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AT_2 receptor-mediated vasodilation in the heart: effect of myocardial infarction

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Schuijt, Martin P., Munesh Basdew, Richard van Veghel, René de Vries, Pramod R. Saxena, Regien G. Schoemaker, and A. H. Jan Danser. AT_2 receptor-mediated vasodilation in the heart: effect of myocardial infarction. *Am J Physiol Heart Circ Physiol* 281: H2590–H2596, 2001.—To investigate the functional consequences of postinfarct cardiac angiotensin (ANG) type 2 (AT_2) receptor upregulation, rats underwent coronary artery ligation or sham operation and were infused with ANG II 3–4 wk later, when scar formation is complete. ANG II increased mean arterial pressure (MAP) more modestly in infarcted animals than in sham animals. The AT_1 receptor antagonist irbesartan, but not the AT_2 receptor antagonist PD123319, decreased MAP and antagonized the ANG II-mediated systemic hemodynamic effects. Myocardial (MVC) but not renal vascular conductance (RVC) was diminished in infarcted versus sham rats. ANG II did not affect MVC and reduced RVC in all rats. MVC was unaffected by irbesartan and PD123319 in all animals. However, with PD123319, ANG II reduced MVC in sham but not infarcted animals, and, with irbesartan, ANG II increased MVC in infarcted but not sham animals. Irbesartan increased RVC and antagonized the ANG II-mediated renal effects in all animals. RVC, at baseline or with ANG II, was not affected by PD123319 in infarcted and sham animals. In conclusion, coronary but not renal AT_2 receptor stimulation results in vasodilation, and this effect is enhanced in infarcted rats.

angiotensin; heart failure; receptors; vasoconstriction/dilation

THE EFFECTS OF ANGIOTENSIN (ANG) II are mediated by specific receptors, of which two major receptor subtypes, termed AT_1 and AT_2, have been characterized to date. AT_1 receptors mediate essentially all of the known effects of ANG II, including vasoconstriction and cell proliferation (37). Much less is known about the physiological role of AT_2 receptors. On the basis of its high expression in fetal tissues, it has been speculated that AT_2 receptors are involved in cell growth and differentiation (21). Indeed, AT_2 receptor stimulation in isolated cells results in growth inhibition and apoptosis (33, 39, 45), thereby antagonizing the AT_1 receptor-mediated growth-stimulatory effects. AT_2 receptor knockout mice are more sensitive to the pressor action of ANG II than wild-type mice (12, 13), suggesting that AT_2 receptors antagonize the AT_1 receptor-mediated rise in blood pressure. However, this increase in sensitivity may also be explained on the basis of the increased vascular AT_1 receptor expression in AT_2 receptor knockout mice (35). Moreover, AT_2 receptor-mediated blood pressure decreases have not been found consistently in normal animals (4, 17, 22, 31).

Although initially it was thought that cardiac AT_2 receptors disappear after birth, it is now widely accepted that both AT_1 and AT_2 receptors are expressed in the normal adult heart, either at equal levels or with AT_1 receptors predominating (3, 5, 19, 26, 32, 34). Pathophysiological conditions such as postinfarct remodeling and heart failure are accompanied by increased AT_2 receptor expression (19, 25, 27, 40, 46) and/or decreased AT_1 receptor expression (2, 11), resulting in a relative AT_2 receptor upregulation (23, 26). Stimulation of cardiac AT_2 receptors results in inhibition of cell growth and fibrillar collagen metabolism, thereby counteracting the AT_1 receptor-mediated effects on cardiac remodeling after myocardial infarction (MI) (18, 38). The contribution of AT_2 receptors to coronary blood flow regulation is currently unknown. A recent study (7) in dogs that underwent a 15-min coronary artery occlusion followed by 4-h reperfusion observed an increase in myocardial blood flow after pretreatment the AT_1 receptor antagonist candesartan. This finding is in agreement with the concept that AT_1 and AT_2 receptors mediate vasoconstriction and vasodilation, respectively.

In the present study, we investigated ANG II-mediated effects in the rat coronary circulation in vivo using the radiolabeled microsphere method. Microspheres are trapped in end arterioles, thus allowing one to obtain information on regional hemodynamics by measuring tissue radioactivity. In view of the relative upregulation of AT_2 receptors after MI, we also studied the effects of ANG II on coronary blood flow in MI rats. These studies were performed at 3–4 wk after coronary ligation, when scar formation is complete (9), i.e., at the compensated stage of cardiac remodeling (30). Fi-

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nally, for comparison, we investigated ANG II-mediated effects on renal hemodynamics in sham and MI rats in view of the AT_2 receptor-mediated vasodilation that has been reported in glomerular arterioles (1). All studies were performed with and without the AT_1 receptor antagonist irbesartan or the AT_2 receptor antagonist PD123319. The effect of combined AT_1 receptor and AT_2 receptor blockade was not investigated, because PD123319 displaces AT_1 receptor antagonists from their plasma protein-binding sites (42–44). Nonspecific displacement of irbesartan would increase its free (effective) plasma concentration, thereby making the interpretation of such combination studies highly complex.

METHODS

The investigation conforms with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, Revised 1996). Experiments were carried out in male Wistar rats (260–280 g body wt) obtained from Harlan (Zeist, The Netherlands). Rats were housed with a 12:12-h light-dark cycle with standard rat chow and water available at libitum.

Myocardial infarction. Rats were subjected to either coronary artery ligation (n = 64) or sham operation (n = 28). Under pentobarbital sodium (60 mg/kg ip, Apharma; Arnhem, The Netherlands) anesthesia, the left anterior descending coronary artery (LADCA) was ligated (30). Briefly, after the trachea had been intubated, an incision was made in the skin, and the muscles overlying the fourth intercostal space were placed aside. The animals were put on positive pressure ventilation (frequency, 65 breaths/min; tidal volume, 3 ml), and the thoracic cavity was opened by cutting the intercostal muscles. The heart was then carefully pushed to the left, and a 6-0 silk suture was looped under the LADCA ~2 mm from its origin. After the heart was returned to its normal position, the suture was tied. The intercostal space was closed by pulling the ribs together with 2-0 silk. Subsequently, the muscles were returned to their normal position, and the skin was sutured. Sham-operated animals underwent the same surgical procedure without the actual LADCA ligation. Proper occlusion of the LADCA resulted in an extensive transmural infarction comprising a major part of the left ventricular tissue, with small variations in size (30).

Systemic and regional hemodynamics. At 3–4 wk after surgery, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). To maintain an adequate depth of anesthesia, intravenous bolus injections of pentobarbital sodium (5–10 mg/kg) were administered via the right external jugular vein every 15 min during the stabilization period. A catheter was placed in the trachea for intermittent positive pressure ventilation with a mixture of oxygen and air using a respiratory pump (Small Animal Ventilator, Harvard Apparatus; Natick, MA). The ventilatory rate was adjusted to keep arterial blood gases within the physiological range. Blood pressure and heart rate (HR) were recorded with a pressure transducer (Combitrans Disposable Pressure Transducer, Braun; Melsungen, Germany) in the left femoral artery. Radioactive microspheres were injected into the left ventricle via a catheter in the right carotid artery. Drugs were administered via the right external jugular vein. The right femoral artery was cannulated to allow the withdrawal of reference blood samples.

After a 1-h stabilization period after the completion of instrumentation, animals were given a 30-min infusion of the AT_1 receptor antagonist irbesartan (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$), the AT_2 receptor antagonist PD123319 (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$, continued throughout the experiment to ensure blockade) (31), or vehicle (saline, 0.1 ml/min). Two consecutive 10-min infusions of ANG II (100 and 300 $ng \cdot kg^{-1} \cdot min^{-1}$) were then given to each animal. At the end of each ANG II infusion, when a steady state had been reached, hemodynamic parameters were measured, and the distribution of aortic blood flow was determined by injecting 15.5 ± 0.1 (means ± SD)-μm-diameter microspheres labeled with ^{141}Ce, ^{103}Ru, or ^{95}Nb (NEN-DuPont; Boston, MA). For each measurement, ~200,000 microspheres suspended in 0.2 ml of saline and labeled with one of the isotopes, were mixed, and injected into the left ventricle over a 15-s period. After each injection, the catheter was thoroughly flushed with 0.5 ml saline. Starting 10 s before microsphere injection and lasting 70 s, an arterial reference blood sample was drawn from the right femoral artery at a constant rate of 0.5 ml/min using a withdrawal pump (model 55, Harvard Apparatus). At the end of the experiment, the animal was euthanized with an overdose of pentobarbital, and the heart and kidneys were removed. The ventricles were separated from atria and large vessels and subsequently divided into right and left ventricular tissue and interventricular septum tissue. The left ventricular tissue of MI hearts was further divided into viable tissue and scar tissue based on macroscopic appearance. Tissues were washed thoroughly to remove radioactive microspheres not trapped in arterioles, weighed, and put into vials. The radioactivity in the reference blood samples and tissues was counted for 5 min in a gamma scintillation counter (Minaxi Auto-Gamma 5000 series, Packard; Downers Grove, IL) using suitable windows, discriminating the different isotopes. Tissues other than the heart and kidney were also removed and counted, but because the findings in these tissues resembled those in the kidney, they will not be discussed here.

Drugs. Irbesartan was the kind gift of Bristol-Myers-Squibb (Princeton, NJ). PD123319 was the kind gift of Parke-Davis (Natick, MA). Irbesartan (330 μg/ml) was dissolved in 1.2 mmol/l KOH as described by Trippodo et al. (36). PD123319 (70 μg/ml) and ANG II (0.33 and 0.99 μg/ml, respectively) were dissolved in saline.

Data presentation and statistical analysis. Data were processed as described previously (28). Cardiac output (CO) and regional blood flow were calculated as follows

$$CO = \frac{\text{amount of radioactivity injected}}{\text{radioactivity of arterial blood sample}} \times \text{withdrawal rate of arterial blood sample} \quad (1)$$

and

$$\text{regional blood flow} = \frac{\text{tissue radioactivity} \times CO}{\text{amount of radioactivity injected}} \quad (2)$$

Systemic and regional vascular conductances [i.e., CO and regional blood flow corrected for mean arterial blood pressure (MAP)] were calculated to quantify the vasoconstrictor effects of ANG II with or without its receptor antagonists.

All data are presented as means ± SE. Duncan's new multiple-range test was used to test differences from baseline once a two-way ANOVA had revealed that differences existed between the consecutive infusions. Student's unpaired t-test

was used to evaluate the effects of the AT receptor antagonists once two-way repeated-measures ANOVA followed by Bonferroni's correction had revealed differences between the groups. To evaluate differences between sham and MI animals, Student's unpaired *t*-test was used. Statistical significance was accepted at $P < 0.05$ (two tailed).

RESULTS

Mortality. All 28 sham animals survived the followup period. Three sham animals were excluded from analysis due to technical failure during microsphere injection. Of the 64 MI animals, 32 animals died within 24 h after LADCA ligation and 8 animals died due to technical failure during microsphere injection. Three MI animals were excluded from analysis because the infarcted area comprised only a minor part (<20%) of the left ventricular free wall.

Systemic hemodynamic effects. Acute administration of irbesartan or PD123319 did not affect CO or HR in sham and MI animals (Fig. 1). Irbesartan, but not PD123319, reduced MAP and tended to increase (P = not significant) systemic vascular conductance in sham and MI rats. ANG II reduced systemic vascular conductance similarly in sham and MI animals. ANG II did not affect HR and reduced CO in MI animals only. The ANG II-induced rise in MAP was larger in sham (44 ± 6 mmHg) animals than in MI (26 ± 5 mmHg) animals ($P < 0.05$). In both sham and MI rats, irbesartan reduced or abolished the systemic hemodynamic effects of ANG II, whereas PD123319 did not affect these effects.

Cardiac hemodynamic effects. MI reduced left ventricular blood flow by 35% but did not significantly affect right ventricular, interventricular septal, or atrial blood flow (Fig. 2). As a consequence, myocardial blood flow (i.e., the sum of left and right ventricular, interventricular septal, and atrial blood flow) and myocardial vascular conductance were lower ($P < 0.05$) in MI animals than in sham animals (Figs. 2 and 3). Irbesartan and PD123319 did not affect myocardial vascular conductance. In sham animals, ANG II, with or without irbesartan, did not affect myocardial vascular conductance. Only in the presence of PD123319 did ANG II infusions decrease myocardial vascular conductance (i.e., caused coronary vasoconstriction) in sham animals. This effect was due to vasoconstriction in the left ventricle (Table 1). In MI animals, ANG II also did not affect myocardial vascular conductance, nor did PD123319 affect the myocardial vascular response to ANG II. In the presence of irbesartan, however, ANG II increased myocardial vascular conductance (i.e., caused vasodilation) in MI animals. These vasodilatory effects were limited to the right ventricle and the viable part of the left ventricle (Table 1).

Renal hemodynamic effects. MI did not affect renal vascular conductance (Fig. 3). Irbesartan, but not PD123319, increased renal vascular conductance. ANG II decreased renal vascular conductance, and this effect was reduced or blocked by irbesartan. PD123319 did not affect ANG II-mediated responses in the kidney.

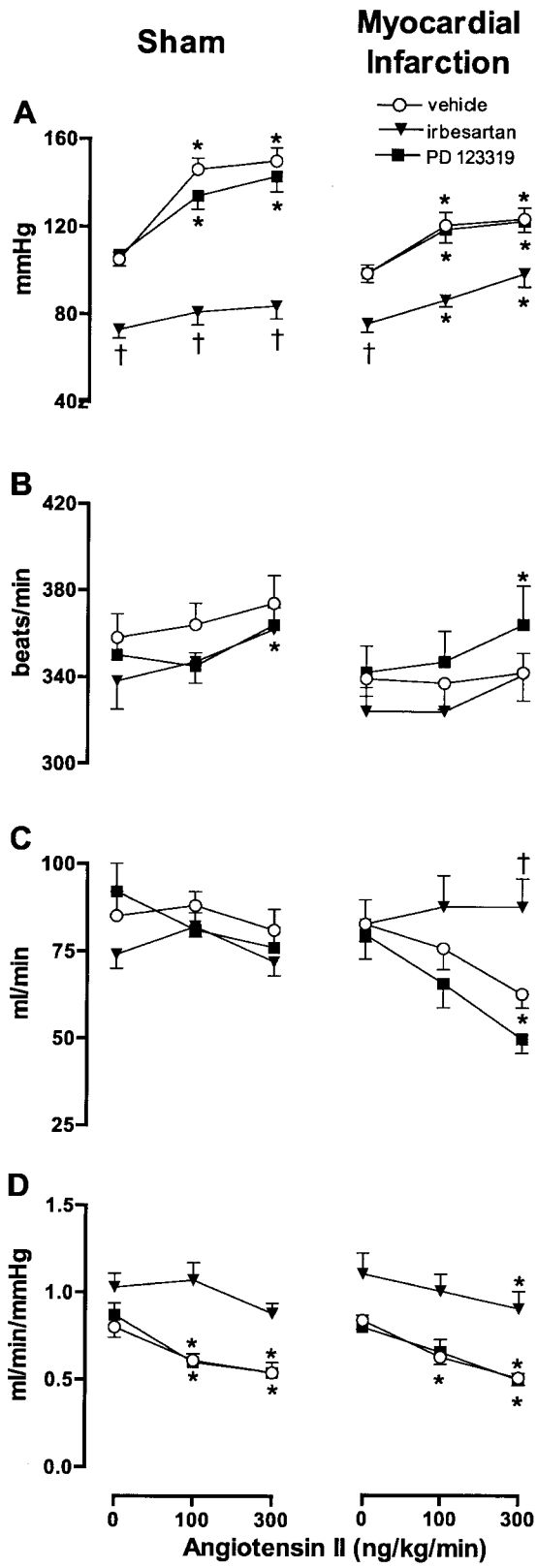

Fig. 1. Effects of 10-min intravenous infusions of angiotensin II on mean arterial blood pressure (*A*), heart rate (*B*), cardiac output (*C*), and systemic vascular conductance (*D*) in sham-operated and myocardial infarcted (MI) rats pretreated with vehicle (0.1 ml/min, $n = 9$ and $n = 7$, respectively), irbesartan (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$, $n = 8$ and $n = 7$, respectively), or PD123319 (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$, $n = 8$ and $n = 7$, respectively). Values are means ± SE. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. vehicle.

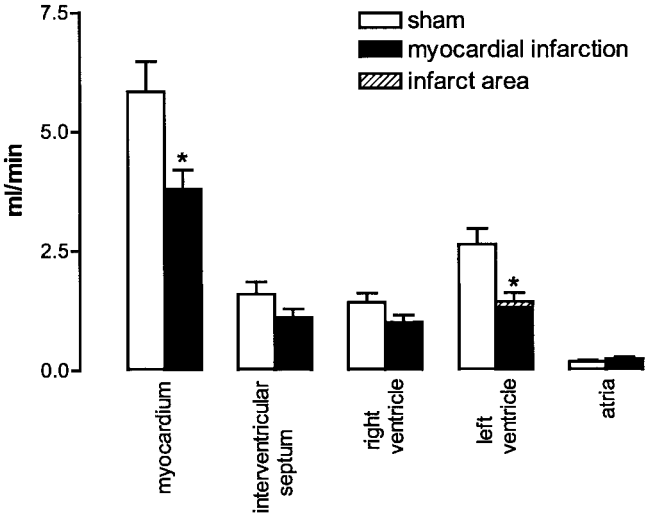

Fig. 2. Baseline regional myocardial blood flow values in sham-operated (n = 9) and MI rats (n = 7). Myocardium represents the sum of interventricular septum, left and right ventricles, and atria. Values are means ± SE. *$P < 0.05$ vs. sham.

DISCUSSION

The present study supports the concept of AT_2 receptor-mediated vasodilation in the rat coronary vascular bed. No such vasodilation was observed in the renal vascular bed or the systemic circulation in either normal or MI animals.

ANG II infusion into sham-operated rats did not affect myocardial vascular conductance despite its significant pressor effects, thereby indicating either a balance between AT_1 and AT_2 receptors in the coronary circulation in the normal heart or autoregulatory mechanisms overruling any ANG II-mediated coronary effects. In support of the first concept, myocardial vascular conductance did decrease (i.e., vasoconstriction occurred) when ANG II was infused in the presence of the AT_2 receptor antagonist PD123319. These data are in agreement with studies (3, 41) providing evidence for the presence of both AT_1 and AT_2 receptors in coronary arteries of normal hearts. Remarkably, however, coronary vasodilation did not occur during ANG II infusion in the presence of the AT_1 receptor antagonist irbesartan at a dose that fully prevented the systemic pressor effects of ANG II. This indicates that, normally, AT_2 receptor-mediated coronary vasodilation is of limited importance and mainly serves to counteract AT_1 receptor-mediated effects. This may be different under pathological conditions, when AT_2 receptors are upregulated (23, 46) relative to AT_1 receptors either because AT_2 receptor density increases and/or because AT_1 receptor density decreases (2, 11, 19, 23, 25–27, 40). Indeed, 3–4 wk after MI, we observed ANG II-mediated coronary vasodilation in the presence of irbesartan. In agreement with a reduced density of AT_1 receptors in the infarcted heart, ANG II did not cause coronary vasoconstriction in the presence of PD123319. An alternative, less likely, explanation for this lack of ANG II-mediated vasoconstriction in the presence of PD123319 is that, due to the upregulation of AT_2 receptors in the infarcted heart, the applied dose of PD123319 was insufficient to obtain full blockade of cardiac AT_2 receptors. Previous studies, however, have demonstrated that this dose of PD123319 is sufficient to result in micromolar blood plasma concentrations (i.e., concentrations that selectively block AT_2 receptors) and that higher doses will lead to concentrations that also interfere with AT_1 receptors (20). Moreover, the previously described increases in AT_2 receptor density are relatively modest, i.e., less than three- to fourfold (23, 24).

We were unable to demonstrate a vasodilator role for AT_2 receptors in the systemic circulation and kidney. The irbesartan-induced increases in systemic and renal vascular conductance in sham-operated and MI rats are suggestive for AT_1 receptor-mediated vasoconstriction by endogenous ANG II in anesthetized animals. No PD123319-induced decreases in systemic or renal vascular conductance were observed in normal or MI rats, nor did the AT_2 receptor antagonist affect the ANG II-induced systemic and renal hemodynamic responses in these rats. Thus MI does not appear to result in AT_2 receptor upregulation in organs other

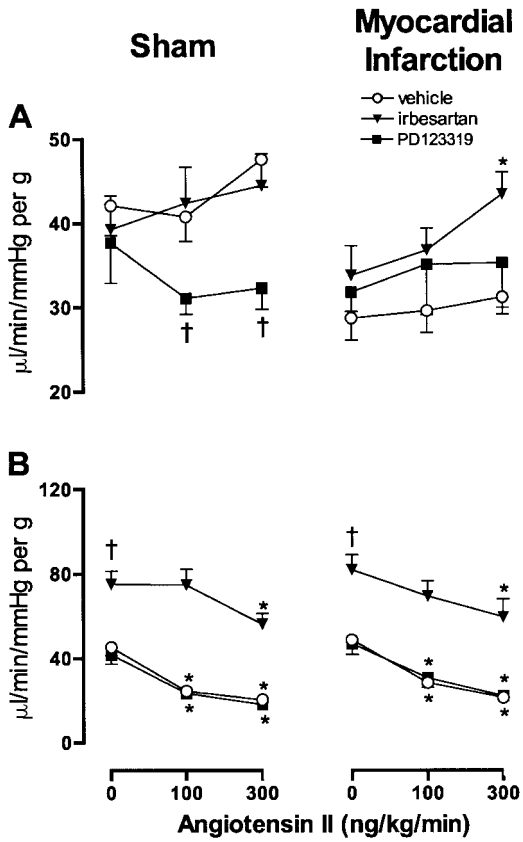

Fig. 3. Effects of 10-min intravenous infusions of angiotensin II on myocardial (A) and renal vascular conductance (B) in sham-operated and MI rats pretreated with vehicle (0.1 ml/min, n = 9 and n = 7, respectively), irbesartan (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 8 and n = 7, respectively), or PD123319 (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 8 and n = 7, respectively). Values are means ± SE. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. vehicle.

Table 1. *Regional myocardial conductances during 10-min intravenous infusions of ANG II in sham-operated and MI rats pretreated with vehicle, irbesartan, or PD123319*

	Sham-Operated Rats, ng ANG·kg^{-1}·min^{-1}			MI Rats, ng ANG·kg^{-1}·min^{-1}		
	0	100	300	0	100	300
Interventricular Septum						
Vehicle	45±5	44±3	48±3	34±4	33±4	37±3
Irbesartan	38±5	43±3	46±4	44±10	47±6	56±7
PD123319	38±6	33±4	33±3	33±2	37±5	40±6
Right ventricle						
Vehicle	47±7	49±6	52±6	34±3	39±4	42±6
Irbesartan	48±13	48±10	47±8	42±4	45±4	54±3*
PD123319	52±6	42±5*	44±6	45±5	49±10	49±9
Left ventricle						
Viable tissue						
Vehicle	47±5	43±3	55±3	32±2	33±3	34±3
Irbesartan	44±2	49±5	53±5	36±4	40±3	48±4*
PD123319	41±6	34±2†	35±2†	34±3	38±6	38±5
Infarct area						
Vehicle				11±3	11±3	9±2
Irbesartan				13±4	13±4	16±3
PD123319				11±3	14±6	10±3
Atria						
Vehicle	12±1	10±1	10±1	16±3	13±2	13±1
Irbesartan	15±3	15±3	14±2	14±3	14±2	16±3
PD123319	11±3	9±2	10±3	15±3	13±4	11±3

Values are means ± SE; n = 9 sham-operated and 7 myocardial infarcted (MI) rats pretreated with vehicle (0.1 ml/min), 8 sham-operated and 7 MI rats pretreated with irbesartan (100 μg·kg^{-1}·min^{-1}), and 8 sham-operated and 7 MI rats pretreated with PD123319 (20 μg·kg^{-1}·min^{-1}). Values of myocardial conductance are in μl·min^{-1}·mmHg^{-1} per g. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. vehicle.

than the heart, including the kidney, at least at 3–4 wk after coronary ligation, i.e., at the compensated stage of cardiac remodeling (30).

Our in vivo data showing no AT_2 receptor-mediated vasodilation in the rat kidney contrast with in vitro data demonstrating AT_2 receptor-dependent vasodilation in microperfused rabbit glomerular afferent and efferent arterioles (1). One explanation for this discrepancy might be a difference in shunting in the kidney compared with the heart as a consequence of the use of microspheres of a single size (15.5 μm in the present study). However, we (29) demonstrated earlier that for the measurement of regional blood flow, for the vast majority of tissues (including the kidney), it does not matter whether one uses microspheres of 10, 15, 25 or 35 μm in diameter (29). It is also unlikely that drawing the reference blood sample from a relatively distal vessel such as the femoral artery and/or non-appropriate admixture of microspheres with blood after their injection into the left ventricle underlie this phenomenon. First, although using femoral arterial blood as a reference may result in a modest overestimation of CO (14), this will not affect regional blood flow or mask regional AT_2 receptor-mediated effects. Second, in this study, as in many previous studies (6, 8, 10), we observed similar blood flow values in the left and right kidney both in sham-operated and infarcted animals (data not shown), thereby supporting the concept of appropriate admixture. A more likely explanation for the lack of renal AT_2 receptor-mediated vasodilation, therefore, is that AT_1 receptors predominate in renal blood vessels other than the glomerular arterioles. Indeed, our results obtained in the whole kidney do not rule out the possibility of regional hemodynamic changes with no change in total renal hemodynamic blood flow.

The MI model used in the present study is well established (30) and results in extensive transmural infarction comprising >20% of the left ventricle. As a consequence, and in full agreement with previous studies (16), baseline left ventricular blood flow was found to be reduced by 35% in MI animals compared with sham-operated animals. No flow reductions were observed in other parts of the heart. The ANG II-induced effects on myocardial vascular conductance were limited to the left ventricle in sham-operated animals and to the right ventricle and viable left ventricle in MI animals, indicating that the MI-induced changes in AT receptor density were most prominent in these areas of the heart. Such changes, which need to be confirmed in future studies, most likely relate to the vascular growth and remodeling processes that occur in the noninfarcted myocardium (15, 16, 18, 24).

ANG II reduced systemic vascular conductance similarly in sham-operated and MI rats. However, in MI rats, this increase in systemic vascular conductance was accompanied by a reduction in CO, thereby attenuating the rise in blood pressure in these rats.

In conclusion, this study is the first to demonstrate the counteracting effect of AT_2 receptors on AT_1 receptor-mediated coronary vasoconstriction. This effect appears to be enhanced after MI and parallels similar findings on AT_2 receptor-mediated growth inhibition opposing AT_1 receptor-mediated growth stimulation (33, 39, 45).

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