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

Paolo Madeddu, Costanza Emanuelli, Roberta Maestri, Maria Bonaria Salis, Alessandra Minasi, Maurizio C. Capogrossi, Giorgio Olivetti

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.1,10–12 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-
RESULTS

In B<sub>2</sub><sup>−/−</sup> treated with AT<sub>1</sub> receptor antagonist did not affect SBP, HR, or BW over time (Figure 1, left). In B<sub>2</sub><sup>−/−</sup> at 40 days of age, treatment resulted in a 7% (P<0.05) reduction in SBP compared with untreated B<sub>2</sub><sup>−/−</sup>, 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 B<sub>2</sub><sup>−/−</sup> increased to the levels found in untreated B<sub>2</sub><sup>−/−</sup>. At the last time point, B<sub>2</sub><sup>−/−</sup> had higher SBP and HR than B<sub>2</sub><sup>+/+</sup>, regardless of the treatment allocation. In B<sub>2</sub><sup>−/−</sup> at 180 days of age, treatment resulted in a 15% reduction in BW (P<0.01) compared with untreated B<sub>2</sub><sup>−/−</sup> (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 cavity diameters did not differ between treated and untreated mice of either strain (Figure 3 and Table). Thus, at this early stage of postnatal development, AT<sub>1</sub> receptor blockade did not alter cardiac and body growth.

In untreated B<sub>2</sub><sup>+/+</sup> from 40 to 180 days of age, no significant change occurred in the ratio of ventricular weights to BW and in cavity 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 B<sub>2</sub><sup>−/−</sup> 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 B<sub>2</sub> receptor–deficient mice from 40 to 180 days of age is indicative of a progressive LV dilatation with time (Figure 3).

The treatment of B<sub>2</sub><sup>+/+</sup> with AT<sub>1</sub> 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 B<sub>2</sub><sup>−/−</sup>, 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 B<sub>2</sub><sup>−/−</sup>. 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 AT<sub>1</sub> receptor antagonist not only prevented LV hypertrophy in B<sub>2</sub><sup>−/−</sup> but also abrogated the tendency toward LV dilatation (Figures 3C and 3D).

In untreated B<sub>2</sub><sup>−/−</sup>, myocardial fibrosis (Figure 4, top) was 5-fold higher than that detected in B<sub>2</sub><sup>+/+</sup> 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 AT<sub>1</sub> blockade (Figure 4, bottom, and Table). Therefore, AT<sub>1</sub> antagonism fully prevented LV remodeling and myocardial damage.
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. Over-expression of the B₁ 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 B₁⁺/⁺ (□, ■) 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 AT₁ receptors, Ang II contributes substantially to the development of LV hypertrophy and chamber dilatation in B₂⁻/⁻/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 B₂⁻/⁻, 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 AT₁ 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 AT₁ 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 AT₁ receptor expression is not altered in untreated B₂⁻/⁻ 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 B₂⁻/⁻.

The counterregulatory influence of kinins on Ang II–induced myocardial growth has also been documented in animal models with renin-dependent hypertension and 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
**Anatomic Parameters**

<table>
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<th>Parameter</th>
<th>40 d</th>
<th>180 d</th>
</tr>
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<tbody>
<tr>
<td>Heart weight, mg</td>
<td>Un-B2+/+ 81±4</td>
<td>Tr-B2+/+ 80±2</td>
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<td></td>
<td>Un-B2−/− 77±6</td>
<td>Tr-B2−/− 79±2</td>
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<tr>
<td>Heart weight/BW, mg/g</td>
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<td>Tr-B2+/+ 4.32±0.10</td>
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<td></td>
<td>Un-B2−/− 4.31±0.15</td>
<td>Tr-B2−/− 4.76±0.20</td>
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<tr>
<td>LV wall thickness, mm</td>
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<td>Tr-B2+/+ 1.23±0.13</td>
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<tr>
<td></td>
<td>Un-B2−/− 1.36±0.02</td>
<td>Tr-B2−/− 1.25±0.13</td>
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<tr>
<td>RV wall thickness, mm</td>
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<td>Tr-B2+/+ 0.58±0.04</td>
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<tr>
<td></td>
<td>Un-B2−/− 0.65±0.03</td>
<td>Tr-B2−/− 0.60±0.10</td>
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<tr>
<td>Perivascular fibrosis (volume fraction, %)</td>
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<td>ND</td>
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<tr>
<td></td>
<td>ND</td>
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<td>Reparative fibrosis (volume fraction, %)</td>
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<td>ND</td>
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Un indicates untreated; Tr, treated; ND, not determined. Values are mean±SEM.

*P<0.05 vs mice of the same strain and treatment at 40 days of age.
†P<0.05 vs untreated B2+/+ of the same age, ‡P<0.05 vs untreated B2−/− of the same age.

An 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.29

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 terms of cell growth and pressure regulation.31

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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**References**


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