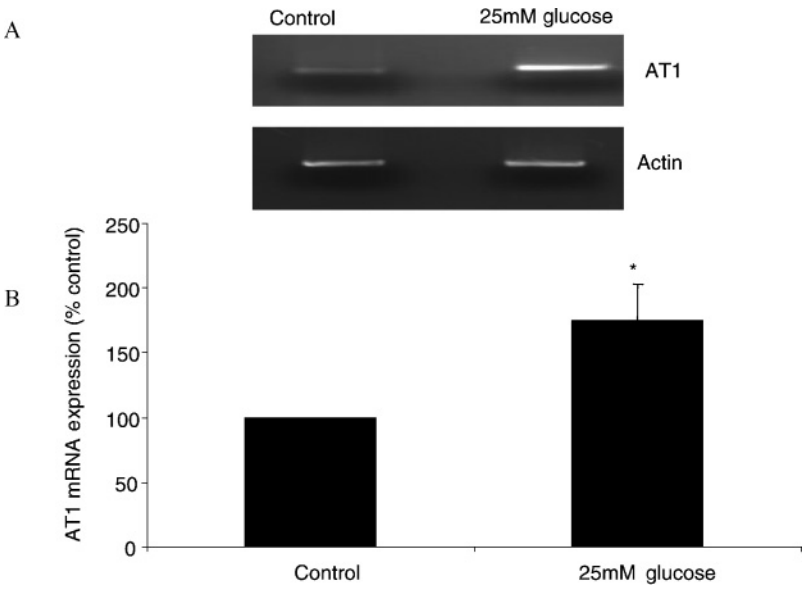

Fig. 2. AT-1 receptor RT-PCR. Cells were treated under either control (5 mM) or high glucose (25 mM) conditions for 24 h. (**A**) Representative PCR demonstrating high glucose induces AT1 expression. (**B**) Graphical representation of AT1 PCR of cells exposed to high glucose. $N = 4$, $^*P < 0.05$ versus control.

protein from cell lysates, cells were firstly washed twice with PBS, then lysed with 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 20 mM sodium orthovanadate, 0.5% Triton-X 100 with proteinase inhibitor cocktail 'Complete' (Boehringer/Roche) for 20 min, followed by centrifugation at 10 000 × *g* for 10 min at 4°C. From lysates 30 μg protein was separated under reduced conditions on a 10% SDS–PAGE gel and transferred to the Hybond ECL nitrocellulose membrane (Amersham Pharmacia, Germany). The membranes were incubated with an antibody to SGK-1 (Cell Signalling, Beverly, MA, USA) or NHE3 (BD Biosciences, Franklin Lakes, NJ, USA) overnight at 4°C, followed by incubation with a peroxidase-labelled anti-rabbit antibody (Amersham Life Science) for 2 h at room temperature. Protein was detected using the ECL western blotting analysis system (Amersham Pharmacia, Germany).

Table 3. siRNA sequences

SGK-1 siRNA	
Sense	GUCCUUCUCAGCAAAUCAAUU
Antisense	UUGAUUUGCUGAGAAGGACUU

A non-specific band of around 120 kDa was detected using the NHE3 antibody. The identity of NHE3 (85 kDa) was confirmed by overexpressing NHE3 (data not shown).

PCR

SGK-1 and NHE3 competitive RT-PCR Competitive RT-PCR was performed to confirm the changes in the SGK-1 and NHE3 mRNA expression level induced by exposure to

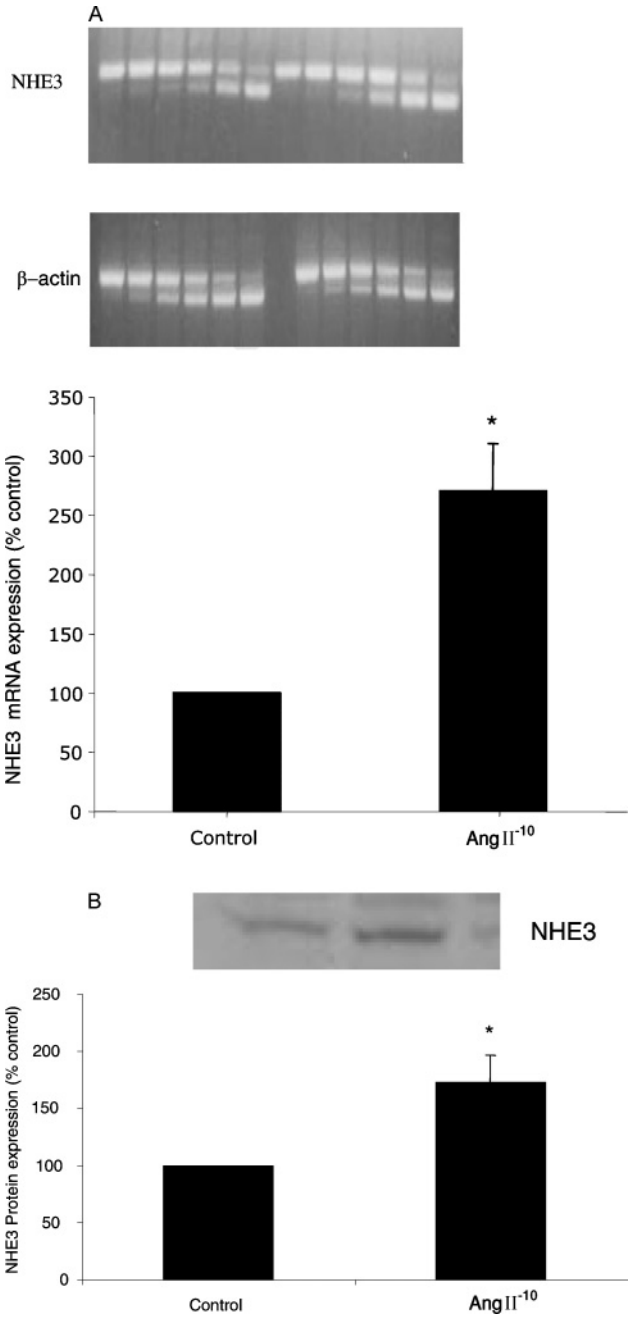

Fig. 3. Effect of Ang II on NHE3 mRNA and protein expression. **(A)** Representative competitive PCR gel and graphical representation of cPCR demonstrating an increase in NHE3 mRNA expression in cells exposed to 10^{-10} M Ang II for 24 h. $^{*}P < 0.02$ versus control. **(B)** Graphical representation of an increase in NHE3 protein expression in cells exposed to Ang II for 48 h. Results are expressed as a percentage of standardized control values and represent the mean ± SEM of experiments from four different culture preparations. $^{*}P < 0.05$ versus control.

Ang II. Primer sequences are given in Table 2. Total RNA was extracted using the RNeasy kit (QIAGEN, Victoria, Australia), according to manufacturer's instructions. RNA was reverse transcribed using the Superscript II Reverse Transcriptase kit (GibcoBRL, MD, USA). Sense and competitive primer sequences are as detailed for both NHE31 and SGK-1 in Table 1.

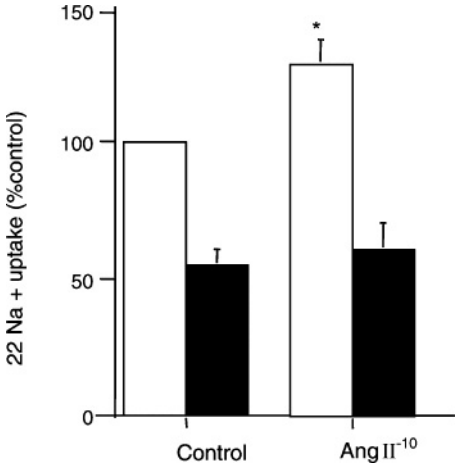

Fig. 4. Effect of Ang II on $^{22}Na^{+}$ uptake. PTCs were treated with Ang II (10^{-10} M) in the absence (□) or presence (■) of EIPA. Results are expressed as a percentage of standardized control values (□) or a percentage of the EIPA sensitive component of uptake (■) and represent the mean ± SEM of six experiments. $^{*}P < 0.005$ versus control.

PCR reactions were performed on cDNA using the sense and competitor primers and the product gel purified and quantitated. For the PCR reaction, the competitor cDNA was used at concentrations of 0.2, 0.5, 1.5, 4.6 and 13.7 fM. The thermal profile consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1.30 min and extension at 72°C for 1.30 min, for 30 cycles using the Expand High Fidelity PCR System (Roche, Mannheim, Germany). The products were run on a 2% agarose gel stained with ethidium bromide and photographed. The photograph was then scanned into a computer and the relative intensities of the individual bands were quantitated using NIH Image software v1.60. It was considered that the concentration of the competitor is equivalent to the concentration of the message for the target gene.

SGK-1 and AT_1 RT-PCR Angiotensin II type I receptor (AT_1 receptor) and SGK-1 (to confirm silencing) expression in PTC was confirmed by RT-PCR. Total RNA was isolated from PTC grown in 35-mm Petri dishes using the Rneasy kit (Invitrogen, Life Technologies, Carlsbad, CA). One microgram of RNA was reverse transcribed to cDNA using SuperScript II^{TM} RNase H Reverse Transcriptase (Life Technologies). The amplification of the resulting cDNA was performed using the Expand High Fidelity PCR System (Roche Molecular Biochemicals, Mannheim, Germany) in a 25-μl reaction volume with 2.5 mM MgCl, 200 μM dNTPs and 50 nM forward and reverse primers. The thermal profile consisted of denaturation at 94°C or for 1 min, annealing at 55°C (AT_1) for 1 min or 58°C (SGK-1) and extension at 72°C for 1 min, for 40 cycles. B actin was used as an internal standard using the same thermal profile as SGK-1 but for 28 cycles.

The products were electrophoresed on a 2% agarose gel. The identity of the PCR product was confirmed as being the AT1 receptor. Primer sequences are shown in Table 2.

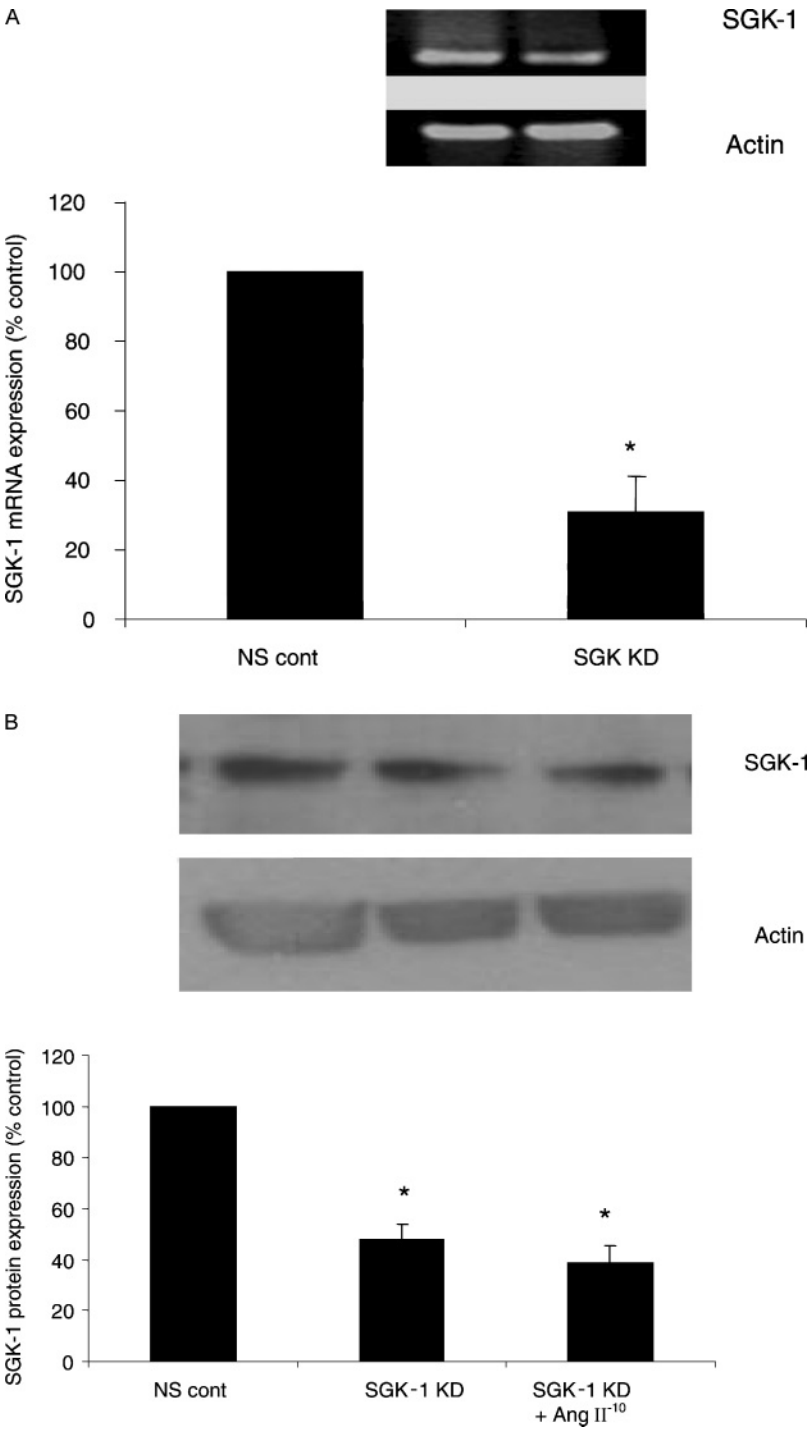

Fig. 5. SGK-1 silencing in PTCs. (**A**) Graphical representation of SGK-1 silencing in PTCs. Representative PCR of SGK-1 silencing, demonstrating a decrease in SGK-1 expression. $^{*}P < 0.05$ versus control. (**B**) Effect of Ang II on SGK-1 protein after silencing. Graphical representation of SGK-1 protein expression subsequent to SGK-1 silencing, representing the mean ± SEM of four experiments. $^{*}P < 0.02$ versus control.

SGK-1 gene silencing Small interfering siRNA was designed specifically to target SGK-1 mRNA sites (Table 3) (Ambion, Austin, TX, USA). PTCs were transfected with SGK-1 siRNA (4 nM final concentration) using the Amaxa Electroporator accompanied by the Basic Nucleofactor kit for primary epethilial cells, as per manufacturer's instructions. Briefly, PTCs were grown to 70–80% confluency, at which time they were gently trypsinized. Then, 1×10^6 cells were gently spun down at 200 rcf for 10 min at room temperature, resuspended in a 100 μL Basic Nucleofactor Solution and then a final concentration of 4 nM siRNA was used. The cells were gently transferred to a cuvette and nucleofected using the program T-20. To each cuvette 500 μl of pre-warmed HDM was added, and the contents were transferred to one of the wells of a six-well plate with pre-warmed HDM. The cells were allowed to recover for 24 h

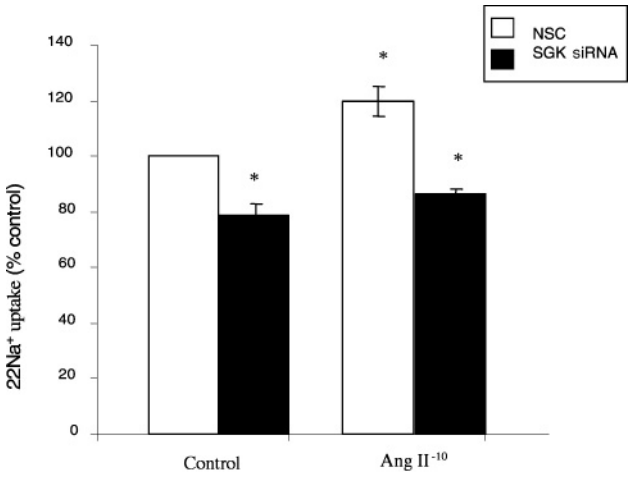

Fig. 6. Effect of SGK-1 silencing on $^{22}Na^+$ uptake. Cells with NSC (□) or SGK-1 siRNA (■) were treated with Ang II (10^{-10} M). Results are expressed as a percentage of standardized control (□) values and represent the mean ± SEM of four experiments. * $P < 0.005$ versus control.

before beginning appropriate treatment. Seventy-two hours following transfection and treatment, mRNA and protein levels were determined. All siRNA experiments included negative non-specific control (NSC) transfection of cells (NSC siRNA, Ambion).

Once SGK-1 knockdown was confirmed, subsequent experiments were conducted to determine the effect of exposure to Ang II on SGK-1, NHE3 expression and sodium uptake in wild-type PTCs and those in which SGK-1 was effectively silenced. Twenty-four hours after SGK-1 knockdown, the cells were treated with control (5 mM glucose) or Ang II^{-10} M for 48 h, at which stage mRNA and cell lysates were collected. PCR was performed as described below to confirm SGK-1 silencing. Western blotting was performed as described above for SGK-1 and NHE3 expression.

$^{22}Na^+$ uptake Na^+ uptake into cells was measured to confirm that alterations in NHE3 mRNA and protein uptake reflected physiological changes in Na transport in both wild-type cells and cells in which SGK-1 was silenced. This was demonstrated using standard $^{22}Na^+$ uptake techniques [8,22]. Quiescent cells were first incubated with 10^{-10} M Ang II for 4 h in a serum-free medium and then washed with a Na^+-free solution (which contained 136.0 mM NMDG, 5.4 mM KCl, 1.2 mM $CaCl_2$, 0.8 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 10.0 mM HEPES, pH 7.4, and 5.0 mM D-glucose). They were then incubated for 30 min in the sodium-free solution containing Ang II and 100 μM ouabain in the presence or absence of 10^{-4} M ethylisopropylamiloride (EIPA). Following this, the cells were exposed to the uptake solution (which was the same as the incubation buffer except for substitution of NMDG and NaH_2PO_4 with NaCl and KH_2PO_4) containing $^{22}Na^+$ (1 mCi/ml as NaCl, NEN Life Science Products Inc., Boston, MA, USA). The experiment was terminated at 20 min by washing four times with ice-cold 0.1 M $MgCl_2$. The cells were solubilized with 0.2 M NaOH and the samples counted in a β-counter (LKB Wallac, Turku, Finland). Zero time values (cells exposed to the uptake solution for 5 s) were subtracted from the experimental values. Initial uptake experiments confirmed that the uptake of ^{22}Na was in the linear range at the time point tested (data not shown).

Statistical analysis

All experimental conditions were replicated at least six times using PTC cultures from at least three separate human donors, unless otherwise stated. Results are expressed as a percentage change from the control value (5 mM glucose). Results are expressed as mean ± SEM. Statistical comparisons between groups were made by analysis of variance (ANOVA), with pairwise multiple comparisons made by Fisher's protected least-significant differences test. Analyses were performed using the software package StatView version 4.5 (Abacus Concepts Inc., Berkley, CA, USA). *P*-values <0.05 were considered significant.

Results

Ang II production by PTCs under control and high glucose conditions

PTCs grown under serum-free conditions were found to produce significant levels of endogenous Ang II in the cell culture supernatant of 4.2 ± 0.89 pg/mg cellular protein ($n = 6$). When translated to culture conditions, these levels correspond to ∼100 pg/ml in the culture medium, which is equivalent to 10^{-10} M Ang II. Following exposure to high glucose, the level of Ang II effectively doubled to 211.9 ± 37.9% of control values ($n = 6$; $P < 0.02$) (Figure 1).

Angiotensin type 1 receptor PCR

We performed AT_1 receptor RT-PCR in order to confirm that these cells in culture continue to express AT_1. This resulted in a 643 bp band, which was the predicted product size for the AT_1R (Figure 2a). PCR products from cultured cells were cloned and sequenced and found to be 100% homologous with the published sequence for the human AT_1R (Gene Bank Accession Number X65699) confirming that the PTCs used in this study express the message for the AT_1 receptor. The AT1 receptor was found to be upregulated by exposure to high glucose to 174.5 ± 28.9% of control levels (Figure 2b, $P < 0.05$).

NHE3 mRNA and protein expression

The effect of Ang II on NHE3 expression was examined. Ang II increased both mRNA and protein expression of NHE3 to 275 ± 30% and 170 ± 23%, respectively (Figure 3a and b; $P < 0.02$). Previously, we have demonstrated that SGK-1 overexpression upregulates NHE3 expression in the proximal tubule [23]. It was therefore considered that Ang II may regulate NHE3 through an SGK-1 dependent mechanism. To confirm that a physiological increase in Na transport occurred as a consequence of an increase in NHE3 mRNA and protein expression, cellular uptake of Na was examined.

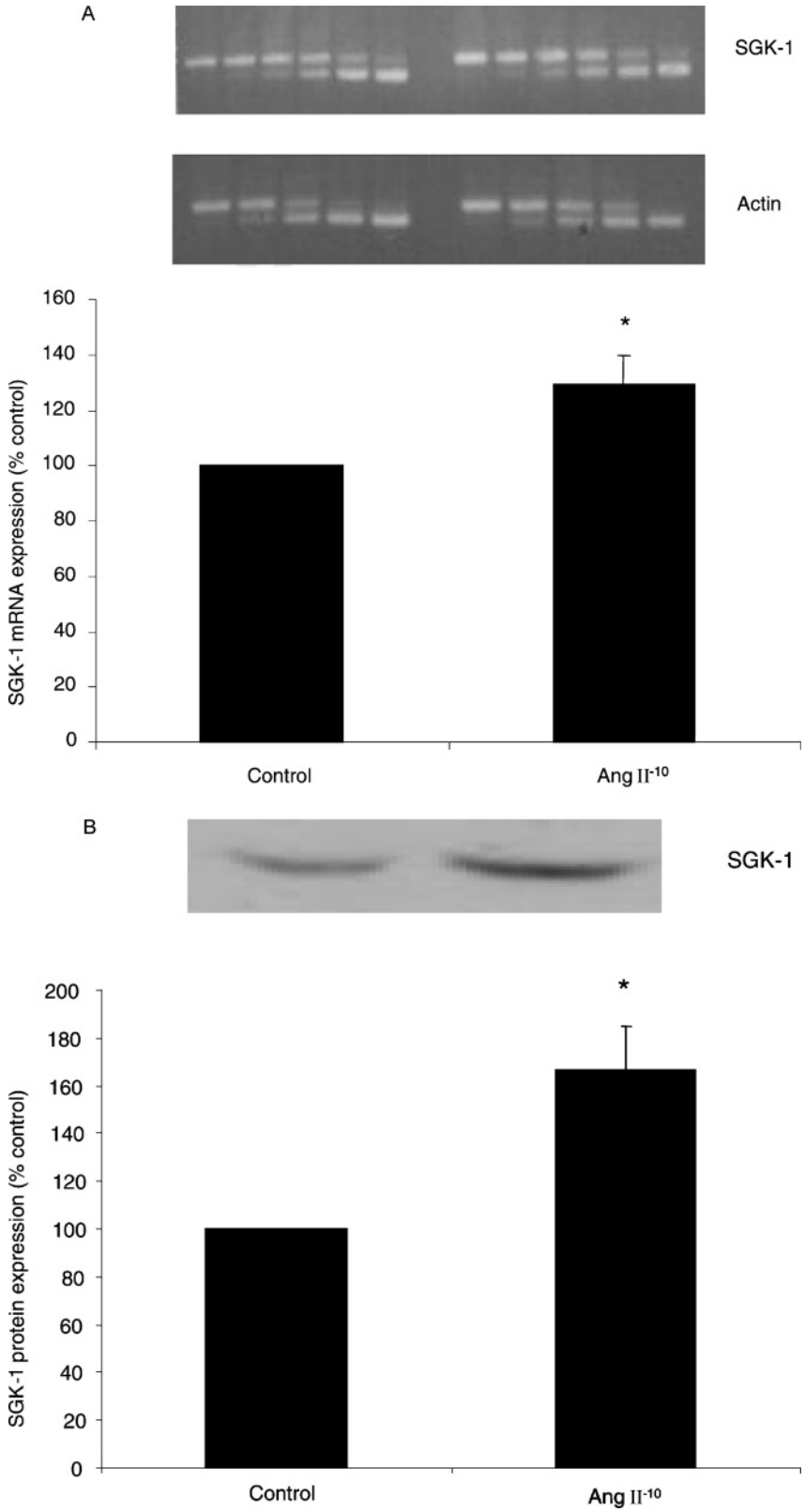

Fig. 7. Effect of Ang II on SGK-1 expression. **(A)** Representative competitive PCR gel and graphical representation of cPCR demonstrating an increase in SGK-1 mRNA expression in cells exposed to 10^{-10} M Ang II for 24 h. **(B)** Graphical representation of western blots demonstrating increased SGK-1 protein expression in cells exposed to 10^{-10} M Ang II. Results are expressed as a percentage of standardized control values and represent the mean ± SEM of experiments from four different culture preparations. $^{*}P < 0.05$ versus control.

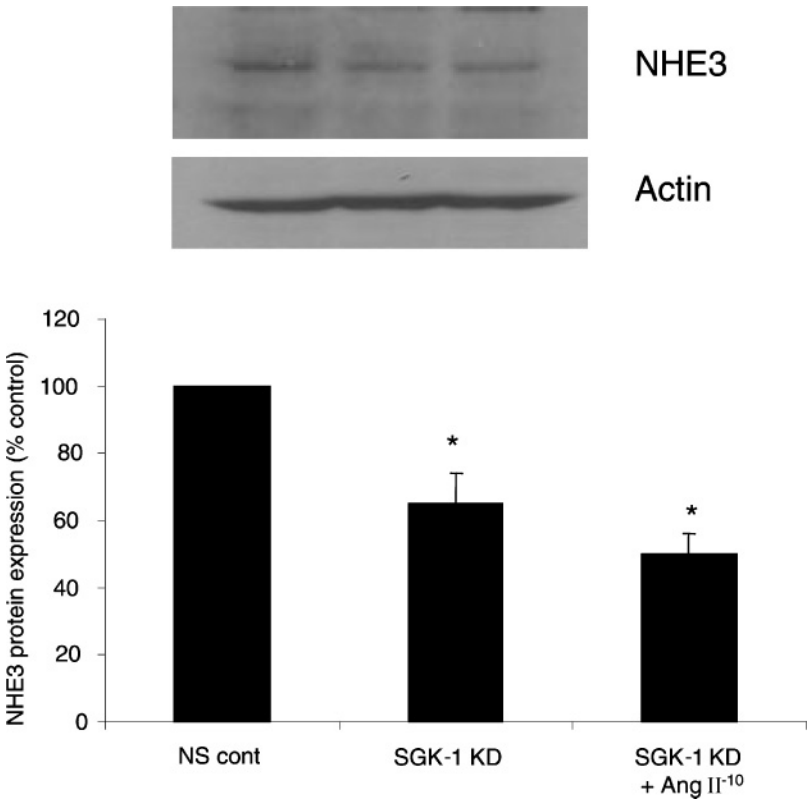

Fig. 8. Effect of SGK-1 silencing on NHE3 protein expression. A representative protein gel and a graphical quantitation of NHE3 protein expression in PTCs subsequent to SGK-1 silencing ± SEM of experiments from four different culture preparations. $^{*}P < 0.05$ versus control.

$^{22}Na^{+}$ uptake

Exposure of PTC to concentrations of Ang II corresponding to those measured in the lumen of the proximal tubule (i.e. 10^{-10} M) resulted in significant stimulations of total $^{22}Na^{+}$ uptake to 129.0 ± 8.5% of control values ($n = 6$; $P < 0.005$) (Figure 4). These increases in total Na^{+} uptake were inhibited by EIPA and therefore attributable to an increase in Na^{+} uptake by NHE3.

We then performed $^{22}Na^{+}$ uptake studies in cells in which SGK-1 was silenced to determine its role in mediating changes seen in Na^{+} transport by Ang II in PTC culture. To confirm SGK-1 silencing, mRNA expression was examined and found to be reduced to 30 ± 10% ($P < 0.05$) compared to control levels (Figure 5a). This translated into effective silencing of SGK-1 protein expression to 47.7 ± 6.2% ($P < 0.02$) of control levels (Figure 5b). In SGK-1-silenced cells, Ang II did not elicit an increase in SGK-1 protein expression (38.66 ± 6.9%; Figure 5b).

NSC siRNA was used to determine the specificity of SGK-1 siRNA. SGK-1 silencing resulted in a reduction in total $^{22}Na^{+}$ uptake to 78.9 ± 3.6% of control values ($P < 0.005$), suggesting a role for SGK-1 in the basal regulation of sodium uptake. When SGK-1-silenced cells were exposed to Ang II, $^{22}Na^{+}$ uptake did not increase being 86.7 ± 1.6% of control similar to values observed in cells in which SGK-1 was silenced ($n = 5$; Figure 6).

The dependence of Ang II-induced increases in NHE3 on SGK-1

As both Ang II and high glucose increased NHE3 mRNA and protein expression, we determined whether this was mediated through an SGK-1-dependent mechanism. Ang II increased SGK-1 mRNA and protein expression to 131 ± 12% and 165 ± 15%, respectively (Figure 7a and b, $P < 0.05$).

We then examined a possible role for SGK-1 in mediating changes seen in NHE3 induced by Ang II. SGK-1 silencing significantly reduced NHE3 protein expression to 65.33 ± 8.95% ($P < 0.05$) control levels (Figure 8). In cells lacking SGK-1 and subsequently treated with Ang II, there was also a significant reduction of NHE3 protein expression to 49.8 ± 6.1% ($P < 0.05$) of control levels (Figure 8).

Discussion

This current study shows that isolated cultures of PTCs produce Ang II, providing direct confirmation that the proximal tubule in the human is a major site regulating the paracrine effects of Ang II. Furthermore, we demonstrate that Ang II alone at intra-renal concentrations stimulates NHE3 protein and mRNA expression reflected in increased Na^{+} reabsorption. This reveals an important role for

'resting' levels of intrarenal Ang II in regulating Na^+ uptake by the human proximal tubule.

It has been shown in diabetic animal models that early diabetes mellitus results in increased renin production by the proximal tubules [14]. Recent studies have measured the ability of the rat proximal tubule to regulate precursors of Ang II in response to high levels of glucose. These studies have demonstrated that high glucose induces angiotensinogen gene expression [12,29], in part through the generation of reactive oxygen species and p38 MAP kinase [3,19]. More recently, there has been data to support that the stimulatory effect of high glucose concentrations on the expression of angiotensinogen mRNA is likely to be mediated via local Ang II in rat immortalized renal proximal tubular cells, probably involving both AT_1R and AT_2R [11].

Importantly, we have shown in PTCs grown under serum-free conditions, that the levels of Ang II production are greatly increased upon exposure to high glucose. The findings of the current study in human cells are therefore consistent with reports in other species where excessive levels of intrarenal Ang II are implicated in the pathogenesis of diabetic nephropathy [20,26]. By using RT-PCR we were able to detect the message for the AT_1 receptor and demonstrate that it is upregulated when acutely exposed to high glucose. The regulation of AT_1 receptors in response to high glucose is controversial with a number of studies suggesting a downregulation in response to high glucose and additional studies suggesting an upregulation as confirmed in our own studies [9,27].

This study highlights the importance of autocrine regulation of Na^+ transport by Ang II in the human proximal tubule. Basal levels of Ang II in the culture media were found to be 10^{-10} M and this concentration was sufficient to increase Na^+ transport. Proximal tubule Na^+ reabsorption and its regulation are of particular relevance in the genesis of hypertension and the Na^+ retention seen in renal diseases. It has been reported that exposure of opossum kidney cells to high glucose results in an increase in NHE3 protein and activity [1,5]. This raises the possibility of a high glucose-mediated potentiation of Ang II-mediated effects on Na^+ reabsorption and therefore a link between high glucose and the Na^+ retention frequently associated with diabetic nephropathy.

Previously we have demonstrated that high glucose increases both SGK-1 and NHE3 protein and mRNA expression [23]. The downstream effects of high glucose and overexpression of SGK-1 are analogous, with high glucose known to induce an increase in tubular growth [15] and NHE3 activity [5]. By using small interfering RNA techniques we have demonstrated that SGK-1 is critically involved in Ang II-mediated Na reabsorption via NHE3. Our prior studies have demonstrated that both high glucose-induced SGK-I activations signal through the EGF receptor (EGF-R). As Ang II has also recently been shown to signal through this receptor [2,7], the upstream link to SGK-1 is further suggested.

The conclusions of the present studies are drawn in light of limitations in the experimental models. It should be recognized that differences in driving forces due predominantly to the prevailing intracellular sodium concentration may influence apical sodium uptake. Although intracellular sodium was not directly measured in the present experiments, there is no physiological reason to suspect that this may have affected the results of the present studies. In particular, the studies were conducted in the linear range of sodium uptake and the dominant basolateral transporter regulating intracellular sodium concentration, i.e. Na, K-ATPase was similarly blocked in these studies. Similarly, the concentration of EIPA used in the present experiments would be expected to additionally block the basolateral NHE1 and the apical NHE2 transporters. However, as these transporters are responsible for minimal transcellular sodium transport, it is reasonable to consider that increased NHE3 activity is responsible for the increase in the EIPA inhibitable increase in sodium uptake.

Hence, the findings of the current study present a possible model for the mechanisms by which high glucose can induce tubulointerstitial damage by autocrine mechanisms. At physiological levels of glucose, Ang II acts in a housekeeping role to mediate sodium reabsorption and maintain intracellular pH. When the glucose concentration increases, NHE3 stimulation by Ang II is likely to result in hypertension and potentially the characteristic increased protein trafficking and cellular cytokine release observed in diabetic nephropathy.

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Conflict of interest statement. None declared.

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