Basic Res Cardiol 95: 404 – 412 (2000)

ORIGINAL CONTRIBUTION

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Influence of the angiotensin II AT_1 receptor antagonist irbesartan on ischemia / reperfusion injury in the dog heart

Received: 6 October 1999
Returned for revision: 16 November 1999
Revision received: 7 February 2000
Accepted: 29 March 2000

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Abstract The aim of the present study was to investigate whether the non-peptide angiotensin II type 1 (AT_1) receptor antagonist irbesartan (SR 47436, BMS 186295, 2-*n*-butyl-3[2´-(1*H*-tetrazol-5-yl)-biphenyl-4-yl)methyl]-1,3-diaza-spiro[4,4]non-1-en-4-one) has myocardial protective effects during regional myocardial ischemia/reperfusion *in vivo*. Eighteen anesthetized open-chest dogs were instrumented for measurement of left ventricular and aortic pressure (tip manometer and pressure transducer, respectively), and coronary flow (ultrasonic flowprobes). Regional myocardial function was assessed by Doppler displacement transducers as systolic wall thickening (sWT) in the antero-apical and the postero-basal wall. The animals underwent 1 h of left anterior descending coronary artery (LAD) occlusion and subsequent reperfusion for 3 hours. Irbesartan (10 mg kg^{-1}, n = 9) or the vehicle (KOH, control, n = 9) was injected intravenously 30 min before LAD occlusion. Regional myocardial blood flow (RMBF) was measured after irbesartan injection and at 30 min LAD occlusion using colored microspheres. Infarct size was determined by triphenyltetrazolium chloride staining after 3 h of reperfusion. There was no recovery of sWT in the LAD perfused area in both groups at the end of the experiments (systolic bulging, –15.1 ± 6.1 % of baseline (irbesartan) vs. –12.3 ± 3.0 % (control), mean ± SEM). Irbesartan led to an increase in RMBF in normal myocardium (2.47 ± 0.40 vs. 1.35 ± 0.28 ml min^{-1} g^{-1}, P < 0.05), and also to an increase in collateral blood flow to the ischemic area (0.27 ± 0.04 vs. 0.17 ± 0.02 ml min^{-1} g^{-1}, P = < 0.05). Infarct size (percent of area at risk) was 24.8 ± 3.2 % in the treatment group compared with 26.9 ± 4.8 % in the control group (P = 0.72). These results indicate that a blockade of angiotensin II AT_1 receptors with irbesartan before coronary artery occlusion led to an increase in RMBF, but did not result in a significant reduction of myocardial infarct size.

Key words Ischemia – reperfusion – infarct size – angiotensin II receptor antagonists – irbesartan

Introduction

The renin-angiotensin system (RAS) not only plays an important role in the regulation of cardiovascular function, but it is also important for the pathophysiology of myocardial ischemia/reperfusion injury (6, 8, 21). The systemic RAS is activated during myocardial infarction (9, 25). Angiotensin II (AII) elicits several physiological effects that may exacerbate ischemia/reperfusion injury, i.e., vasoconstriction, positive inotropy and noradrenaline release. Angiotensin-converting enzyme (ACE) inhibitors reduce the formation of AII from angiotensin I, and protective effects of ACE inhibitors against ischemia/reperfusion injury have been demonstrated previously in different animal models (12, 13, 24). This protection was mediated primarily by an action on the kinin-prostaglandin-nitric oxide pathway (12, 13, 24, 28). A second strategy to reduce the effects of AII stimulation is the use of selective AII receptor antagonists (38). At least two AII receptor subtypes, the AT_1 and AT_2 receptors, have been identified, and cardiovascular effects of AII are mostly generated by an action on the AT_1 receptor (15, 38). Results from studies investigating the effects of AT_1 receptor blockade on myocardial infarct size are contradictory. While several studies failed to show a protective effect of AT_1 receptor antagonism by losartan in rats (24, 36), rabbit (5, 11, 22) and dog hearts (31), recent published studies demonstrated a reduction of reperfusion injury by candesartan in isolated rat hearts (43) and an infarct size reduction by candesartan and losartan in anesthetized pigs (16, 35).

The present study was designed to investigate the effects of the new AT_1 receptor antagonist irbesartan (SR 47436, BMS 186295, 2-*n*-butyl-3[2´-(1*H*-tetrazol-5-yl)-biphenyl-4-yl) methyl]-1,3-diaza-spiro[4,4]-non-1-en-4-one) on regional myocardial blood flow (RMBF, colored microspheres), regional myocardial function (Doppler displacement transducers), and myocardial infarct size (triphenyltetrazolium staining). Therefore, anesthetized open-chest dogs underwent 1 h of left anterior descending coronary artery (LAD) occlusion followed by 3 h of reperfusion. Irbesartan or the vehicle (KOH) was given intravenously 30 min before LAD occlusion.

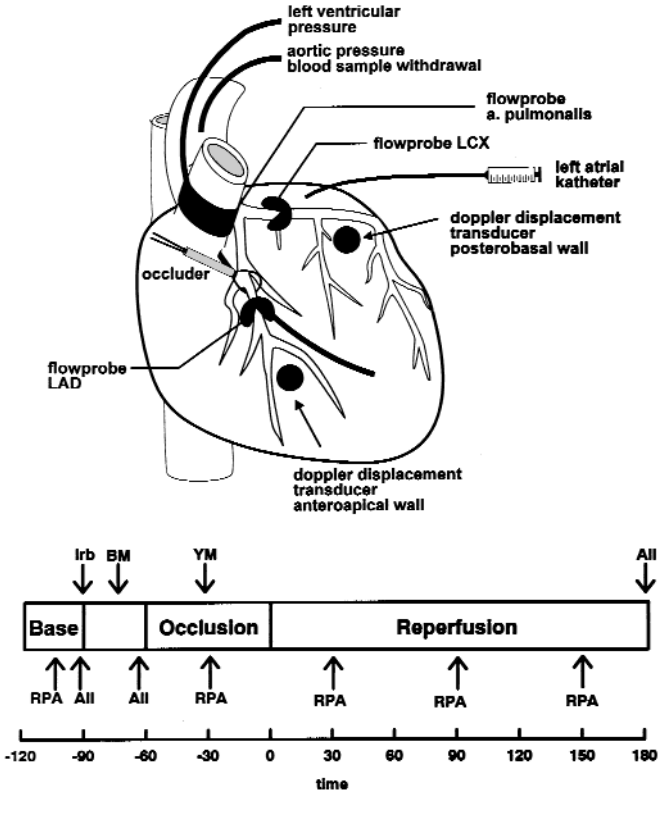

Fig. 1 Experimental preparation (top) and experimental protocol (bottom); *LAD* left anterior descending coronary artery; *LCX* left circumflex coronary artery; *Irb* intravenous injection of irbesartan; *BM* and *YM* are regional myocardial blood flow measurements using blue and yellow microspheres, respectively; *PRA* determination of plasma renin activity; *AII* intravenous injection of 100 µg/kg angiotensin II.

Materials and methods

The study conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Bioethic Committee of the District of Düsseldorf.

Animal preparation

The experimental model has been described in detail previously (29, 34). In brief, a total of 20 mongrel dogs of either sex weighing 23–39 kg were anesthetized and instrumented for measurement of aortic pressure using a 3 mm outer diameter Teflon catheter introduced from the right femoral artery and connected to a pressure transducer (Statham PD23; Gould, Cleveland, OH, U.S.A.). This catheter was also used for the withdrawal of the reference blood probe for measurement of RMBF. After left thoracotomy and pericardiotomy had been performed, left ventricular (LV) pressure (LVP) was monitored by using a catheter-tip manometer (Micro-Tip Pressure Transducer PC-350 A; Millar Instruments, Houston, TX, U.S.A.) introduced from the left atrium. A polyethylene catheter was introduced into the left atrium for taking blood samples (blood gas analysis, plasma renin activity) and for injection of colored microspheres for measurement of RMBF. The pulmonary artery, the LAD and the left circumflex coronary artery (LCX) were dissected free and instrumented with ultrasonic flowprobes connected to a flowmeter (4SB, T 208; Transonic Systems Inc., Ithaca, NY, U.S.A.) for measurement of cardiac

output (CO) and coronary artery flow, respectively. Doppler displacement transducers were fixed epicardially in both, the LAD-perfused as well as the LCX-perfused region and connected to Doppler displacement modules (Triton Technology, San Diego, CA, U.S.A.) to assess regional myocardial function. The hearts were paced via the left atrium at a rate of 150 beats per minute (USM 30; Biotronik, Düsseldorf, Germany). Fig. 1 illustrates the experimental preparation and the experimental protocol.

Experimental protocol

After surgical preparation, sufficient time was allowed to reach steady-state conditions. AII (100 µg kg^{-1}, Sigma-Aldrich Chemie, Deisenhofen, Germany) was injected intravenously after baseline measurements had been performed and the hemodynamic effects were determined. Thereafter, animals received either 10 mg kg^{-1} irbesartan or the respective volume of the vehicle solution (stoichiometric equivalent of KOH, followed by acid neutralization; controls). Fifteen minutes after irbesartan injection, RMBF flow was measured by the colored microspheres technique (20) using blue microspheres (Dye Trak Microspheres; Triton Technology, San Diego, CA, U.S.A.). Again, 100 µg kg^{-1} AII was injected to investigate whether the blockade of the AII AT_1 receptor was effective. After this recording, the animals underwent 60 min of LAD occlusion. RMBF to the ischemic region was measured at 30 min LAD occlusion using yellow microspheres. After 60 min of coronary artery occlusion, the myocardium was reperfused for three hours. At the end of the reperfusion period, the hemodynamic effects of 100 µg kg^{-1} AII were again determined. Thereafter, the heart was arrested in diastole by cardioplegic perfusion of the coronary arteries via the aortic root. After cardioplegic arrest, the LAD area was perfused with 1 % dextran in normal saline, while the rest of the myocardium was perfused via the aortic root with 0.2 % Evans Blue added to the same perfusion medium. This treatment identifies the area at risk as unstained. The heart was then excised and cut into ten to twelve 4-mm-thick transverse slices and stained in buffered 0.75 % triphenyltetrazolium chloride solution (37 °C) to identify viable and necrotic tissue within the area at risk (18). The area at risk and the infarcted area were determined by planimetry. Then the area at risk was further processed for determination of RMBF (20).

In five animals of the treatment group, the plasma renin activity (radio immuno assay, Sorin Biomedica; Germany) was determined at different time points: immediately before irbesartan injection (I), at 30 min of coronary artery occlusion (II), and after 30 (III), 90 (IV) and 150 (V) min of reperfusion.

Substances and solutions: Irbesartan was obtained from Sanofi-Recherche, France. To prepare the injection solution, 655 mg potassium hydroxide was dissolved in 100 ml normal saline. Of this potassium solution 240 µl was added slowly to 10 mg irbesartan. Normal saline was then administered to dilute the solution to the desired concentration. The solutions were prepared immediately before use.

Data analysis and statistics

LVP, its first derivative dP/dt, aortic pressure, coronary blood flow in the LAD and the LCX, cardiac output, and antero-apical and postero-basal wall thickening were continuously recorded on an ink-recorder (Recorder 2800; Gould, Cleveland, OH, U.S.A.) and stored on a videotape recorder (SL-C 30 PS; Sony, Tokyo, Japan) using pulse code modulation (VPMD 8-12; Fa. Heim, Bergisch Gladbach, Germany) for later playback and computation. The data were digitized with an AD/DA converter (Data Translation; Marlboro, MA, U.S.A.) at a sampling rate of 500 Hz and processed on a personal computer. Twenty sequential cardiac cycles were averaged to compensate for respiratory variations. Regional myocardial contractile function was assessed as systolic wall thickening (sWT; i.e., systolic wall excursion as percentage of end-diastolic wall thickness).

All data are expressed as mean ± standard error of the mean (SEM). Analysis was done by two-way analysis of variance (ANOVA) for time and group effects. If an overall significance for time was found, comparison between baseline and each point of time was done using Dunnett's test as a post-hoc test. Comparison between the groups was done by Student's *t*-test as a post-hoc test. In the case of unequal standard deviations, a Welch *t*-test was used. Differences with $P < 0.05$ were regarded as significant.

Results

Twenty dogs were studied in ischemia/reperfusion experiments. One dog of each group died because of ventricular fibrillations, so that eighteen dogs were included into data analysis (control, n = 9, irbesartan, n = 9).

Plasma renin activity and effects of intravenous AII after irbesartan administration

The plasma renin activity was 0.38 ± 0.2 ng ml^{-1} h^{-1} at baseline and increased 120 min after blockade of the AII receptor to 5.44 ± 1.8 ng ml^{-1} h^{-1} (n = 5, P < 0.05). Intravenous injection of AII before irbesartan administration increased LV peak systolic pressure (LVPSP) from 141 ± 5 to 160 ± 6 mmHg (P < 0.05) and MAP from 128 ± 5 to 147 ± 6 mmHg (P < 0.05). LVPSP (140 ± 5 vs. 142 ± 5 mmHg, P = 0.8) and MAP (123 ± 6 vs. 124 ± 4 mmHg, P = 0.9) did not change after intra-

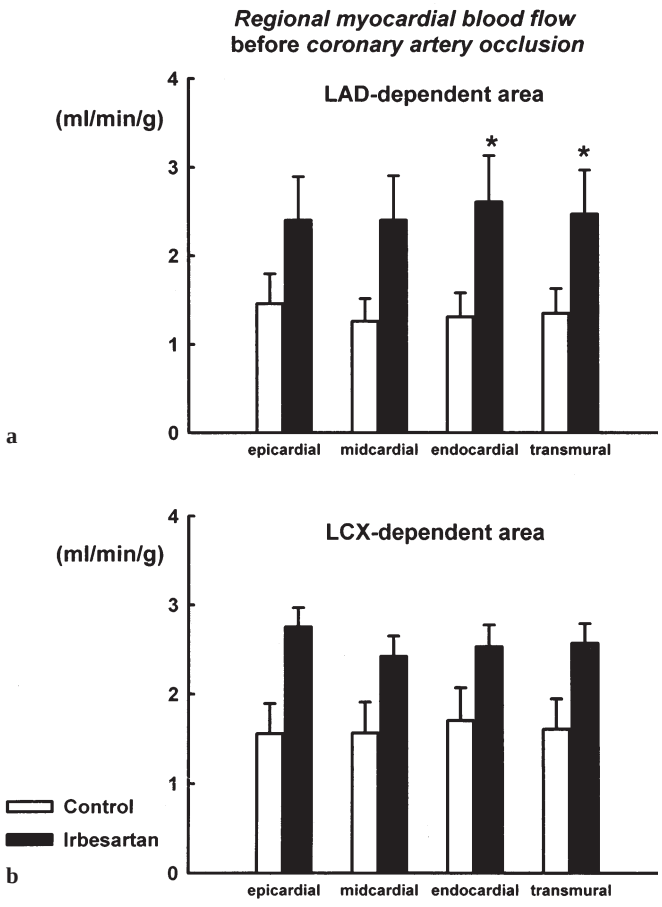

Fig. 2 Regional myocardial blood flow after irbesartan administration before coronary artery occlusion in the left anterior descending coronary artery (LAD, **a**) and left circumflex coronary artery (LCX, **b**) dependent area. Mean ± SEM. *, P < 0.05 vs. control.

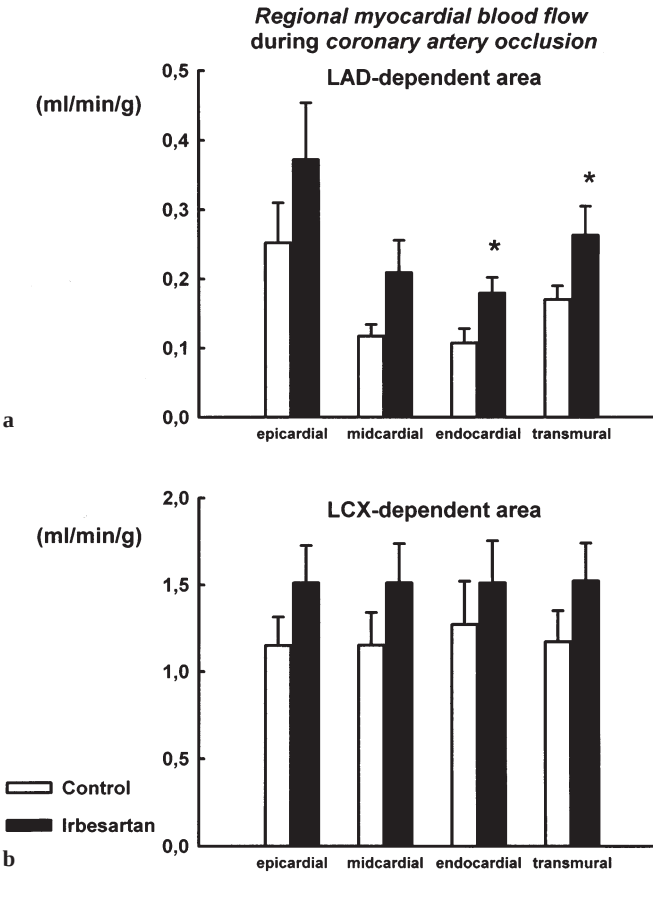

Fig. 3 Regional myocardial blood flow during coronary artery occlusion in the left anterior descending coronary artery (LAD, **a**) and the left circumflex coronary artery (LCX, **b**) dependent area. Mean ± SEM. *, P < 0.05 vs. control.

venous AII injection in the irbesartan group, suggesting an effective blockade of the AT_1 receptor. The response was still diminished at the end of the reperfusion period (LVPSP: 124 ± 7 vs. 131 ± 9 mmHg; MAP: 109 ± 8 vs. 117 ± 10 mmHg).

Regional myocardial blood flow

After irbesartan administration, RMBF tended to be higher in the LAD dependent (area at risk, Fig. 2a) as well as in the LCX dependent (Fig. 2b) myocardium (significantly different in endocardial and transmural probes of the LAD area). During coronary artery occlusion, a higher collateral blood flow towards the ischemic area was seen in irbesartan-treated animals compared with controls (Fig. 3a). In irbesartan treated animals, there was still an elevated RMBF in the LCX dependent area during occlusion of the LAD compared to controls (Fig. 3b).

Ischemia/reperfusion experiments

The hemodynamic variables are shown in Table 1 and Table 2. During baseline conditions, the groups were comparable with regard to LVPSP, dP/dt_{max}, and mean aortic pressure (MAP). After administration of irbesartan, MAP was reduced by about 6 mmHg. During LAD occlusion, LVPSP and MAP further declined by 10 ± 4 and 15 ± 4 % in irbesartan-treated animals, respectively. DP/dt_{max} was slightly reduced during coronary artery occlusion. In controls, a small reduction of all three global hemodynamic variables was observed during LAD occlusion. During reperfusion, these variables remained reduced and they did not reach baseline values in either group until the end of the experiments. However, there were no significant differences between the treatment and the control group.

Baseline values of CO and coronary flow were similar in both groups. There was only a small increase in coronary flow

Table 1 Hemodynamic parameters

	LVPSP (mm Hg)		dP/dt_{max} (mm Hg s^{-1})		MAP (mm Hg)	
	Control	Irbesartan	Control	Irbesartan	Control	Irbesartan
Baseline	139.8 ± 5.5	141.2 ± 5.3	3032 ± 179	3158 ± 148	124.2 ± 5.9	128.1 ± 5.4
Irb/vehicle	143.5 ± 4.8	141.0 ± 4.2	3092 ± 208	3567 ± 294	126.1 ± 5.2	122.7 ± 4.4
Occlusion						
30 min	135.6 ± 6.3	125.8 ± 6.0	2693 ± 207	2685 ± 224	117.9 ± 6.1	107.6 ± 5.3
60 min	134.1 ± 6.9	125.5 ± 5.0	2682 ± 183	2820 ± 191	117.2 ± 7.0	108.2 ± 4.8
Reperfusion						
3 min	124.6 ± 5.7	112.1 ± 5.6*	2654 ± 156	2670 ± 254	105.5 ± 5.3	90.1 ± 5.7*
10 min	126.0 ± 7.2	118.1 ± 7.0*	2477 ± 169	2570 ± 225	109.5 ± 7.9	99.8 ± 6.6*
15 min	119.9 ± 5.4	115.7 ± 5.1*	2356 ± 187*	2538 ± 169	101.2 ± 4.3	98.4 ± 5.1*
30 min	120.8 ± 6.9	110.3 ± 6.9*	2333 ± 181*	2426 ± 233	100.5 ± 5.8	92.3 ± 6.9*
1h	125.7 ± 6.6	119.9 ± 5.2	2443 ± 143	2565 ± 194	108.6 ± 6.4	103.3 ± 5.2*
2h	125.5 ± 7.2	125.8 ± 7.9	2396 ± 137	2554 ± 161	108.5 ± 7.7	110.2 ± 8.5
3h	123.3 ± 6.2	124.2 ± 7.3	2338 ± 144*	2468 ± 141	105.1 ± 6.9	109.0 ± 8.1

Values are means ± SEM; *Irb/vehicle* measurement after irbesartan or vehicle injection; *LVPSP* left ventricular peak systolic pressure; dP/dt_{max} maximum rate of left ventricular pressure increase; *MAP* mean aortic pressure; * , $P < 0.05$ vs. baseline.

Table 2 Cardiac output and LAD and LCX flows

	Cardiac output (l min^{-1})		LAD flow (ml min^{-1} g^{-1})		LCX flow (ml min^{-1} g^{-1})	
	Control	Irbesartan	Control	Irbesartan	Control	Irbesartan
Baseline	3.3 ± 0.3	3.3 ± 0.4	1.03 ± 0.12	0.86 ± 0.10	0.55 ± 0.06	0.57 ± 0.06
Irb/vehicle	3.5 ± 0.3	3.2 ± 0.4	1.02 ± 0.12	0.94 ± 0.12	0.54 ± 0.07	0.63 ± 0.06
Occlusion						
30 min	2.9 ± 0.3	2.5 ± 0.3	0	0	0.69 ± 0.08	0.71 ± 0.07
60 min	2.8 ± 0.2	2.7 ± 0.3	0	0	0.68 ± 0.09	0.69 ± 0.06
Reperfusion						
3 min	2.7 ± 0.3	2.3 ± 0.2	2.38 ± 0.34*	1.72 ± 0.28*	0.53 ± 0.09	0.56 ± 0.09
10 min	2.9 ± 0.2	2.7 ± 0.3	1.63 ± 0.23*	1.64 ± 0.16*	0.53 ± 0.09	0.64 ± 0.05
15 min	2.4 ± 0.3	2.7 ± 0.2	1.40 ± 0.09	1.30 ± 0.15	0.53 ± 0.06	0.59 ± 0.04
30 min	2.5 ± 0.3	2.5 ± 0.2	1.04 ± 0.16	0.97 ± 0.09	0.48 ± 0.08	0.61 ± 0.04
1 h	2.6 ± 0.3	2.5 ± 0.3	1.05 ± 0.04	0.98 ± 0.14	0.52 ± 0.04	0.62 ± 0.06
2 h	2.7 ± 0.3	2.4 ± 0.2	0.85 ± 0.06	0.78 ± 0.09	0.54 ± 0.07	0.54 ± 0.04
3 h	2.6 ± 0.3	2.3 ± 0.2	0.88 ± 0.07	0.73 ± 0.09	0.59 ± 0.08	0.58 ± 0.06

Values are means ± SEM; *Irb/vehicle* measurement after irbesartan or vehicle injection; *LAD* left anterior descending coronary artery; *LCX* left circumflex coronary artery; *, $P < 0.05$ vs. baseline.

in the LAD (from 86 ± 10 to 94 ± 12 ml min^{-1} 100 g^{-1}) as well as in the LCX (from 57 ± 6 to 63 ± 6 ml min^{-1} 100 g^{-1}) in irbesartan-treated animals. During coronary occlusion, the LAD flow fell to zero, paralleled by a small initial increase of the LCX flow. Coronary occlusion was accompanied by a reduction of CO from 3.5 ± 0.3 to 2.9 ± 0.3 l min^{-1} in controls and from 3.3 ± 0.4 to 2.5 ± 0.3 l min^{-1} in irbesartan-treated animals. CO remained reduced during the reperfusion period. During reperfusion, LAD flow showed a short time of reactive hyperemia, but the LAD flow then fell to values not different from baseline. The reactive hyperemia was attenuated in the irbesartan group.

Irbesartan injection had no influence on regional myocardial function in the area at risk as well as in the control area. sWT in the LAD-dependent myocardium (antero-apical wall) became negative with the onset of coronary occlusion in both groups (systolic bulging) (Fig. 4). sWT remained negative until the end of the experiments in both groups (control: –12.3 ± 3.0 % of baseline, irbesartan: –15.1 ± 6.1 %, P = 0.69). In the postero-basal wall, sWT during baseline conditions was similar in both groups. During LAD occlusion, sWT in the LCX-dependent area was markedly increased in control animals and it remained elevated until the end of the experiments. This increase of regional myocardial function during LAD

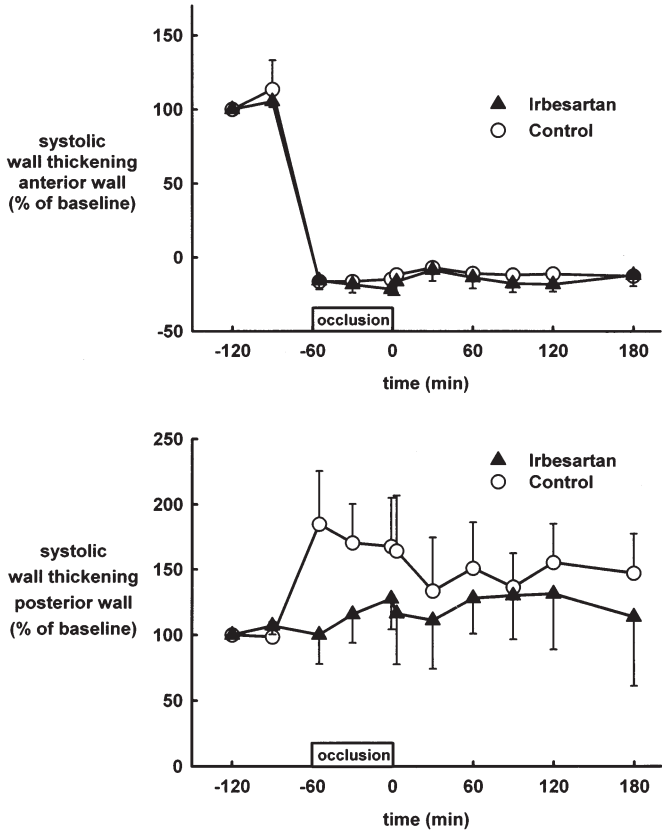

Fig. 4 Systolic wall thickening (sWT) of the antero-apical and the postero-basal wall: occlusion of the left anterior descending coronary artery changed sWT of the antero-apical wall into systolic bulging (negative sWT). sWT did not recover during reperfusion in both groups. Mean ± SEM. (Baseline values antero-apical wall: control, 77.5 ± 14.7 %; irbesartan, 37.5 ± 3.1 %; postero-basal wall: control, 28.6 ± 6.4 %; irbesartan, 20.5 ± 3.2 %.)

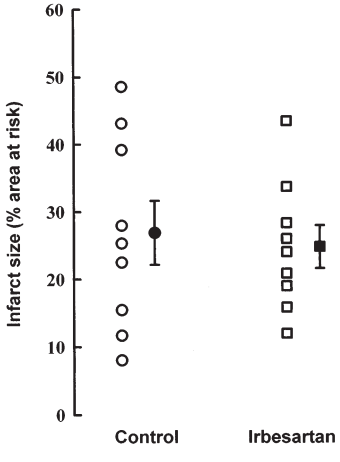

Fig. 5 Infarct size as percent of the area at risk. Single data and mean ± SEM

occlusion was attenuated in irbesartan-treated animals.

The LV weight (control: 99.7 ± 6.3 g; irbesartan: 106.2 ± 5.0 g) and the size of the area at risk (control: 33.4 ± 2.9 % of the LV weight; irbesartan: 37.0 ± 3.0 % of the LV weight) were similar in both groups. Infarct size, expressed as percentage of the area at risk, was 26.9 ± 4.8 % in the control group and 24.8 ± 3.2 % in the irbesartan-treated group (P = 0.72, Fig. 5).

Discussion

Critique of methods

Variables that are considered to be important determinants for development of myocardial injury are the duration of ischemia and the collateral blood flow towards the ischemic area. In the present study, a period of 60 min coronary artery occlusion was chosen to investigate the effects of irbesartan on infarct size reduction. Because of this long period of ischemia, the effects of infarction and stunning on recovery of contractile function cannot be distinguished in our data. The dog is known to have a collateral myocardial circulation (30), and it is necessary to assess collateral blood flow in ischemia/reperfusion experiments because high collateral transmural blood flows (> 0.30 ml min^{-1} g^{-1}) may reduce infarct size (1). In the present study, transmural collateral blood flow was lower than 0.20 ml min^{-1} g^{-1} in controls, but was increased by irbesartan treatment (Fig. 3). However, there was no animal with a transmural collateral blood flow greater than 0.30 ml min^{-1} g^{-1}.

Reperfusion of the ischemic myocardium was carried out for 3 h. It has been shown previously that this period is sufficiently long for determination of infarct size by triphenyltetrazolium chloride staining (10). However, a greater difference in infarct size after a longer time of reperfusion can not be excluded in this short-term animal experimental setup.

AII was injected in single doses prior to ischemia to assess an effective blockade of the AT_1 receptor by irbesartan. Since AII can alter the supply-demand relationship in myocardium, there is the possibility that in the present study AII injection caused ischemia and thus produced ischemic preconditioning. Pre-treatment with AII for 5 min before ischemia has been shown to activate protein kinase C and to mimic ischemic preconditioning (22). Although these effects of the AII injections on ischemia/reperfusion injury can not be excluded, they are unlikely because infarct size in controls was similar to that observed in previous studies from our laboratory without AII injection (29, 34).

Interpretation of results

The present study was designed to investigate the effect of a blockade of AII AT_1 receptors with irbesartan on regional myocardial blood flow, regional myocardial function and

myocardial infarct size. The main finding is that administration of 10 mg kg^{-1} irbesartan iv. 30 min before coronary artery occlusion elevated RMBF and collateral blood flow towards the ischemic area, but failed to improve post-ischemic functional recovery and to reduce infarct size in the dog in vivo.

It has been well documented that the RAS plays an important role in cardiovascular homeostasis, and it is activated during myocardial infarction. AII is a powerful vasoconstrictor peptide and AII concentrations are elevated during ischemia (28) and following acute myocardial infarction (9, 25, 33). Increased production of AII would increase coronary vessel resistance and increase contractility. These effects of AII could jeopardize ischemic myocardium by increasing oxygen demand while decreasing oxygen supply. Blockade of the RAS with either ACE-inhibitors or angiotensin receptor blockers is a beneficial approach for the treatment of hypertension, heart failure and left ventricular dysfunction after myocardial ischemia. Protective effects of ACE inhibitors in ischemia/reperfusion situations have been demonstrated previously (12, 13, 24). However, experimental studies have shown that complete blockade of the RAS is not achieved with ACE inhibitors (2, 27). AII receptor antagonists represent a new class of drugs that provide a site-specific blockade of the effects of AII. Unlike ACE inhibitors, AII receptor antagonists were thought to not inhibit bradykinin metabolism or enhance prostaglandin synthesis, but recent studies showed that AT_1 receptor blockers involve angiotensin II type 2 receptor activation, bradykinin and prostaglandins (16, 24). Several studies investigated the effects of AT_1 receptor blockade in myocardial ischemia/reperfusion, but the results are inconsistent. These conflicting results might be caused by the use of different AT_1 receptor blockers [losartan (11, 22, 24, 31), candesartan (16, 43), valsartan (14, 42)], different dosages of the receptor blockers, differences in pharmacodynamic properties of the AT_1 receptor blockers, differences of duration and severity of ischemia, and different animal species used in the experiments. For example, losartan or its active metabolite, EXP3174, did not reduce infarct size in rabbits (5, 11, 22), rats (24, 36), nor dogs (31), but did in pigs (35). The dosage of EXP3174 was 0.1 mg kg^{-1} iv in dogs but was 10-fold higher (1 mg kg^{-1} iv) in pigs, which might be the reason for the different results in these large-animal studies. Candesartan, another AT_1 receptor blocker, reduced CK release after global ischemia in isolated rat hearts (43), and reduced infarct size in pigs at a dosage of 1 mg kg^{-1} iv (16). In the present study, irbesartan was used at a single dose of 10 mg kg^{-1} iv, which was derived from previous studies using irbesartan (3, 4, 26, 32). This dosage effectively blocked the AII pressure response and led to a slight reduction of mean aortic pressure (Table 1). In irbesartan-treated animals, the increase in posterior systolic wall thickening during LAD occlusion was nearly abolished (Fig. 4), probably reflecting a prevention of the positive inotropic action of AII. Based on the antagonism of AII pressure effects or antihypertensive effects, 10 mg kg^{-1} irbesartan is equally effective as 1 mg kg^{-1} candesartan (41). However, we did not observe a significant reduction of infarct size after coronary artery occlusion and reperfusion in dogs. Besides species differences, different models of ischemia – coronary artery occlusion in dogs versus hypoperfusion in pigs (16) – might be responsible for these conflicting results.

Sudhir an co-workers demonstrated that AT_1 receptor blockade in the coronary circulation resulted in vasodilation of greater magnitude than ACE inhibition (37). The coronary vasodilation by losartan was partly endothelium dependent. AT_1 receptor blockade also increased coronary blood flow during ischemia in dogs (17) and during reperfusion in rats (39), indicating an attenuation of the severity of ischemia. In accordance with these previous findings, we observed an increase in RMBF as measured by colored microspheres and an elevated collateral blood flow to the ischemic area after irbesartan administration. In addition, irbesartan attenuated reactive hyperemia during early reperfusion (Table 2). Dörge et al. observed an increase of subendocardial blood flow in normal myocardium after candesartan administration, but there was no effect on RMBF to the ischemic myocardium during coronary artery occlusion (7). In contrast to our findings, Richard et al. did not observe any effect of ACE inhibitors or AT_1 receptor antagonists on myocardial blood flow and on infarct size in dogs (31), suggesting that activation of the RAS during myocardial ischemia does not contribute significantly to myocardial necrosis in this animal species.

AT_1 receptor blockers have been demonstrated to improve functional and metabolic recovery (40, 43) and to attenuate ventricular dilatation (19, 23) after myocardial infarction. Candesartan, given before coronary artery occlusion, attenuated myocardial stunning during reperfusion in dogs (7). In the present study, irbesartan failed to improve post-ischemic recovery of myocardial function, but in our model it is not possible to distinguish an impairment of regional function resulting from necrosis and resulting from myocardial stunning (Fig. 4). There was a sustained negative wall thickening fraction (systolic outward bulging) in the ischemic-reperfused area at the end of the experiment in both groups. It remains unclear how irbesartan attenuated the compensatory increase in regional myocardial function of the non-ischemic myocardium. Dörge et al. observed a small but insignificant increase of regional myocardial function in the control wall after candesartan administration during LCX occlusion, similar to the placebo group (7).

In summary, the AII AT_1 receptor antagonist irbesartan increased collateral blood flow to the ischemic myocardium, but had no cardioprotective actions with regard to post-ischemic myocardial function and infarct size in the dog heart. Because of frequent occurrence of myocardial infarction and increasing clinical use of thrombolysis, percutaneous balloon angioplasty, and coronary bypass surgery, it is of great practi-

cal interest to determine if additional therapeutic intervention during the ischemia/reperfusion period can lead to a reduction of ischemia and reperfusion injury. Up to now it remains uncertain whether AII AT_1 receptor antagonists offer protective effects in such situations in the human heart and further clinical studies with these new antihypertensive drugs are necessary.

Acknowledgment We thank Mrs. E. Hauschildt for excellent technical assistance. This work is part of the M.D. thesis of M. Gonzàlez.

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