Tissue Kallikrein Is Involved in the Cardioprotective Effect of AT1-Receptor Blockade in Acute Myocardial Ischemia

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ABSTRACT

Angiotensin-converting enzyme inhibitors limit infarct size in animal models of myocardial ischemia-reperfusion injury. This effect has been shown to be due to inhibition of bradykinin degradation rather than inhibition of angiotensin II formation. The purpose of this study was to determine whether angiotensin AT1 receptor blockade by losartan or its active metabolite EXP3174 protects against myocardial ischemia-reperfusion injury in mice and whether this protection is mediated by the kallikrein kinin system. We subjected anesthetized mice to 30 min of coronary artery occlusion followed by 3 h of reperfusion and evaluated infarct size immediately after reperfusion. Losartan (Los) or EXP3174 [2-n-buty1-4-chloro-1-{[2"-(1H-tetrazol-5-yl)biphenyl-4-y1]imidazo[5,1-b]pyridine-6-carboxylic acid} were administered 5 min before starting reperfusion at dosages determined by preliminary studies of blood pressure effect and inhibition of angiotensin pressor response. Compared with saline, both drugs significantly reduced myocardial infarct size by roughly 40% (P < 0.001). Pretreatment of mice with the selective AT2 receptor antagonist PD123,319 [S-(+)-1-{[4-(dimethylamino)-3-methylphenyl](methyl)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid] did not affect infarct size in the absence of losartan but abolished the reduction in infarct size provided by losartan. In tissue kallikrein gene-deficient mice (TK−/−), losartan no longer reduced infarct size. Pretreatment of wild-type mice with the B2 receptor antagonist icatibant reproduced the effect of TK deficiency. We conclude that AT1 receptor blockade provides cardioprotection against myocardial ischemia-reperfusion injury through stimulation of AT2 receptors. Kallikrein and B2 receptor antagonists are major determinants of this cardioprotective effect of losartan. Our results support the hypothesis of a coupling between AT2 receptors and kallikrein during AT1 receptor blockade, which plays a major role in cardioprotection.

Myocardial ischemia followed by reperfusion of the ischemic tissue results in myocardial damage (Hearse and Bolli, 1992). In patients with acute myocardial ischemia, ischemia-

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ABBREVIATIONS: IR, ischemia-reperfusion injury; RAS, renin angiotensin system; ACE, angiotensin-converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; AT1R, AT1 receptor; AT2R, AT2 receptor; ARB, AT1 receptor blocker; TK, tissue kallikrein; TK−/−, tissue kallikrein-deficient mice; TK+/−, wild-type littermates; AUC, area under curve; TTC, 2,3,5-triphenyltetrazolium chloride; IS, infarct size; AR, area at risk; LV, left ventricle; ANOVA, analysis of variance; MAP, mean arterial pressure; B1R, B1 receptor; B2R, B2 receptor; EXP3174, 2-n-buty1-4-chloro-1-{[2"-(1H-tetrazol-5-yl)biphenyl-4-y1]imidazo[5,1-b]pyridine-6-carboxylic acid; Ang II, angiotensin II; Los, losartan; ECG, electrocardiogram; PCR, polymerase chain reaction; HOE140, icatibant; PD123,319, S-(+)-1-{[4-(dimethylamino)-3-methylphenyl](methyl)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid.
able to limit infarct size (Ertl et al., 1982; Dickstein and Kjekshus, 2002). However, several lines of evidence indicate that the protective effects of ACEIs in ischemia-reperfusion injury are the result of the protection of endogenous kinins from degradation rather than inhibition of Ang II formation (Martorana et al., 1990; Liu et al., 1996; Griol-Charhhbili et al., 2005). With regard to AT1 receptor (AT1R) blockers (ARBs), their cardioprotective effect is not universally recognized in the setting of IR. Liu et al. (1996) failed to demonstrate infarct size reduction by losartan after IR in rats, and Harada et al. (1998) could not provide evidence of any significant infarct size reduction in mice deficient in AT1R receptor subtype. In contrast, it has been reported that losartan reduced infarct size after IR in rats (Lee et al., 1997; Ozer et al., 2002) or in pigs (Schwarz et al., 1997).

The aim of this study was to investigate the cardioprotective effectiveness of losartan and its active metabolite EXP3174 in an in vivo model of IR in mice, focusing on infarct size reduction. In addition, we attempted to determine whether the kallikrein kinin system contributes to the effect of AT1R blockade by losartan in IR by comparing the effect of losartan in tissue kallikrein (TK) gene-deficient mice (TK−/−) and in their wild-type littermates (TK+/+). Tissue kallikrein is a serine protease synthesized in many organs, including the heart and arteries (Nolly et al., 1994; Bergaya et al., 2001; Meneton et al., 2001). TK plays an important role through kinin release in endothelial function in arteries and participates in arterial adaptation to blood flow (Bergaya et al., 2001; Azizi et al., 2005). We recently showed that TK plays a critical role in the cardioprotective effect of ACEIs in acute myocardial ischemia (Griol-Charhhbili et al., 2005). By taking advantage of genetically engineered mice deficient in TK and kinins, we ascertained the role of TK in the cardioprotection elicited by AT1R blockade in IR.

Materials and Methods

Animals

The TK gene-deficient mice were generated in our laboratory as described previously (Meneton et al., 2001). The mouse mutants have been backcrossed on a C57BL/6 genetic background (Charles River Laboratories, L’Arbresle, France) for over 10 generations before heterozygous crossing to obtain the mutated TK−/− mice and their wild-type littermates used in the experiments (Trabold et al., 2002).

All animals had unrestricted access to standard chow (A03; Scientific Animal Food and Engineering, Augy, France) and drinking water, and they were housed at constant room temperature (24 ± 1°C) with a 12-h light/12-h dark cycle. All experimental procedures were performed in accordance with the Institute of Laboratory Animal Resources (1996).

Measurement of Systemic Blood Pressure and Vascular Reactivity

We initially conducted a vascular reactivity study to determine the dose of losartan to use, taking into account its hypotensive effect and its potency to block Ang II pressor responses. Anesthetized (60 mg/kg sodium pentobarbital) mice (body weight, 25–35 g) were placed on a thermally controlled heating pad (37 ± 1°C). After tracheotomy, a catheter was inserted into the left carotid artery for blood pressure recordings. After a 10-min stabilization period, blood pressure and heart rate were continuously recorded (MP100, Biopac systems; Cerom, Paris, France). The blood pressure response to norepinephrine (1 µg/kg) was first determined as a control. The maximal blood pressure changes triggered by increasing doses of angiotensin I (Ang I, 0.3–30 µg/kg) at 1 µl/g body weight bolus injected at 5-min intervals via a catheter inserted into the jugular vein then were measured. Vascular reactivity to Ang I was assessed in TK−/− or TK+/+ mice to examine whether it was influenced by the genotype. Wild-type mice were treated with losartan at different dosages (1, 5, 8, or 10 mg/kg), with EXP3174 (0.4 mg/kg), with ramiprilat (50 µg/kg), or with saline (1 µl/g, control). The maximal hypotensive effects of the different drugs were determined, and then vascular reactivity to increasing doses of Ang I of the pretreated mice was assessed as described previously in untreated animals. The maximal hypotensive effect of 8 mg/kg losartan was also determined in TK−/− mice. For analyzing pressor responses, area under curves (AUCs) versus log dose of Ang I were calculated in each mouse according to the trapezoidal rule and averaged within each experimental group.

In Vivo Mouse Model of Myocardial Infarction

Surgical Preparation. Mice were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Additional doses of pentobarbital were administered during the protocol when maintaining anesthesia was required. The animals were intubated and ventilated with 100% oxygen (200 µl per breath at a rate of 170 breaths/min), using a Harvard rodent ventilator (model 845; Harvard Apparatus, Les Ulis, France). Drugs were administered via a catheter inserted into the jugular vein. Body temperature was monitored with a rectal probe connected to a digital thermometer and maintained at 37°C using a heating pad. The electrocardiogram (ECG) was recorded throughout the experiments on a Gould TA240 recorder (ECC Biotech; Gould Instruments, Cleveland, OH). A left thoracotomy was performed to expose the heart, and the pericardium was removed. The left anterior descending coronary artery was occluded with an 8.0 prolene suture, 2 mm from the tip of the left atrium for 30 min. Successful coronary occlusion was verified by the development of a pale color in the distal myocardium and by S-T segment elevation and QR-S complex widening on the ECG. After 30 min of sustained ischemia, the blood flow was restored by loosening the suture. Successful reperfusion was confirmed when the bright red color of the left ventricle (LV) and a normal ECG were restored. The lungs were then inflated by increasing positive end expiratory pressure, and the chest was closed. Reperfusion was maintained for a 3-h period, and the animals were kept on the heating pad throughout the experiment (Griol-Charhhbili et al., 2005).

Experimental Protocols. All animals were subjected to the same myocardial IR injury. To assess the role of kallikrein in AT1R blockade, groups of animals of each genotype (TK−/− or TK+/+) received either saline (control) or the ARB Los (8 mg/kg) or its active metabolite, EXP3174 (EXP, 0.4 mg/kg), given as an i.v. bolus 5 min before reperfusion.

To assess the role of the B2 receptors in losartan effect, TK−/− mice were pretreated with the B2 receptor antagonist icatibant (500 µg/kg i.v.) 5 min before the onset of ischemia. Five minutes before reperfusion, one group received saline, and the other group received 8 mg/kg losartan. A control group received losartan in the absence of icatibant pretreatment.

To investigate the role of AT2R, two additional experimental groups of TK−/− mice were subjected to IR. The mice were pretreated with PD123,319 (10 mg/kg i.v. bolus 5 min before starting ischemia): one group receiving losartan and the other receiving saline 5 min before starting reperfusion. In an ancillary experiment designed to assess the blood pressure effect of PD123,319, either alone or in association with losartan, TK−/− mice were pretreated with PD123,319 (10 mg/kg i.v.), and then one group received saline and the other group received losartan (8 mg/kg) 5 min after PD123,319. Blood pressure was recorded in these mice up to 30 min, and the maximal blood pressure variations were determined. In a fourth set of experiments designed to study angiotensin II receptor mRNAs in the hearts, we used eight additional experimental groups: four groups of TK−/− mice and four groups of TK+/+ mice submitted to the Institute of Laboratory Animal Resources (1996).
IR or sham operation, each with and without saline or losartan (8 mg/kg).

Measurement of Infarct Size. After 3 h of reperfusion, the chest was reopened, and the coronary artery was recollared. Evans Blue (5%) solution (0.5 ml) was then injected as a bolus into the jugular vein to delineate the area at risk (AR), which remained unstained by the Evans Blue solution. The heart was excised, and the LV was isolated, weighed, and sliced into four transverse pieces from base to apex, the first cutter blade positioned at the site of the coronary occlusion. The slices were weighed, and color digital images of both sides of each slice were obtained with a DC120 zoom digital camera (Kodak Digital Science, Rochester, NY) connected to a microscope (Leica MZ 75; Leica Microsystems, Rueil-Malmaison, France), using the Adobe Photoshop software. The slices were then incubated at 37°C with buffered 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 20 min. Viable myocardium, which contained dehydrogenases, reacted with TTC and was stained brick red, whereas any necrotic tissue remained unstained due to the lack of active enzymes. The tissue sections were then fixed in a buffered 10% formalin solution for 24 h before being photographed again to delineate the infarct size (IS) (Griol-Charhbili et al., 2005).

The cross-sectional area, the lumen area, the AR (unstained by Evans Blue), and the IS (unstained by TTC) of the LV were outlined on each color image and quantified by a blinded observer using the Scion Image software (Scion Image for Windows; Scion Corporation, Frederick, MD). The absolute weights of AR and IS were then calculated for each slice. The sum of the absolute weight values of AR and IS of the three ischemic slices of each heart was calculated and expressed as a percentage of the total weight of the slice. The ratio of IS to AR was calculated from these absolute weight evaluations and expressed as a percentage of AR.

Quantification of Angiotensin Receptors mRNAs in the Left Ventricle. AT1R and AT2R mRNA was studied by real-time PCR in the hearts from wild-type mice in basal conditions (sham-operated) or after IR, pretreated or not with losartan. At the end of reperfusion or sham operation, the hearts were quickly excised and preserved in the RNAlater solution (Ambion Inc., Austin, TX). Left ventricles were dissected, and total RNAs were isolated using RNAeasy extraction kit (QIAGEN, Valencia, CA). First-strand cDNA synthesis was performed on 5 μg of total RNAs using random hexamers (Superscript III first strand; Invitrogen, Carlsbad, CA). cDNA synthesis reactions were stored at −20°C to be used for real-time PCR.

Real-time PCR was carried out in a 7000 Sequence Detector (ABI Prism) and the TaqMan PCR core reagent kit (Applied Biosystems, Foster City, CA) using 18S as an internal control. References for TaqMan probes for 18S, AT1R, and AT2R are available upon request. Conditions were as follows: 1 cycle of 95°C for 10 min and then 40 cycles of 94°C for 10 s, 60°C for 30 s, and 72°C for 30 s and a final cooling step at 10°C. The relative quantification of gene expression was analyzed according to the 2−ΔΔCT method (Livak and Schmittgen, 2001).

Drugs

Ramiprilat and the selective B2-receptor antagonist icatibant (HOE140, registered as JEO49) were kindly provided by Dr. J. Punter (Aventis Pharma Deutschland GmbH, Frankfurt, Germany); losartan and EXP3174 were kindly provided by Merck Inc. (Rahway, NJ). PD123,319, a specific AT2R antagonist (Bumpus et al., 1991), was purchased from Sigma-Aldrich (St. Louis, MO).

Statistical Analysis

Results are expressed as means ± S.E.M. Comparisons between genotypes and between treatments in each genotype were performed by one-way ANOVA followed by post hoc analysis using the JMP software system (JMP; SAS Institute Inc., Cary, NC). For Ras blockade evaluation, the Ang I dose-response (variations in mean arterial pressure (MAP) expressed in absolute values) curves obtained in the different experimental groups were compared by ANOVA for repeated measurements with the Greenhouse-Geisser adjustment (Ludbrook, 1994). AUCs for pressor responses versus log dose Ang I were compared by ANOVA. Values of P < 0.05 were considered to be statistically significant.

Results

Effects of Losartan on Blood Pressure and Vascular Reactivity to Pressor Agents. Mean baseline values for body weight, blood pressure, or heart rate of mice assigned to the various experimental groups are presented in Table 1. In TK+/− mice, losartan, EXP 3174, and ramiprilat, at the doses used, significantly decreased blood pressure; however, the maximal hypotensive responses to these drugs, occurring between 10 and 20 min, were less than 15% in all cases and did not significantly differ from the response observed in the control group receiving only saline (Fig. 1A). In TK−/− mice, the blood pressure-lowering effect of losartan was not altered compared with TK+/−. MAP after losartan was the same in Los-TK+/− (45.4 ± 2.1 mm Hg, n = 11) and in Los-TK−/− (45.4 ± 3.2 mm Hg, n = 8).

Pressor responses (absolute variations) to norepinephrine were similar in TK+/− (+37.3 ± 3.5 mm Hg) and TK−/− mice (+34.6 ± 4.6 mm Hg). The increases in blood pressure triggered by Ang I were similar in TK−/− and TK+/− mice. The pressor effect of Ang I was reduced in wild-type mice pretreated with losartan, EXP3174, or ramiprilat compared with untreated mice (Fig. 1, B and C). The inhibition of Ang I-pressor responses by losartan was dose-dependent, as documented by the comparison of the AUCs (Fig. 1C). Losartan (8 mg/kg) appeared to significantly block Ang I responses compared with saline and be equipotent to 50 μg/kg ramiprilat (Fig. 1B). EXP3174 (0.4 mg/kg) also suppressed the pressor effect of Ang I and was more effective than losartan or ramiprilat (Fig. 1B).

Effect of Losartan on Infarct Size. Role of the Kallikrein Kinin System. Mean values for body weight, LV weight, AR/LV ratio, or heart rate of TK+/− or TK−/− mice receiving either saline or the different investigated drugs are presented in Table 2. In all groups, heart rate remained unchanged throughout the IR experiments, and AR/LV ratios did not differ among the different experimental groups. After IR, IS in saline-treated mice averaged 38.9 ± 3.3% AR in TK+/− mice and 38.4 ± 2.3% AR in TK−/− mice, with no difference between genotypes (Fig. 2A).

Losartan and EXP3174 both exhibited cardioprotection in TK+/− mice and reduced IS/AR to a similar extent (−41%, both p < 0.001) (Fig. 2A). In contrast, in TK−/− mice, the

### TABLE 1

<table>
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<th>Group</th>
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<th>BW (g)</th>
<th>MAP (mm Hg)</th>
<th>HR (bpm)</th>
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<tr>
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<td>27.3 ± 0.5</td>
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<td>25.0 ± 0.5</td>
<td>67.3 ± 2.1</td>
<td>368 ± 20</td>
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BW, body weight; bpm, beats per minute; HR, heart rate; Los, losartan, 8 mg/kg; EXP, EXP3174, 0.4 mg/kg; Ram, ramiprilat, 50 μg/kg.
Effect of AT2R Blockade on Infarct Size. In wild-type mice, blockade of AT2R by PD123,319 had no effect on IS (Fig. 3). However, the cardioprotective effect of losartan was suppressed by pretreatment with the AT2R antagonist PD-123,319 (PD-Los-TK+/+), IS/AR = 33.6 ± 2.6% versus Los-TK+/+, IS/AR = 23.1 ± 1.6%, *p < 0.01) (Fig. 3). Infarct size of TK−/− mice treated by losartan was similar to that of wild-type mice treated with PD123,319 + losartan (Los-TK−/−, 32.5 ± 2.8% versus PD-Los-TK−/−, 33.6 ± 2.6%) (Figs. 2A and 3).

PD123,319 had no effect on arterial pressure in wild-type mice. MAP was 62.0 ± 2.1 mm Hg in mice receiving PD123,319 versus 65.6 ± 1.4 mm Hg in mice receiving saline. When injected before losartan, PD123,319 did not significantly alter the maximal blood pressure-lowering effect of losartan (saline + losartan, MAP = 50.2 ± 2.4 mm Hg versus PD123,319 + losartan, MAP = 46.0 ± 3.4 mm Hg).

Effect of Losartan on Angiotensin Receptors mRNAs in the Left Ventricle. AT1 receptor mRNAs were readily detected and quantified by real-time PCR in mice heart. The level of AT1 receptor mRNA was increased by IR injury. Losartan suppressed this induction (Fig. 4). AT2R mRNA level was at or below the detection limit of our method and could not be accurately quantified.

Discussion

Our study shows that losartan and its active metabolite EXP3174 reduce IS after acute coronary occlusion/reperfusion in mice. We show that this effect is mediated by the AT2R. Furthermore, we demonstrated for the first time that the IS-limiting effect of AT1R blockade involves TK.

When coronary artery is occluded, the cardiac oxygen supply/demand imbalance and subsequent cardiac dysfunction reduces cardiac output causing reflex vasoconstriction, which in turn increases heart work and worsens the cardiac condition. Besides an increase in sympathetic tone and the release of catecholamines, an activation of the RAS occurs, resulting in increased Ang II formation. Increased amounts of Ang II have been measured in the coronary effluent from postischemic myocardium (Sato et al., 2000). Ang II elicits coronary vasoconstriction by activation of postsynaptic AT1R as well as presynaptic AT1R inducing catecholamine release that could exacerbate ischemic injury. Cardiac AT1R gene expression increased after IR in our experimental conditions, contrary to Xu et al. (2002) who observed a decrease in AT1R mRNA, but consistent with other observations of cardiac AT1R induction in IR (Sun and Weber, 1994; Yang et al., 1997). The induction of AT1R may contribute to the increase in coronary vascular resistance and cardiac dysfunction. In this context, several studies investigated the effects of AT1R blockade in myocardial IR, but the results are not consistent across these studies. Losartan or its active metabolite EXP3174 did not reduce IS after myocardial IR in vivo in rabbits (Hartman, 1995), rats (Liu et al., 1996), or dogs (Richard et al., 1993). Moreover, Harada et al. (1998) could not prove any IS reduction in AT1a receptor-deficient mice after IR injury. In contrast, it has been reported that losartan reduced IS after IR in rats in vivo (Lee et al., 1997; Ozer et al., 2002) or in vitro (Sato et al., 2000; Flynn and Akers, 2003) or in pigs in vivo (Schwarz et al., 1997). In the present study, using an in vivo mouse model of IR, we showed that AT1R blockade by losartan largely reduced IS after IR. We used...
Bumpus et al., 1991; Bivalacqua et al., 1999) totally blocked activation. Indeed, selective AT2R blockade by PD123,319 experimental conditions, and the extent of AT2 receptor activation may be explained by differences in species, drug dosage, Charhbili et al., 2005). Discrepancy among published studies provided the same level of cardioprotection in this model (Griol- the decrease induced by ramiprilat (50 μg/kg), which pro- vided the same level of cardioprotection in this model (Griol- Charhbili et al., 2005). Discrepancy among published studies may be explained by differences in species, drug dosage, experimental conditions, and the extent of AT2 receptor activation. Indeed, selective AT2R blockade by PD123,319 (Bumpus et al., 1991; Bivalacqua et al., 1999) totally blocked the losartan-induced cardioprotection. As AT1R blockade activ- ates renin secretion by the kidney, our data suggest that increased Ang II level and subsequent stimulation of AT2R are responsible for the cardioprotection afforded by losartan. The activation of AT2R has not always been considered as resulting in cardioprotection (Lévy, 2004), but consistent with our observation, it has been proposed that AT2R activation plays a role in the cardioprotective effect of ARBs in acute myocardial ischemia (Jalowy et al., 1998). In humans, this mechanism could be even more important, because the relative abundance of AT2R compared with AT1R in the

<table>
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<th>Genotype</th>
<th>Group</th>
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<th>BW (g)</th>
<th>LVW/BW</th>
<th>AR/LV (%)</th>
<th>HR before Occlusion (bpm)</th>
<th>HR after Occlusion (bpm)</th>
<th>HR after Reperfusion (bpm)</th>
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BW, body weight; LVW/BW, left ventricular weight to body weight ratio; HR, heart rate; Los, losartan, 8 mg/kg; EXP, EXP3174, 0.4 mg/kg; Icat, icatibant, 500 μg/kg; PD, PD123,319, 10 mg/kg.

Fig. 2. Effect of losartan on infarct size after ischemia-reperfusion injury: the role of the kallikrein kinin system. A, role of tissue kallikrein. ISAR was measured in TK+/- or TK-/- mice receiving saline, losartan (8 mg/kg), or EXP3174 (0.4 mg/kg) 5 min before reperfusion. Data are mean ± S.E.M. For n, see Table 2. * p < 0.001 versus control TK+/-; §, p < 0.05 versus losartan-TK+/-; †, p < 0.001 versus EXP3174-TK+/-, B, role of B2 receptors. ISAR was measured in TK+/- mice receiving icatibant (500 μg/kg) (n = 5), losartan (8 mg/kg) (n = 4), or icatibant (500 μg/kg) + losartan (8 mg/kg) (n = 6). Data are mean ± S.E.M. * p < 0.001 versus icatibant-TK+/-; §, p < 0.05 versus losartan-TK+/-.
Because we observed that PD123,319, administered at a dose that blocks AT2 receptors and has no significant effect on arterial blood pressure in agreement with Bivalacqua et al. (1999), had no cardioprotective effect in the absence of losartan, we propose that in the presence of a functional renal AT1R, angiotensin II levels, while increased during IR, remain too low to trigger AT2-mediated cardioprotection. Another hypothesis is that any cardioprotective effect of AT2 stimulation is counterbalanced by deleterious AT1-mediated effects in the setting of AT1R induction. We were unable to quantify accurately AT2R mRNA in mouse heart by our real-time PCR technique because of a low level of gene expression. Limited information is available on AT2R gene expression in normal heart and in acute cardiac ischemia, but AT2R mRNA levels have been found by several techniques to be very low compared with AT1R or even undetectable (Yang et al., 1997; Bivalacqua et al., 1999). However, because PD123,319 had a dramatic effect on IS in losartan-treated mice, the AT2R was present in the heart of our mice, although it may have had a low turnover; this receptor clearly plays a role in cardioprotection. A peripheral hemodynamic effect of PD123,319 influencing IS seems unlikely because of the lack of effect of this compound on blood pressure.

We observed that the cardioprotective effect of AT1R blockade by losartan was abolished in TK-deficient mice, despite a similar blood pressure-lowering effect of losartan in TK\textsuperscript{+/−} and TK\textsuperscript{−/−} mice. The AT1R is normally functional in these mice (Fig. 1), and the AT2R is also present (Bergaya et al., 2004). This observation shows that the cardioprotective effect of AT2R stimulation in IR requires the presence of TK. Previous studies, using kinin B\textsubscript{2} receptor (B2R) blockers, have documented a functional coupling between AT2R and B2R during AT1R blockade mediating, at least in part, the pharmacological effect of AT1R blockers in the kidney (Siragy et al., 2000), heart (Sato et al., 2000), and arteries (Gohlke et al., 1998). The cardioprotective effects of B\textsubscript{2} receptor stimulation in cardiac ischemia have been well documented (Oldenburg et al., 2004). The mechanism of the AT2R-B2R coupling has been proposed to be related to receptor heterodimerization (Abadir et al., 2006), to stimulation of kinin production by unidentified kininogenases (Sun and Weber, 1994; Yang et al., 1997) triggered by AT2R activation (Siragy et al., 2000), or to potentiation of kinin action secondary to AT2R-mediated ACE down-regulation (Hunley et al., 2000). Our data cast some light on this question by showing that TK is required for the cardioprotective effect of AT2R stimulation in cardiac ischemia. TK can either directly activate the B2R (Hequet et al., 2000) or release kinins, which in turn activate B\textsubscript{1} and B\textsubscript{2} receptors. The cardioprotective effect of losartan was suppressed by the B2R antagonist icatibant. This observation shows that B2R rather than B1R is involved in the cardioprotective effect of losartan in acute myocardial ischemia as that for ACE inhibitors (Griol-Charbhilli et al., 2005). However, it cannot distinguish between direct or kinin-mediated B2R activation by kallikrein, because both actions are antagonized by icatibant (Hequet et al., 2000). In favor of a mechanism related to kinin release, however, is the observation of increased kinin concentration in the kidney and coronary effluent of losartan-treated rats (Sato et al., 2000; Siragy et al., 2000) or in plasma of losartan-treated human subjects (Campbell et al., 2005). The present data indicate that TK rather than other kinin-forming enzymes, including plasma kallikrein, is responsible for the AT2-mediated kinin formation in the ischemic heart. Our study further documents the importance of the tissue kallikrein-kinin pathway in cardioprotection in acute cardiac ischemia. A role for kallikrein has also been established for the cardioprotection afforded by ischemic preconditioning (Griol-Charbhilli et al., 2005) and for the cardioprotective effects of ACEIs, whereas in this latter case, AT\textsubscript{2}R activation is not involved (Griol-Charbhilli et al., 2005). Induction of B1R and B2R occurs in the heart in IR (Tschope et al., 2000; Griol-Charbhilli et al., 2005); however, the physiological mechanisms by which kinins protect the myocardium are not yet all fully understood. One plausible hypothesis would be that kallikrein and kinins exert their cardioprotective effect,


