http://www.kidney-international.org

Proximal tubule sphingosine kinase-1 has a critical role in A_1 adenosine receptor-mediated renal protection from ischemia

Sang W. Park[1], Mihwa Kim[1], Joo Y. Kim[1], Kevin M. Brown[1], Volker H. Haase[2], Vivette D. D'Agati[3] and H. Thomas Lee[1]

[1]*Department of Anesthesiology, Columbia University, New York, New York, USA;* [2]*Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University, Nashville, Tennessee, USA and* [3]*Department of Pathology, Columbia University, New York, New York, USA*

Renal ischemia–reperfusion injury is a major cause of acute kidney injury. We previously found that renal A_1 adenosine receptor (A_1AR) activation attenuated multiple cell death pathways including necrosis, apoptosis, and inflammation. Here, we tested whether induction of cytoprotective sphingosine kinase (SK)-1 and sphingosine-1-phosphate (S1P) synthesis might be the mechanism of protection. A selective A_1AR agonist (CCPA) increased the synthesis of S1P and selectively induced SK1 in mouse kidney and HK-2 cells. This agonist failed to protect SK1-knockout but protected SK2-knockout mice against renal ischemia–reperfusion injury indicating a critical role of SK1 in A_1AR-mediated renal protection. Inhibition of SK prevented A_1AR-mediated defense against necrosis and apoptosis in HK-2 cells. A selective $S1P_1R$ antagonist (W146) and global *in vivo* gene knockdown of $S1P_1Rs$ with small interfering RNA completely abolished the renal protection provided by CCPA. Mice selectively deficient in renal proximal tubule $S1P_1Rs$ ($S1P_1R^{f/f}$ $PEPCK^{Cre/-}$) were not protected against renal ischemia–reperfusion injury by CCPA. Mechanistically, CCPA increased nuclear translocation of hypoxia-inducible factor-1α in HK-2 cells and selective hypoxia-inducible factor-1α inhibition blocked A_1AR-mediated induction of SK1. Thus, proximal tubule SK1 has a critical role in A_1AR-mediated protection against renal ischemia–reperfusion injury.

Kidney International (2012) **82,** 878–891; doi:10.1038/ki.2012.224; published online 13 June 2012

KEYWORDS: apoptosis; hypoxia-inducible factor-1α; inflammation; necrosis; sphingosine-1-phosphate

Correspondence: *H. Thomas Lee, Department of Anesthesiology, Anesthesiology Research Laboratories, Columbia University, P&S Box 46 (PH-5) 630 West 168th Street, New York, New York 10032-3784, USA.*
E-mail: tl128@columbia.edu

Received 6 December 2011; revised 15 February 2012; accepted 27 March 2012; published online 13 June 2012

Renal ischemia–reperfusion (IR) injury is a frequent cause of acute kidney injury (AKI).[1] AKI a major clinical problem often leading to multi-organ dysfunction and systemic inflammation with extremely high mortality.[2] Unfortunately, the severity and incidence of AKI has been increasing without any improvements in therapy or patient survival over the past 50 years.[3] Currently, the incidence of renal dysfunction after major surgery in high-risk patients has been reported to be as high as 70–80%.[4–6] Despite continued research on renal protective strategies, there are no proven therapies to reduce AKI in the perioperative setting.

We have previously demonstrated that A_1 adenosine receptor (A_1AR) activation reduced necrosis and apoptosis in cultured proximal tubule cells.[7,8] Moreover, A_1AR activation immediately before renal ischemia protected against renal IR injury in rats and mice *in vivo*.[9,10] In addition to reduction in renal necrosis and apoptosis,[9,11] we also observed a surprising reduction in renal inflammation (reduced leukocyte influx and pro-inflammatory cytokine upregulation) as an important component of renal protection with A_1AR activation. Unlike the better characterized and traditionally understood anti-inflammatory mechanisms of A_{2a}ARs, the anti-inflammatory mechanisms of A_1ARs remain unclear. Therefore, we hypothesized that A_1AR activation may induce additional mediator(s) that produce direct anti-inflammatory effects to protect against renal IR.

Phosphorylation of sphingosine by two subtypes of sphingosine kinase (SK1 and SK2) leads to the formation of sphingosine-1-phosphate (S1P), a lysophospholipid targeting G-protein coupled receptors with diverse extracellular as well as intracellular effects.[12–14] In particular, renal tubular SK1 activation has been shown to produce renoprotection after IR.[15–17] Moreover, of five G-protein coupled S1P receptors (S1PRs), $S1P_1R$ activation has been shown to counteract against cardiac[18,19] and renal IR injury[17,20] and attenuates T-lymphocyte-mediated tissue inflammation.[13,21,22] Furthermore, $S1P_1R$ agonists produce renal protection via direct activation of $S1P_1Rs$ in renal proximal tubules.[23]

In this study, we tested the hypothesis that renal tubular A_1AR activation produces anti-inflammatory and cytoprotective S1P via activation of renal proximal tubule SK. We utilized genetically modified strains of mice lacking SK1 ($SK1^{-/-}$) and SK2 ($SK2^{-/-}$) enzyme. In addition, we tested that activation of $S1P_1R$ via A_1AR–mediated S1P generation is critical in renal protection utilizing mice treated with a selective $S1P_1R$ antagonist (W146) as well as mice treated with small interfering RNA (siRNA) designed for *in vivo* targeting of $S1P_1R$. To investigate the direct involvement of proximal tubule $S1P_1R$ in A_1AR agonist-mediated renal protection *in vivo*, we generated mice with proximal tubule-specific deletion of $S1P_1R$ by crossing mice carrying the floxed $S1P_1R$ gene[12] with mice expressing Cre recombinase under the control of the phosphoenolpyruvate carboxykinase promoter.[24] Finally, we examined the mechanisms of A_1AR–mediated renal proximal tubule SK activation. Utilizing cultured human proximal tubule cells *in vitro*, we tested the hypothesis that hypoxia-inducible factor-1α (HIF-1α) has a critical role in mediating A_1AR–mediated induction of SK1.

RESULTS

A_1AR activation or overexpression increases SK1 synthesis and induces SK activity in mouse kidney

We initially tested whether A_1AR activation increased SK1 expression and activity in mouse kidney (cortex and corticomedullary junction). Figure 1a and b show that a selective A_1AR agonist 2-chloro-N6-cyclopentyladenosine (CCPA) (0.1 mg/kg, intraperitoneal (i.p.)) increased SK1 mRNA (measured at 6 h) and protein expression (measured at 16 h) and upregulated SK activity (measured at 6 h) in mouse kidney. In contrast, the A_1AR agonist CCPA failed to increase SK2 synthesis in mouse kidney. We measured preferentially SK1 activity by adding Triton x-100 in our SK activity assay as described by Vessey *et al.*[25]

Next, we aimed to determine whether overexpression of renal A_1ARs increased SK1 expression in mouse kidney. We previously demonstrated that intrarenal A_1AR–enhanced green fluorescent protein (EGFP) lentivirus transduction resulted in a selective and robust A_1AR overexpression in the injected kidneys within 48 h.[26] In this study, we show that kidneys injected with A_1AR–EGFP lentivirus 48 h prior showed increased SK1 mRNA and protein expression compared with EGFP lentivirus-injected kidneys (Figure 1c). SK2 mRNA expression did not change with A_1AR–EGFP lentivirus injection.

SK1 activation is critical for A_1AR–mediated protection against renal IR

Next, we tested whether SK1 activation is critical in A_1AR–mediated protection against renal IR. Plasma creatinine values were similar between sham-operated (anesthesia, laparotomy, right nephrectomy and recovery) wild-type (WT) creatinine (Cr) = 0.4 ± 0.03 mg/dl, $N = 3$), $SK1^{-/-}$ (SK1 knockout, Cr = 0.4 ± 0.06 mg/dl, $N = 3$), and $SK2^{-/-}$ (SK2 knockout, Cr = 0.4 ± 0.09 mg/dl, $N = 3$) mice. Plasma creatinine increased in WT, $SK1^{-/-}$, and $SK2^{-/-}$ mice subjected to 30-min renal IR compared with sham-operated mice (Figure 2a). As we described previously, $SK1^{-/-}$ and $SK2^{-/-}$ mice had slightly increased and decreased renal injury, respectively, compared with WT mice.[17] Treatment with CCPA significantly attenuated the increases in plasma creatinine in WT and $SK2^{-/-}$ mice but not in $SK1^{-/-}$ mice (Figure 2a). Figure 2b demonstrates increased histological

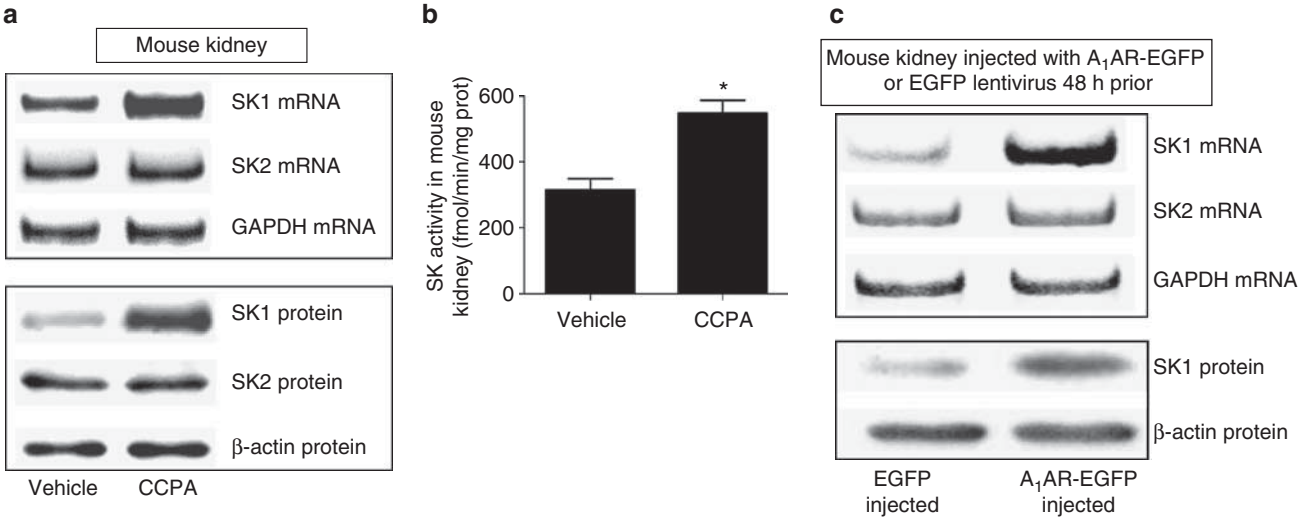

Figure 1 | A_1 adenosine receptor (A_1AR) activation or overexpression increases sphingosine kinase (SK)-1 expression and activity in mouse kidney. (**a**) Representative bands for SK1 and SK2 mRNA (reverse transcription (RT)-PCR, 6 h) and protein (immunoblotting, 16 h) expression in mouse kidney treated with vehicle (0.4% dimethyl sulfoxide (DMSO) in saline) or 2-chloro-N6-cyclopentyladenosine (CCPA) (0.1 mg/kg, intraperitoneal (i.p.)) ($N = 4$ for each group). (**b**) Mouse kidney SK activity 6 h after vehicle (0.4% DMSO) or CCPA (0.1 mg/kg, i.p.) treatment ($N = 4$ for each group). Data are presented as means ± s.e.m. *$P < 0.05$ vs. vehicle-treated mice. (**c**) Representative bands for SK1 and SK2 mRNA (RT-PCR) and SK1 protein (immunoblotting) expression in mouse kidney injected with enhanced green fluorescent protein (EGFP) or A_1AR–EGFP lentivirus 48 h prior ($N = 4$ for each group). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.