Chronic Bradykinin Infusion and Receptor Blockade in Angiotensin II Hypertension in Rats

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Abstract—The influence of endogenous bradykinin (BK) on the control of arterial pressure and the development of cardiac hypertrophy was assessed in chronically angiotensin II (Ang II)–infused rats (200 ng \( \cdot \) kg\(^{-1} \) \( \cdot \) min\(^{-1} \)) through the effects of concomitant infusion of 3 doses of BK (15 ng \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \), 100 ng \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) and 100 ng \( \cdot \) kg\(^{-1} \) \( \cdot \) min\(^{-1} \)) or BK-blockade by Hoe140 (300 \( \mu \)g \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \)) for 10 days. In Ang II–infused rats, tail-cuff pressure increased from 124 ± 3 to 174 ± 6 mm Hg (\( P < 0.001 \)). The pressor effect of Ang II was not affected by simultaneous infusion of BK or Hoe140. At the end of the experiments, cardiac mass was higher in rats infused with Ang II alone (3.56 ± 0.10 versus 2.89 ± 0.05 mg/g in untreated controls, \( P < 0.01 \)) and the development of cardiac hypertrophy was not modified by administration of the 3 doses of BK or Hoe140. In addition, the fall in cardiac output associated with Ang II was prevented only by the moderate and high doses of BK, mainly through an increase in stroke volume and a decrease in total peripheral resistance. In the same way, the renal vasoconstrictor effect of Ang II was abolished by the medium and high dose of BK. Hoe140 did not affect cardiac output or renal blood flow in this model. No influence of BK or Hoe140 on the increase in albuminuria induced by Ang II was detected. In conclusion, exogenous BK may oppose the effect of Ang II on vascular tone, but it cannot prevent hypertension and target-organ damage associated with this experimental model of hypertension, even at a very high dose. (Hypertension. 1999;33:830-834.)

Key Words: albuminuria ■ angiotensin II ■ bradykinin ■ hypertrophy ■ hemodynamics

Kinins are vasodilator peptides that may act systemically and/or locally as a paracrine system through the stimulation of endothelial B\(_2\)-receptors with subsequent release of nitric oxide and prostaglandins.\(^1\) Several studies using acute infusions of various bradykinin(BK) antagonists have suggested that endogenous kinins may be involved in the control of arterial pressure in normotensive\(^2\) as well as spontaneously hypertensive rats.\(^3\) Moreover, chronic blockade of BK B\(_2\)-receptors was shown to enhance the progressive pressor effect of chronic angiotensin II (Ang II)–infusion in rats.\(^4\) Due to the identity between angiotensin I-converting enzyme and kininase II, the issue of whether accumulation of endogenous kinins participates in the antihypertensive and antihypertrophic effects of angiotensin converting enzyme (ACE) inhibitors is still a matter of much debate.

Recent studies have demonstrated that cardiac tissue synthesizes and releases both kallikrein and kininogen\(^5\) and that BK is continuously formed in isolated perfused rat heart,\(^6\) thus suggesting that an independent kallikrein-kinin system is present in rat heart. Endogenous BK may directly influence the effect of ACE inhibitors on left ventricular hypertrophy, irrespective of their antihypertensive effect as suggested by the effect of the BK B\(_2\)-receptor antagonist, Hoe140,\(^7\) in rats with aortic banding treated by nonantihypertensive doses of ramipril\(^8\) as well as in Ang II–infused rats treated by enalapril.\(^9\) In the sole study evaluating the effect of chronic infusion of BK, it was reported that a subcutaneous infusion of very low-dose BK (15 ng \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) for 6 weeks) had no effect on arterial pressure, but totally prevented the development of left ventricular hypertrophy in rats with aortic banding.\(^10\)

Increasing evidence suggests that Ang II acts as a growth factor and induces hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts.\(^11\) In rats with chronic infusion of Ang II (200 ng \( \cdot \) kg\(^{-1} \) \( \cdot \) min\(^{-1} \)), the development of cardiac hypertrophy is associated with a shift to the fetal phenotype of cardiomyocytes, despite a normalization of arterial pressure by concomitant hydralazine treatment.\(^12\) The aim of the present study was to assess, in Ang II–infused rats, the effects of chronic administration of very low, moderate, and very high doses of BK on the development of hypertension and cardiac hypertrophy, and on the systemic and renal vascular changes associated with Ang II–induced hypertension. In addition, the contribution of endogenous kinins to the effect of Ang II was assessed through concomitant blockade of B\(_2\)-receptors by Hoe140.

Methods
Experiments were carried out in 54 male Sprague-Dawley rats (Iffa-Credo, L’Arbresle, France) weighing 260 to 280 g at the.
beginning of studies and maintained on a normal sodium diet throughout the experiments (sodium-free rat chow containing 0.5 mmol sodium per kilogram and distilled water containing 77 mmol sodium per liter as drinking fluid). Animals were placed in individual metabolic cages at least 1 week before studies began and were randomly assigned to the 6 experimental groups. After a 3-day control period, Ang II, infused for 10 days at the dose of 200 ng · kg⁻¹ · min⁻¹, was given alone (n=10), or combined with BK at doses of 15 and 100 ng · kg⁻¹ · min⁻¹ (n=10 in each group), and 100 ng · kg⁻¹ · min⁻¹ (n=8). Ang II and BK (Sigma Chemical Co, France) were infused subcutaneously via separate osmotic pumps (model 2002, Alza Corp, Calif). An additional group of 8 rats was infused with Ang II and the BK B₂-receptor antagonist, Hoe140, given at the dose of 300 µg · kg⁻¹ · d⁻¹. Such a dose of Hoe140 was previously shown to provide complete blockade of the acute vasodepressor effect of exogenous BK in rats. A group of 8 rats receiving a subcutaneous infusion of distilled water served as the untreated control group.

Body weight, food and water intake, urine volume, and excretion of creatinine and electrolytes was measured daily, whereas urinary excretion of albumin was determined before and at the end of the treatment period. Systolic arterial pressure (SAP) was recorded before treatment and at the end of studies in conscious animals (tail-cuff–sphygmonanometric method, Model PE-300, Narco Bio-Systems).

Systemic and Renal Hemodynamics in Conscious Rats
At the end of experiments, rats were prepared for cardiac output and renal blood flow determination in the conscious state using the microsphere method as previously reported. Animals were then killed by intraventricular injection of sodium pentobarbital. Kidneys were removed and weighed for radioactivity counting. The heart was cleared of the pericardium and large vessels and the right and left ventricles were then carefully separated and weighed. All procedures were designed in accordance with the French law and institutional guidelines for the care and use of laboratory animals.

Analytical Methods and Statistical Analysis
In all samples, concentrations of sodium and potassium were measured by flame photometry and creatinine concentration was measured by a colorimetric method. In all groups except the low-dose BK group, albuminuria, calculated as the average of values of 2 consecutive 24-hour urine collections, was measured by laser nephelometry. Total peripheral resistance was calculated as mean arterial pressure/cardiac output indexed for body weight (kg), and renal vascular resistance as mean arterial pressure/renal blood flow indexed for kidney weight (g). Results were expressed as mean±SEM and analyzed by ANOVA or ANOVA for repeated measures when appropriate. Differences between groups were assessed by the Fisher Paired Least Significant Difference test for multiple comparisons, and within-group differences were determined with the Student’s t test for paired values. P< 0.05 was considered significant.

Results
Tail-Cuff Pressure
Tail-cuff SAP increased from 124±3 to 174±6 mm Hg (P<0.001) in rats infused with Ang II alone, whereas no significant change was observed in the vehicle–infused group (129±2 to 126±2 mm Hg). BK infusion did not modify the pressor effect of Ang II and the final level of SAP was similar in rats treated by the low, moderate or high dose of BK (174±9, 174±7 and 179±7 mm Hg, respectively). In Ang II–infused rats receiving Hoe140, SAP increased from 112±2 mm Hg to a final level of 164±5 mm Hg, a value comparable to that achieved in rats infused with Ang II alone.

Change in Systolic Arterial Pressure (mm Hg)

Figure 1. Changes in tail-cuff SAP induced by a 10-day infusion of 200 ng · kg⁻¹ · min⁻¹ Ang II and influence of superimposed BK and Hoe140. *P<0.05 vs control group.

As shown in Figure 1, no significant difference was detected between the increase in SAP observed in all Ang II–treated groups.

Metabolic Data
Administration of Ang II had a marked dipsogenic effect (640±85 mL/10 days in Ang II versus 371±16 mL/10 days in the control group, P<0.05) that tended to be blunted by the lowest dose of BK (514±49 mL/10 days), and almost abolished by the intermediate dose of BK (432±40 mL/10 days). Surprisingly, the effect of Ang II was restored in the presence of the highest dose of BK (659±43 mL/10 days, P<0.05 versus control group). Ang II infusion was associated with sodium retention (0.55±0.11 in Ang II versus 0.22±0.03 mmol · 10 d⁻¹ · g⁻¹ of body weight gain in control group, P<0.05). This effect was clearly suppressed by concomitant BK treatment at low and moderate doses (0.25±0.04 and 0.37±0.15 mmol · 10 d⁻¹ · g⁻³ of body weight gain respectively), but not at high dose (0.50±0.20 mmol · 10 d⁻¹ · g⁻¹ of body weight gain, P=nonsignificant. versus Ang II). No influence of blockade of B₂-receptors on the effects of Ang II was detected.

Serum potassium concentration was slightly but not significantly lower in Ang II–treated rats (3.3±0.1 versus 3.5±0.1 mmol/L in the vehicle-treated group). No influence of BK was detected; however, Hoe140 treatment resulted in a serum potassium mean value of 3.8±0.1 mmol/L (P<0.01 and 0.05 when compared with the Ang II and vehicle groups respectively). Serum creatinine concentration was similar in all groups.

Systemic Hemodynamics in Conscious Rats
As shown in the Table, at the end of the 10-day period of treatment, mean intra-arterial pressure was significantly higher in rats infused with Ang II given alone when compared with control animals. No influence of the 3 doses of BK and Hoe140 was detected. Heart rate was not significantly different in Ang II–infused rats. Cardiac output and stroke volume were lower and total peripheral resistance was higher in rats infused with Ang II alone compared with vehicle-treated rats.
animals. These parameters were restored to values observed in vehicle-treated animals in rats concomitantly infused with BK given at the moderate and high doses. No effect of blockade of BK receptors by Hoe140 on the effect of Ang II was detected.

**Renal Hemodynamics in Conscious Rats**

As presented in the Table, chronic Ang II infusion was associated with a consistent reduction in renal blood flow (4.8±0.3 versus 7.4±0.2 mL.min⁻¹.g⁻¹ kidney weight in the vehicle-treated group, P<0.01). The lowest dose of BK did not modify the renal vasoconstrictor effect of chronic Ang II infusion (5.2±0.8 mL.min⁻¹.g⁻¹). However, the Ang II–induced reduction in renal blood flow was blunted to a similar extent by the 2 highest doses of BK (6.9±0.8 and 7.4±0.6 mL.min⁻¹.g⁻¹ respectively, P<0.01 versus Ang II group). Coadministration of Hoe140 did not influence the effect of Ang II infusion on renal hemodynamics.

**Cardiac Mass**

At the end of the experiments, cardiac mass was significantly higher in rats infused with Ang II alone (3.56±0.11 mg/g) when compared with untreated controls (2.89±0.05 mg/g, P<0.01). As shown in Figure 2, the increase in cardiac mass was essentially due to a rise in left ventricular weight. The degree of cardiac hypertrophy was altered neither by administration of the 3 doses of BK nor infusion of the BK antagonist Hoe140.

**Urinary Albumin Excretion**

As shown in Figure 3, urinary albumin excretion increased in Ang II–infused rats (from 215±39 to 2082±832 µg/24 hours, P<0.001). No change was observed in vehicle-treated rats (from 125±16 to 91±7 µg/24 hours, P=NS.). BK did not prevent the rise in albuminuria induced by Ang II. No influence of Hoe140 on the effect of Ang II was noted.

**Discussion**

In the present experiments, it was demonstrated that concomitant administration of BK at the moderate and high doses used in this study, but not at the low dose, totally prevented the increase in systemic and renal vascular resistance induced by chronic infusion of Ang II. The vasodilatory influence of BK was accompanied by an increase in cardiac output, whereas tail-cuff as well as intra-arterial systemic pressure were unaffected. In fact, the maximal effect was obtained with the moderate (100 ng·kg⁻¹·d⁻¹) dose of BK and no additional effect was observed with the highest dose (144 000 ng·kg⁻¹·d⁻¹). Moreover, neither the development of cardiac hypertrophy nor the increase in albuminuria induced by the 10-day period of Ang II infusion was affected by BK administration. Interestingly, chronic blockade of BK B₂-receptor by Hoe140 did not modify the effect of Ang II on systemic and renal hemodynamics as well as cardiac weight and albuminuria, thus suggesting a poor role of endogenous BK in this experimental model of hypertension.

A role of endogenous kinins in the control of arterial pressure was suggested by the potentiation by a B₂-receptor antagonist of the pressor effect of chronic administration of Ang II. In Ang II–treated rats, the slow pressor effect of Ang II was enhanced by concomitant chronic (4-week) treatment by Hoe140, suggesting that BK may prevent the chronic pressor effect of Ang II. Moreover, the rise in arterial pressure induced by a 10-day period of dietary sodium loading was prevented by kallikrein infusion in a rat strain inbred for low urinary kallikrein. The influence of chronic (6-week) BK infusion at the dose of 15 ng·kg⁻¹·d⁻¹ was assessed in rats made hypertensive by aortic banding and no effect of BK on arterial pressure was observed. Despite a lack of sustained effect on arterial pressure, chronic intrarenal administration of BK was associated with renal vasodilation, no change in glomerular filtration rate, a decrease in filtration fraction, and no effect on urinary water and sodium excretion in normotensive dogs.
The smallest dose of BK had only a slight effect on total peripheral resistance without any other effect on cardiac output or renal vascular resistance, probably because it was far too low. However, the 2 highest doses of BK unequivocally blunted the rise in systemic and renal vascular resistance associated with Ang II infusion, due to an increase in cardiac index, stroke volume, and renal blood flow, respectively. These results indicate that BK may counteract the effect of Ang II infusion on vascular tone as previously suggested in isolated rat kidney.\textsuperscript{18} The lack of antihypertensive influence of systemic BK infusion observed in the present study might be related to stimulation of sympathetic nervous system activity.\textsuperscript{19} However, the absence of change in heart rate argues against this hypothesis. Another explanation for the increase in cardiac output might be an increase in venous return through BK-induced venous constriction.\textsuperscript{20}

In experimental models of hypertension, the contribution of endogenous kinins to the development of hypertension and left ventricular hypertrophy and the effect of ACE inhibitors are unclear. In hypertension associated with aortic banding (a renin-dependent model), it was reported that subcutaneous infusion of BK (15 ng·kg\textsuperscript{-1}·d\textsuperscript{-1} for 6 weeks) begun on the day of aortic banding prevented the development of left ventricular hypertrophy despite a lack of reduction in arterial pressure.\textsuperscript{10} In the present study, identical as well as markedly higher doses of BK, which had no detectable effect on systemic pressure, did not influence the increase in cardiac mass associated with chronic Ang II administration. Discrepancies between these studies may be related to the shorter duration (10 days versus 6 weeks) of BK administration. Using the BK antagonist Hoe140, it was suggested that kinins may contribute to the beneficial effects of nonantihypertensive doses of ACE inhibitors on the development of left ventricular hypertrophy in rats with aortic banding.\textsuperscript{8,10} In contrast, Rhaleb et al.\textsuperscript{21} reported that a nonantihypertensive dose of ramipril (0.01 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}), begun on the day after aortic coarctation above the left renal artery and continued for 6 weeks, failed to alter significantly the development of left ventricular hypertrophy; however, a dose of ramipril that prevented the rise in arterial pressure (1 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) normalized left ventricular weight, an effect not modified by the concomitant administration of Hoe140. No obvious explanation for these 2 contrasting observations could be proposed. In Ang II-induced hypertension, the prevention by an ACE inhibitor of the development of cardiac hypertrophy was abolished by concomitant administration of the BK antagonist Hoe140, in the absence of influence on blood pressure.\textsuperscript{9} Although the lack of effect of BK infusion on arterial pressure observed in the present study probably contributed to the failure of BK to prevent or lessen left ventricular hypertrophy associated with chronic Ang II administration, a role for BK–induced sympathetic activation remains to be demonstrated. In a recent study,\textsuperscript{22} it was demonstrated that BK induced an increase in protein synthesis in cultured ventricular cardiomyocytes, and this effect was abolished when cardiomyocytes were cocultured with endothelial cells. Moreover, BK abolished the Ang II-induced increase in protein synthesis by cardiomyocytes, only in the presence of endothelial cells, suggesting that intact endothelial cells are required for the antihypertrophic effect of BK.

The rise in albuminuria associated with Ang II infusion was not influenced by concomitant administration of BK at the 3 doses used in the present study. Proteinuria induced by
chronic infusion of Ang II, in our experiments as well as during acute Ang II administration, may result from an increase in systemic and intraglomerular capillary pressure and an increase in the glomerular permeability to albumin and possibly other macromolecules.  

Ang II-induced alterations in systemic and renal hemodynamics as well as cardiac weight and albuminuria were not affected by the kinin receptor antagonist. Consequently, the present results do not favor a major role for endogenous kinins in the regulation of blood pressure as well as the development of Ang II–induced target-organ damage. Administration of Hoe140 failed to potentiate the pressor response to acute infusion of Ang II, phenylephrine, and ET-1, whereas chronic inhibition of B₂-receptors enhanced the slow vasopressor response to Ang II. Discrepancies between the present experiments and the above-mentioned studies are unlikely related to the dose of Hoe140 (77 versus 79–84). 

In conclusion, chronic infusion of exogenous BK exerted a systemic and renal vasodilatory effect in rats with Ang II–induced hypertension. However, despite systemic vasodilatation, arterial pressure was not affected. Neither cardiac hypertrophy nor albuminuria associated with chronic Ang II administration were modified by concomitant BK treatment. The systemic and renal hemodynamic as well as structural cardiac alterations associated with Ang II hypertension were not influenced by the BK receptor antagonist Hoe140. Therefore, it is suggested that exogenous BK may oppose the effect of Ang II on vascular tone but, even at a very high dose, BK did not prevent target-organ damage in the absence of effect on arterial pressure in this experimental model of hypertension. 

References

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