Published in final edited form as:

Can J Physiol Pharmacol. 2008 September ; 86(9): 633–642. doi:10.1139/y08-068.

Simvastatin reverses cardiac hypertrophy caused by disruption of the bradykinin 2 receptor¹

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Abstract

Bradykinin 2 receptor (B2R) deficiency predisposes to cardiac hypertrophy and hypertension. The pathways mediating these effects are not known. Two-month-old B2R knockout (KO) and wild-type (WT) mice were assigned to 4 treatment groups ($n = 12-14/\text{group}$): control (vehicle); nitro-L-arginine methyl ester (L-NAME) an NO synthase inhibitor; simvastatin (SIM), an NO synthase activator; and SIM+L-NAME. Serial echocardiography was performed and blood pressure (BP) at 6 weeks was recorded using a micromanometer. Myocardial eNOS and mitogen-activated protein kinase (MAPK, including ERK, p38, and JNK) protein expression were measured. Results showed that (i) B2RKO mice had significantly lower ejection fraction than did WT mice ($61\% \pm 1\%$ vs. $73\% \pm 1\%$), lower myocardial eNOS and phospho-eNOS, normal systolic BP, and higher LV mass, phospho-p38, and JNK; (ii) L-NAME increased systolic BP in KO mice (117 ± 19 mm Hg)

¹Presented in part at the American College of Cardiology meeting March 2003 in Orlando, USA, and the Society for Pediatric Research meeting May 2007 in Toronto, Canada.

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but not in WT mice and exacerbated LV hypertrophy and dysfunction; and (iii) in KO mice, SIM decreased hypertrophy, p38, and JNK, improved function, increased capillary eNOS and phospho-eNOS, and prevented L-NAME-induced LV hypertrophy without lowering BP. We conclude that disruption of the B2R causes maladaptive cardiac hypertrophy with myocardial eNOS downregulation and MAPK upregulation. SIM reverses these abnormalities and prevents the development of primary cardiac hypertrophy as well as hypertrophy secondary to L-NAME-induced hypertension.

Keywords

cardiomyopathy; hypertrophy; bradykinin; nitric oxide; simvastatin; mitogen-activated protein kinases; echocardiography

Introduction

Cardiac hypertrophy is a multifactorial condition that is caused by hemodynamic, neurohormonal, and genetic stimuli and requires the activation of complex transcriptional programs. Progressive cardiac hypertrophy can be maladaptive, resulting in cellular phenotypic changes in the myocytes and eventually causing cardiac fibrosis, dilatation, and dysfunction. The hypertrophic response to hemodynamic load may be influenced by individual genotype. In this regard, the bradykinin 2 receptor (B2R) genotype has emerged as an important determinant of the hypertrophic response to load. Two mammalian genes encoding bradykinin receptors have been identified. The B2R subtype is found in healthy smooth muscle and neurons, whereas the bradykinin 1 receptor (B1R) subtype is only detected after tissue injury. The B2R binds bradykinin with high affinity. The activation of B2R by bradykinin activates endothelial nitric oxide synthase (eNOS) and stimulates the synthesis of NO in endothelial cells (Luckhoff et al. 1987; Yang et al. 2003). Bradykinin also mediates the antihypertrophic effects of angiotensin-converting enzyme (ACE) inhibitors and improves left ventricular (LV) diastolic function in response to ACE inhibitors during heart failure, suggesting an interaction with other hypertrophy signaling pathways (Ishigai et al. 1997; Fujii et al. 2002). Several studies have highlighted the important role of the B2R in human studies in conditions that predispose to LV hypertrophy (Cui et al. 2005; Dhamrait et al. 2003; Fu et al. 2004). Studies have shown that an inactivating (loss of function) mutation in the gene encoding the B2R is associated with greater susceptibility to physiologic and pathologic LV hypertrophy, as seen during exercise training of healthy army recruits as well as in patients with systemic hypertension (Brull et al. 2001; Fu et al. 2004). The molecular basis of cardiac hypertrophy in this population is not known, however.

Emanueli and colleagues (1999) studied the cardiac phenotype in mice with targeted disruption of the B2R and reported the development of LV hypertrophy in the animals by 2 months of age that progressed to overt heart failure with a dilated cardiomyopathic phenotype by 6 months. The molecular pathways that mediate this phenotype are unknown. Bradykinin stimulates NO synthesis by Ca^{2+} -calmodulin-mediated eNOS activation and also by promoting protein kinase B (Akt)-mediated eNOS phosphorylation (Harris et al. 2001). B2R can also decrease eNOS activity in aortic endothelial cells, however, by activating extracellular signal-regulated kinase (ERK), a mitogen-activated protein kinase (MAPK) (Bernier et al. 2000). Besides their opposing effects on eNOS activation, both Akt and MAPKs have an important and independent role in cell growth and survival. Although a role for reduced NO or increased MAPK activation has been independently demonstrated in some forms of heart failure, the role of these pathways in mediating cardiac remodeling in B2R-deficient states has not been studied (Hayashida et al. 2001). Duka et al. (2008) further

reported that ACE inhibition worsens cardiac function in mice lacking the B2R, possibly related to overexpression of B1R that can have noxious proinflammatory effects. Because simvastatin (SIM) acts downstream of the B2R through direct activation of eNOS, we hypothesized that SIM would exert a beneficial effect on cardiac function in mice lacking the B2R.

The availability of mice with targeted disruption of the B2R provides an opportunity to study the signaling pathways that mediate hypertrophy in this genetic model. The objectives of the study were (i) to determine the role of alterations in eNOS and MAPK signaling on development of cardiac hypertrophy and hypertension in B2R knockout mice, and (ii) to determine the effect of SIM administration on reversal of the cardiac phenotype. The rationale for SIM use was based on previous evidence that SIM increases eNOS expression and phosphorylation and can also reduce MAPK activation independently of its lipid-lowering effect (Dechend et al. 2001; Laufs et al. 1998; Liu et al. 1999).

Materials and methods

The studies were performed in 2-month-old B2R knockout mice (Bdkrb2) and wild-type controls (B6129/F2). Mice were obtained from Jackson Laboratory (Bar Harbor, Me.). Animals were housed in cages as littermates with alternate light and dark cycles and free access to food and water. Wild-type (WT) and knockout (KO) mice were assigned to 4 treatment groups ($n = 12\text{--}14$ per group): control (vehicle); nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, $0.14\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ per 100 mg body weight once per day in drinking water; simvastatin (SIM), a NOS activator, $10\text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ by oral gavage; and SIM+L-NAME. Treatment was administered for 6 weeks. The doses of L-NAME and SIM were chosen on the basis of demonstrable safety and efficacy in previous studies (Madeddu et al. 1997; Mital et al. 2000a, 2000b). Echocardiographic evaluation was performed at baseline and after 6 weeks of treatment in all mice. At the end of the treatment period, half the mice in each group underwent hemodynamic measurement of blood pressure (BP) using a micromanometer catheter and the remainder were sacrificed for in vitro studies to measure protein expression in explanted myocardium. The protocols were approved by the institutional animal care and use committee and conform to the *Guide to the Care and Use of Experimental Animals*.

Echocardiographic assessment

After training the mice for 3 days, the left hemithorax was shaved, and transthoracic echocardiographic images were obtained in conscious animals using an Acuson Sequoia ultrasound equipped with a 13 MHz linear transducer (15L8) in a phased-array format with real-time digital acquisition, storage, and review capabilities. The heart was imaged in 2-dimensional (2D) mode using standard parasternal short axis, apical 4-chamber, and parasternal long-axis views to measure LV chamber dimensions and wall thickness and to calculate LV mass, volume, and ejection fraction. Three beats were averaged for each measurement. The details of these techniques have been previously published (Abraham et al. 2004). All calculations were derived using standard formulas (Suehiro et al. 2001).

LV chamber dimensions and wall thickness—LV end-diastolic (LVEDD) and end-systolic (LVESD) diameters and wall thickness were measured from M-mode tracings.

LV volume and ejection fraction—LV volume and ejection fraction were calculated from the 2D parasternal long-axis view using the single-plane area-length method.

LV mass—LV mass was calculated according to uncorrected cube assumptions using the following equation: $LV\ mass = 1.055 (IVST + LVEDD + PWT)^3 - LVEDD^3$, where 1.055 is the specific gravity of the myocardium, IVST is the inter-ventricular septal thickness, and PWT is the posterior wall thickness.

Hemodynamic assessment

After 6 weeks of treatment, systemic BP was measured. Briefly, mice were anesthetized with isoflurane (induction, 5%; maintenance, 2%) and placed in a supine position. The neck region was clipped and prepped with betadine for surgery. A small vertical paramedian incision was made aseptically in the neck. With careful superficial and deep dissection, the right carotid artery was visualized and exposed. It was then prepared for catheterization with a 1.4 F high-fidelity micromanometer catheter model SPR-671 (Millar Instruments, Houston, Tex.). The catheter was introduced into the carotid artery and stabilized with the help of a 4-0 silk suture when it had been advanced into the arch of the aorta. The catheter was connected to a pressure transducer box through which systolic, diastolic, and mean BP were monitored and recorded on a PowerLab/800 once a stable reading was achieved (generally within 5–10 min). The mice were euthanized after BP measurements.

Myocardial protein expression

Mice were anesthetized 6 weeks after the initiation of SIM treatment using an intraperitoneal injection of pentobarbital (65 mg/kg), hearts were removed by thoracotomy, and myocardium was isolated for measurement of protein expression of eNOS, phosphorylated eNOS (phospho-eNOS), Akt, and MAPKs, including total and phosphorylated ERK1/2, c-Jun N-terminal kinase (JNK), p38 MAPK, and B1R. Frozen heart tissues were ground under liquid nitrogen, then suspended in lysis buffer containing 10 mmol/L HEPES (pH 7.4), 50 mmol/L $Na_4P_2O_7$, 50 mmol/L NaF, 50 mmol/L NaCl, 5 mmol/L EDTA, 5 mmol/L EGTA, 2 mmol/L Na_3VO_4 , 0.1% Triton X-100, 10 µg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride. Western blot was performed as previously described (Mital et al. 2000b). Proteins were separated on 10% precast Tris–HCl gels (Bio-Rad, Melville, N.Y.). Separated proteins were transferred by a semi-dry method onto a polyvinylidene fluoride (PVDF) membrane (0.2 µm). The membranes were immunoblotted using the following primary antibodies in a buffer containing 10 mmol/L Tris–HCl, 100 mmol/L NaCl, 0.1% Tween 20, 5% bovine serum albumin, pH 7.5: anti-phospho-Ser¹¹⁷⁷ (pSer¹¹⁷⁷) eNOS antibody (1:700) and anti-eNOS antibody (1:500) (EMD Chemicals, San Diego, Calif.); anti-phospho-Ser⁴⁷³ Akt antibody (1:500), Akt (1:500), anti-ERK, anti-p38 MAPK, and anti-JNK antibodies (phospho-specific and total, 1:1000) (Cell Signaling Technology, Beverly, Mass., and Santa Cruz Biotechnology, Santa Cruz, Calif.); and B1R antibody (A-16) (1:300) (Santa Cruz Biotechnology). β-actin was used as a loading control. After overnight incubation, the membranes were washed and incubated with the secondary antibody anti-rabbit IgG (1:2000) (Cell Signaling Technology). Protein abundance was quantified by scanning developed immunoblots containing known amounts of total homogenate protein. Signals were detected by enhanced chemiluminescence. Maximum light emission at a wavelength of 428 nm was detected by short exposure to blue-light sensitive autoradiography film, and optical density of scanned blots was measured using ImageQuant software.

Myocyte size

Myocyte size was measured from myocardial sections stained with hematoxylin–eosin. Myocyte cross-sectional images were obtained at a magnification of × 40, and myocyte surface area was measured in 5 fields per section using GSA Image Analyser (GSA Bansemer and Scheel GbR). Average myocyte area was expressed in micrometres (µm²).

Myocardial capillary eNOS expression

Myocardial samples from WT and KO mice were formalin-fixed and paraffin-embedded. After deparaffinization and dehydration, 5 μ mol/L thick sections were heated in a steam water bath for 40 min with an epitope retrieval solution (Dako). Sections were incubated with primary antibody, eNOS/NOS type III (BD Biosciences) overnight at 4 °C. Sections were then visualized with goat anti-mouse IgG antibodies conjugated with TRITC (red) and FITC (green) (Sigma-Aldrich). Slides were mounted with fluorescent mounting solution containing the nuclear stain 4',6-diamidino-2-phenylindole (DAPI) (Vector, Burlingame, Calif.) and examined under a Nikon E400 microscope equipped with excitation and barrier filter for eNOS-stained capillaries. In addition, to enable localization of eNOS, paraffin-embedded sections of LV myocardium were immunostained with monoclonal anti-human eNOS antibody using a peroxidase/DAB method. Anti-eNOS, a murine monoclonal antibody to human eNOS (1:100 dilution) (Transduction Laboratories, Lexington, Ky.), was used as the primary antibody, and anti-mouse immunoglobulin was used as the secondary antibody. eNOS staining was identified in tissue sections using 400 $\times$ magnification.

Statistical analysis

All data were expressed as means $\pm$ SE. ANOVA and paired or unpaired Student *t* tests were used to compare change in echocardiographic data from baseline to 6 weeks within and between treatment groups and differences in myocyte size and protein expression between groups. The Student–Newman–Keuls post hoc analysis was used to identify which groups differed in their means (SigmaStat 2.03). A value of $p < 0.05$ was considered statistically significant.

Results

Controls

Echocardiographic assessment—At baseline, B2RKO mice ($n = 32$) had higher indexed LV septal and posterior wall thickness, higher LV mass, higher LVESD, and lower ejection fraction compared with age-matched WT mice ($n = 24$) (Table 1), suggesting early onset of maladaptive LV hypertrophy in KO mice. During 6 weeks follow-up, there was no significant change in LV mass or ejection fraction in the WT mice. At 6 weeks follow-up in the KO mice, LV mass remained higher and LV ejection fraction remained lower compared with WT (Figs. 1*a*, 1*b*). The heart weight to body weight ratio was also higher in knockout (5.1 ± 0.1 mg/g) compared with wild-type mice (4.6 ± 0.1 mg/g, $p < 0.05$).

Hemodynamic assessment—Systolic BP was not different between the WT (95 ± 7 mm Hg) and KO (98 ± 9 mm Hg) mice at 6 weeks follow-up (Fig. 1*c*). Diastolic and mean BPs were also not different (data not shown).

Myocardial protein expression—Cardiac hypertrophy and dysfunction in KO mice were associated with lower expression of eNOS, phospho-eNOS, phospho-Akt, and phospho-ERK, and higher expression of phospho-p38 and phospho-JNK than those in WT mice (Figs. 2, 3).

Effect of L-NAME

In KO mice, treatment with NOS inhibitor, L-NAME, increased systolic BP to 117 ± 9 mm Hg ($p < 0.05$ vs. untreated KO controls) (Fig. 1*c*). This was associated with a further increase in LV mass and decrease in ejection fraction but only in the KO mice ($p < 0.05$ vs. untreated KO controls) (Fig. 1*b*). L-NAME had no effect on LV size, function, or BP in WT mice, that is, WT mice were protected from the development of hypertension despite NO

blockade. L-NAME did not alter myocardial eNOS, Akt, or MAPK expression in either WT or KO mice (Figs. 2, 3).

Effect of SIM

SIM treatment decreased LV mass and increased LV ejection fraction in the KO mice without causing any significant change in BP (Fig. 1). This was associated with increased eNOS and phospho-eNOS expression, and reduced p38 and JNK expression in the KO (Figs. 2, 3). SIM had no effect on LV size, function, BP, or eNOS and MAPK expression in WT mice. When SIM was administered concomitantly with L-NAME, SIM prevented the increase in LV mass and decrease in LV function without reversing L-NAME-induced systemic hypertension. Therefore, SIM treatment prevented cardiac hypertrophy secondary to systemic hypertension in the KO mice.

Myocyte size and myocardial capillary eNOS, CD31 staining

Myocyte size was significantly greater in the KO mice than in the WT ($0.49 \pm 0.06 \mu\text{mol/L}^2$ vs. $0.33 \pm 0.01 \mu\text{mol/L}^2$, $p = 0.002$). After treatment with SIM, myocyte hypertrophy decreased in the KO mice to $0.32 \pm 0.01 \mu\text{mol/L}^2$ ($p = 0.002$ vs. KO controls). SIM had no effect on myocyte size in the WT mice (Fig. 4). Myocardial eNOS capillary staining was significantly reduced in the KO mice compared with the WT mice. eNOS density increased in the KO mice after SIM treatment (Fig. 5). Myocardial CD31 staining was significantly lower in capillaries in KO mice than in WT. Intensity of CD31 staining increased in both WT and KO mice after SIM treatment (Fig. 6).

Bradykinin 1 receptor expression

B1R expression was not different between B2RKO and WT mice. SIM did not significantly alter B1R expression in either group (Fig. 7).

Discussion

The B2R genotype has emerged as an important determinant of hypertrophic response to load in humans. This study reports several important findings: (i) B2R disruption predisposed to early onset on maladaptive cardiac hypertrophy, which is likely related to reduced myocardial eNOS and (or) increased p38 and JNK activation; (ii) B2R disruption also predisposed to the development of systemic hypertension in conditions of reduced NO bioavailability; and finally, (iii) SIM prevented the development of cardiac hypertrophy and dysfunction caused by B2R disruption by reversing the downstream effects of disruption of the B2R on myocardial eNOS and MAPK activation. These results indicate that targeted pharmacologic intervention can be used to reverse the phenotypic effects of a single gene defect.

Mechanisms of maladaptive cardiac hypertrophy with B2R disruption

Mice lacking the B2R developed primary cardiac hypertrophy at a relatively young age in the absence of any alterations in baseline BP. This is consistent with a previous study that reported that elevation in BP in B2RKO mice is usually moderate and plateaus at 6 months, whereas cardiac remodeling progresses despite no further increases in BP, thus indicating that the cardiac remodeling in this gene defect is a primary process unrelated to systematic hypertension (Emanuelli et al. 1999).

The antihypertrophic effect of the B2R has been described in previous studies and appears to be mediated by NO-dependent mechanisms although emerging evidence points to a role for MAPKs in mediating the antihypertrophic effects of B2R as well (Ishigai et al. 1997; Fujii et al. 2002). The B2R has been shown to oppose the prohypertrophic effects of angiotensin II

possibly through eNOS activation via receptor-coupled Ca^{2+} -calmodulin-dependent pathways (Ishigai et al. 1997). Bradykinin can also activate eNOS directly via Akt-mediated eNOS phosphorylation at Ser¹¹⁷⁹ (Bell and Yellon 2003; Harris et al. 2001). Besides NO activation, an interaction of the B2R with MAPK activation was first reported in kidney cells and in vascular smooth muscle cells (Adomeit et al. 1999; Mukhin et al. 2003; Takenaka et al. 2003). The MAPK family (ERK, JNK, p38) is an important family of protein kinases that regulate cell growth and survival by phosphorylating other signaling proteins, notably transcription factors and other protein kinases (Sugden 2001). While ERK promotes cell growth and survival, p38 and JNK activation decreases cell survival and can cause progressive cardiac dilatation and dysfunction (Liang and Molkentin 2003). The reduced myocardial eNOS and phospho-eNOS in B2RKO mice may contribute to cardiac dysfunction by causing endothelial dysfunction. Disruption of the B2R can also unmask the effects of other hormone systems. In particular, disruption of the B2R increases the susceptibility to angiotensin II-induced hypertension as well as to endothelin-1 (Brochu et al. 2002; Cervenka et al. 2001). Whether upregulation of these hormones in the B2RKO mice contributes to MAPK activation requires further study.

In the present study, disruption of the B2R was associated with lower expression of eNOS and Akt (antihypertrophic factors) and increased expression and phosphorylation of p38 and JNK (prohypertrophic factors). In addition, p38 and JNK activation has been associated with the transition from compensated to decompensated cardiac hypertrophy. The adverse cardiac remodeling seen in the present study appears to be related to an imbalance between the prohypertrophic and antihypertrophic factors.

Predisposition to early onset systemic hypertension

Besides the development of primary hypertrophy, mice lacking the B2R also demonstrated worsening of cardiac hypertrophy when subjected to L-NAME-induced hypertension. Interestingly, systemic hypertension in response to L-NAME was seen only in the KO, not in the WT mice. In other words, B2RKO mice were unable to compensate for reduction in eNOS activation, resulting in unmasking of hypertension. In contrast to 2 previously published studies, our study failed to show an increase in BP in WT mice treated with L-NAME. This may be related to the younger age of mice used in our study and (or) to the lower dose of L-NAME compared with that used in the study by Cervenka et al. (Cervenka et al. 2001; Madeddu et al. 1997). We did not perform a dose-response study with higher doses of L-NAME because we wanted to assess the differential effect of L-NAME between the WT and the KO mice. We used younger mice since our goal was to assess the cardiovascular phenotype before onset of overt heart failure that typically manifests in older B2RKO mice. The mechanism of hypertension appears to be related to disruption of NO signaling. In normal mice, NO is activated by both receptor-mediated (for example, B2R-coupled) and other pathways including Akt-mediated pathways. The WT mice have the ability to compensate for reduced NO by increasing B2R-mediated activation of eNOS. This compensation is expected to be impaired in the B2RKO mice.

Pharmacotherapy for reversal of cardiac phenotype

Although studies have shown the ability of statins to improve LV function and decrease LV hypertrophy after myocardial infarction or in conditions of systemic hypertension (Bauersachs et al. 2001; Hayashidani et al. 2002), this is the first study to show that a statin can decrease cardiac hypertrophy in a genetically induced cardiomyopathy. In addition, it showed the ability of SIM to provide end-organ protection that was independent of BP lowering. SIM prevented the development of cardiac hypertrophy and dysfunction in the KO mice without altering BP. The lack of a BP-lowering effect is consistent with previous animal and human studies that have shown no significant effect of SIM on systolic BP

(Bauersachs et al. 2001; Borghi et al. 2000; Tonolo et al. 2000). The mechanism of this protection may be manifold. SIM promotes postnatal neovascularization and angiogenesis through eNOS phosphorylation and NO production as well as through recruitment of circulating bone marrow-derived endothelial progenitor cells. In our study, SIM increased eNOS expression and phosphorylation, as reported in other studies (Abraham et al. 2004; Laufs et al. 1998; Laufs and Liao 1998), suggesting that the mechanism included both post-transcriptional and post-translational increase in eNOS. In particular, the increase in capillary eNOS and in endothelial cell-specific markers suggests a role for increased potential for neoangiogenesis in enhancing endothelial function and reducing cardiac hypertrophy and dysfunction. In addition, SIM decreased p38 and JNK activation, both of which cause progressive cardiac remodeling and dysfunction. This dual effect may have contributed to the beneficial effect of SIM in this population. Moreover, SIM prevented cardiac hypertrophy in response to L-NAME-induced hypertension, again without lowering BP. Liesma et al. (2007) showed that lovastatin induces the expression of B2Rs in cultured human coronary artery endothelial cells through a cholesterol-independent pleiotropic mechanism that involves RhoA kinase inhibition and cyclooxygenase (COX)-2 activation. While statins may exert benefit through a B2R-dependent pathway in endothelial cells, the benefit in B2RKO mice suggests that the predominant benefit of SIM in B2RKO mice may be primarily at the tissue or end-organ level related to correction of abnormalities in eNOS and MAPK activation downstream of B2R.

Clinical relevance

Our findings have several clinical implications. They identify the B2R genotype as a risk factor for early-onset cardiac disease and an important determinant of maladaptive cardiac hypertrophy in response to systemic load and NO deficiency. A previous study reported that the polymorphism (−58 T/C) in the promoter region of B2R that reduces receptor function was a risk factor for development of LV hypertrophy in hypertensive patients (Fu et al. 2004). Our study provides a possible mechanism, that is, reduced NO and altered MAPK signaling, to explain this effect. The B2R genotype can also influence response to therapy. Hallberg et al. (2003) reported that patients with the B2R +9/+9 genotype (impaired receptor function) demonstrate incomplete reversal of LV hypertrophy in response to standard antihypertensive therapy using angiotensin receptor blockers or beta blockers. Duka et al. (2008) reported that ACE inhibition worsens cardiac function in mice lacking the B2R, possibly related to overexpression of B1R that can have noxious proinflammatory effects. In our study, B1R expression was not different in WT compared with KO mice and was unaffected by SIM treatment. The lack of effect on the B1R suggests that SIM may be better tolerated in B2RKO mice. Our findings suggest an alternate choice of pharmacotherapy that may be both safer and more beneficial in clinical practice since it specifically targets molecular pathways disrupted downstream of the single gene defect. A drug like SIM that normalizes both NO signaling as well as the abnormalities in MAPK signaling may be more effective in subjects with B2R genotype abnormalities by specifically targeting the downstream pathways disrupted by B2R inactivation. SIM also provides end-organ protection independent of hemodynamic effects. This has implications for the tailored use of medical therapy to rescue the cardiac phenotype caused by a single gene defect.

Limitations

(i) We did not study the effect of modulation of the MAPK pathways on cardiac function and remodeling. Experiments to determine the functional effect of administration of MAPK inhibitors in B2RKO mice will help determine the relative contribution of altered MAPK signaling versus NO signaling in causing the cardiac phenotype. (ii) We did not directly measure NO production in the myocardium in response to agonists and antagonists. (iii) We did not measure apoptosis as a possible mechanism for the antihypertrophic effect of SIM.

In this context, it is important to note that the antihypertrophic effect of SIM was associated with improvement in LV function, which makes it unlikely that the antihypertrophic effect of SIM was related to increased cell death. Also, we did not measure the activity of the upstream regulator of MAPKs, apoptosis signal-regulating kinase 1 (ASK1). (iv) The influence of other regulatory hormones, like the renin-angiotensin system, in mediating cardiac hypertrophy in the B2RKO mice was not investigated. (v) Blood pressure was only measured at the end of the experiments. Therefore daily variations in blood pressure were not assessed.

In summary, the present study is consistent with recent evidence that hypertrophy is not mediated by a unified molecular program but is transduced through multiple pathways that are fairly specific to the hypertrophy-inducing stimulus (Aronow et al. 2001). A normally functioning B2R is essential for cardiovascular homeostasis. The disruption of the B2R is a risk factor for early-onset cardiovascular disease. Early initiation of pharmacotherapy tailored to the genotype can reverse the phenotypic effects of B2R disruption by reversing the abnormalities in downstream signaling pathways caused by B2R disruption.

Acknowledgments

We acknowledge Catharine S. Forster for her assistance with the hemodynamic studies. The current affiliation of the senior author, Seema Mital, M.D., is Division of Cardiology, Department of Pediatrics, Hospital for Sick Children, Toronto, Canada. This work was supported by a Scientist Development Award from the American Heart Association, Heritage Affiliate (2002–2005).

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Fig. 1.

Comparison of echocardiographic and hemodynamic findings between wild-type (WT) and B2R knockout (KO) mice after 6 weeks of treatment with simvastatin (SIM, grey bars), a NOS activator, and L-NAME (black bars), a NOS inhibitor. White bars, control (vehicle); hatched bars, SIM+ L-NAME . (a) LV mass indexed was higher in KO than in WT. In KO mice, LV mass increased after L-NAME treatment and decreased after SIM treatment. (b) LV ejection fraction (EF) was lower in KO than in WT. LV EF decreased after L-NAME and increased after SIM. (c) Systolic blood pressure (BP) was not different between KO and WT. In KO mice, BP increased after L-NAME and was not altered by SIM treatment. Significant at *, $p < 0.05$ from baseline; #, $p < 0.05$ vs. WT; †, $p < 0.05$ vs. KO control; ‡, $p < 0.05$ vs. KO SIM; §, $p < 0.05$ vs. KO L-NAME . WT, $n = 6-11$ each group; KO, $n = 6-13$ each group. NOS, nitric oxide synthase; L-NAME , nitro- L-arginine methyl ester; LV, left ventricular.

Fig. 2.

Comparison of expression of phospho-eNOS and phospho-Akt between wild-type (WT) and B2R knockout (KO) mice after 6 weeks of treatment with simvastatin (SIM, grey bars), and L-NAME (black bars). White bars, control; hatched bars, SIM+L-NAME. (a) Phospho-eNOS/eNOS expression was lower in KO than in WT. eNOS phosphorylation increased in SIM-treated KO mice. L-NAME alone had no effect. (b) Phospho-Akt/Akt expression was lower in KO than in WT. SIM decreased Akt phosphorylation in WT mice. L-NAME had no effect. Significant at #, $p < 0.01$ vs. WT; †, $p < 0.01$ vs. corresponding control (vehicle), $n = 4$ per group (except L-NAME, $n = 3$). eNOS, endothelial nitric oxide synthase; Akt, protein kinase B; L-NAME, nitro-L-arginine methyl ester; AU, arbitrary units.

Fig. 3.

Comparison of expression of phospho-p38, phospho-JNK, and phospho-EPK between wild-type (WT) and B2R knockout (KO) mice after 6 weeks of treatment with simvastatin (SIM, grey bars), and L-NAME (black bars). White bars, control; hatched bars, SIM+L-NAME. (a) Phospho-p38/p38 was higher in KO than in WT. SIM and SIM+L-NAME treatments decreased phospho-p38 in the KO mice. L-NAME alone had no effect. (b) Phospho-JNK/JNK was higher in KO than in WT. SIM decreased JNK phosphorylation in the WT and KO mice. L-NAME had no effect. (c) Phospho-ERK/ERK was higher in KO mice than in WT. SIM and L-NAME had no effect on ERK expression. Significant at #, $p < 0.01$ vs. WT; †, $p < 0.01$ vs. corresponding control (vehicle), $n = 4$ per group (except L-NAME, $n = 3$). JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; L-NAME, nitro-L-arginine methyl ester; AU, arbitrary units.

Fig. 4.

Quantitative assessment of myocyte size (left) revealed that myocyte area was greater in the KO mice than in WT mice. This decreased significantly in the KO mice after SIM treatment ($n = 6$ each group). Representative myocardial sections are shown on the right (hematoxylin–eosin). Significant at *, $p < 0.01$ vs. WT; #, $p < 0.01$ vs. KO-Con. WT, wild-type; KO, B2R knockout; Con, control (vehicle); SIM, simvastatin.

Fig. 5.

Myocardial capillary eNOS staining. (a) eNOS immunofluorescent staining reveals red staining of capillaries; blue staining (DAPI) represents myocyte nuclei. In contrast to WT, there was no detectable eNOS staining in the KO control mouse hearts. SIM treatment was associated with an increase in myocardial capillary eNOS staining (arrow) in the KO mice. (b) eNOS staining by immunoperoxidase (brown) revealed that vascular eNOS staining was lower in KO than in WT mice and increased in both groups after SIM treatment. WT, wild-type; KO, B2R knockout; SIM, simvastatin. Representative images are shown ($n = 6$ each group); original magnification $\times 400$.

Fig. 6. Endothelial cell-specific CD31 staining (green) of blood vessels (arrows) was lower in KO than in WT mice ($n = 6$ each group). SIM increased CD31 staining in both groups. WT, wild-type; KO, B2R knockout; SIM, simvastatin. Original magnification $\times 400$.

Fig. 7. Bradykinin 1 receptor (B1R) expression by Western blot was not different between WT and KO mice or between SIM-treated and untreated mice. WT, wild-type; KO, B2R knockout; SIM, simvastatin.

Table 1

Baseline echocardiographic findings in bradykinin 2 receptor wild-type and knockout mice.

	B2R wild type (n = 24)	B2R knockout (n = 32)
Age, months	2.1±0.2	2.0±0.5
Weight, g	27±2	23±3*
Heart rate, bpm	634±72	626±56
Left ventricle		
Posterior wall thickness, cm/m ²	6.5±2	8±0.2***
Septal thickness, cm/m ²	7.1±0.3	8.1±0.2**
End-systolic diameter, cm/m ²	14.9±0.7	16.5±0.4*
End-diastolic diameter, cm/m ²	32.5±0.9	33.6±0.7
Mass (indexed), mg/m ²	5.8±0.2	6.5±0.3*
Diastolic volume, mL/m ²	8.7±0.4	8.9±0.4
Mass/volume, mg/mL	0.68±0.01	0.73±0.01**
Ejection fraction, %	73±1	61±1***

Note: B2R, bradykinin 2 receptor; LV, left ventricular. Significant difference at

*
 $p < 0.05$,**
 $p < 0.01$, and***
 $p < 0.001$ from wild type.