Renovascular Hypertension in Bradykinin B₂-Receptor Knockout Mice

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Abstract—We evaluated whether kinins exert a protective action against the development of two-kidney, one clip (2K1C) hypertension, a model characterized by an activated renin-angiotensin system in the ischemic kidney and increased expression of the bradykinin (BK) B₂ receptor in the contralateral kidney. BK B₂-receptor knockout (B₂⁻/⁻), wild-type (B₂⁺/+), and heterozygous (B₂⁺/⁻) mice underwent clipping of the left renal artery, with the other kidney remaining untouched. Basal systolic blood pressure (SBP, via tail-cuff plethysmography) was higher in B₂⁻/⁻ mice than in B₂⁺/+ (121±2 versus 113±2 and 109±1 mm Hg; P<0.05 for both comparisons). SBP did not change from basal values after sham operation, but it increased in mice that underwent clipping. The increase in SBP was greater in 2K1C B₂⁻/⁻ mice than in B₂⁺/+ or B₂⁺/⁻ mice (28±2 versus 14±2 and 14±2 mm Hg, respectively, at 2 weeks; P<0.05 for both comparisons). Blockade of the BK B₂ receptor by Icatibant enhanced the pressure response to clipping in B₂⁺/⁻ mice (29±2 mm Hg at 2 weeks). Intra-arterial mean blood pressure (MBP) was higher in 2K1C than in respective sham-operated mice, with the MBP difference being higher in B₂⁻/⁻ mice (32 and 38 mm Hg, at 2 and 4 weeks, respectively), and higher in B₂⁺/⁻ mice given Icatibant (30 and 32 mm Hg) than in B₂⁺/+ mice without Icatibant (17 and 18 mm Hg). At 4 weeks, acute injection of an angiotensin type 1 receptor antagonist normalized the MBP of 2K1C hypertensive mice. A tachycardic response was observed 1 week after clipping in B₂⁻/⁻ and B₂⁺/⁻ mice, but this effect was delayed in B₂⁺/+ mice. However, the HR response to clipping in B₂⁺/+ mice was enhanced by Icatibant. Within each strain, heart weight to body weight ratio was greater in 2K1C hypertensive mice than in sham-operated control animals (B₂⁻/⁻: 5.7±0.1 versus 5.2±0.1; B₂⁺/+: 5.1±0.1 versus 4.5±0.1; P<0.01 for both comparisons). The clipped kidney weight to nonclipped kidney weight ratio was consistently reduced in mice with 2K1C hypertension. Our results indicate that kinins acting on the BK B₂ receptor exert a protective action against excessive blood pressure elevation during early phases of 2K1C hypertension. (Hypertension. 1998;32:503-509.)

Key Words: angiotensin I hypertension, renovascular I mice, kinin B₂-receptor, knockout I hypertrophy, myocardial I kallikrein-kinin system

The hypothesis that arterial hypertension is the consequence of an imbalance between vasoconstrictor and vasodilator mechanisms is now widely accepted. Recently, evidence that a defective activity of the vasodilator and natriuretic kallikrein-kinin system (KKS) is implicated in the pathogenesis of various forms of hypertension has been provided by a series of epidemiological surveys,1 gene-polymorphism-cosegregation analyses,2 and studies on animal models with long-term blockade or disruption of the bradykinin (BK) B₂ receptor.3–6 The elevated blood pressure of these models is reportedly caused by blunted basal activation of the nitric oxide (NO) pathway that leads to the amplification of vasoconstrictor activity.5 In particular, the slow pressor response to angiotensin II (Ang II) is exaggerated in animals with pharmacological or genetical deficiency of the KKS, thus indicating that endogenous kinins could buffer the long-term pressure effect of an excess of Ang II.3,5,7 Together, these results reinforce the notion that the KKS and renin-angiotensin system (RAS) are functionally interrelated in the regulation of blood pressure and total body fluid volume homeostasis.8

The Goldblatt hypertensive rat model was the first animal model shown to have a reduction in the urinary excretion and the vascular content of tissue kallikrein.9–12 In particular, the two-kidney, one clip (2K1C) hypertension model is characterized, early after clipping, by activation of the RAS in the ischemic kidney and by suppression of renin release in the contralateral one, whereas kallikrein activity is reduced principally in the compromised kidney, with a normal or mildly decreased level of expression in the contralateral one.13 The

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reason or reasons for changes in system activity in opposite directions are not yet explained. Nevertheless, the differential pattern of expression of the 2 systems, leading to unopposed vasoconstriction, could represent an important pathogenetic factor in renovascular hypertension. This theory has been further elaborated by Emond et al.\textsuperscript{11} in their study determining renal B\textsubscript{2}-receptor density in 2K1C rats. They reported increased levels of expression of the B\textsubscript{2} receptor in both kidneys, particularly in the nonclipped one, which also showed augmented BK-stimulated prostaglandin E\textsubscript{2} release. In a recent review, Margolius\textsuperscript{8} hypothesized that “these increases could signify an augmented kinin-induced production of eicosanoids and nitric oxide in response to the higher perfusion pressure, that the nonclipped kidney faces.” These changes could also contribute to the augmented renal blood flow and sodium excretory ability typical of the nonclipped kidney, aiming to prevent hypervolemia and excessive increase in blood pressure. If this were the case, abrogation of kinin function should worsen the development of renovascular hypertension.

Disruption of genes that control hormones or receptors related to the regulation of arterial blood pressure has been used recently to assess the role of the targeted mechanism in the pathogenesis of certain forms of hypertension.\textsuperscript{6,14–19} The “knockout” mouse model B\textsubscript{2}\textsuperscript{-/-}, in which the gene encoding for the B\textsubscript{2} receptor has been targeted for disruption, appears to be an ideal tool to address the hypothesis that kinins exert a protective role in renovascular hypertension. Therefore, we evaluated whether 2K1C hypertension develops more severely in B\textsubscript{2}\textsuperscript{-/-} than in wild-type and heterozygous mice. In addition, we determined whether chronic blockade of the BK B\textsubscript{2} receptor by Icatibant also worsens the development of renovascular hypertension.

Methods

The knockout strain was developed by gene targeting and homology recombination on a J129 Sv genetic background.\textsuperscript{20} Injection of 129Sv/Ev embryonic cells, carrying the targeted mutation, into C57Bl/6J blastocysts produced highly chimerical mice. They were mated with 129Sv/Si mice (a substrain closely related to 129Sv/Ev), and only the offspring (F1) that were heterozygous for the knockout (thus having both sets of chromosomes of 129Sv origin) were used for subsequent mating to homozygous (F2) mice. This strategy has important advantages over the practice of breeding the chimerical F2 mice derived from these hybrids may suffer from the mutation. B2\textsuperscript{-/-} mice were a generous gift of Dr Fred Hess (Merck Laboratories, West Point, Pa). B\textsubscript{2}\textsuperscript{-/-} (129Sv/Ev strain) were purchased from Jackson Laboratories (Bar Harbor, Maine). Heterozygous mice were obtained by breeding pairs of B\textsubscript{2}\textsuperscript{+/-} and B\textsubscript{2}\textsuperscript{-/-} mice.

Animals (male, 28 to 35 g body wt [BW]) were housed at a constant room temperature (24±1°C) and humidity (60±3%) with a 12-hour light/dark cycle. During experiments, mice had free access to chow (Mucedola, Settimo Milanese) and tap water. All procedures complied with the standards for the care and use of animal subjects as stated in \textit{Guide for the Care and Use of Laboratory Animals} (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md) and were approved by the local animal care and use committee.

\textit{D-Arg,[Hyp,Thr]D-Tic,Oic\textsubscript{2}}-BK (Icatibant) and 2-[(N-propyl-N-[2'[(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]amino]pyridine-3-carboxylic acid (A-81988) were generous gifts from Hoechst AG (Frankfurt, Germany) and Abbott Laboratories (Abbott Park, Ill), respectively: 2,2,2-tribromoethanol and tert-amyl alcohol were purchased from Sigma-Aldrich.

Clipping

Mice were anesthetized with 2,2,2-tribromoethanol (88 mmol/100 g BW, IP) dissolved in tert-amyl alcohol. The left kidney was exposed through a small flank incision, and the renal artery was individualized over a short segment by blunt dissection. A U-shaped stainless steel clip (3×2×1 mm with a 2-mm-long cleft and 0.12-mm opening width, Exidel SA) was placed around the renal artery close to the aorta, according to the procedure described by Wiesel et al.\textsuperscript{21} Then the kidney was gently pushed back into the retroperitoneal cavity, and the muscle layer and the skin incision were sutured. Sham-operated mice underwent the same surgical procedure except without clipping.

Blood Pressure Measurements

Measurements of systolic blood pressure (SBP) and heart rate (HR) were performed in unanesthetized mice by the tail-cuff plethysmography method. They were warmed for 10 minutes at 37°C in a thermostatically controlled heating cabinet for better detection of tail artery pulse. With the mouse kept gently wrapped in a cotton hand towel, the tail was passed through a miniaturized cuff (1 cm long, 0.8 cm internal diameter) and a tail-cuff sensor that was connected to an amplifier (Recorder 8002, Ugo Basile). The amplified pulse was recorded during automatic inflation and deflation of the cuff. SBP was defined as the cuff inflation pressure at which the waveform becomes indistinguishable from baseline noise. As an inclusion criterion, we required that at least 10 of 12 measurements were successful. Final SBP value was obtained by averaging 10 to 12 successful readings. Experimental measurements were performed between 10 AM and 2 PM by a single investigator, and then they were judged by an independent investigator in a blind fashion. HR was recorded automatically by counter triggered by the pulse wave.

To measure mean blood pressure (MBP) directly, a polyethylene catheter (a PE-10 soldered to a PE-50, Clay Adams) was inserted into the left carotid artery and advanced into the thoracic aorta of anesthetized mice. For drug injections, another catheter was inserted into the left jugular vein. Catheters (filled with 5% heparinized saline solution) were then tunneled under the skin and exteriorized at the back of the neck. The following day, a Statham transducer was connected to the carotid catheter, and intra-arterial MBP of unanesthetized free-moving mice was recorded continuously on Quartet polygraph (Ugo Basile).

Experimental Protocols

Basal measurements of SBP, HR, and BW were performed on 3 occasions (3 days apart). Mice then were randomly assigned to control or experimental groups that underwent sham operation or clipping of the left renal artery. In an additional group of 2K1C B\textsubscript{2}\textsuperscript{-/-} mice, we tested the cardiovascular effects of the BK B\textsubscript{2}-receptor antagonist Icatibant (50 nmol in 10 µL saline/100 g BW twice a day, SC). The dose and timing of administration were chosen on the basis of preliminary experiments in B\textsubscript{2}\textsuperscript{-/-} mice showing the ability of Icatibant to antagonize the vasodepressor effect of BK but not that of acetylcholine or prostaglandin E\textsubscript{2} (PGE\textsubscript{2}).

SBP, HR, and BW were measured every week for 2 weeks (n=8 per group) or 4 weeks (B\textsubscript{2}\textsuperscript{-/-}: 2K1C n=18, Icatibant-treated 2K1C n=6, and sham-operated n=8; B\textsubscript{2}\textsuperscript{+/-}: 2K1C n=16, Icatibant-treated 2K1C n=6, and sham-operated n=8). Standard ECG was recorded in anesthetized mice on the occasion of clipping, and it was repeated at the end of the experiment. On the last day of the experimental period, animals were instrumented with carotid and jugular catheters (as indicated above) and allowed to recover for 5 hours. MBP was then continuously measured before and for 30 minutes after the acute intravenous injection of 10 µg A-81988, a nonpeptidic antagonist of angiotensin type 1 (AT\textsubscript{1}) receptors. This dose of A-81988 is able to block the vasopressor action of 10 pmol IV Ang II. Then mice received an
overdose of anesthesia, and the heart and both kidneys were removed, cleaned, washed in saline, blotted, and weighed. Cardiac weight index was calculated as heart wet weight (mg)/BW (g). The left kidney weight to right kidney weight ratio was also calculated. Kidneys were fixed in neutral formaldehyde, and hematoxylin–eosin–stained sections were examined by conventional microscopy. At the end of the experimental period, we observed a homogeneous distribution of renal infarction (14% in average) among groups that underwent clipping. This percentage is similar to that reported from another group previously.21 Because functional exclusion of the clipped kidney is generally not accompanied by the development of hypertension, and because it was impossible to recognize the exact time when infarction had occurred, we decided to exclude these animals from the study. Therefore, group number (n; see above) represents the animals that were actually considered for statistical analysis.

Statistical Analysis
All data are expressed as mean±SEM. Multivariate repeated-measures ANOVA was performed to test for interaction between time and grouping factor. Univariate ANOVA then was used among groups and over time. Differences within and between groups were determined using paired or unpaired Student’s t tests, respectively, with the Bonferroni multiple comparisons adjustment.

Results
Basal SBP levels were higher in B2+/− than in B2+/+ or B2−/− mice before sham operation (122±2 versus 112±2 and 113±1 mm Hg; P<0.05 for both comparisons) or clipping (121±2 versus 113±2 and 109±1 mm Hg; P<0.05 for both comparisons). As shown in Figure 1, SBP remained unchanged after sham operation. After clipping, it increased more in B2+/− than in B2+/+ or B2−/− mice (28±2 versus 14±2 and 14±2 mm Hg, respectively, at 2 weeks; P<0.05 for both comparisons). Chronic blockade of the BK B2 receptor by Icatibant enhanced the pressure response to clipping in B2+/− mice (29±2 versus 14±2 mm Hg at 2 weeks in mice without Icatibant; P<0.05), thus leading to the hypertensive blood pressure levels of B2−/− mice.

Under basal conditions, B2−/− showed accelerated HR compared with B2+/− and B2+/+ mice (before sham operation: 497±16 versus 443±11 and 420±14 bpm, P<0.05 for both comparisons; before clipping: 467±11 versus 431±11 and 419±10 bpm, P<0.05 for both comparisons). As shown in Figure 1, no significant change in HR was observed after sham operation. A prompt tachycardic response was detected 1 week after clipping in B2+/− and B2+/+ mice, whereas tachycardia was delayed in B2−/− mice. However, the HR response to clipping in B2+/+ mice was enhanced by Icatibant.

ANOVA did not detect any difference among groups as far as BW gain over time is concerned. As shown in Figure 2, within each group the MBP of 2K1C mice was higher than that of respective sham-operated controls, with this difference being more pronounced in B2−/− mice (32 and 38 mm Hg at 2 and 4 weeks, respectively) and in B2+−/− mice given Icatibant (30 and 32 mm Hg) than in B2+/− mice without blockade of the B2 receptor (17 and 18 mm Hg). The pressure level observed in B2+−/− mice was similar to that of B2−/− mice.

As shown in Figure 2, clipping increased heart weight to BW ratio in all groups. However, 2K1C B2+/+ mice given

Figure 1. Absolute changes in SBP (top) and HR (bottom) after sham operation (○) or clipping of the left renal artery in mice with (▲) or without Icatibant treatment (●). Values are mean±SEM. *P<0.05 vs sham-operated mice; ++P<0.05 vs 2K1C mice without Icatibant.
Icatibant showed a greater increase compared with mice of the same strain without blockade of the BK B\textsubscript{2} receptor.

As shown in Figure 2, left kidney weight to right kidney weight ratio was diminished consistently in all groups with 2K1C hypertension (averaging from 0.73 to 0.78).

As shown in Figure 3, acute AT\textsubscript{1} receptor blockade induced a more pronounced MBP decrease in 2K1C mice than in sham-operated mice ($P < 0.05$). There was no significant difference in the MBP decrease induced by AT\textsubscript{1} receptor blockade in B\textsubscript{2}⁻/⁻ and B\textsubscript{2}⁺/⁺ mice with 2K1C hypertension.

No change from basal ECG was detected in B\textsubscript{2}⁺/⁺ at 4 weeks after clipping. By contrast, as shown in Figure 4, both the duration and the amplitude of QRS were increased in B\textsubscript{2}⁻/⁻ mice.

Histological examination did not reveal any major alteration in the kidneys of mice that underwent sham operation or clipping, except in those showing renal infarction. Changes included increased collagen content, glomerular sclerosis, tubular atrophy, and monocyte infiltration of the interstitium. As stated in “Methods,” these animals were excluded from statistical examination.
than spontaneously hypertensive rats and resemble more hypertensive mice also tend to have lower blood pressure levels left renal artery and leaving the other kidney untouched. renovascular hypertension induced in mice by clipping of the other paracrine and endocrine systems. Despite reduced and renal hemodynamics and by interacting with the RAS and electrolyte homeostasis by directly affecting tubular function these potent vasodilators are known to modulate water and hypertension. Our attention was focused on kinins because of the neurohumoral mechanisms that regulate the function of blood pressure. Therefore, it is conceivable that any alteration preventing hypervolemia and further incremental rises of 0.12-mm clips (identical to those used by Wiesel et al. in C57BL/6 mice), we were able to show that 2K1C hypertension can be induced also in mice of J129 background. Consistent with other reports, the magnitude of blood pressure increase was less than that observed in renovascular hypertensive rats. Interestingly enough, genetically hypertensive mice also tend to have lower blood pressure levels than spontaneously hypertensive rats and resemble more closely hypertensive rabbit models, which develop target organ damage even with modestly elevated blood pressure. In fact, a mild pressure overload is sufficient to increase cardiac mass in wild-type mice with Goldblatt hypertension. A direct effect of Ang II on the heart could have amplified the adaptive hypertrophic response. This possibility appears to be likely in consideration of the fact that Ang II is able to stimulate protein and DNA synthesis in cardiac cells. Similar to other species, murine 2K1C hypertension is sustained by a continuously activated RAS. The contralateral kidney is able to maintain an efficient natriuresis, thus especially in the contralateral kidney. It is tempting to interpret the main finding of the present study, ie, the accelerated development of 2K1C hypertension in mice with chronic blockade or genetic disruption of the B2 receptor, as a consequence of a defective compensatory excretory response to increased perfusion pressure by the untouched kidney, leading to total body fluid expansion. Unfortunately, our attempts to obtain consistent measurements of separate renal excretory function failed because of the high variability of renal perfusion pressure in mice under the effects of general anesthesia (P.M., unpublished observations, 1998).

In the mouse, resistance to vasoconstriction induced by acute renin infusion has been reported, possibly due to low angiotensinogen concentration, which represents a rate-limiting factor for the enzymatic reaction catalyzed by renin. No difference exists between B2 receptor knockout mice and wild-type controls with regard to plasma renin activity and to the tissue expression of various components of the RAS, including angiotensinogen and angiotensin-converting enzyme. This discounts the possibility that the accelerated development of hypertension in B2−/− is due to more favorable kinetic conditions for Ang II generation.

In contrast to rats, in which different levels of hypertension can be obtained depending on the size of the clip opening, an all-or-nothing blood pressure response to clipping is observed in mice, ie, sizes >0.13 mm do not cause renal damage but are not able to induce hypertension, whereas sizes of <=0.10 mm always result in infarction of the clipped kidney. The rate of occurrence of a very small white kidney, indicating loss of excretory capacity, was consistently low in both B2−/− and B2+/− mice, similar to that reported previously by Wiesel et al., who used the same solid stainless steel clip to cause hypertension in C57BL/6 mice. Renal infarction was not associated with hypertension, probably because of the functional exclusion of the stenotic kidney. In our study, successful development of renovascular hypertension led to consistent reduction of clipped/nonclipped kidney weight ratio in B2−/− and B2+/− mice. Four weeks after clipping, plasma renin activity was similarly elevated in the 2 strains (P.M., unpublished observations, 1998), and no significant difference was detected in the MBP response to acute AT1 receptor blockade. Together, these results speak against the possibility that in knockout mice, hypertension was worsened by more severe constriction of the renal artery and/or by a greater degree of activation of the RAS.

In vitro studies have shown that Ang II stimulates NO production in vascular endothelial cells by enhancing the synthesis and release of BK. In the early phase of 2K1C hypertension, when increased blood pressure is sustained by elevated Ang II, endothelium-dependent vasodilation is not reduced and may actually be increased. In fact, in 2K1C hypertensive rats, inhibition of NO synthase results in an exaggerated increase in blood pressure and a decrease in contralateral renal blood flow. This suggests that increased NO release could exert a compensatory response on the vasoconstrictor activity of activated RAS. The same vasodilator mechanism could contribute to buffering of angiotensin-independent vasoconstriction during chronic phases of 2K1C hypertension. 

Discussion

We found that pharmacological blockade or genetic disruption of the BK B2 receptor accelerates the development of renovascular hypertension induced in mice by clipping of the left renal artery and leaving the other kidney untouched.

Successful development of 2K1C and 1K1C hypertension has been recently reported by 2 independent groups. Using 0.12-mm clips (identical to those used by Wiesel et al. in C57BL/6 mice), we were able to show that 2K1C hypertension can be induced also in mice of J129 background. Consistent with other reports, the magnitude of blood pressure increase was less than that observed in renovascular hypertensive rats. Interestingly enough, genetically hypertensive mice also tend to have lower blood pressure levels than spontaneously hypertensive rats and resemble more closely hypertensive rabbit models, which develop target organ damage even with modestly elevated blood pressure. In fact, a mild pressure overload is sufficient to increase cardiac mass in wild-type mice with Goldblatt hypertension. A direct effect of Ang II on the heart could have amplified the adaptive hypertrophic response. This possibility appears to be likely in consideration of the fact that Ang II is able to stimulate protein and DNA synthesis in cardiac cells.

Similar to other species, murine 2K1C hypertension is sustained by a continuously activated RAS. The contralateral kidney is able to maintain an efficient natriuresis, thus preventing hypervolemia and further incremental rises of blood pressure. Therefore, it is conceivable that any alteration of the neurohumoral mechanisms that regulate the function of the nonclipped kidney can accelerate the development of hypertension. Our attention was focused on kinins because these potent vasodilators are known to modulate water and electrolyte homeostasis by directly affecting tubular function and renal hemodynamics and by interacting with the RAS and other paracrine and endocrine systems. Despite reduced levels of expression of tissue kallikrein in the ischemic kidney of 2K1C hypertensive rats, this defect is reduced or even absent in the contralateral kidney in the early phases of hypertension. These changes may only be secondary to the increase in B2 receptor expression that reportedly occurs, especially in the contralateral kidney. It is tempting to interpret the main finding of the present study, ie, the accelerated development of 2K1C hypertension in mice with chronic blockade or genetic disruption of the B2 receptor, as a consequence of a defective compensatory excretory response to increased perfusion pressure by the untouched kidney, leading to total body fluid expansion. Unfortunately, our attempts to obtain consistent measurements of separate renal excretory function failed because of the high variability of renal perfusion pressure in mice under the effects of general anesthesia (P.M., unpublished observations, 1998).

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Deficiency in NO release, due to the
interrupted signaling by endogenous kinins, could have exacerbated the development of renovascular hypertension in our mouse model lacking the BK B2 receptor. Dysfunctional endothelium-dependent vasodilation in these mice is documented by the observation that they show a reduced blood pressure response to chronic NO synthase inhibition and a rightward shift in the dose-pressure response curve to acetylcholine. In addition to NO, other relaxant factors such as PGE2 and endogenous cannabinoid derived from arachidonate are known to mediate the vascular effects of BK. Further studies are necessary to determine whether deletion of the B2-receptor gene worsens 2K1C hypertension by affecting the production of PGE2 and/or endogenous cannabinoid. Other unforeseen adaptation mechanisms taking place in animals that are subjected to higher blood pressures during development might have also contributed to the amplification of the pressor response to clipping in B2−/− mice.

The finding that heterozygosity did not confer the same blood pressure phenotype after clipping was unexpected, since a condition of partial deficiency has been shown to be sufficient to elicit increased sensitivity to chronic Ang II infusion. However, heterozygous mice share with B2−/− mice the feature of a tachycardic response during the first 2 weeks after clipping. The reasons for such discrepancies remain unknown. Chronic infusion allows one to evaluate the effects of Ang II as a blood-borne hormone, but it might not reflect the paracrine actions of an excess of endogenous peptide generated endogenously. In addition, redundancy of the B2 receptor, known to be a spare receptor, might compensate for heterozygosity, thus allowing full response to kinins.

A puzzling aspect of our results is that at 4 weeks after clipping, the MBP of 2K1C B2−/− mice given Icatibant was higher compared with 2K1C B2−/− mice without concomitant blockade of the BK B2 receptor, but the difference in SBP was lost at the same time. This discrepancy could be due to the different experimental conditions used to measure direct or indirect blood pressure. In addition, the pattern of SBP might reflect a progressive decrease in cardiac output with persistently elevated total peripheral resistances. Interestingly, SBP was maintained at high levels in Icatibant-treated 2K1C knockout mice. Thus, the pattern observed in wild-type mice could be related to the long-term interaction between the antagonist and its receptor (ie, receptor upregulation) rather than other unforeseen effects of Icatibant.

The mechanisms mediating the exaggerated HR responses of mice with genetic or pharmacological manipulation of the BK B2 receptor remain unknown. Previous studies showed that BK can modulate the reflex response to increments in blood pressure by interacting with other peptidergic systems. The accelerated HR found in B2−/− mice after clipping could be the consequence of an unbalanced central effect of Ang II.

Recent reports have suggested a direct inhibitory action of kinins on myocardial and vascular proliferation, instrumental for cardiovascular protection of converting enzyme inhibitors. We found that under basal conditions, heart weight is increased in B2−/− mice compared with wild-type mice. In addition, analysis of transverse chamber diameter showed that hypertrophy is associated with dilation of the left ventricle, suggesting that the heart of adult B2−/− mice could have almost reached the limit for a compensatory hypertrophic response (P. Madeddu and G. Olivetti, unpublished results, 1998). This could explain why in B2−/− mice, despite a greater pressure load, the increase in heart weight after clipping was similar to that observed in B2+/− mice. By contrast, the potential for further hypertrophic adaptation in wild-type mice is revealed by the finding that long-term blockade of the BK B2 receptor brought heart weight to the levels of knockout mice. Changes in ECG observed after clipping in B2−/− mice probably reflect augmented duration of ventricular depolarization, consequent to heart enlargement. Collectively, our findings indirectly favor the possibility that activation of the KKS can prevent target organ damage in renovascular hypertension. The potential protective role of kinins in cardiac function is documented by the observation that adenovirus-mediated kallikrein gene delivery causes a reduction of the left ventricular mass and cardiac myocyte size in 2K1C hypertensive rats.

In conclusion, our results indicate that kinins are part of a homeostatic response that buffer the vasoconstrictor activity of Ang II. Development of renin-dependent renovascular hypertension could be accelerated by the concomitance of genetic defects relevant to this counterbalancing mechanism.

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