

Role of the B₁ Kinin Receptor in the Regulation of Cardiac Function and Remodeling After Myocardial Infarction

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Abstract—Kinins exert cardioprotective effects via 2 G-protein-coupled receptors, B₁ and B₂. Using B₁ kinin receptor gene knockout mice (B₁^{−/−}), we tested the hypotheses that the B₁ receptor plays an important role in preservation of cardiac function, whereas lack of B₁ may accelerate cardiac remodeling and dysfunction after myocardial infarction, and that B₂ receptors may compensate for lack of B₁, whereas blockade of B₂ receptors in B₁^{−/−} mice may cause further deterioration of cardiac function and remodeling. Female B₁^{−/−} mice and wild-type controls (C57BL/6J, B₁^{+/+}) underwent sham surgery or myocardial infarction and were treated with either vehicle or B₂-antagonist (icatibant, 500 μg/kg per day, subcutaneous) for 8 weeks. We found that in sham myocardial infarction, B₁^{−/−} mice had a larger left ventricular diastolic chamber dimension both initially and at 4 to 8 weeks compared with B₁^{+/+}. Left ventricular mass and myocyte size were also larger in B₁^{−/−} with sham operation than in B₁^{+/+}, although cardiac function did not differ between strains. After myocardial infarction, cardiac remodeling and function were similar in both strains, although B₁^{−/−} mice tended to have lower blood pressure. Blockade of B₂ receptors tended to worsen cardiac remodeling and dysfunction in B₁^{−/−} but not in B₁^{+/+}. These results may suggest that B₂ receptors play an important role in compensating for lack of B₁ receptors in mice with myocardial infarction. Dual blockade of both B₁ and B₂ eliminates this compensation, leading to further deterioration of cardiac dysfunction and remodeling after myocardial infarction. (*Hypertension*. 2005;45[part 2]:747-753.)

Key Words: kinins ■ myocardial infarction ■ mice

Kinins are vasodilator polypeptides released from low- and high-molecular-weight kininogens by plasma and tissue kallikreins and hydrolyzed by angiotensin-converting enzyme (ACE, also called kininase II), neutral endopeptidase-24.11, and other peptidases.^{1,2} The biological action of kinins is mediated by activation of at least 2 known G-protein-coupled receptors, B₁ and B₂.^{3,4} B₂ receptors are constitutively expressed in most tissues, whereas B₁ receptors are weakly expressed under physiological conditions but strongly induced in response to pathological stimuli such as inflammation or tissue injury.^{5,6} Recent studies suggest that the B₁ receptor is involved in regulation of vasodilatation, inflammation, and tissue repair, including myocardial infarction.^{6–9} Lamontagne et al¹⁰ reported that activation of the B₁ receptor by intravenous infusion of des-Arg⁹-bradykinin caused a profound hypotensive response, which was partially blocked by the nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine, suggesting an NO-mediated mechanism. Su et al showed that intracoronary infusion of des-Arg⁹-bradykinin produced dose-dependent coronary vasodilatation, as evidenced by increased coronary diameter and blood flow;

this was not affected by a B₂ kinin receptor antagonist (B₂-ant) but was attenuated by NOS blockade.⁵ Activation of the B₁ receptor has also been shown to promote angiogenesis in vivo and endothelial cell proliferation in vitro through the NOS pathway.^{11,12} Emanuelli et al reported that hindlimb ischemia in mice induced B₁ gene overexpression accompanied by increased muscular capillary density, and this angiogenesis was blunted by a B₁ receptor antagonist but unaffected by B₂ blockade.¹³

It has also been reported that both B₁ and B₂ receptors are upregulated in the left ventricle (LV) after myocardial infarction (MI).⁷ However, the pathophysiological relevance of upregulation of both receptor subtypes remains unclear. We recently showed that mice lacking B₂ kinin receptors (B₂^{−/−}) did not exhibit altered blood pressure (BP) or cardiac phenotype under normal conditions; however, the therapeutic effect of ACE inhibitors (ACEi) and angiotensin II type 1 receptor antagonists on cardiac dysfunction and remodeling was diminished in B₂^{−/−} mice subjected to MI.¹⁴ Duka et al¹⁵ reported that the B₁ receptor is upregulated in B₂^{−/−} mice, which exhibited a hypotensive response to a selective B₁

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agonist and an acute hypertensive response to a selective B₁ antagonist. In the present study, using mice with targeted deletion of B₁ receptors (B₁^{-/-}), we further studied whether B₁ receptors play an important role in cardiac remodeling after MI, whether lack of B₁ may accelerate cardiac remodeling and dysfunction after MI, or whether the B₂ receptor may act as a compensatory mechanism for lack of the B₁ receptor. Simultaneous blockade of B₁ and B₂ eliminates this compensation and causes further deterioration of cardiac dysfunction and remodeling after MI.

Materials and Methods

Animals and Procedures

Two breeding pairs of B₁ kinin receptor knockout mice (B₁^{-/-}) on a C57/J6 genetic background were obtained from Dr Michael Bader's laboratory (Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany)¹⁶ and bred in our Mutant Mouse Facilities. Wild-type C57BL/6J^{+/+} purchased from Jackson Laboratories (Bar Harbor, Me) served as controls. Animals were housed in an air-conditioned room with a 12-hour light/dark cycle, received standard mouse chow, and drank tap water. For induction of MI, female mice 10 to 12 weeks of age were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). MI was surgically induced by ligating the left anterior descending coronary artery as described previously.¹⁷ This study was approved by the Institutional Animal Care and Use Committee of Henry Ford Health System, and all procedures involving animals were performed in accordance with institutional guidelines.

Measurement of BP and Cardiac Function

Systolic BP (SBP) and heart rate (HR) were measured weekly in conscious mice using a noninvasive computerized tail-cuff system (BP-2000; Visitech Systems, Apex, NC) as described previously.^{18,19} Left ventricular diastolic dimension (LVDD), mass, and shortening fraction (SF), an index of LV systolic performance, were measured monthly with a Doppler echocardiographic system equipped with a 15-MHz linear transducer (Acuson c256, Mountain View, Calif) as described previously.^{20,21} All studies were performed on awake mice.

Histopathological Study

Mice were euthanized after 8 weeks of MI. The heart, lungs, and liver were weighed to assess hypertrophy and congestion. The LV was sectioned transversely into 3 slices from apex to base and rapidly frozen in isopentane precooled in liquid nitrogen, then stored at -70°C to measure infarct size, myocyte cross-sectional area, interstitial collagen fraction, and oxygen diffusion distance as described previously.^{22,23}

Experimental Protocols

To determine the inhibitory effect of B₂-ant on the BP response to exogenous bradykinin (BK), B₁^{+/+} mice received either vehicle or B₂-ant (icatibant) at 100 or 500 µg/kg per day. B₂-ant was injected daily subcutaneously for 2 weeks. Mice were then anesthetized with sodium pentobarbital (50 mg/kg, IP), placed on a heating pad, and the carotid artery and jugular vein were cannulated to measure mean blood pressure and administer BK (25, 50, and 100 ng/mouse as a bolus) at 15-minute intervals. As shown in Figure 1, B₂-ant at 500 µg/kg per day almost completely blocked the vasodepressor effect of BK; thus, this dose was chosen for the study.

To determine whether lack of B₁ receptors alters cardiac morphology and function at baseline and after MI, and whether blockade of B₂ receptors causes cardiac function and remodeling to deteriorate further after MI in B₁^{-/-} mice, both B₁^{+/+} and B₁^{-/-} mice were divided into sham MI; MI plus vehicle; and MI plus B₂-ant (500 µg/kg per day, subcutaneous). Vehicle or B₂-ant was started 3 days before MI and continued for 8 weeks after MI.

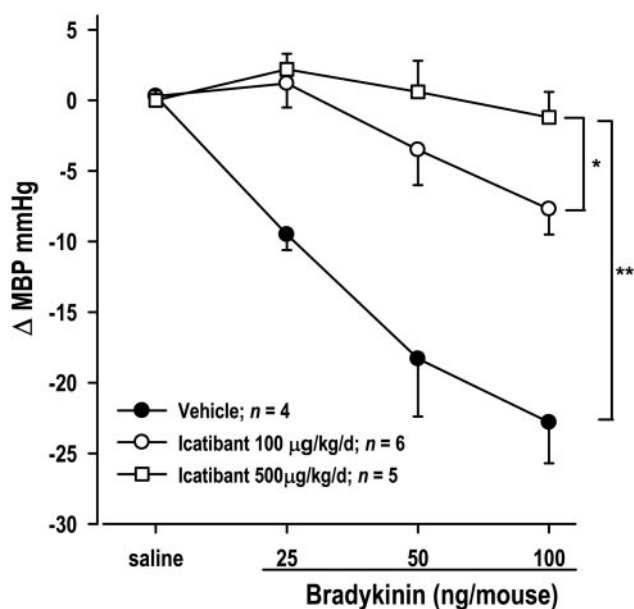


Figure 1. Dose-dependent inhibitory effect of B₂ kinin receptor antagonist icatibant on bradykinin-induced vasodilatation. MBP indicates mean blood pressure. **P*<0.05, slopes between icatibant 100 µg/kg per day and vehicle; ***P*<0.001, slopes between icatibant 500 µg/kg per day and vehicle.

Data Analysis

Data are expressed as mean±SE. Student's 2-sample *t* test was used to compare differences between groups, either between strains or between treatments within strains. When multiple comparisons were performed, Hochberg's step-up procedure was used to adjust probability values.²⁴ The type I error rate was set at 0.05.

Results

Inhibitory Effect of B₂-ant on Mean BP Response to Bradykinin

We tested the inhibitory effect of B₂-ant on BK-induced vasodilatation. BK caused dose-dependent reduction of mean blood pressure. B₂-ant at 100 µg/kg per day partially but significantly blocked the vasodilator response to BK, whereas at 500 µg/kg per day it almost completely prevented the mean blood pressure response to BK (Figure 1).

Mortality, Tissue Weight, and Infarct Size

Within 24 hours after MI, 4 out of 22 mice died in the B₁^{-/-} vehicle group and none died in the B₁^{+/+} group (n=21), but the difference was not statistically significant. Otherwise, the mortality rate was similar and no cardiac rupture was found in either strain. Body and organ weight in sham MI groups were no different between strains. MI increased LV and total heart weight to a similar extent in both B₁^{+/+} and B₁^{-/-} but had no effect on lung or liver weight. B₂-ant increased total heart weight further in B₁^{-/-} compared with B₁^{+/+}. There was no significant difference in infarct size among groups or between strains (Table).

SBP and HR

Basal SBP and HR were similar between strains in all groups (Table). After MI, SBP did not change significantly in B₁^{+/+}, whereas B₁^{-/-} tended to have lower SBP. B₂-ant per se had no

Effect of B₂-ant Icatibant on SBP, HR, Tissue Weight, and Infarct Size 8 Weeks After MI in B₁^{+/+} and B₁^{-/-} Mice

| Parameters | B ₁ ^{+/+} | | | B ₁ ^{-/-} | | |
|----------------|-------------------------------|----------------|------------------|-------------------------------|----------------|------------------|
| | MI | | | MI | | |
| | Sham MI (n=15) | Vehicle (n=13) | Icatibant (n=13) | Sham MI (n=16) | Vehicle (n=13) | Icatibant (n=11) |
| BW, g | 23.0±0.2 | 23.0±0.3 | 22.2±0.3 | 24.0±0.5 | 24.1±0.7 | 23.8±0.5 |
| SBP, mm Hg | 109.4±2.2 | 113.4±1.8 | 113.4±2.2 | 105.7±3.7 | 104.1±3.1 | 101.1±2.9 |
| HR, beats/min | 690±12.9 | 697±13.1 | 675±12.2 | 713±7.8 | 702±17.1 | 713±13.2 |
| LV, mg/10 g | 31.4±0.6 | 42.3±1.2* | 43.2±1.4 | 32.9±0.8 | 42.0±1.2* | 45.9±1.8 |
| THW, mg/10 g | 42.1±0.8 | 56.7±1.9* | 54.3±1.2 | 43.9±0.8 | 55.6±1.9* | 60.3±2.7† |
| Lungs, mg/10 g | 69.5±2.1 | 71.2±4.0 | 65.7±2.9 | 74.4±2.4 | 71.3±4.6 | 78.7±6.9 |
| Liver, mg/10 g | 455.3±10.2 | 468.4±12.2 | 413.9±8.3 | 433.7±10.3 | 460.6±14.0 | 452±14.7† |
| IS, % | — | 31.8±2.5 | 31.7±1.2 | — | 33.8±13 | 34.6±1.4 |

B₂-ant indicates B₂ kinin receptor antagonist; BW, body weight; HR, heart rate; IS, infarct size; LV, left ventricular weight corrected by body weight; MI, myocardial infarction; RV, right ventricular weight; SBP, systolic blood pressure; sham, sham operation; THW, total heart weight corrected by body weight.

Lung and liver weight corrected by body weight.

**P*<0.001 vs sham within strains.

†*P*<0.05 between strains receiving the same treatment.

effect on SBP in either strain. MI did not cause a significant increase in HR in either strain.

Cardiac Function and LV Chamber Dimension

Lack of B₁ receptors had no effect on cardiac performance under basal conditions. LV SF was similar between B₁^{+/+} and B₁^{-/-} subjected to sham operation (Figure 2, left panel). However, LVDd in B₁^{-/-} mice with sham MI was significantly greater than B₁^{+/+} either initially or at 4 and 8 weeks (Figure 2, right panel). After MI, SF was decreased and LVDd increased significantly but similarly in both strains receiving vehicle (Figures 3 and 4). Blockade of B₂ receptors had no additional effect on SF and LVDd in B₁^{+/+}; however, it tended to worsen cardiac function and increase chamber dilatation further in B₁^{-/-} compared with vehicle within strains, although the difference was not statistically significant.

Histological Measurements

In sham-operated mice, LV mass, myocyte cross-sectional area, and oxygen diffusion distance were larger in B₁^{-/-} than in B₁^{+/+}, whereas interstitial collagen fraction was similar between strains (Figures 5, 6, and 7). MI increased LV mass, myocyte cross-sectional area, and oxygen diffusion distance to a similar extent in both strains receiving vehicle, whereas collagen deposition was more severe in B₁^{-/-}. The B₂-ant increased myocyte hypertrophy and oxygen diffusion distance further in B₁^{-/-} and these effects were not seen in B^{+/+} (Figures 5, 6, and 7).

Discussion

We found that lack of the B₁ receptor in mice did not affect SBP and cardiac performance under basal conditions. However, B₁^{-/-} with sham MI had larger LV chamber dimension, myocyte size, and LV mass compared with wild-type con-

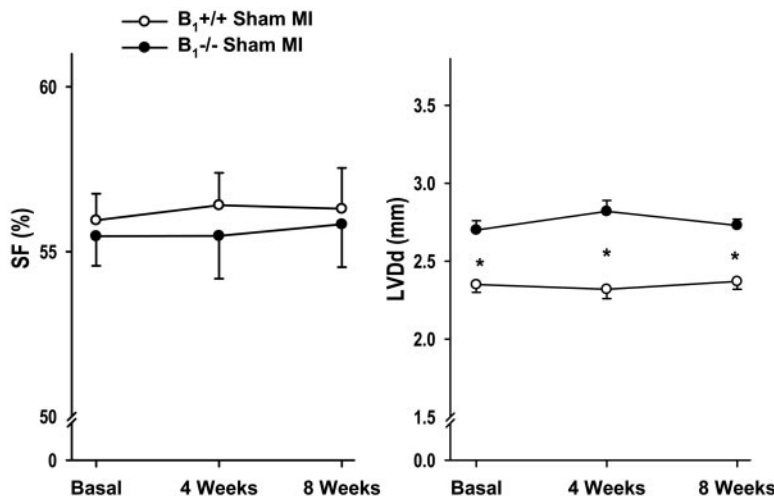


Figure 2. Left ventricle (LV) shortening fraction (SF) and diastolic dimension (LVDd) in B₁^{+/+} and B₁^{-/-} mice with sham myocardial infarction (MI). **P*<0.001 between strains.

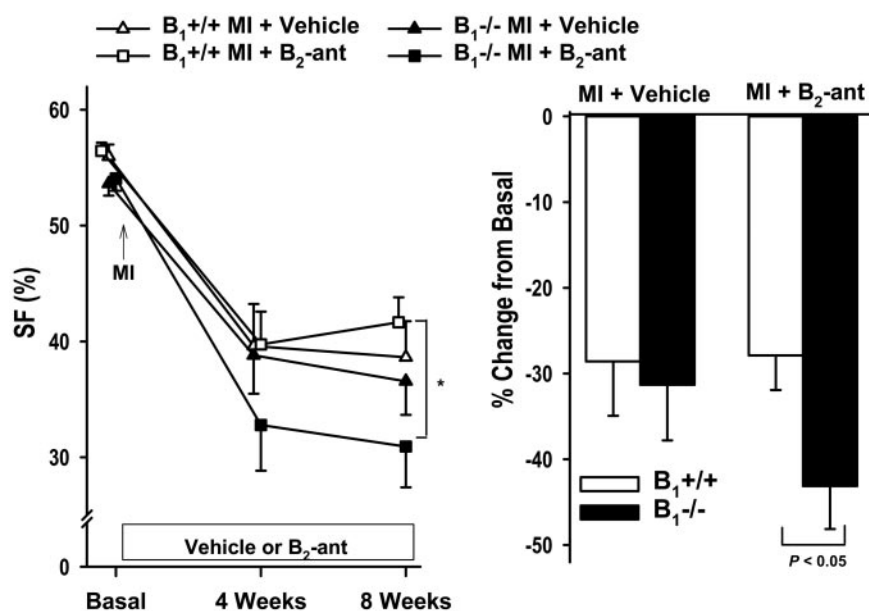


Figure 3. LV shortening fraction (SF) in $B_1^{+/+}$ and $B_1^{-/-}$ mice with MI treated with either vehicle or B_2 receptor antagonist (B_2 -ant). Bar graph shows average percent change after vehicle or B_2 -ant. * $P < 0.05$ between strains treated with B_2 -ant.

trols. The larger LV dimension and myocyte hypertrophy in $B_1^{-/-}$ mice did not have a significant impact on structural and functional remodeling after MI, because LV chamber dilation, cardiac hypertrophy, and collagen deposition, as well as LV dysfunction, were similar between $B_1^{+/+}$ and $B_1^{-/-}$ receiving vehicle. Blockade of B_2 receptors tended to cause further reduction of SF and increase in LV dilation and myocyte hypertrophy in $B_1^{-/-}$, effects not seen in $B_1^{+/+}$. These findings may indicate that the B_1 receptor is involved in regulation of structural homeostasis, because $B_1^{-/-}$ mice had increased heart weight, myocyte size, and LV chamber dimension under basal conditions. However, the B_1 receptor does not seem to play an important role in remodeling after MI, because lack of B_1 did not worsen cardiac function and remodeling compared with wild-type controls. Moreover, B_2 may play a compensatory role for lack of B_1 , because blocking the B_2 receptor tended to exaggerate LV dysfunction and remodeling in $B_1^{-/-}$.

Evidence indicates that a local or tissue kallikrein-kinin system exists in the heart, because kininogens, kininogenases (kallikreins), and kinins are all found in the heart.^{3,25} The biological actions of kinins are mediated via 2 subtypes of G-protein-coupled receptors, B_1 and B_2 .^{3,4} B_2 receptors are constitutively expressed in most tissues and are sensitive to bradykinin and kallidin, whereas B_1 receptors are induced in response to pathological stimuli, such as inflammation or tissue injury, and are sensitive to des-Arg⁹-bradykinin and des-Arg¹⁰-kallidin, two carboxypeptidase metabolites of kinins.^{5,6} Recent studies have shown that activation of the B_1 receptor by des-Arg⁹-bradykinin causes vasodilator and angiogenic responses via an NO-mediated mechanism.^{5,11} However, the B_1 receptor is only weakly expressed under physiological conditions, and its role in the regulation of cardiovascular homeostasis remains unclear. In the present study, we investigated whether targeted deletion of the B_1 receptor could influence cardiac structure and function under

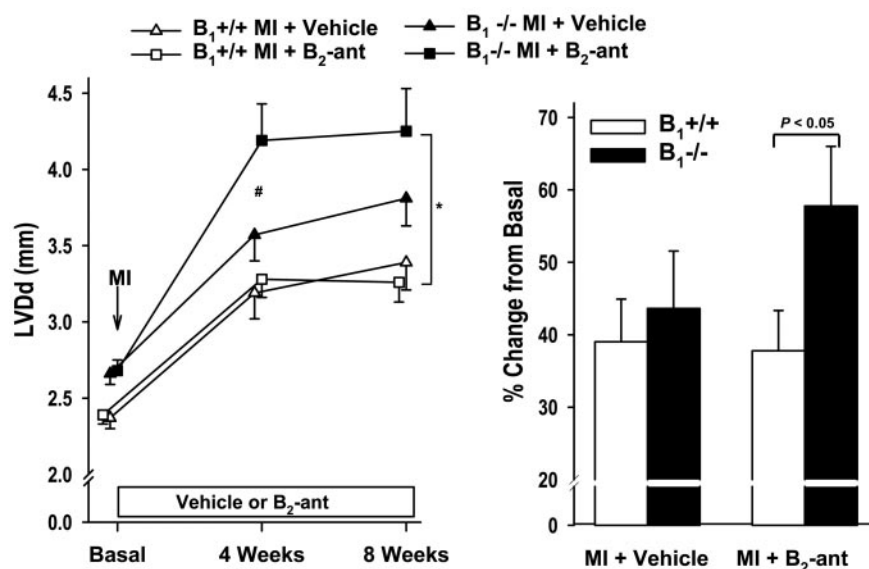


Figure 4. LV diastolic dimension (LVDd) in $B_1^{+/+}$ and $B_1^{-/-}$ mice with MI treated with either vehicle or B_2 receptor antagonist (B_2 -ant). Bar graph shows average percent change after vehicle or B_2 -ant. # $P < 0.05$, vehicle vs B_2 -ant in $B_1^{-/-}$ mice. * $P < 0.05$ between strains treated with B_2 -ant.

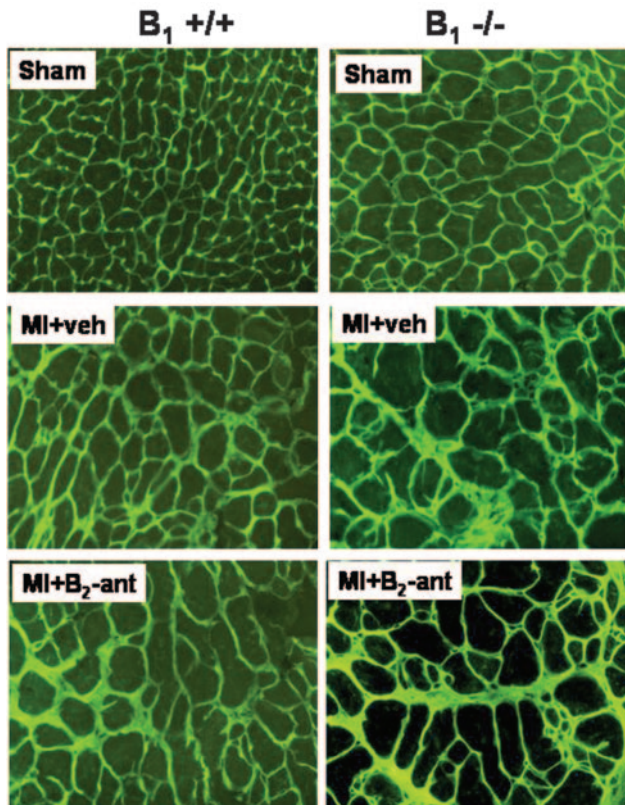


Figure 5. Representative slides showing increased myocyte cross-sectional area and interstitial collagen deposition (green staining) in mice with heart failure induced by MI. B₂ receptor antagonist (B₂-ant) caused a further increase in myocyte cross-sectional area and collagen deposition in B₁^{-/-} mice compared with B₁^{+/+}. Veh indicates vehicle.

physiological conditions. We found that lack of B₁ receptors did not affect blood pressure and cardiac function in sham-operated mice. However, B₁^{-/-} have an enlarged LV chamber associated with increased LV mass, myocyte size, and oxygen diffusion distance. Collagen deposition tended to be higher in B₁^{-/-} compared with B₁^{+/+}. These data may suggest that B₁ receptors are involved in maintaining the integrity of cardiac structure. However, the mechanisms responsible for such a response need to be studied further.

It has been shown that B₁ receptors are upregulated in the heart when it is subjected to myocardial ischemia or pressure overload.⁷ Using a Langendorff mouse heart preparation, Lagneux et al²⁶ showed that infarct size was smaller in B₁^{-/-} mice; blockade of B₁ receptors in wild-type controls also reduced ischemic injury, indicating a detrimental effect of the B₁ receptor on cardiac ischemia. However, Agata et al¹² found that local delivery of an adenovirus carrying the kallikrein gene reduced vascular injury caused by balloon angioplasty, as evidenced by reduction of neointima formation and regeneration of endothelium. These effects were blocked by a B₁ antagonist, indicating that activation of B₁ receptors exerts a vascular protective effect. Because B₁ receptors may participate in the inflammatory response and aid in tissue repair and wound healing,^{7,27} in our *in vivo* study we tested the hypothesis that lack of B₁ receptors may accelerate cardiac remodeling and dysfunction after MI.

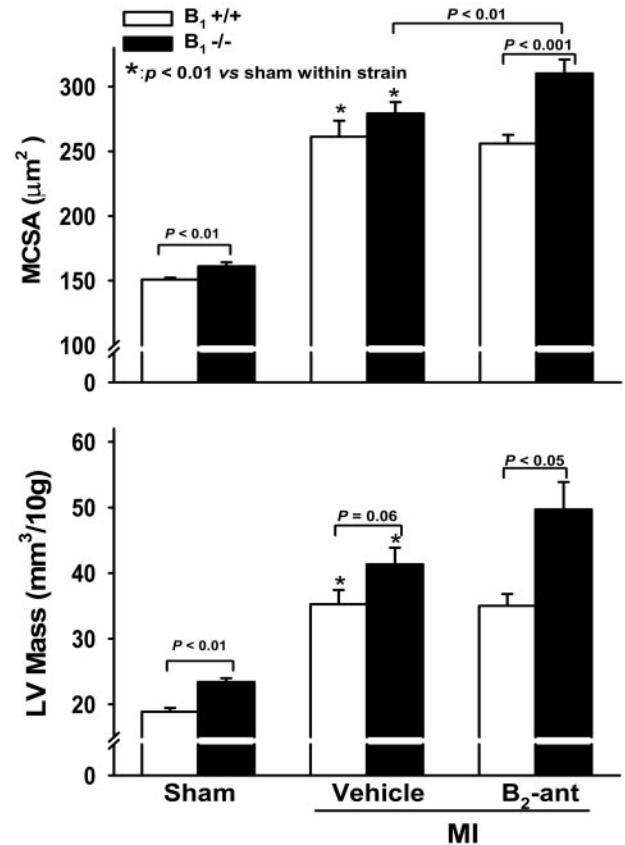


Figure 6. Myocyte cross-sectional area (MCSA) and LV mass (corrected by body weight) in B₁^{+/+} and B₁^{-/-} mice with sham MI or MI treated with either vehicle or B₂ receptor antagonist (B₂-ant). **P* < 0.01 vs sham within strain.

However, our data showed that lack of B₁ receptors neither benefits nor deteriorates remodeling after MI. Infarct size, LV function, chamber dilatation, and myocyte hypertrophy were similar between B₁^{-/-} and B₁^{+/+}, except that collagen deposition was more severe in B₁^{-/-}. Tschöpe et al⁷ and Spillmann et al²⁸ showed that B₁ receptors were induced as early as 6 hours after MI, reached a maximum at 24 hours, decreased to a level comparable to 6 hours MI, and were then weakly expressed until 3 weeks. Early induction of the B₁ receptor may be actively involved in infarct healing and scar formation in the acute phase of MI. However, we are not able to answer this question based on the current study, because this was a chronic experiment and mice were euthanized after 8 weeks MI. Although there were a few more deaths in the B₁^{-/-} group during the first 24 hours after MI, mortality thereafter was similar in both strains. We also saw no deaths caused by cardiac rupture in the first week of MI, which agrees with our previous observation that female mice rarely have rupture.²⁹ Nevertheless, our findings do not support the hypothesis that lack of B₁ worsens cardiac remodeling after MI.

Duka et al¹⁵ recently reported that the B₁ receptor was upregulated in B₂^{-/-} mice, which exhibited a hypotensive response to a selective B₁ agonist and an acute hypertensive response to a selective B₁ antagonist. We previously showed that lack of B₂ receptors does not alter BP or cardiac

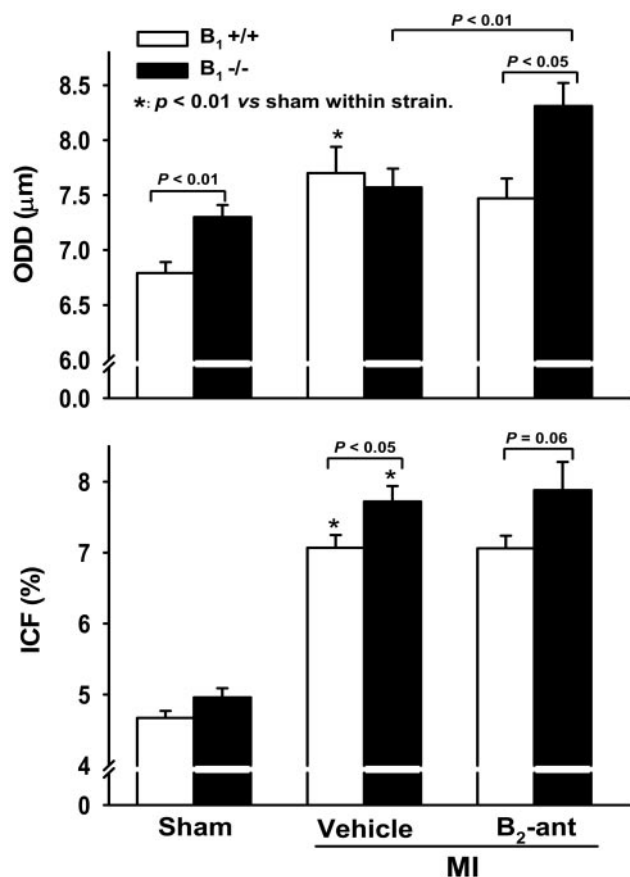


Figure 7. Oxygen diffusion distance (ODD) and interstitial collagen fraction (ICF) in $B_1^{+/+}$ and $B_1^{-/-}$ mice with sham MI or MI treated with either vehicle or B_2 receptor antagonist (B_2 -ant). * $P < 0.01$ vs sham within strain.

phenotype either under normal conditions or after MI.^{14,30} Taken together, these data may indicate a compensatory function of B_1 receptors in maintaining hemodynamic homeostasis when B_2 receptors are absent. Thus, in the present study, we questioned whether dual blockade of B_1 and B_2 worsens cardiac dysfunction and remodeling after MI. Our data showed that in $B_1^{-/-}$ mice, the B_2 -ant icatibant tended to cause further LV dilatation, cardiac hypertrophy, interstitial fibrosis, and reduction of the SF, which were not seen in $B_1^{+/+}$ controls after 2 months MI. Infarct size was similar among groups, indicating that the effect of B_2 -ant was independent of infarct size. Its effect may primarily involve the viable myocardium. Thus we believe it is essential that at least 1 kinin receptor remain intact, which compensates for functional loss of the other receptor, particularly in pathological conditions such as MI. Inactivation of both B_1 and B_2 eliminates such compensation and leads to further deterioration of cardiac dysfunction and remodeling after MI.

Activation of B_1 receptors may also contribute to the cardioprotective effect of ACEi or angiotensin II receptor blockers. Ignjatovic et al^{31,32} recently reported that the ACEi enalaprilat at nanomolar concentrations directly activated the B_1 receptor in cultured human and bovine endothelial cells, independent of endogenous kinins or ACE activity. This interaction led to prolonged release of NO, which was

blocked by a B_1 antagonist but not the B_2 antagonist icatibant. Tschöpe et al³³ showed that the angiotensin II receptor blocker irbesartan improved cardiac function and upregulated B_1 receptors in rats with MI, and this effect was blocked by a B_1 antagonist. However, using an isolated rabbit aorta or mouse stomach preparation, Fortin et al³⁴ failed to demonstrate that ACEi act as B_1 receptor agonists to stimulate a tissue response. Such controversial findings may call for further studies to clarify the role of the B_1 receptor in the therapeutic effect of ACEi and angiotensin II receptor blockers, particularly in vivo.

Limitations

We demonstrated that blockade of the B_2 receptor worsened cardiac remodeling after MI when B_1 was absent, whereas lack of B_1 per se had no effect on the remodeling process. To confirm the existence of a compensatory mechanism between the 2 kinin receptors, we need to block the B_1 receptor in B_2 knockout mice and see whether cardiac dysfunction and remodeling are aggravated.

Perspectives

A number of studies have shown that both B_1 and B_2 kinin receptors are upregulated in the heart after MI.^{7,35} However, the pathophysiological relevance of kinin receptor upregulation remains unclear. Previously, we have shown that lack of the B_2 receptor did not affect cardiac remodeling after MI.¹⁴ In the present study, we further showed that targeted deletion of B_1 receptors did not aggravate remodeling after MI, whereas blockade of B_2 receptors in $B_1^{-/-}$ mice exaggerated LV dysfunction and remodeling. Taken together, these data may suggest that either B_1 or B_2 compensates for absence of the other receptor. Furthermore, our finding that dual inactivation of B_1 and B_2 (gene deletion plus receptor blockade) caused deterioration of cardiac function and remodeling after MI may indicate that the kallikrein-kinin system acting via the B_1 or B_2 receptor plays an important role in protecting the heart against dysfunction and remodeling after MI.

Acknowledgments

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