Selective Kinin Receptor Agonists as Cardioprotective Agents in Myocardial Ischemia and Diabetes

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Received February 7, 2013; accepted April 13, 2013

ABSTRACT
Cardiac ischemia is a leading cause of death, especially in diabetic patients. The diabetic ischemic heart is resistant experimentally to established cardioprotective treatments. New pharmacological approaches to cardiac protection are warranted. The kallikrein-kinin system is involved in myocardial protection in ischemia. Respective roles of B1 (B1R) and B2 (B2R) receptors remain controversial. We tested whether pharmacological activation of kinin receptors may have therapeutic effect in cardiac ischemia-reperfusion in nondiabetic (NDiab) and diabetic (Diab) mice. We assessed effect on infarct size (IS) and signaling pathways involved in myocardial protection of potent selective pharmacological agonists of B1R or B2R given at reperfusion. In NDiab mice, a B2R agonist reduced IS significantly by 47%, similarly to ramiprilat or ischemic postconditioning, via activation of phosphoinositide 3 kinase/Akt pathway leading to inhibition of glycogen synthase kinase-3β (GSK-3β). B1R agonist had no effect on IS. In contrast, in Diab mice, the B2R agonist, ramiprilat, or ischemic postconditioning failed to reduce IS but a B1R agonist significantly reduced IS by 44% via activation of phosphoinositide 3 kinase/Akt and extracellular signal-regulated kinase 1/2, both leading to GSK-3β inhibition. Differential effect of B2R or B1R agonists in NDiab and Diab mice can be linked to inactivation of B2R signaling and induction of B1R in heart of Diab mice. Thus, a pharmacological B2R agonist is cardioprotective in acute ischemia in nondiabetic animals. B1R agonist overcomes resistance of diabetic heart to cardioprotective treatments. Pharmacological activation of B1R and B2R may become a treatment for diabetic and nondiabetic patients, respectively, in acute coronary syndromes.

Introduction
Coronary heart disease is a leading cause of death worldwide and coronary occlusion is a major complication of coronary heart disease, leading to ischemia, myocardial infarction, heart failure, arrhythmia, and death (White and Chew, 2008). Diabetes is currently one of the main causes of coronary heart disease, while early and late prognosis of myocardial infarction is dramatically worsened in diabetes, so that coronary heart disease accounts for up to 50% of premature deaths in diabetic populations (Kannel and McGee, 1979; Danaei et al., 2006; Donahoe et al., 2007). The poor prognosis of coronary heart disease in patients with diabetes is unlikely to be improved solely by improvement of glycemic control, since intensive glycemic control failed to reduce cardiovascular events and mortality rate in recent clinical trials (Patel et al., 2008; Duckworth et al., 2009). Early myocardial reperfusion remains the most effective therapy for limiting infarct size (IS) after coronary occlusion. However, restoring blood flow to the ischemic myocardium can result in myocardial injury and partly offset the beneficial effect of reperfusion (Yellon and Hausenloy, 2007). Experimental maneuvers such as ischemic pre- or postconditioning or administration after coronary occlusion of several bioactive substances or pharmacological agents can reduce myocardial ischemia-reperfusion (IR) injury in nondiabetic animals and in man (Zhao et al., 2003; Piot et al., 2008; Gerczuk and Kloner, 2012). However, diabetes is known...
to impair response of the myocardium to cardioprotective interventions. In diabetic animals the myocardium is resistant to the cardioprotective effect of ischemic or pharmacological pre- and postconditioning (Gross et al., 2007; Bouhidel et al., 2008; Miki et al., 2009; Ozvize et al., 2010; Zhu et al., 2012). In humans, the ischemia-reducing effect associated with prodomal angina was not observed in patients with diabetes, suggesting that diabetes prevents ischemic preconditioning (Ishihara et al., 2001). Novel approaches for protecting and salvaging the ischemic myocardium, especially in the setting of diabetes are needed and can lead to significant improvement in prognosis of coronary heart disease.

Kinins are potent vascular endothelium activators and trigger release of a number of endothelial mediators promoting smooth muscle relaxation, inhibition of platelet aggregation, and fibrinolysis (Furchgott and Vanhoutte, 1989; Brown et al., 2000). Kinins are released in vivo by tissue kallikrein, synthesized in several organs, including arteries and heart (Meneton et al., 2001), and are mainly inactivated in the circulation by the angiotensin I-converting enzyme (ACE)/kininase II. Kinins activate two receptor subtypes B1 (B1R) and B2 (B2R). B2R is considered as the only receptor constitutively synthetized in tissues, whereas B1R is induced in pathologic situations, especially in diabetes and ischemia (Leeb-Lundberg et al., 2005). Lack of kallikrein and kinins, or suppression of kinin and kallikrein, can have deleterious consequences in experimental cardiac and renal ischemic diseases, suggesting that kinin actions afford organ protection in these diseases (Yang et al., 1997; Grial-Charrhili et al., 2005; Kakoki et al., 2007). Previous studies in nondiabetic animals suggest that kinin effects in cardiac ischemia are mainly triggered through B2R activation, (Grial-Charrhili et al., 2005; Xi et al., 2008), whereas the effect of B1R remains controversial (Gross and Gross, 2006; Yin et al., 2007). The role of the two kinin receptors in cardiac ischemia in the setting of diabetes is unknown.

Bioavailability in kinins is probably a limiting factor in cardiac protection, even after ACE inhibition (Alhenc-Gelas et al., 2011). Recently, potent selective and long-acting kinin receptor agonists (B1R agonist: SarLys[Hyp3, Igl5, DPhe8]desArg9-bradykinin, B2R agonist: [Hyp3,Thi5]bradykinin) have been synthesized (Belanger et al., 2009; Cote et al., 2009). The B2R agonist: [Hyp(3),Thi(5),(N)Chg(7),Thi(8)]-bradykinin have been described previously (Belanger et al., 2009; Cote et al., 2009). Dose-response effects on blood pressure and heart rate (MP100; Biopac Systems, Cerom, Paris, France). The effect on blood pressure of increasing doses of B1R or B2R agonist from 0.01 to 10 nmol/kg (1 μg/kg body weight bolus injected at 5–10-minute intervals via the jugular vein) was followed (n = 9–11 mice/dose). The effect of bradykinin at the same doses was also studied for comparison. HOE140 (N-Arg-[Hyp3, Thii5, Thii7, Oic8]bradykinin) (Icatibant; Aventis Pharma, Frankfurt, Germany), a specific B2 receptor antagonist (Hock et al., 1991), was injected (500 μg/kg) in mice 5 minutes prior to B2R agonist injection. Mean arterial pressure (MAP) was calculated as [(2 x diastolic blood pressure) + systolic blood pressure]/3. Doses of agonists to be used in cardiac IR experiments were determined from these studies.

**Myocardial IR Injury Procedure.** The procedure was done as previously described (Grial-Charrhili et al., 2005; Messadi et al., 2010). The left anterior descending coronary artery was occluded 2 mm from the tip of the left atrium for 30 minutes. Reperfusion was maintained for a 2-hour period, except as otherwise specified. At the end of the experiment, the coronary artery was reoccluded and 5% Evans Blue solution was injected into the jugular vein to delineate the area at risk (AR). The heart was then removed and the left ventricle (LV) was isolated, weighed, and cut transversely into four slices from base to apex. The slices were then incubated with buffered 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 20 minutes to determine IS. The tissue sections were then fixed in buffered 10% formalin solution for 24 hours and photographedin. Viable myocardium in the AR appeared stained brick red, whereas necrotic tissue was pale white. Area at risk and IS were quantified by an observer kept unaware of treatment using the Scion Image software (Scion Image for Windows; Scion Corporation, Frederick, MD). Infarct size was expressed as percentage of AR.

**Effect of Kinin Receptor Agonists on IR Injury.** Animals received either saline or the B1R or B2R agonist given as an i.v. bolus (1 or 3 nmol/kg for B1R agonist, 0.01 or 0.1 nmol/kg for B2R agonist), administered into jugular vein 2 minutes before reperfusion followed by 2 hours of infusion (0.5 or 1.5 nmol/kg/h for B1R agonist, 0.05 or 0.05 nmol/kg/h for B2R agonist). To test specificity of the cardioprotective effect of the agonists, additional mice receiving B1R or B2R agonist were pretreated either with a B2R antagonist HOE140 (Icatibant, 500 μg/kg i.v.) or a B1R antagonist (SSR240612: [2R]-2-[(3R)-3-(1,3-benzodioxol-5-yl)-3-[(6-methoxy-2-naphthyl)sulfonyl]amino]propanoyl)amino]-3-(4-[[2R,6S]-2,6-dimethylpiperidinyl]methyl]phenyl)-N-isopropyl-N-methylpropanamide hydrochloride), 300 μg/kg kindly provided by J. Gougat, Sanofi, Montpellier, France) (Gougat et al., 2004), 5 minutes before the onset of ischemia.

To compare the effects of kinin receptor agonists to established cardioprotective treatments, additional groups of NDiab and Diab mice received the ACE inhibitor, ramiprilat (50 μg/kg) (Aventis Pharma) 2 minutes before reperfusion or were submitted to ischemic postconditioning (IPostC), performed at the end of the 30-minute occlusion period by a sequence of three successive cycles, each consisting of 1 minute of coronary occlusion followed by 1 minute of reperfusion, followed by 2-hour reperfusion according to Roubille et al. (2011).

**Quantification of Kinin Receptor mRNA.** Relative changes in mRNA level of kinin receptors were quantified in dedicated series of mice, diabetic and nondiabetic, submitted to ischemia-reperfusion injury or sham-operated, using real-time polymerase chain reaction (Bodin et al., 2009). After 10 minutes of reperfusion, the heart was excised, washed in cold isotonic saline, and AR was dissected and rapidly frozen in liquid nitrogen. Total RNA was isolated from the LV
using TRIzol (Life Technologies, St. Aubin, France). The cDNAs were synthesized, amplified and quantified using TaqMan Gene Expression Assays and Assays-on-Demand Gene Expression Probes for B1R and B2R (Applied Biosystems, Courtaboeuf, France) in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Data were normalized to 18S rRNA. Changes in the target gene relative to the mean expression in the NDiab sham-operated group were calculated by the 2^{-ΔΔCT} comparative method for each sample (Livak and Schmittgen, 2001).

Analysis by Western-Blot of Reperfusion Injury Salvage Kinases and GSK-3β. Protein level and phosphorylation status of kinases of the RISK pathway (Ovize et al., 2010) and of GSK-3β were studied early after reperfusion in dedicated series of diabetic and nondiabetic mice treated with agonist or saline as described above and in sham-operated animals. After 10 minutes of reperfusion, AR was dissected and sampled as described above. Membranes were blotted with P-Akt (Ser473), Akt (Ser473), P-extracellular signal-regulated kinase 1/2 (ERK1/2) (Thr202/Tyr204), ERK1/2 (Thr202/Tyr204). P-GSK-3β (Ser9), GSK-3β (Ser9) (all from Cell Signaling Technology, Danvers, MA). Signals were detected in ImageQuant LAS 4000 (GE Healthcare, Vélizy, France) and quantified using Multi Gauge software 2.0 (FujiFilm, Tokyo, Japan).

Statistical Analysis. Data are presented as means ± S.E.M. Effect of ischemia-reperfusion and agonist treatment on infarct size and signaling pathways was assessed by using one-way analysis-of-variance followed, when appropriate, by the Fisher protected least significant difference post hoc test. Student’s t test was used to assess effect of B1R and B2R blockers on agonist action. Effects of diabetes and ischemia on receptor mRNA level were assessed by two-way analysis-of-variance. Significance was set at P < 0.05.

Results

Dose Response Effect of Kinin Receptor Agonists on Blood Pressure and Heart Rate. The B2R agonist [Hyp(3),Thi(5),(N)Chg(7),Thi(8)]-bradykinin decreased MAP in a dose-dependent manner (Fig. 1A). Tachycardia occurred only at 1 nmol/kg and above (Fig. 1B). The effect of the B2R agonist on blood pressure was up to 2.5 times stronger and lasted 3 times longer than the effect of bradykinin at equimolar doses (not shown). It was inhibited >90% by pretreatment with the B2R antagonist HOE140 (not shown). The hypotensive effect was similar in NDiab and Diab mice (Fig. 1A). The B1R agonist SarLys[Hyp3, Ig5, DPhe8]desArg9-bradykinin had no effect on MAP and heart rate for doses up to 10 nmol/kg, in NDiab or Diab mice (not shown). Doses of 0.01 and 0.1 nmol/kg for B2R agonist that did not induce tachycardia and of 1 and 3 nmol/kg for B1R agonist were chosen for IR studies.

B2R Agonist Reduces Infarct Size in Nondiabetic Mice and B1R Agonist in Diabetic Mice. Glycemia, body weight, LV weight, and AR of mice receiving either saline or the different investigated drugs are presented in Table 1. Body weight and relative LV weight were lower in Diab than in NDiab mice (P < 0.05). Mean fasting glycemia was similar in all groups of Diab mice. AR/LV ratios did not differ among experimental groups. Infarct size of saline-treated mice was similar in NDiab and Diab mice (Figs. 2 and 3).

In NDiab mice, the B2R agonist at 0.01 and 0.1 nmol/kg markedly reduced IS by 37 and 47%, respectively, compared with saline (P < 0.05), as did ramiprilat or IPostC (32 and 52%, respectively, P < 0.05). The B2R antagonist HOE140 totally suppressed the cardioprotective effect of the B2R agonist. The IS was not significantly altered by B1R agonist treatment at 1 and 3 nmol/kg (Fig. 2).

In Diab mice, the B2R agonist had no cardioprotective effect at both doses. Similarly, ramiprilat and IPostC failed to reduce IS. However, the B1R agonist decreased IS by 44 and 43% at 1 and 3 nmol/kg, respectively (P < 0.05). This effect was abolished by pretreatment with the B1R antagonist SSR240612 (Fig. 3).

Kinin Receptors in the Heart. Cardioprotective Effect of Agonists Is Associated with Activation of Reperfusion Ischemia Salvage Kinases and Inhibition of GSK-3β. Diabetes induced a 3.5-fold increase in cardiac B1R mRNA level (P < 0.05) but had no effect on B2R mRNA. Ischemia-reperfusion had no effect on B1R or B2R mRNA (Table 2). Total immune-reactive Akt, ERK1/2, and GSK-3β levels were not changed by IR, B1R, or B2R agonist administration compared with sham-operated mice. Ischemia-reperfusion induced a 2.5-fold increase in phosphorylation of Akt and ERK1/2 in heart of NDiab and Diab mice compared with sham-operated mice (P < 0.05), whereas phosphorylation of GSK-3β was not significantly altered (Fig. 4). In NDiab mice, the B2R agonist increased phospho-Akt and phospho-GSK-3β compared with saline-treated group (P < 0.05) but had no effect on phospho-ERK1/2. No effect on phosphorylation of
effect on kinase phosphorylation was observed.

Glycemia, body weight, and cardiac parameters of nondiabetic and diabetic mice subjected to IR injury

Data are mean ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>FG (mg/dl)</th>
<th>BW (g)</th>
<th>LV (mg/g)</th>
<th>AR</th>
<th>% LV</th>
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<tr>
<td>Saline</td>
<td>9</td>
<td>113 ± 9</td>
<td>24.9 ± 0.2</td>
<td>3.3 ± 0.1</td>
<td>30.9 ± 1.9</td>
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<tr>
<td>Ramiprilat</td>
<td>10</td>
<td>116 ± 5</td>
<td>25.4 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>30.7 ± 1.9</td>
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<tr>
<td>IPostC</td>
<td>7</td>
<td>132 ± 7</td>
<td>25.4 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>29.9 ± 1.8</td>
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<td>B2R 0.01 nmol/kg</td>
<td>8</td>
<td>108 ± 8</td>
<td>24.5 ± 0.2</td>
<td>3.5 ± 0.1</td>
<td>28.3 ± 2.1</td>
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<tr>
<td>B2R 0.1 nmol/kg</td>
<td>8</td>
<td>123 ± 12</td>
<td>24.8 ± 0.2</td>
<td>3.4 ± 0.1</td>
<td>28.8 ± 1.3</td>
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<tr>
<td>B2R + HOE140</td>
<td>4</td>
<td>112 ± 6</td>
<td>24.5 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>27.6 ± 1.1</td>
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<tr>
<td>B1R 1 nmol/kg</td>
<td>5</td>
<td>114 ± 10</td>
<td>24.0 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>30.6 ± 2.5</td>
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<tr>
<td>B1R 3 nmol/kg</td>
<td>5</td>
<td>127 ± 14</td>
<td>25.9 ± 0.3</td>
<td>3.3 ± 0.2</td>
<td>30.5 ± 2.5</td>
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<tr>
<td>Diabetic</td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>13</td>
<td>408 ± 6</td>
<td>23.4 ± 0.6</td>
<td>3.1 ± 0.1</td>
<td>27.9 ± 1.1</td>
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<tr>
<td>Ramiprilat</td>
<td>7</td>
<td>404 ± 9</td>
<td>22.1 ± 0.3</td>
<td>3.3 ± 0.1</td>
<td>29.4 ± 1.6</td>
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<tr>
<td>IPostC</td>
<td>5</td>
<td>412 ± 12</td>
<td>22.4 ± 0.4</td>
<td>3.0 ± 0.1</td>
<td>28.8 ± 2.2</td>
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<tr>
<td>B2R 0.01 nmol/kg</td>
<td>8</td>
<td>400 ± 9</td>
<td>24.5 ± 0.3</td>
<td>3.2 ± 0.1</td>
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<td>B2R 0.1 nmol/kg</td>
<td>10</td>
<td>385 ± 8</td>
<td>23.8 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>30.5 ± 1.4</td>
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<tr>
<td>B1R 1 nmol/kg</td>
<td>9</td>
<td>401 ± 8</td>
<td>22.0 ± 0.6</td>
<td>3.0 ± 0.1</td>
<td>29.4 ± 1.6</td>
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<td>B1R 3 nmol/kg</td>
<td>6</td>
<td>457 ± 13</td>
<td>22.2 ± 0.5</td>
<td>3.3 ± 0.1</td>
<td>26.8 ± 0.9</td>
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<td>B1R + SSR240612</td>
<td>12</td>
<td>418 ± 6</td>
<td>21.7 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>27.8 ± 0.9</td>
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FG, fasting glycemia; BW, body weight; LV (mg/g), left ventricle weight to body weight ratio.

Akt, ERK1/2, and GSK-3β was observed in B1R-treated Diab mice. In contrast, in Diab mice, B1R agonist increased phospho-Akt, phospho-ERK1/2, and phospho-GSK-3β compared with saline (P < 0.05). In B2R-treated Diab mice, no effect on kinase phosphorylation was observed.

Discussion

The present study documents cardioprotective effects of synthetic specific kinin receptor agonists given at reperfusion in IR in mice. We demonstrate differential sensitivity to either B2R or B1R agonism of the ischemic heart of nondiabetic and diabetic animals. Pharmacological activation of B1R has a unique feature among cardioprotective agents, as it overcomes diabetes-induced resistance to established cardioprotective treatments, activates the RISK pathway, and reduces IS.

In the present study, there was no difference in IS between saline-treated nondiabetic and diabetic mice. Previous studies of IR injury in diabetic animals have brought conflicting results concerning effect of diabetes on IS. Some studies have suggested that hyperglycemia increases IS in IR (Marrella et al., 2004; Di Filippo et al., 2005), while others reported an IS-reducing effect or no effect at all (Liu et al., 1993; Mozaffari and Schaffer, 2003; Gross et al., 2007). Duration of diabetes and level of insulin may influence myocardial tolerance against ischemia (Miki et al., 2012). Our data indicate that one month of insulinopenic diabetes in mice has no influence on IS in our experimental conditions but impairs effect of cardioprotective treatments.

B2R Agonism. A number of studies have suggested a major role for kinins and B2R in myocardial protection during IR using mice or rats with genetic inactivation of the kallikrein-kinin system or pharmacological B2R blockade (Linz et al., 1996; Yang et al., 1997; Griel-Charbhilli et al., 2005; Penna et al., 2007; Xi et al., 2008). In the present study, we describe the cardioprotective effect of direct and specific activation of the B2R in mice. The IS-reducing effect of the B2R agonist was similar to the effect of IPostC or the ACE inhibitor ramiprilat. These findings further document the role of B2R signaling in cardioprotection during ischemia by using a gain-of-function approach. They are consistent with previous observation of a prominent role of kinins and B2R in the IS reducing effect of ACE inhibition, angiotensin II AT1 receptor blockade, or ischemic pre- and postconditioning in nondiabetic mice. Pharmacological or genetic inactivation of B2R suppresses this effect (Linz et al., 1996; Griel-Charbhilli et al., 2005; Messadi-Laribi et al., 2007; Xi et al., 2008). Our observations are also consistent with ex vivo studies documenting prevention of IR injury by bradykinin in isolated perfused rodent hearts (Bell and Yellon, 2003; Yang et al., 2004). However, in vivo, when administered intravenously, bradykinin is quickly inactivated by lung peptidases. The B2 agonist [Hyp(3),Thi(5),(N)Chg(7),Thi(8)]-bradykinin is resistant to this inactivation. Kallikrein gene therapy has been attempted in IR but the procedure is not adapted to treatment of established ischemia (Yoshida et al., 2000).

Most studies on cardioprotection in IR have been undertaken in nondiabetic animals. Interestingly, available reports showed that rodents with diabetes are resistant to ischemic or pharmacological pre- or postconditioning in cardiac ischemia (Gross et al., 2007; Bouhidel et al., 2008; Miki et al., 2009; Zhu et al., 2012). Consistent with these previous studies, we observed that the IS-reducing effect of IPostC or ramiprilat was lost in diabetic mice. We also show that a B2R agonist is also ineffective in these mice. Cardiac B2R mRNA level was not decreased in the diabetic heart. However, B2R activation did not trigger Akt or GSK-3β phosphorylation, contrary to B1R activation. This observation suggests that B2R signaling is impaired upstream of Akt in the diabetic heart.

B1R Agonism. In contrast with the well-established role of B2R in kinin-mediated myocardial protection in ischemia, the role of B1R in this setting remained uncertain. Detrimental implication of B1R during IR has been suggested in isolated perfused mouse hearts by using pharmacological blockade of B1R and B1R gene knockout mice (Lagneux et al., 2002; Xi et al., 2008), whereas a cardioprotective effect of B1R activation in the rat, mediated partially by a reduction in sympathetic outflow, has been reported (Chahine et al., 1993). Another study showed that Arg9-BK, the natural agonist of B1R, had no cardioprotective effect in cardiac ischemia in nondiabetic rats (Yin et al., 2007). We previously observed that pharmacological B1R blockade had no effect on IR injury in nondiabetic mice, contrary to B2R blockade (Griel-Charbhilli et al., 2005). Consistent with these reports, the present study shows that a B1R agonist has no effect on cardiac necrosis in nondiabetic mice. Thus, different experimental approaches relying on either loss or gain of function of B1R suggest that this receptor is not importantly involved in cardioprotection in acute ischemia in nondiabetic mice.

However, our findings highlight a major cardioprotective effect of a pharmacological B1R agonist in acute ischemia in diabetic mice. Diabetes has been associated with upregulation of B1R synthesis in different organs including the heart (Bodin et al., 2009; Westermann et al., 2009). Consistent with these studies, we show that B1R mRNA was increased in myocardium of diabetic mice, with no additive effect of ischemia reperfusion, at least at the early time of reperfusion studied. However, to our knowledge, the role of B1R in the diabetic and
ischemic heart has not been studied. Our observation of B1R-mediated cardioprotection in diabetic animals can be linked to the hypothesis of impaired B2R signaling in the diabetic heart discussed in the B2R Agonism section. Indeed, in nondiabetic mice genetically deficient in B2R, the B1R was shown to take over kinin-triggered cardioprotection (Griol-Charhbili et al., 2005). Moreover, the B1R is known to be resistant to agonist-induced desensitization (Leeb-Lundberg et al., 2005). Given functionality of the B1R in diabetic heart, the lack of cardioprotective effect of ACE/kininase II inhibitors in the diabetic heart may appear surprising. However, the natural agonist of B1R is Des-arg9 bradykinin released from bradykinin by the action of carboxypeptidase N. Reduced kinin-forming and -converting activities in the diabetic heart may explain this resistance to ACE inhibition (Spillmann et al., 2006). In any case, the B1R agonist is, to the best of our knowledge, the only pharmacological agent reported so far to exert strong cardioprotective action in the setting of IR in diabetic animals. It is not known however whether B1R activation can provide cardioprotection against ischemia-reperfusion injury at a more advanced stage of diabetic cardiomyopathy and myocardial inflammation, where detrimental effect of the B1R gene on cardiac function has been documented (Westermann et al., 2009).

**Signaling.** Recent data suggest that bradykinin protects the heart at reperfusion by modulating mitochondrial permeability transition pore opening through inhibition of GSK-3β (Juhaszova et al., 2004). In isolated perfused hearts bradykinin exerts cardioprotection through PI3-kinase/Akt activation leading to GSK-3β inactivation (Bell and Yellon, 2003; Yang et al., 2004). Our data suggest a critical role for this pathway in myocardial protection afforded by the agonists (Hausenloy and Yellon, 2004). Indeed cardioprotection by B1R or B2R agonist was consistently associated with Akt and GSK-3β phosphorylation, whereas lack of IS-reducing effect of the agonists (B1R agonist in nondiabetic or B2R agonist in diabetic mice) was associated with lack of GSK-3β phosphorylation. Tsang et al. (2005) have reported that the threshold for ischemic preconditioning-induced cardioprotection was increased in diabetic rats due to impairment of prosurvival signaling cascades. B1R induction in the diabetic heart may allow the threshold for RISK pathway activation...
and myocardial protection to be reached during B1R agonist stimulation.

The streptozotocin-induced diabetes model is closely related to type 1 diabetes but not to type 2. We cannot extrapolate our results to type 2 diabetes models where other mechanisms of cardioprotection resistance, like endoplasmic reticulum stress, have been described (Miki et al., 2009). Further studies are needed to explore the effect of B1R agonist in type 2 diabetes models.

In conclusion, we identified a novel cardioprotective approach in acute cardiac ischemia based on pharmacological agonism of kinin receptors. Pharmacological activation of B2R reduces IS during cardiac IR in nondiabetic mice via activation of PI3-kinase/Akt pathway. We uncovered a unique and strong cardioprotective effect of B1R agonist in diabetic mice through activation of PI3-kinase/Akt and mitogen-activated protein kinase pathway, while diabetes induces resistance to other cardioprotective agents, including B2R agonist. Thus, individualized pharmacological intervention may provide therapeutic benefit in acute coronary syndrome, using B1R or B2R agonists in patients with and without diabetes, respectively. Occurrence of unwanted side effects like hypotension or edema is however a concern, albeit hypotension does not occur in mice during B1R agonist administration (Alhenc-Gelas et al., 2011).

### Table 2

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<tr>
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<th>B1R</th>
<th>B2R</th>
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<tbody>
<tr>
<td>Nondiabetic Sham</td>
<td>1.00 ± 0.15 (7)</td>
<td>1.00 ± 0.20 (6)</td>
</tr>
<tr>
<td>Nondiabetic IR</td>
<td>1.26 ± 0.32 (8)</td>
<td>0.86 ± 0.12 (7)</td>
</tr>
<tr>
<td>Diabetic Sham</td>
<td>3.56 ± 1.10 (7)</td>
<td>1.18 ± 0.14 (6)</td>
</tr>
<tr>
<td>Diabetic IR</td>
<td>2.53 ± 1.11 (7)</td>
<td>1.20 ± 0.20 (7)</td>
</tr>
<tr>
<td>Two-Way ANOVA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes effect</td>
<td><em>P &lt; 0.05</em></td>
<td>NS</td>
</tr>
<tr>
<td>IR effect</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

ANOVA, analysis-of-variance; NS, not significant.
Authorship Contributions

Participated in research design: Potier, Richer, Marre, Alhenc-Gelas, Bouby.

Conducted experiments: Potier, Waeckel, Vincent, Chollet.

Contributed new reagents or analytic tools: Gobeil.

Performed data analysis: Potier, Waeckel, Bruneval, Richer, Roussel, Bouby.

Wrote or contributed to the writing of the manuscript: Potier, Gobeil, Marre, Richer, Roussel, Alhenc-Gelas, Bouby.

References


Diabetes 54:203–810.


