Angiotensin II–Induced Hypertension in Bradykinin B₂ Receptor Knockout Mice

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Abstract—The present study was performed to examine the role of endogenous bradykinin (BK) in the development of angiotensin II (Ang II)–induced hypertension in mice. BK B₂ receptor knockout (B₂R⁻/⁻) and wild-type (B₂R⁺/⁺) mice (22 to 26 g) were infused with either saline (SAL) or Ang II (40 ng/min) via an osmotic minipump implanted intraperitoneally. On day 12 after implantation, there was no difference in systolic blood pressure (SBP, tail-cuff plethysmography) between SAL/B₂R⁺/⁺ and SAL/B₂R⁻/⁻ mice (128±5 versus 133±6 mm Hg, n=24/group). In contrast, SBP was higher on day 12 of infusion in Ang II/B₂R⁻/⁻ than in Ang II/B₂R⁺/⁺ mice (173±6 versus 156±5 mm Hg; P<0.05, n=27 and 28). Mean arterial pressure (MAP) was also higher in anesthetized Ang II/B₂R⁻/⁻ mice than in Ang II/B₂R⁺/⁺ mice (139±3 versus 124±3 mm Hg; P<0.05, n=16 and 14). Unlike Ang II, long-term norepinephrine (NE) infusion via an osmotic minipump (45 ng/min) caused equivalent increases in SBP in B₂R⁺/⁺ and B₂R⁻/⁻ mice measured on day 12 after implantation (151±4 versus 149±5 mm Hg, n=9 and 8). MAP also did not differ on day 13 after implantation between NE/B₂R⁺/⁺ and NE/B₂R⁻/⁻ mice (120±6 versus 122±4 mm Hg, n=9 and 8).

There were no differences in glomerular filtration rate and urinary sodium excretion among the groups. However, renal plasma flow (RPF) was lower in Ang II/B₂R⁺/⁺ mice than in Ang II/B₂R⁺/⁺ mice (2.34±0.06 versus 4.33±0.19 mL·min⁻¹·g⁻¹; P<0.05). Acute inhibition of NO synthase (NOS) with nitro-L-arginine-methyl ester (0.5 μg·g⁻¹·min⁻¹) in SAL/B₂R⁺/⁺ and SAL/B₂R⁻/⁻ mice caused equal increases in MAP (142±1 versus 145±1 mm Hg) and decreases in RPF (2.06±0.06 versus 2.12±0.15 mL·min⁻¹·g⁻¹). However, short-term NOS inhibition caused a greater increase in MAP of Ang II/B₂R⁺/⁺ mice than of Ang II/B₂R⁻/⁻ mice, such that MAP after NOS inhibition in Ang II/B₂R⁺/⁺ approached that of Ang II/B₂R⁻/⁻ mice (156±2 versus 159±2 mm Hg). These changes were associated with a decrease in RPF in Ang II/B₂R⁺/⁺ mice to values similar to those of Ang II/B₂R⁻/⁻ mice before NOS inhibition (2.12±0.09 versus 2.34±0.06 mL·min⁻¹·g⁻¹). These results demonstrate that the kallikrein-kinin system selectively buffers the vasoconstrictor activity of Ang II. Furthermore, the enhanced susceptibility of B₂R⁻/⁻ mice to Ang II–induced hypertension and renal vasoconstriction is likely due to an impaired ability to release NO by endogenous kinins. (Hypertension. 2001;37:967-973.)

Key Words: kallikrein-kinin system ■ angiotensin II ■ nitric oxide ■ blood pressure ■ norepinephrine ■ blood flow

The 2-kidney, 1-clip (2K1C) Goldblatt model of hypertension is a form of secondary hypertension that is similar in many ways to human renovascular hypertension and is highly dependent on the increased activity of the renin-angiotensin system (RAS) (for a review, see Mitchell et al). It is generally agreed that the long-term infusion of subpressor doses of angiotensin II (Ang II) leads to a slowly developing hypertension that resembles 2K1C Goldblatt hypertension. Previous studies have postulated that the decreased activity of the intrarenal kallikrein-kinin system (KKS) contributes to the development and maintenance of 2K1C Goldblatt hypertension. Bradykinin (BK), the enzymatic product of the action of kallikrein on kininogen, causes vasodilatation and natriuresis by releasing endothelium-derived relaxing factors and prostaglandins (for a review, see Carretero et al). At least 2 types of kinin receptors exist, B₁ and B₂, and both belong to the G proteincoupled receptor family. The B₂ receptor (B₂R) activation mediates the majority of the cardiovascular and renal actions of BK. Acute B₂R blockade decreases renal blood flow, papillary blood flow, and the natriuretic response to saline (SAL) loading. It has been shown that long-term pharmacological blockade of B₂R combined with subpressor doses of Ang II leads to hypertension in rats. Moreover,
Brown Norway Katholiek rats, which are kininogen deficient, are highly sensitive to Ang II. Furthermore, inhibition of intrarenal kinin degradation attenuates the development of deoxycorticosterone acetate salt–induced hypertension in rats. Because there remain some uncertainties regarding the role of KKS in the pathogenesis of hypertension, we performed studies on the newly developed mice with targeted disruption of the B2 R gene. These mice provide a suitable model in which to assess the contribution of KKS to the pathogenesis of Ang II–dependent hypertension.

Three groups of investigators, including our group, independently reported that B2 R knockout mice exhibit salt-sensitive hypertension that develops early in life and is accompanied by reduced renal blood flow. In addition, Madeddu et al have shown that B2 R knockout mice fed a normal salt diet exhibit a slightly higher resting blood pressure (BP) than did wild-type control animals and that this elevation is associated with impaired NO activity.

It was recently shown that the development of hypertension in B2 R knockout mice is accelerated during the early phases of 2K1C hypertension and that NO activity is increased in the nonclipped kidney of 2K1C Goldblatt hypertensive rats as well as the kidneys of Ang II–infused hypertensive rats. Moreover, it was recently suggested that renal kinins and NO may be responsible for mediating the pressure-natriuresis mechanism and therefore significantly contribute to the long-term control of arterial BP.

In view of this information, we tested the hypothesis that disruption of the B2 R gene enhances the development of Ang II–induced hypertension by limiting the availability of endothelium-derived NO. Experiments were performed in wild-type and knockout mice to (1) examine the impact of B2 R disruption on the development of Ang II–induced hypertension and on renal function and (2) evaluate the effects of the short-term inhibition of NO synthase (NOS) on BP, renal hemodynamics, and sodium excretion in Ang II–infused B2 R knockout mice.

In addition, to determine whether the B2 R gene disruption leads to a specific increase in vasoconstrictor sensitivity to Ang II or a generalized increase in BP responsiveness to other vasoconstrictors, additional experiments were performed to examine BP responses to long-term infusion of norepinephrine (NE).

Methods
The study described here was performed in accordance with the guidelines and practices established by the Institute for Clinical and Experimental Medicine Animal Care and Use Committee.

Animals
Targeting and disruption of the B2 R gene were accomplished by homologous recombination in embryonic stem cells as described by Borkowski et al. B2 R knockout mice (B2 R homozygous recombination in embryonic stem cells as described by Borkowski et al.), originally provided by Drs Hess and Chen (Merck Research Laboratory, Rahway, NJ), were bred on a C57BL/6J background to the eighth generation at Tulane School of Medicine (New Orleans, La). Breeders from this colony of mice were transferred to the Institute for Clinical and Experimental Medicine, Prague, Czech Republic, and bred to the tenth generation. Wild-type C57BL/6J (B2 R ) mice were obtained from Charles Rivers Laboratories. Animals were housed in a temperature- and light-controlled room and allowed access to standard chow (SEMED; containing 0.75% salt and 19% protein) and water ad libitum.

General Procedures
B2 R and B2 R mice (22 to 26 g) were anesthetized with a combination of pentobarbital sodium (50 mg/kg IP) and ketamine (10 mg/kg IP) to allow the implantation of osmotic minipumps. Osmotic minipumps (model 1002; Alzet Co) containing Ang II (Sigma Chemical Co) at concentrations sufficient to allow an infusion rate of 40 ng/min were implanted into the abdominal cavity in B2 R (Ang II/B2 R , n=28) and B2 R (Ang II/B2 R , n=27) mice. This dosage of Ang II was chosen on the basis of our pilot studies that determined the lowest dose of Ang II that induced the development of hypertension in mice in a similar temporal pattern as observed in Ang II–infused rats. Osmotic minipumps containing SAL solution were implanted in the abdominal cavity of the control groups (SAL/B2 R , n=24; SAL/B2 R , n=24).

In an additional series of experiments, acute mean arterial pressure (MAP) responses to a bolus dose of NE (100 ng) in B2 R and B2 R mice (n=5/group) were evaluated. In a separate group of mice, osmotic minipumps (model 1002; Alzet Co) containing NE (Sigma Chemical Co) at concentrations sufficient to allow an infusion rate of 45 ng/min were implanted into the abdominal cavity in B2 R (NE/B2 R , n=9) and B2 R (NE/B2 R , n=8) mice. This dosage of NE was chosen on the basis of published results and modified in accordance with the results of our pilot studies. The aim of this protocol was to examine the long-term BP responses to subpressor doses of NE and to clarify whether B2 R gene disruption confers enhanced sensitivity to vasoconstrictor agents other than Ang II.

BP Measurement
Systolic BP (SBP) was measured via a tail-cuff apparatus (RTBP 1007; Kent Scientific Co) in conscious mice 2 days before and then on days 2, 5, 9, and 12 after the implantation of minipumps. SBP values were derived from an average of 6 to 8 measurements per animal at each time point. Three preliminary training sessions were performed during 1 week before starting the experiment.

Renal Function Studies
Thirteen days after implantation of the minipumps, mice were prepared for acute clearance experiments as previously described. Mice were anesthetized with a combination of pentobarbital sodium (50 mg/kg IP) and ketamine (10 mg/kg IP). Supplemental doses of anesthesia (ketamine 5 mg/kg) were administered intramuscularly as required. The mice were placed on a servo-controlled surgical table that maintained body temperature at 37°C, and a tracheostomy was performed with PE-90 tubing. The surgical field was then air enriched with O2 by placing the exterior end of tracheal cannula inside a small plastic chamber into which humidified 95% O2/5% CO2 was continuously passed. This procedure markedly improves the stability of arterial pressure in anesthetized mice. The right carotid artery was cannulated with PE-10 tubing for continuous arterial BP measurement and blood sampling. MAP was monitored with a Tesla pressure transducer (model LMP 102; Tesla) and recorded on a charter recorder (model TZ 4100; Laboratori Přístroje Praha). The right jugular vein was catheterized with PE-10 tubing for fluid infusion. The bladder was catheterized with PE-50 tubing via a suprapubic incision to allow timed urine collections. During surgery, an isotonic SAL solution containing 6% albumin (bovine; Sigma Chemical Co) was infused at a rate of 4 mL/min via the right jugular vein. After surgery, the intravenous infusion was changed to isotonic SAL containing 1% albumin, 7.5% polyfructosan (Inu Pep; Laevosan), and 1.5% para-aminobenzoic acid (PAH; Merck Sharp & Dohme) and infused at the same rate. After a 60-minute equilibration period, 3 consecutive 30-minute urine collections and an arterial blood sample (200 mL) were obtained to determine whole kidney hemodynamic and excretory function. PAH, polyfructosan clearances, and urinary sodium excretion were determined in SAL/B2 R (n=10); SAL/B2 R (n=10), Ang II/B2 R .
Statistical analyses were performed with 1-way ANOVA or with a Student–Newman–Keuls test. Significant difference was defined as $P<0.05$.

**Results**

Basal values of body, kidney, and heart weights in B2R+/− and B2R−/− mice are summarized in the Table. Although there is a trend for heart weight to be higher in B2R−/− than in B2R+/− mice (119±2 versus 110±2 mg, n=59 and 61), these values did not reach statistical significance.

**BP Under Basal Conditions and in Response to Ang II or NE Infusion and Acute NOS Inhibition**

Basal SBP levels did not differ between B2R+/− and B2R−/− mice (130±4 versus 133±5 mm Hg). As shown in Figure 1A, SBP remained unchanged in SAL/B2R+/− and SAL/B2R−/− mice for the duration of the study. SBP in Ang II/B2R−/− exhibited progressive increases during the infusion period, reaching a value of 156±5 mm Hg on day 12. The increase in SBP in Ang II/B2R−/− mice was enhanced compared with that in Ang II/B2R+/− mice at days 9 and 12 (168±3 versus 151±4 and 173±6 versus 156±5 mm Hg; $P<0.05$ for both comparisons). Chronic NE infusion caused similar SBP increases in B2R+/− and B2R−/− mice (151±4 and 149±5 mm Hg). MAP values for SAL/B2R+/− and SAL/B2R−/− mice, measured on day 13 in anesthetized animals, were similar (105±1 versus 108±2 mm Hg) (Figure 1B). However, Ang II/B2R−/− mice had a significantly higher MAP compared with Ang II/B2R+/−, NE/B2R+/−, and NE/B2R−/− mice (139±3 versus 124±3, 120±6, and 122±4 mm Hg, respectively; $P<0.05$).

Acute NOS inhibition caused a significant increase in MAP in all experimental groups. Acute NOS inhibition increased MAP in SAL/B2R+/− and SAL/B2R−/− mice to similar values (141±3 versus 144±3 mm Hg) (Figure 1C). Ang II/B2R−/− mice responded to short-term NO blockade with a more profound increase in MAP than Ang II/B2R+/− mice, such that the MAP levels after NO blockade in Ang II/B2R−/− mice were similar to those of Ang II/B2R+/− mice (155±4 versus 158±5 mm Hg).

As shown in Figure 1D, a bolus injection of NE (100 ng IV) caused similar increases in MAP in B2R+/− and B2R−/− mice (51±6 and 48±8 mm Hg).

**Effects of Long-Term Ang II Infusion and Acute NOS Inhibition on Renal Hemodynamic Function**

Figure 2 summarizes the results of clearance studies performed 13 days after implantation of minipumps in SAL- and Ang II–infused animals. As shown in Figure 2A, the values for GFR did not differ among SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (0.78±0.03, 0.73±0.03, 0.67±0.03, and 0.67±0.05 mL·min⁻¹·g⁻¹, respectively). Acute NOS inhibition did not influence GFR in SAL/B2R+/−, SAL/B2R−/−, and Ang II/B2R−/− mice (0.64±0.04, 0.62±0.03, and 0.71±0.05 mL·min⁻¹·g⁻¹, respectively). However, NOS blockade significantly decreased GFR in Ang II/B2R−/− mice compared with all other groups (to 0.40±0.02 mL·min⁻¹·g⁻¹; $P<0.05$, Figure 2B).

As shown in Figure 2C, the values of RPF measured as PAH clearance did not differ in SAL/B2R+/−, SAL/B2R−/−, and Ang II/B2R−/− mice (4.41±0.2, 4.42±0.24, and 4.33±0.19 g·min⁻¹·100 g BF⁻¹·100 g−1 mL⁻¹, respectively).
mL · min⁻¹ · g⁻¹, respectively). In contrast, Ang II/B₂R⁻/⁻ mice exhibited significantly lower RPF compared with the other groups (2.34±0.06 mL · min⁻¹ · g⁻¹; P<0.05). Acute NOS inhibition caused equivalent decreases in RPF in SAL/B₂R⁺/⁺, SAL/B₂R⁻/−, and Ang II/B₂R⁻/⁻ mice to levels similar to those found in Ang II/B₂R⁻/− mice before NOS blockade (to 2.06±0.06, 2.12±0.15, 2.12±0.09, and 2.34±0.06 mL · min⁻¹ · g⁻¹) (Figure 2D). NOS blockade caused a further

Figure 1. A, Changes in SBP in SAL/B₂R⁺/⁺ (○), SAL/B₂R⁻/− (□), Ang II (ANGII)/B₂R⁺/⁺ (●), ANGII/B₂R⁻/− (▲), NE/B₂R⁺/⁺ (▼), and NE/B₂R⁻/− (◆) mice. B, MAP measured 13 days after the implantation of minipumps in anesthetized mice. C, MAP after short-term NOS inhibition measured on day 13 in anesthetized mice. D, BP responses to intravenous bolus administration of NE (100 ng) in wild-type (filled columns) and knockout mice (hatched columns). Values are mean±SEM. *P<0.05 vs SAL-infused mice. #P<0.05 vs all other groups.

Figure 2. A, GFR in mice 13 days after the implantation of minipumps. B, GFR in mice after short-term NOS inhibition. C, RPF in mice 13 days after the implantation of minipumps. D, RPF in mice after short-term NOS inhibition. Values are mean±SEM. *P<0.05 vs all other groups.
reduction in RPF in Ang II/B2R/−/− mice (to 1.32±0.08 mL·min⁻¹·g⁻¹). FF was significantly higher in Ang II/B2R/−/− mice than in the other groups (Figure 3A), and short-term NOS inhibition caused an increase in FF in SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (1.21±0.13, 1.35±0.14, 1.01±0.1, and 1.06±0.17 μmol·min⁻¹·g⁻¹, respectively). Acute NOS blockade led to similar increases in absolute sodium excretion in SAL/B2R+/−, SAL/B2R−/−, and Ang II/B2R+/− mice (2.76±0.13, 3.13±0.23, and 2.42±0.1 μmol·min⁻¹·g⁻¹, respectively). However, short-term NOS inhibition caused smaller increases in sodium excretion in Ang II/B2R−/− mice compared with the other groups (1.69±0.23 μmol·min⁻¹·g⁻¹). There were no significant differences in fractional sodium excretion among SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (0.66±0.04%, 0.75±0.13%, 0.63±0.07%, and 0.74±0.1%, respectively). In contrast to absolute sodium excretion rates, no significant changes were observed in the fractional sodium excretion in response to short-term NOS blockade in SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (2.63±0.15%, 2.74±0.12%, 2.21±0.17%, and 2.05±0.55%, respectively). Changes in urinary flow rates occurred in the same pattern as changes in fractional sodium excretion; there were no significant differences in urine flow among SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (7.36±0.21, 7.15±0.51, 7.43±0.42, and 6.33±0.5 μL·min⁻¹·g⁻¹, respectively). Moreover, short-term NOS inhibition caused similar increases in urinary flow rate of all of the groups (13.24±1.13, 16.85±1.77, 16.38±0.87, and 12.67±0.98 μL·min⁻¹·g⁻¹, respectively).

**Effects of Long-Term Ang II Infusion and Acute NOS Blockade on Urinary Flow and Sodium Excretion**

Figure 4 summarizes the results of urinary sodium excretion on day 13 after the implantation of minipumps. As shown in Figure 4A, there were no differences in absolute sodium excretion among SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (1.21±0.13, 8.35±0.14, 8.10±0.1, and 8.19±0.17 μmol·min⁻¹·g⁻¹, respectively). Changes in urinary flow rates occurred in the fractional sodium excretion among SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (0.66±0.04%, 0.75±0.13%, 0.63±0.07%, and 0.74±0.1%, respectively). In contrast to absolute sodium excretion rates, no significant changes were observed in the fractional sodium excretion in response to short-term NOS blockade in SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (2.63±0.15%, 2.74±0.12%, 2.21±0.17%, and 2.05±0.55%, respectively). Changes in urinary flow rates occurred in the same pattern as changes in fractional sodium excretion; there were no significant differences in urine flow among SAL/B2R+/−, SAL/B2R−/−, Ang II/B2R+/−, and Ang II/B2R−/− mice (7.36±0.21, 7.15±0.51, 7.43±0.42, and 6.33±0.5 μL·min⁻¹·g⁻¹, respectively). Moreover, short-term NOS inhibition caused similar increases in urinary flow rate of all of the groups (13.24±1.13, 16.85±1.77, 16.38±0.87, and 12.67±0.98 μL·min⁻¹·g⁻¹, respectively).

**Effects of Ang II Infusions on PRA**

PRA, measured in the anesthetized state, did not differ between SAL/B2R+/−, SAL/B2R−/− mice (36.9±1.3 versus 41.5±0.5 ng Ang I·ml⁻¹·h⁻¹). Both groups of Ang II–infused mice exhibited significant suppression of PRA (8.3±0.3 versus 9.4±0.3 ng Ang I·ml⁻¹·h⁻¹; P<0.05).

**Discussion**

The KKS participates in the regulation of salt and water homeostasis and vascular resistance and therefore may play an important role in the pathogenesis of hypertension (for a review, see Carretero et al). The recent development of genetically engineered mice that lack the bradykinin B2R gene has provided a unique opportunity to investigate the physiological role of the KKS in BP regulation. In this regard, studies in B2R-knockout mice have shown that lack of B2Rs predisposes to salt-sensitive hypertension.14–16 However, there remains some debate regarding the beneficial role of exogenous BK in prevention of the development of Ang II–dependent hypertension and the associated target-organ damage24 or the effects B2R gene disruption on the establishment of hypertension induced by deoxycorticoste-
In the present study, we used a genetic approach to evaluate the role of the KKS in protection from Ang II–induced hypertension and the interplay between BK and NO.

The major finding of the study is that disruption of the B2R gene in mice exacerbates the hypertension induced by the long-term administration of initially subpressor doses of Ang II. In contrast, the disruption of the B2R gene did not enhance the short- or long-term BP responses to the vasoconstrictor agent NE. We interpret these results that the disruption of B2R gene specifically exacerbates BP responses to long-term infusion of subpressor doses of Ang II. The present findings are in good agreement with the recent findings that the development of 2K1C hypertension is accelerated in B2R knockout mice. On the other hand, we found that basal BP was not different in wild-type mice compared with that in B2R knockout mice in both conscious and anesthetized animals, confirming our previous results in these mice and those of Alfie et al and Rhaieb et al. No significant differences were detected in plasma Ang II levels and renal Ang II type 1 (AT1) receptor gene expression between B2R+/− and B2R−/− mice, and kidney Ang II levels are appropriately suppressed in B2R−/− mice that received high-salt diet. Taken together, these data indicate that B2R gene disruption itself does not affect the basal activity of the RAS. This may help explain why unstressed B2R−/− mice have a normal BP. However, we did not measure plasma Ang II levels in this study. Thus, it might be possible that B2R−/− mice exhibit reduced clearance of exogenous Ang II and that this can be at least in part responsible for the enhanced susceptibility of B2R−/− mice to Ang II–induced hypertension. Kinins are endogenous vasodilators that act as local hormones by activating the release of endothelium-derived relaxing factors and prostaglandins (for a review, see Carretero et al). We hypothesized that the accelerated hypertension in Ang II–infused B2R−/− mice was the result of impaired NO-mediated vasodilatation. We found that short-term NOS inhibition caused similar increases in the BP of SAL-infused B2R−/− and B2R+/− mice, suggesting that basal NO activity is not different in B2R−/− compared with B2R+/− mice. In contrast, in Ang II–infused mice, short-term NOS blockade caused a more profound increase in BP of B2R−/− compared with B2R+/− mice, suggesting that Ang II–stimulated NO release is diminished in B2R−/− compared with B2R+/− mice. Moreover, RPF was significantly lower in Ang II/B2R−/− than in Ang II/B2R+/− mice, and NOS inhibition led to reductions in RPF in Ang II/B2R−/− mice to levels approaching those found in Ang II/B2R+/− mice. These results imply that impaired NO release in response to Ang II as a result of interrupted signaling by endogenous kinins contributes to the accelerated hypertension in B2R-deficient mice.

The finding that NOS inhibition causes a decrease in GFR in Ang II/B2R−/− mice seems at odds with the conclusion that B2R−/− mice exhibit impaired NO activity in response to long-term Ang II infusion. Thus, these data indicate that NO production is somehow increased in Ang II/B2R−/− mice in the region of afferent arterioles. These findings are clearly in contrast to our claim that B2R−/− mice exhibit impaired NO release via B2R activation in response to long-term Ang II infusion. However, it is likely that other factors, such as prostaglandins, are also involved in the regulation of renal hemodynamic functions in Ang II/B2R−/− mice. Moreover, short-term NOS inhibition may differentially influence activity of the RAS in B2R−/− and B2R+/− mice and thus cause different renal functional responses.

The conclusion that B2R−/− mice exhibit impaired NO release in response to Ang II infusion is supported by other studies. For example, Ang II stimulates NO production in vascular endothelial cells by enhancing the synthesis and release of BK. Overexpression of B2Rs in transgenic mice is associated with activation of the NO-cGMP pathway. A recent study by Tsutsumi et al has shown that Ang II, acting via the AT2 receptors, activates a kinin-NO-cGMP pathway in the vascular wall. Sigmund and Beierwaltes demonstrated an important role for endothelin-derived NO in the non-clipped kidney of 2K1C hypertensive rats. Furthermore, NOS inhibition–induced decreases in RPF and GFR are significantly greater in Ang II–infused rats and in transgenic hypertensive rats that harbor the mouse Ren-2 renin gene. The exact mechanism by which the long-term subpressor infusion of Ang II can increase NO due to the activation of B2Rs is not known. There is evidence to suggest that the activation of AT2 receptors somehow leads to an increase in NO release secondary to endothelial B2R activation (for a review, see Carey et al). The interplay of AT2 receptors and B2Rs in Ang II–induced hypertension requires further investigation.

It is of interest that the induction of hypertension via long-term Ang II infusion in mice requires ~6-fold higher doses than are required to induce the same hypertensive response in rats (after correction for body weight). The reasons for the differential sensitivity of mice versus rats to Ang II–induced hypertension are not completely known.

We did not observe any significant difference in heart weight between B2R−/− and B2R+/− mice that would indicate accelerated cardiac hypertrophy in B2R−/− mice, as was found in some previous studies. However, there are 2 possible explanations to reconcile this discrepancy. First, we used whole heart weight as a marker of cardiac hypertrophy, which may not allow the detection of mild left ventricular hypertrophy. Second, we infused Ang II for 13 days in relatively young animals; thus, it is conceivable that to unmask the full effects of B2R gene disruption on the cardiovascular phenotype, a longer period of Ang II infusion may be required.

We also found that although urinary sodium excretion rates under basal conditions were similar in the SAL- and Ang II–infused groups, short-term NOS blockade elicited significantly smaller increases in sodium excretion in Ang II/B2R−/− mice compared with the other groups. A possible explanation for the latter finding is that the smaller increase in BP and reduction in GFR in response to NOS inhibition in Ang II/B2R−/− compared with the other groups. This hypothesis is supported by previous studies that show the response of urinary sodium excretion to NOS inhibition is influenced by the concomitant increase in systemic BP. Other studies have demonstrated that NOS inhibition decreases sodium excretion and urinary flow rate when the increase in renal perfusion pressure was held constant.

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In summary, Ang II–dependent hypertension is exacerbated in mice that lack the gene that encodes the bradykinin B$_2$R and is accompanied by lower RPF in these animals. In addition, the data demonstrate that the disruption of B$_2$R gene does not augment the BP responses to NE, suggesting that the enhanced BP responsiveness in B$_2$R$^{-/-}$ mice is likely specific for Ang II. Although B$_2$R gene disruption in itself does not affect basal BP, renal function, or NO activity, enhanced NO activity mediated via B$_2$R activation appears to play an important role in buffering the vasoconstrictor influence of elevated Ang II levels.

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