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Angiotensin II diminishes the effect of SGK1 on the WNK4-mediated inhibition of ROMK1 channels

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ROMK1 channels are located in the apical membrane of the connecting tubule and cortical collecting duct and mediate the potassium secretion during normal dietary intake. We used a perforated whole-cell patch clamp to explore the effect of angiotensin II on these channels in HEK293 cells transfected with green fluorescent protein (GFP)-ROMK1. Angiotensin II inhibited ROMK1 channels in a dose-dependent manner, an effect abolished by losartan or by inhibition of protein kinase C. Furthermore, angiotensin II stimulated a protein kinase C-sensitive phosphorylation of tyrosine 416 within c-Src. Inhibition of protein tyrosine kinase attenuated the effect of angiotensin II. Western blot studies suggested that angiotensin II inhibited ROMK1 channels by enhancing its tyrosine phosphorylation, a notion supported by angiotensin II's failure to inhibit potassium channels in cells transfected with the ROMK1 tyrosine mutant (R1Y337A). However, angiotensin II restored the with-no-lysine kinase-4 (WNK4)-induced inhibition of R1Y337A in the presence of serum–glucocorticoids-induced kinase 1 (SGK1), which reversed the inhibitory effect of WNK4 on ROMK1. Moreover, protein tyrosine kinase inhibition abolished the angiotensin II-induced restoration of WNK4-mediated inhibition of ROMK1. Angiotensin II inhibited ROMK channels in the cortical collecting duct of rats on a low sodium diet, an effect blocked by protein tyrosine kinase inhibition. Thus, angiotensin II inhibits ROMK channels by two mechanisms: increasing tyrosine phosphorylation of the channel and synergizing the WNK4-induced inhibition. Hence, angiotensin II may have an important role in suppressing potassium secretion during volume depletion.

Kidney International (2011) **79,** 423–431; doi:10.1038/ki.2010.380; published online 6 October 2010

KEYWORDS: AT1 receptor; collecting duct; K channels; K secretion; Src-family protein tyrosine kinase

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Received 20 January 2010; revised 6 August 2010; accepted 10 August 2010; published online 6 October 2010

ROMK1 channels are located in the apical membrane of the connecting tubule and the cortical collecting duct (CCD) and have an important role in mediating K secretion under normal dietary K intake.[1–3] Our previous study has demonstrated that Src-family protein tyrosine kinase (PTK) is involved in increasing inhibition of ROMK1 channels during K restriction.[3] The effect of PTK on ROMK1 channels is at least partially the result of increasing tyrosine phosphorylation of ROMK1 channels on tyrosine residue 337.[4,5] Consequently, tyrosine-phosphorylated ROMK1 channels are subjected to endocytosis.[6] In addition, ROMK1 channels are also inhibited by with-no-lysine kinase (WNK) family kinases including WNK1, WNK3, and WNK4.[7–10] It has been demonstrated that WNK1 and WNK4 stimulate the internalization of ROMK channels by a mechanism involving intersection, a scaffolding protein, and clathrin.[10] Recently, we have reported that Src-family PTK is also involved in modulating the interaction between serum–glucocorticoids-induced kinase 1 (SGK1) and WNK4 by diminishing the SGK1-mediated phosphorylation of WNK4.[11] Consequently, SGK1 failed to reverse the inhibitory effect of WNK4 on ROMK channels in the presence of Src-family PTK. However, the physiological factor that stimulates Src-family PTK is not known. Our previous study has demonstrated that stimulation of angiotensin II (AngII) type I receptor (AT1R) inhibited ROMK channel activity through a PTK-dependent pathways in the native CCD of rats on a low K diet.[12] Moreover, AngII has been shown to stimulate Src-family PTK in vascular tissue.[13] Therefore, we speculate that AngII may be one of the upstream factors that stimulate Src-family PTK and modulate the effect of SGK1 on WNK4 under physiological conditions. Hence, the goal of this study is to test whether AngII also inhibits ROMK channels by enhancing the inhibitory effect of WNK4 on ROMK1 channels and to test whether AngII decreases ROMK channels in the native CCD.

RESULTS

We used HEK293T cells transfected with green fluorescent protein (GFP)-ROMK1 to study the effect of AngII on ROMK channels. Figure 1a is a typical western blot carried out with lysates from HEK293T cells and rat kidney (cortex) and demonstrates the expression of endogenous AT1R in HEK 293T cells. We measured Ba^{2+}-sensitive K currents with the perforated whole-cell recording technique under control

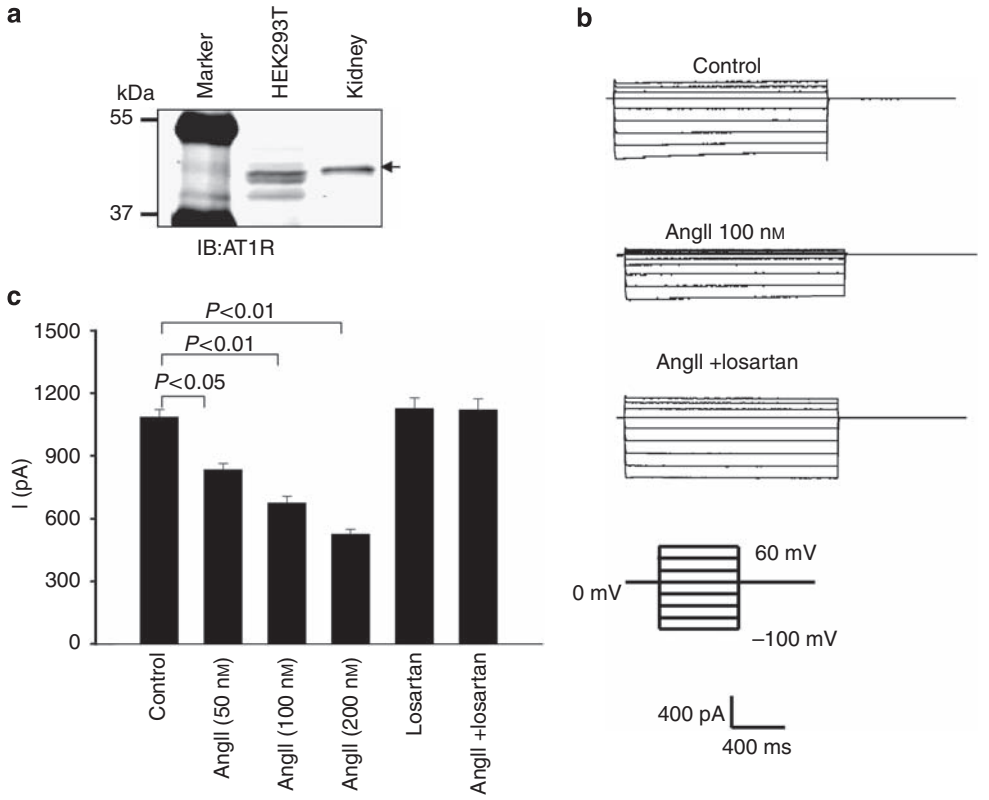

Figure 1 | Angiotensin II (AngII) inhibits ROMK1 channels. (**a**) A western blot showing the expression of type 1 Ang II receptor (AT1R) in HEK293T cells and the rat kidney. (**b**) Whole-cell Ba^{2+}-sensitive K currents in HEK293T cells transfected with green fluorescent protein (GFP)-ROMK1 in the absence of AngII (control) and in the presence of 100 nM AngII and AngII + losartan (20 μM), respectively. K currents were measured with the perforated whole-cell patch clamp from −100 to 60 mV at a step of 20 mV. (**c**) A bar graph summarizes the inhibitory effect of AngII on ROMK1 channels at different concentrations in the absence of or presence of losartan (20 μM). K currents were measured with perforated whole-cell patch clamp at −100 mV in HEK293T cells transfected with GFP-ROMK1. I (pA), currents (pA).

conditions and in the cells treated with AngII (100 nM) for 20 min. Figure 1b is a whole-cell patch recording showing that K currents in cells transfected with GFP-ROMK1 were inward rectified and that application of AngII (100 nM) inhibited ROMK1 channels. We also measured the cell capacitances, which varied between 24.5 and 26 pF. The value was used to normalize the K currents to that of a cell with 25 pF capacitance. The effect of AngII on ROMK channels was dose dependent: application of 50, 100, and 200 nM AngII decreased K currents from 1086 ± 35 pA ($n = 6$) to 833 ± 30 pA ($n = 6$), 678 ± 30 pA ($n = 6$), and 527 ± 22 pA ($n = 6$), respectively (Figure 1c). The effect of AngII on ROMK channels was the result of stimulation of AT1R because losartan completely abolished the inhibitory effect of AngII on the ROMK channels (Figure 1b). Data summarized in Figure 1c show that losartan (20 μM) *per se* has no significant effect on K currents (1127 ± 50 pA, $n = 6$) in comparison with the control value. However, 100 nM AngII failed to decrease K currents (1120 ± 52 pA, $n = 6$) in the presence of losartan.

After demonstrating that stimulation of AT1R inhibited ROMK channels, we examined the effect of AngII (100 nM) on ROMK1 channels in HEK293T cells pretreated with protein kinase C (PKC) inhibitors, calphostin C (100 nM), for 20 min. Although inhibition of PKC had no significant effect on ROMK channel activity, it abolished the effect of AngII. As calphostin C may have an effect other than inhibiting PKC, we repeated the experiments with GF109203x (5 μM). Data summarized in Figure 2 show that treatment of HEK293T cells transfected with ROMK1 with either calphostin C (100 nM) or GF109203x (5 μM) abolished the inhibitory effect of AngII on ROMK channels (control, 1090 ± 40 pA; calphostin C + AngII, 1110 ± 40 pA. GF109203x + AngII, 1100 ± 40 pA). Thus, the present results are in consistence with experiments performed in the rat CCD, in which inhibition of PKC blocked the effect of AngII on ROMK channels.[12] In that study the effect of AngII was also suppressed by blocking PTK.[12] To examine the role of PTK in mediating the effect of AngII on ROMK channels, we studied whether AngII increases tyrosine phosphorylation of c-Src on tyrosine residue 416, an indication of activated c-Src, which was used as a representative member of Src-family PTK. Figure 3a is a typical western blot from five experiments demonstrating that application of AngII at 5, 10, 25, 50, and 100 nM within 30 min enhanced the tyrosine phosphorylation of c-Src on the residue 416, whereas AngII did not change the total c-Src expression. Also, inhibition of PKC with calphostin C (100 nM) attenuated the