Angiotensin II Type 1 Receptor Blockade Prevents Cardiac Remodeling in Bradykinin B2 Receptor Knockout Mice

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Abstract—Knockout mice (B2−/−) lacking the bradykinin (BK) B2 receptor gene develop mild hypertension, cardiac hypertrophy, and myocardial damage. We hypothesized that these effects are due to the hypertrophying and damaging actions of angiotensin II (Ang II) in the absence of the balancing protection of BK. To verify this hypothesis, B2−/− or wild-type mice (B2+/+) were administered a nonpeptide antagonist of Ang II type 1 (AT1) receptors (A81988) from conception through 180 days of age. Untreated B2+/+ and B2−/− served as controls. Blood pressure (BP) and heart rate were monitored with the use of tail-cuff plethysmography at regular intervals. Ventricular weights, diameters, wall thickness, chamber volume, and myocardial fibrosis were measured at 40 and 180 days. No differences were observed in BP, heart rate, and cardiac weight and dimensions between treated and untreated B2+/+. The BP of AT1 antagonist–treated B2−/− was reduced until 70 days; then, it increased to the levels found in untreated B2−/−. AT1 receptor blockade resulted in a reduction in left ventricular mass, chamber volume, and wall thickness and abrogated myocardial fibrosis in B2−/−. These results indicate that Ang II is the major factor responsible for ventricular remodeling and myocardial damage in mice with disruption of BK B2 receptor signaling. The interaction of Ang II and BK appears to be essential for the development of a normal heart. (Hypertension. 2000;35[part 2]:391-396.)

Key Words: bradykinin ■ angiotensin II ■ myocardium ■ hypertrophy ■ heart failure ■ blood pressure ■ genes

The kallikrein-kinin system constitutes one of the vasodilator mechanisms that can counterbalance angiotensin II (Ang II)–induced vasoconstriction.1–4 Kinins, cleaved by kallikrein from the substrate kininogen, stimulate the release of NO and prostacyclin through the activation of bradykinin (BK) B2 receptors.5 Local kinin generation with a blunted NO formation has been reported in failing human hearts.6 Furthermore, icatibant, a selective antagonist of the BK B2 receptor, reduces coronary blood flow and myocardial contractility and increases left ventricular end-diastolic pressure in pacing-induced heart failure.7 Recently, we found that disruption of the gene that encodes for the B2 receptor causes left ventricular hypertrophy, myocardial damage, and cardiac failure.8 This evolution is quite similar to the natural history of the hypertrophic hypertensive cardiomyopathy seen in men,9 confirming the essential role of kinins in the preservation of myocardial structure and function.10–12 The hypothesis was advanced that cardiac phenotype of B2 receptor gene knockout mice (B2−/−) was the result of Ang II preponderance in the absence of a balancing action of BK.8 In fact, through Ang II type 1 (AT1) receptors, Ang II stimulates myocyte growth independently of or in addition to peripheral vasoconstriction.13–16 Furthermore, Ang II administration may determine myocyte cell death in vivo and in vitro.17–20 When Ang II formation is suppressed by ACE inhibition or is blocked by selective AT1 receptor antagonism, the hypertrophying and damaging effects of Ang II are prevented.1,21–24

In the present study, we tested whether hypertension and myocardial remodeling in B2−/− mice can be avoided through the chronic blockade of Ang II AT1 receptors. Our results indicate that myocardial pathology is completely prevented in animals lacking the B2 receptor due to the blockade of Ang II AT1 receptors. These results imply that the opposite effects of Ang II and BK are needed for the normal development of cardiovascular phenotype.

Methods

All procedures complied with the standards for the care and use of animal subjects as stated in the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academy of Sciences).

B2−/− mice were kindly provided by Dr Fred Hess (Merck Research Labs), and wild-type J129Sv controls (B2+/+) were obtained from Jackson Laboratories. Pregnant mice were administered the nonpeptidic antagonist of Ang II AT1 receptors, A-81988 (2-[(N-propyl-N-[2′(1H-tetrazol-5-yl)-biphenyl-4-yl]-methyl)amino]-pyridine-3-carboxylic acid; 1.7 mg/kg BW per day in drinking water) or vehicle. At the dose indicated above, A-81988 (Abbott Labora-
Hemodynamic Measurements
Body weight (BW), systolic blood pressure (SBP), and heart rate (HR) were measured every 10 days in unanesthetized mice from 40 to 180 days of age. SBP and HR were determined with tail-cuff plethysmography, as previously described.12

Heart Morphology
Treated or untreated mice were anesthetized, and the hearts were arrested in diastole with cadmium chloride (100 nmol) at 40 (n = 10 per group) and 180 (n = 10 per group) days of age. The ventricles were dissected free, rinsed in saline, blotted, and fixed in 10% buffered formalin.

Ventricular Wall and Chamber Diameter Measurements
The free walls of the right ventricle (RV) and the left ventricle (LV) inclusive of the septum were dissected free, and their weights were recorded. The major cavitary axis of the LV from the apex to the aortic valve was measured under a stereo microscope (Wild M 600) with a calibrated ocular accurate to 0.1 mm. LV transverse chamber diameters and RV and LV wall thickness were determined with an analyzer (Image Pro Plus 3.0; accuracy 0.01 mm) with the use of images acquired with a video camera (Sony) through the stereomicroscope at ×16 magnification. LV chamber volume was computed according to the Dodge equation.

Analysis of Ventricular Fibrosis
Transverse slices were embedded in paraffin, and 5-μm-thick sections were cut and stained with Masson’s trichrome. Sections were examined at a calibrated magnification of ×100 with an ocular reticle containing 42 sampling points (Wild Heerbrugg Instruments). This reticle defines a tissue area of 0.85 mm². The points overlying the foci of perivascular or reparative fibrosis were counted separately to compute the volume fraction of myocardial fibrosis.

Reverse Transcription–Polymerase Chain Reaction Analysis
RNA was isolated from frozen hearts (n = 3 each group) according to the RNAzol B method. cDNA was made from RNA according to the manufacturer’s instructions (Stratagene).

Statistical Analysis
Data were expressed as mean±SEM. Multivariate repeated measures ANOVA was performed to test for interaction between time and grouping factor. In multiple comparisons among independent groups in which ANOVA and F test indicated significant differences, the statistical value was determined according to Bonferroni’s method. Differences within and between groups were determined with a paired or an unpaired Student’s t test, respectively. A value of P<0.05 was considered statistically significant.

Results
In B2+/− treatment with AT1 receptor antagonist did not affect SBP, HR, or BW over time (Figure 1, left). In B2−/− at 40 days of age, treatment resulted in a 7% (P<0.05) reduction in SBP compared with untreated B2−/−, whereas HR was unaffected (Figure 1, right). The difference in SBP persisted at 50, 60, and 70 days after birth. From 80 to 180 days of age, the SBP of treated B2−/− increased to the levels found in untreated B2−/−. At the last time point, B2−/− had higher SBP and HR than B2+/−, regardless of the treatment allocation. In B2−/− at 180 days of age, treatment resulted in a 15% reduction in BW (P<0.01) compared with untreated B2−/− (Figure 1F).

At 40 days, ventricular weights and their ratios to BW of wild-type or knockout mice were not affected by the treatment (Figure 2). Similarly, LV wall thickness and cavitary diameters did not differ between treated and untreated mice of either strain (Figure 3 and Table). Thus, at this early stage of postnatal development, AT1 receptor blockade did not alter cardiac and body growth.

In untreated B2+/− from 40 to 180 days of age, no significant change occurred in the ratio of ventricular weights to BW and in cavitary dimensions (Figures 2 and 3), whereas LV wall thickness increased by 16% (Table, P<0.001). During the same period, the ratio of LV weight to BW and LV wall thickness of untreated B2−/− increased by 33% and 29%, respectively (P<0.001 for both comparisons, Figure 3 and Table). In addition, a 144% increase in LV chamber volume (P<0.001) was observed, due to both enlargement and elongation of internal diameters (Figures 2 and 3). The 48% (P<0.001) reduction in the ratio of LV thickness to chamber volume that occurred in B2−/− receptor–deficient mice from 40 to 180 days of age is indicative of a progressive LV dilatation with time (Figure 3).

The treatment of B2+/− with AT1 receptor antagonist did not result in any significant effect on cardiac weights (Figure 2) or dimensions (Figure 3) at 180 days of age. In contrast, in treated B2−/−, a 32% reduction (P<0.0001) in LV weight was seen in comparison with untreated animals (Figure 2A). As shown in Figure 2B, because RV weight decreased only 19% (P<0.01), the entire heart was 30% (P<0.001) smaller than that of untreated B2−/−. However, when these values were corrected for BW changes, it became apparent that the reduction in RV weight paralleled that in BW, whereas the treatment effectively reduced LV weight/BW and heart rate/BW ratios by 20% and 17%, respectively (P<0.001 for both comparisons). The AT1 receptor antagonist not only prevented LV hypertrophy in B2−/− but also abrogated the tendency toward LV dilatation (Figures 3C and 3D).

In untreated B2−/−, myocardial fibrosis (Figure 4, top) was 5-fold higher than that detected in B2+/− at 180 days of age (Table). This type of LV myocardial damage was the consequence of a 6-fold (P<0.002) increase in reparative fibrosis and a 3.5-fold (P<0.02) increase in perivascular lesions. Myocardial fibrosis was completely avoided with AT1 blockade (Figure 4, bottom, and Table). Therefore, AT1 antagonism fully prevented LV remodeling and myocardial damage.

...is able to antagonize the vasopressor effect of 10 pmol intravenous Ang II by 75% in mice. At 2 days after birth, the gender of the pups was determined, and each litter was culled to 5 male pups. Mice whose mother was treated during pregnancy continued to receive the antagonist until 180 days of age (treated B1+/+ and B1−/−, n=25 each group). Untreated controls of each strain (n=25 per group) were provided regular tap water. The animals were housed at a constant room temperature (24±1°C) and humidity (60±3%).

The primers used for amplification of a 287-bp product of Ang AT1 receptor isoform were AT1 L (GAT AAT TAT GCC GAT TGT GC) and AT1 R (TGC TCA TTT TCG TAG ACA G). The primers for amplification of a 303-bp product of Ang AT1b receptor were AT1a L (ATT CAG TTT TCT GGA TGT GC) and AT1a R (TCC ACT TCA AAA CAA TAC GC). Polymerase chain reaction amplification was performed under the following conditions: denaturation at 95°C, annealing at 56°C, and elongation at 72°C for 30 cycles. RNA levels were normalized through the amplification of GAPDH.
in B2−/−, although protection from the development of hypertension was limited in time.

The expression of AT1 receptor isoforms was similar among untreated groups at all ages examined (data not shown).

**Discussion**

The results of the present study demonstrate that chronic Ang II AT1 receptor blockade prevents LV hypertrophy and myocardial damage in B2−/−. However, the treatment only temporarily delayed the tendency of these mice to develop hypertension. These findings support the notion that in the absence of the BK B2 receptors, Ang II has a harmful effect on the myocardium.

Previous studies indicate that genetically determined changes in the level of expression of the BK B2 receptor directly affect the BP of mice during development. Overexpression of the B2 receptor is associated with hypotension, and the lack of the B2 receptor is accompanied by hypertension. In addition, the development of ventricular remodeling leading to myocardial dysfunction and failure has been documented in B2−/− on the basis of anatomic, functional, and

**Figure 1.** Changes in SBP (A and B), HR (C and D), and BW (E and F) in B2+/+ (□, ■) and B2−/− (○, ●). Mice of each strain were provided tap water to drink alone (□, ○) or added to nonpeptidic antagonist of AT1 receptors A-81988 (■, ●). Values are mean±SEM. *P<0.05 vs untreated mice.
biochemical data. The results of the present study confirm the working hypothesis that through AT_1 receptors, Ang II contributes substantially to the development of LV hypertrophy and chamber dilatation in B_2^{-/-}. One possibility is that the increase in cardiac mass and LV thickness is the consequence of progressive enhancement by Ang II of peripheral vascular resistances and LV overload. The distribution of myocardial fibrosis mainly in the subendocardium, the layer with the greatest exposure to the increased intraventricular pressure, seems to confirm the importance of mechanical factors in the development of the structural and functional damage of the heart in this model. Kinins have been shown to exert favorable effects on myocardial metabolism. A shortage of myocardial energy reserves, previously documented in B_2^{-/-}, might have exaggerated the consequences of the pressure overload on the heart, leading to an abnormal growth response and accelerated myocyte death. However, a load-independent mechanism, possibly related to a paracrine action of Ang II, may be also operative. Myocardial hypertrophy is already present in heterozygous mice at 180 days of age, when BP is just starting to diverge from normal. Furthermore, as shown here, pharmacological blockade of the AT_1 receptor exerted a complete inhibitory effect on cardiac growth but only a transient protection against hypertension. The discrepancy between the effects of treatment on heart and BP after 10 weeks might be due to differential changes in vascular and myocardial AT_1 receptor density caused by development of heart failure or by treatment. The number of myocardial Ang II receptors is reportedly normal in moderate heart failure but downregulated in association with a decrease in the mRNA level at the end stage of cardiac disease in humans. Our study indicates that myocardial AT_1 receptor expression is not altered in untreated B_2^{-/-} during the development of cardiac remodeling. Unfortunately, the limited amount of vascular tissue available in the mouse precluded the possibility of an evaluation of whether AT1 receptor expression in the vasculature is altered by the disease state or chronic AT1 receptor blockade. Another possibility is that different vasoconstrictor mechanisms play a role in the pathogenesis of hypertension in adult B_2^{-/-}.

The counterregulatory influence of kinins on Ang II–induced myocardial growth has also been documented in animal models with renin-dependent hypertension and in in vitro preparations of cardiomyocytes cocultured with endothelial cells. The latter results further support the view that the interaction between BK and Ang II on phenotype and growth of cardiac cells is independent of loading conditions. In this regard, a comparable antihypertrophic effect was seen in prevention studies with a low dose of ACE inhibitor in rats with cardiac overload. After 1 year, this treatment had no
effect on BP but prevented LV hypertrophy and myocardial fibrosis and preserved the energy state of the heart. Interestingly, the BK B₂ receptor antagonist icatibant was able to counteract the antihypertrophic effect of low doses of ACE inhibitor, thus suggesting that kinins are involved in the cardiac protection exerted by this class of compounds. The protective effect of kinins may be mediated by the activation of NO/cGMP and prostaglandin I₂/cAMP pathways, which are known to be antimitogenic and antihypertrophic in vitro and in vivo.

Myocyte loss and the consequent myocardial fibrosis were completely prevented by the treatment with AT₁ receptor antagonist in either B₂⁺/⁺ or B₂⁻/⁻. These data suggest that through AT₁ receptors, Ang II is responsible for myocyte death and reparative processes that occur since relatively early stages of development, and this effect is amplified when the protective action of the kallikrein-kinin system is disrupted. However, it cannot be excluded that Ang II AT₁ blockade may leave an excess of unbound Ang II that, in turn, may exert different action through AT₂ receptor stimulation. Although the precise role of Ang II AT₂ receptor in the adult cardiovascular tissue is still unclear, it has been suggested that AT₁ and AT₂ receptor subtypes may exert opposite effects in term of cell growth and pressure regulation.

In conclusion, our results indicate that the lack of the BK B₂ receptors induces distinct cardiac abnormalities in the heart and that chronic Ang II AT₁ receptor blockade prevents the occurrence of pathological changes, demonstrating that Ang II is responsible for the cardiovascular phenotype resulting from the BK B₂ gene defect. Thus, a correct balance between Ang II and BK is essential for the development and maintenance of a normal heart.

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