Kinin Infusion Prevents Renal Inflammation, Apoptosis, and Fibrosis via Inhibition of Oxidative Stress and Mitogen-Activated Protein Kinase Activity

Julie Chao, Huey-Jiun Li, Yu-Yu Yao, Bo Shen, Lin Gao, Grant Bledsoe, Lee Chao

Abstract—The progression of renal disease displays several characteristics, including proteinuria, apoptosis, inflammation, and fibrosis. In this study, we investigated the effect of long-term infusion of kinin in protection against salt-induced renal damage in Dahl salt-sensitive rats. Dahl salt-sensitive rats were fed a high-salt diet for 2 weeks and were then infused with bradykinin (500 ng/h) via subcutaneously implanted minipumps for 3 weeks. Kinin infusion attenuated salt-induced impaired renal function as evidenced by reduced proteinuria, serum creatinine, and blood urea nitrogen levels without apparent effect on blood pressure. Morphological analysis indicated that kinin administration reduced salt-induced glomerular sclerosis, tubular dilatation, luminal protein cast formation, and interlobular arterial thickness. Kinin also significantly lowered collagen I, III, and IV deposition and their mRNA levels. Moreover, kinin reduced interstitial monocyte/macrophage accumulation, as well as tubular cell apoptosis and caspase-3 activity. Protection of renal injury by kinin was accompanied with increased renal NO levels and reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate oxidase activities and superoxide generation. Suppression of oxidative stress by kinin was accompanied by reduced transforming growth factor-β1 protein and mRNA levels, as well as decreased phosphorylation of mitogen-activated protein kinases. This is the first study to demonstrate that kinin infusion can directly protect against salt-induced renal injury without blood pressure reduction by inhibiting apoptosis, inflammation, and fibrosis via suppression of oxidative stress, transforming growth factor-β1 expression, and mitogen-activated protein kinase activation. (Hypertension. 2007;49:490-497.)

Key Words: kinin ■ fibrosis ■ kidney ■ oxidative stress ■ inflammation ■ apoptosis ■ mitogen-activated protein kinases

Tissue kallikrein cleaves low molecular weight kininogen to produce potent vasoactive kinin peptides.1 Intact kinins, such as bradykinin (BK), bind to kinin B2 receptors, whereas kinin metabolites, such as Des-Arg(9)-BK or Des-Arg(10)-Lys-BK, bind to kinin B1 receptors.2 The binding of kinins to their respective receptors activates second messengers such as NO, cGMP, prostacyclin, and cAMP, which then elicit various biological responses.2,3 It has been reported that human patients with severe renal disease excrete low levels of kallikrein, suggesting a relationship between kallikrein and abnormal kidney function.4–6 However, Cumming and Lamrie5 demonstrated an increase in renal kallikrein release from functioning nephrons in patients with chronic renal failure. Thus, these contradictory findings require further examination.

Tissue kallikrein has been reported to attenuate salt-induced renal damage in Dahl salt-sensitive (DSS) rats through kinin B2 receptor activation.7,8 The protective effect of the kinin B2 receptor against kidney injury and fibrosis has also been demonstrated in B2 receptor knockout mice.9,10 However, the in vivo results conflict with studies of kinins in vitro. For example, BK induced collagen type I production via activation of transforming growth factor (TGF)-β and mitogen-activated protein kinases (MAPKs) in cultured vascular smooth muscle cells.11 Similarly, BK increased TGF-β type II receptor, connective tissue growth factor, and collagen expression in cultured mesangial cells.12 Whether kinin plays a detrimental role in the pathogenesis of renal injury and fibrosis in vivo has not been demonstrated.

The progression of renal disease displays several characteristics, including inadequate filtration of proteins (proteinuria), apoptosis, inflammatory cell recruitment, and accumulation of extracellular matrix (ECM) proteins in the interstitium.13 Renal fibrosis is the final contributing factor to kidney failure. Oxidative stress has been shown to play an important role in the development of renal injury, because it can stimulate the expression of proinflammatory and profibrotic molecules.14 A recent study reported that Tempol, a superoxide dismutase mimetic and an antioxidant, attenuates glomerular injury in association with reduced MAPK activity...
in DSS rats. In addition, our recent studies show that tissue kallikrein gene delivery attenuates and reverses salt-induced renal fibrosis by suppression of reactive oxygen species formation through a kinin B2 receptor–mediated event. The results suggest that kinin B2 receptor activation is necessary in the renal protective effects of kallikrein. Whether kallikrein/kinin administration can protect against salt-induced renal injury by suppression of MAPK activation has not been investigated. In this study, we examined the effects of long-term kinin infusion on salt-induced renal injury, inflammatory cell infiltration, apoptosis, and interstitial fibrosis in DSS rats. Our data demonstrate that kinin has a direct effect on the protection of salt-induced renal injury independent of its blood pressure–lowering effect by suppression of oxidative stress, TGF-β expression, and MAPK activation.

Methods
An expanded Methods section is available in an online supplement at http://hyper.ahajournals.org.

Results

Kinin Infusion Attenuates Salt-Induced Renal Dysfunction Independent of Its Blood Pressure–Lowering Effect

The Table shows the effects of kinin infusion for 3 weeks on physiological parameters in DSS rats 5 weeks after high salt loading. Compared with rats on a normal salt diet, a high-salt diet caused an elevation in heart weight/body weight and kidney weight/body weight ratio, water intake and urine volume, and levels of urinary protein, serum creatinine, and blood urea nitrogen. However, kinin infusion prevented the rise in these values. DSS rats in the high-salt groups had significantly higher systolic blood pressure compared with the normal salt group. However, no differences in blood pressure were observed between rats on a high-salt diet with and without kinin infusion.

Kinin Infusion Attenuates Salt-Induced Glomerulosclerosis, Tubular Damage, Collagen Fraction Volume, and Interlobular Arterial Thickness

The effects of kinin on renal injury were identified by periodic acid-Schiff, Masson’s trichrome, Sirius red, and hematoxylin/eosin staining (Figure 1A). Periodic acid-Schiff staining showed that kidneys of DSS rats on a normal salt diet had normal morphology. However, DSS rats on a high-salt diet for 5 weeks displayed glomerular sclerosis, tubular dilatation, and luminal protein cast formation. In contrast, kidneys in the kinin group were nearly comparable to those in the normal salt group. Rats infused with kinin seemed to have significantly less sclerotic glomeruli, tubular damage, and fewer protein casts compared with rats in the high-salt group. Moreover, kidney sections stained with Masson’s trichrome showed that high salt intake induced tubulointerstitial, perivascular, and glomerular fibrosis, whereas kinin infusion for 3 weeks reduced ECM accumulation. Similarly, Sirius red staining showed that kinin infusion reduced salt-induced collagen deposition in the kidney. Quantitative analysis of Sirius red–stained kidney sections indicated that collagen fraction volume was significantly increased after salt loading and reduced after kinin infusion (Figure 1B). It was also apparent from hematoxylin/eosin staining that a high-salt diet increased the thickness of interlobular arterial walls. Accompanying this increased thickness was noticeable inflammatory cell infiltration in the adventitia, increased numbers of smooth muscle cell layers in the media, and decreased diameters of the vessel lumen. However, interlobular arterial thickness was reduced by kinin infusion. These observations were confirmed by quantitative analysis (Figure 1C).

Kinin Infusion Prevents Salt-Induced Collagen Deposition and Expression

Immunohistochemical staining showed that high-salt diet induced collagen type I accumulation in both glomeruli and the interstitium. However, kinin infusion for 3 weeks prevented collagen deposition to the level comparable to the kidney of DSS rats on a normal salt diet (Figure 2A). Similarly, kinin infusion prevented interstitial deposition of collagen types III and IV. The effect of kinin on collagen accumulation was further verified by its inhibitory effect on collagen I and III mRNA levels (Figure 2B).

Kinin Infusion Prevents Salt-Induced Interstitial Inflammatory Cell Accumulation

The effect of high salt on inflammatory cell recruitment was analyzed by ED-1 immunohistochemical staining, used to detect the presence of monocytes and macrophages (Figure 3A). In DSS rats fed a normal salt diet, only a small number of these cells were observed. However, high salt intake for 5 weeks caused a significant accumulation of ED-1–positive cells. Monocytes/macrophages were localized in the interstitium of the cortex and medulla, surrounding blood vessels, and occasionally within glomeruli. Infusion of kinin markedly attenuated salt-induced inflammatory cell accumulation in the kidney, and this observation was verified by quantitative analysis (Figure 3B).

Effect of Kinin Infusion on B1 and B2 Receptor Expression

The effect of high salt loading on kinin B1 and B2 receptor expression was evaluated by real-time PCR (Figure 3C and 3D). Both B1 and B2 receptor mRNA expression was

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NS</th>
<th>HS</th>
<th>HS/Kinin</th>
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<tr>
<td>HW/BW, g/100 g BW</td>
<td>0.36±0.01</td>
<td>0.56±0.03</td>
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<td>BP, mm Hg</td>
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<td>Water intake, mL/100 g BW</td>
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<td>Urine volume, mL/100 g BW</td>
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<td>11.56±2.38</td>
<td>6.98±1.19</td>
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<td>Urinary protein, mg/day/100 g BW</td>
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<td>Serum creatinine, mg/dL</td>
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<td>1.41±0.03</td>
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<tr>
<td>Blood urea nitrogen, mg/mL</td>
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<td>1.60±0.16</td>
<td>0.48±0.05</td>
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</tbody>
</table>

NS indicates normal salt; HS, high salt; HW, heart weight; BW, body weight; KW, kidney weight; BP, blood pressure. Values are expressed as mean±SEM (n=5 to 6 per group).

*P<0.05 vs HS.
increased in the kidneys of DSS rats receiving a high-salt diet. Kinin infusion significantly decreased B1 receptor mRNA expression but had no effect on B2 receptor mRNA levels.

**Kinin Infusion Reduces Salt-Induced Renal Cell Apoptosis**

Kidney sections underwent TUNEL and propidium iodide staining to determine the degree of renal cell apoptosis after high salt loading (Figure 4A). Rats in the normal salt group had negligible TUNEL-positive apoptotic cells. However, apoptotic cells were observed in rats on the high-salt diet. Although apoptosis was seen in the kinin infusion group, it was dramatically less compared with the high-salt group. These observations were quantified by counting the number of TUNEL-positive cells in the tissue sections (Figure 4B). Furthermore, the induction of renal cell apoptosis by high salt loading correlated with increased renal caspase-3 activity, the final step contributing to DNA fragmentation (Figure 4C). The increase in capase-3 activity was reduced by kinin infusion.

**Kinin Infusion Increases Renal NO Levels and Reduces Oxidative Stress**

As shown in Figure 5A, kinin infusion significantly increased renal nitrate/nitrite levels in DSS rats compared with rats in the high-salt group. To determine the effect of high salt on oxidative stress in the kidney, reduced nicotinamide adenine dinucleotide (NADH)/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and superoxide levels were measured in renal extracts. DSS rats on a high-salt diet had significantly higher NADH and NADPH oxidase activities than both rats on a normal salt diet and those infused with kinin (Figure 5B and 5C). Superoxide formation paralleled NADH/NADPH oxidase activity (Figure 5D). Superoxide levels were elevated in the high-salt group above those in the normal salt group. However, kinin infusion significantly lowered salt-induced superoxide formation.

**Kinin Infusion Reduces TGF-β Expression and MAPK Phosphorylation**

Western blot analysis showed that high salt intake markedly increased TGF-β1 levels, whereas kinin infusion inhibited the
rise in TGF-β1 levels without affecting GAPDH levels (Figure 6A). To confirm the effect of kinin on TGF-β1 expression, mRNA was subjected to real-time PCR analysis (Figure 6B). High salt loading caused an increase in TGF-β1 mRNA expression, whereas kinin infusion decreased the relative expression levels. Because MAPK signaling can mediate the effect of TGF-β receptor activation, the effect of a high-salt diet on the phosphorylation/activation of p38 MAPK, c-jun N-terminal kinase (JNK), and extracellular signal regulated kinase (ERK) was also investigated. Representative Western blots showed that a high-salt diet caused significant increases in p38 MAPK, JNK, and ERK phosphorylation (Figure 6C). However, kinin infusion reduced phosphorylation of the MAPKs. Neither a high-salt diet nor kinin had an effect on total p38 MAPK, JNK, and ERK levels.

**Discussion**

Our data demonstrate that a subdepressor dose of kinin exerts renoprotective effects against salt-induced renal injury in the DSS rat model by inhibiting cellular apoptosis, inflammatory cell recruitment, and fibrosis through suppression of oxidative stress and MAPK activation. The tissue kallikrein–kinin system is well known to exert a blood pressure–lowering effect. However, this is the first study to demonstrate that kinin has a direct effect on protection against salt-induced kidney injury independent of its ability to lower blood pressure. Infusion of kinin at 100 ng/h (data not shown) and 500 ng/h for 3 weeks improved renal function with no effect on blood pressure in hypertensive DSS rats. In addition, we reported previously that kallikrein gene delivery protects against ischemia- and drug-induced organ injury in the heart, kidney, and brain of normotensive animal models independent of blood pressure reduction. Similarly, long-term infusion of tissue kallikrein into DSS rats has been shown to attenuate salt-induced renal injury without an apparent effect on blood pressure. These combined data indicate that the tissue kallikrein–kinin system has the ability to exert organ protection independent of its blood pressure-lowering ability.

In this study, kinin infusion (500 ng/h) prevented salt-induced renal dysfunction, glomerulosclerosis, tubular damage, and perivascular remodeling in DSS rats. Similar effects, although to a lesser extent, were observed on infusion of kinin at a dose of 100 ng/h (data not shown). Histological analyses revealed severe renal cortical and medullary damage in animals on a high-salt diet. The pathological development of kidney injury was verified indirectly by elevated urinary protein excretion and blood urea nitrogen. Proteinuria is a causative factor in renal disease progression, and the reduction of protein excretion by kinin demonstrates the possibility of an early protective effect of kinin against salt-induced kidney damage.

Oxidative stress, characterized by superoxide production, is related to the development of salt-induced hypertension and nephrosclerosis in DSS rats. A high-salt diet was observed to provoke an increase in NADH/NADPH oxidase activity and superoxide levels in the kidney. We showed that kinin increased renal NO levels, in the form of nitrate/nitrite,
although NO levels were not altered by high salt alone. This result is supported by the finding that renal medullary administration of high salt significantly elevates inducible NO synthase activity, which could generate NO in the high-salt group. NO plays an important role in protection against hypertension and glomerulosclerosis in DSS rats. NO may attenuate oxidative stress through several mechanisms. For example, NO can scavenge superoxide anions to form peroxynitrite, rendering superoxide biologically ineffective. In addition, NO is capable of inhibiting the assembly of NADH/NADPH oxidase subunits, which would explain the decrease in the enzyme’s activity by kinin infusion.

Figure 3. Kinin infusion reduces salt-induced monocyte/macrophage accumulation in the renal interstitium. A, Immunohistochemical staining for ED-1, a specific marker for monocytes and macrophages. Magnification is ×200. B, The number of ED-1-positive cells was quantified in the kidney sections. C, Relative B1 receptor and (D) B2 receptor mRNA levels were determined by real-time PCR. Values are expressed as mean±SEM (n=5 to 6 per group). *P<0.05 vs high salt (HS). NS indicates normal salt.

Figure 4. Kinin infusion reduces salt-induced renal cell apoptosis. A, TUNEL and PI double staining of kidney sections. Red is PI staining, green is TUNEL staining, and yellow is overlay. B, The number of TUNEL-positive cells was quantified in the kidney. C, Caspase-3 activity in renal extracts. Values are expressed as mean±SEM (n=5 to 6 per group). *P<0.05 vs high salt (HS). NS indicates normal salt.
thereby potentially attenuating superoxide generation.\textsuperscript{25} Kinin decreased salt-induced NADH/NADPH oxidase activity and superoxide generation to similar levels found in DSS rats fed a normal salt diet. Because oxidative stress can stimulate the expression of proinflammatory and profibrotic molecules,\textsuperscript{14} our results indicate that increased NO production by kinin infusion could decrease oxidative stress and, thus, attenuate inflammatory and fibrotic responses.

Previous studies have shown a correlation between salt-sensitive hypertension and inflammatory cell infiltration.\textsuperscript{14} In this study, we found that high salt intake caused a significant infiltration of mononuclear cells into the renal interstitium and that kinin infusion reduced inflammatory cell recruitment. Our observations, however, conflict with the findings that kinins and their receptors induce proinflammatory responses in vivo.\textsuperscript{26} This discrepancy may be because of the distinctive roles of B2 and B1 receptors in the acute and chronic phases of the inflammatory response, respectively. Under physiological conditions, there is little B1 receptor expression in most tissues, but its expression may be induced by stress signals, such as shock and inflammation.\textsuperscript{27} Inflammatory responses after ischemia/reperfusion injury are significantly reduced in B1 receptor–deficient mice, but the effect is reversed on pretreatment with a kinin B2 receptor antagonist.\textsuperscript{28} Thus, the B1 receptor is likely proinflammatory, whereas the B2 receptor is protective against tissue injury. Interestingly, we found that kinin infusion reduced salt-induced B1 receptor expression but had no effect on the B2 receptor. This suggests that elevated levels of kinin may reduce proinflammatory effects by lowering B1 receptor expression.

By inhibiting inflammatory cell infiltration, kinin may be able to attenuate the fibrogenic process, as activated macrophages secrete profibrotic molecules, such as TGF-\(\beta\).\textsuperscript{13} Augmented production of TGF-\(\beta\) has been shown recently to contribute to the development of nephrosclerosis in DSS rats.\textsuperscript{29} We observed that accumulation of collagens induced by high salt loading was abolished by kinin infusion. As kinin treatment reduces TGF-\(\beta\) levels, kinin may be able to prevent the downstream effects of TGF-\(\beta\) 1 on promoting ECM protein accretion. The expression of TGF-\(\beta\) 1 has been shown to be upregulated by oxidative stress and inhibited by NO in renal cells.\textsuperscript{29,30} Therefore, it is likely that the protective effects of kinin against salt-induced renal fibrosis are mediated by elevated NO levels and decreased oxidative stress–induced TGF-\(\beta\) 1 expression.

Perspectives

Previous investigations of tissue kallikrein gene or protein delivery in various animal models have demonstrated that tissue kallikrein has antioxidant, antiapoptotic, and anti-inflammatory effects in the ischemic heart, limb, blood
vessel, kidney, and brain pathologies. Although supporting a beneficial role of tissue kallikrein and kinin, these studies cannot entirely rule out the contribution of a direct action of tissue kallikrein. The current study was designed to eliminate any ambiguity by testing the direct role of kinin in a model of chronic renal disease to determine whether kinin is beneficial or detrimental in intact animals. We demonstrated that long-term infusion of BK peptide at a subpressor dose can prevent salt-induced renal injury by reducing tubular cell apoptosis, interstitial inflammation, and ECM formation in DSS rats. Renal protection by kinin was associated with suppression of oxidative stress, TGF-β1 expression, and MAPK activation. To a large degree, the benefits of kinin infusion were dependent on the elevated levels of NO, thus explaining the need for an intact endothelium to produce these effects. The results of the current study suggest that pharmacological manipulations to increase BK B2 receptor signaling or elevate NO levels could have beneficial effects in treating chronic renal diseases.

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Disclosures

None.

References


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