Basic Res Cardiol 100: 48 – 56 (2005)
DOI 10.1007/s00395-004-0497-5

ORIGINAL CONTRIBUTION

Rainer Schulz
Petra Gres
Ina Konietzka
Gerd Heusch

Regional differences of myocardial infarct development and ischemic preconditioning

Received: 21 September 2004
Returned for 1. revision: 27 September 2004
1. Revision received: 4 October 2004
Accepted: 18 October 2004
Published online: 9 November 2004

Prof. Dr. J. D. Schipke, Düsseldorf, Germany, served as guest editor for the manuscript and was responsible for all editorial decisions, including the selection of reviewers. The policy applies to all manuscripts with authors from the editor's institution.

R. Schulz, MD · P. Gres
I. Konietzka · G. Heusch, MD
Institute of Pathophysiology
Center of Internal Medicine
University of Essen, Medical School
Essen, Germany

Prof. Dr. R. Schulz (✉)
Institut für Pathophysiologie
Zentrum für Innere Medizin
Universitätsklinikum Essen
Hufelandstr. 55
45122 Essen, Germany
Tel.: +49-201/7234-521
Fax: +49-201/7234-481
E-Mail: rainer_schulz@uni-essen.de

Abstract The spatial and temporal development of myocardial infarction depends on the area at risk (AAR), the severity and duration of blood flow reduction (energy supply) as well as on heart rate and regional wall function (energy demand). Both supply and demand can vary within the AAR of a given heart, potentially resulting in differences in infarct development. We therefore retrospectively analyzed infarct size (IS, %AAR, TTC) in 24 anesthetized pigs in vivo following 90 min hypoperfusion and 120 min reperfusion of the LAD coronary artery, which supplies parts of the LV septum (LVS) and anterior free wall (LVAFW). The total LAD perfusion territory averaged 49.8 ± 14.2 (SD) g (49.2 ± 8.4% of LV); 61.4 ± 8.1% of the AAR was LVAFW. IS within the LVS was 25.3 ± 15.1%, while IS within the LVAFW was 16.6 ± 10.1% ($p<0.05$). While ischemic blood flow (radiolabeled microspheres) did not differ between LVS (0.05 ± 0.02 ml/min/g) and LVAFW (0.05 ± 0.03 ml/min/g), perivascular connective tissue (56 ± 9 vs. 38±7 μm^2, $p < 0.05$) and the capillary-to-myocyte distance (1.65 ± 0.23 vs. 1.18 ± 0.23 mm, $p < 0.05$) were larger in LVS than in LVAFW. Interestingly, IS in LVS (9.3 ± 9.6%, n = 24) and LVAFW (9.2 ± 9.1%) were reduced to the same absolute extent by ischemic preconditioning with one cycle of 10 min ischemia and 15 min reperfusion, suggesting that a similar regional difference exists also in the protection afforded by ischemic preconditioning. The mechanism(s) for that remain(s) to be established. *Conclusion* In pigs, regional differences in infarct development and protection from it exist in the LAD perfusion territory, which are independent of ischemic blood flow but apparently related to pre-existing structural differences.

Key words Myocardial infarction – heterogeneity – ischemic preconditioning

Introduction

Regional myocardial blood flow depends on the driving pressure, the diastolic duration, the number and diameter of blood vessels and their vasomotor tone [4]. Both under physiological conditions and during ischemia, there exists a substantial heterogeneity of blood flow within the left ventricular (LV) myocardium (for review, see [2, 5, 23]), which has been attributed to differences in the metabolic rate [13, 14, 16, 42, 46] and/or altered protein expression [22].

The spatial and temporal development of myocardial infarction depends on the size of the area at risk as well as regional oxygen supply and demand during ischemia. Regional oxygen supply to the cardiomyocytes during ischemia depends on the duration and severity of blood flow reduction and the diffusion distance for oxygen [53];

regional oxygen demand – in turn – depends on heart rate [8] and regional wall stress [30]. Below a certain blood flow threshold [28, 40], infarct size – for a given area at risk and duration of ischemia – is inversely related to ischemic myocardial blood flow [7, 29, 31]; a further increase in regional oxygen demand by inotropic stimulation, however, increases infarct size for a given ischemic myocardial blood flow [40].

Ischemic preconditioning delays the development of myocardial infarction [26]. The relationship between infarct size and ischemic blood flow observed in non-preconditioned myocardium is lost or almost lost with ischemic preconditioning [26]. Thus, the protection achieved by ischemic preconditioning , i.e., the extent of infarct size reduction appears to be independent from regional myocardial blood flow and might also differ between different perfusion territories.

To analyze whether or not infarct size following ischemia/reperfusion within the left ventricle (LV) demonstrate regional differences, infarct size within the perfusion territory of the left anterior descending coronary artery (LAD) in pigs, which perfuses parts of the LV anterior free wall and the LV septum [18, 19] was retrospectively analyzed. Resting myocardial blood flow in the LV septum and LV anterior free wall differs in humans [12], and differences in myocardial fiber diameter within the LV septum and LV anterior free wall have also been reported [20], the latter contributing to the diffusion distance for oxygen.

Materials and methods

In previous experiments, in which triggers and mediators involved in ischemic preconditioning's protection were defined [38, 39, 41, 49, 50], it appeared that infarct size in the LV septum was always greater than in the LV anterior free wall. To obtain more detailed data on infarct development, viable and infarcted tissue pieces of the LV septum and the LV anterior free wall were cut out separately in subsequent experiments [35, 36, 43, 44], and only those experiments were taken from previously published data sets and included in the present, retrospective analysis. For the histological analysis additional experiments were performed to obtain tissue samples from both the LV septum and the LV anterior free wall.

Experimental model

Fourty-eight Göttinger minipigs were instrumented as described previously [39]. In brief, the pigs were anesthetized with enflurane and N_2O, and both common carotid arteries were cannulated, one to measure arterial pressure and one to supply blood for an extracorporeal circuit. A micromanometer was placed into the left ventricle for pressure recording. The LAD was cannulated and perfused from an extracorporeal circuit including a roller pump. Perfusion at constant low flow permits the measurement of regional myocardial blood flow with an intracoronary infusion of microspheres.

Regional myocardial blood flow

Radiolabeled microspheres (15 µm diameter, ^{141}Ce, ^{114}In, ^{103}Ru, ^{95}Nb or ^{46}Sc; NEN, Du Pont Co, Boston, MA, USA) were injected into the coronary perfusion circuit (1.5 – 3×10^5 suspended in 1 ml saline) to determine regional myocardial blood flow. Regional myocardial blood flow for a given tissue sample was calculated as the ratio of tissue sample activity to total activity within the myocardium times coronary inflow (given by the roller pump). This procedure for the determination of blood flow has been validated extensively [37]. The number of microspheres in each tissue sample was calculated with the predetermined number of radioactive counts per sphere. For each isotope, samples contained more than 400 microspheres as long as ischemic blood flow was higher than 0.02 ml/min/g and sample weight was greater than 0.5 g, resulting in an error of less than 10% [6, 10].

Experimental protocols

In all experiments, heart rate under baseline conditions was set to 10 beats/min above the spontaneous sinus rhythm by left atrial pacing.

90 min ischemia (group 1, n = 24) After control measurements of systemic hemodynamics and regional myocardial blood flow, pigs were subjected to 90 min ischemia. Coronary inflow was reduced to decrease mean coronary arterial pressure to 30 – 35 mmHg and then maintained until the end of the ischemic period. At 5 and 85 min of ischemia, further sets of hemodynamic measurements were obtained. At 5 min ischemia, regional myocardial blood flow was also measured. The myocardium was reperfused for 120 min.

Ischemic preconditioning with 10 min ischemia and 15 min reperfusion preceding 90 min ischemia (group 2, n = 24) After control measurements the pigs underwent a cycle of 10 min ischemia of a severity to decrease mean coronary arterial pressure to 30 – 35 mmHg and 15 min reperfusion at constant normal coronary arterial pressure. Measurements were performed at the end of the preconditioning ischemia and at 15 min reperfusion. Thereafter, the protocol was identical to that of group 1.

Preparation of tissue At the end of each experiment fol-

lowing 120 min reperfusion the heart was excised, sectioned into five to six slices parallel to the atrioventricular groove, and incubated in 1% TTC solution (25 min, 37 °C) to demarcate infarcted areas [15]. TTC positive (viable) tissue was subdivided into subendocardial, midmyocardial and subepicardial layers of approximately equal weight. Tissue without TTC staining was carefully dissected from viable areas.

Histology

In a subset of experiments (n = 8), tissue samples from both the LV anterior free wall and LV septum were fixed in formalin and embedded in paraffin (for details, see [1, 34]). Sections of 4 μm thickness were cut, stained with hematoxylin and eosin and examined by light microscopy. Images at a magnification ×1000 were taken (Leica DMLB and Leica DC 100, Leica Bensheim, Germany), and the distance from the inner wall of a capillary to the surrounding myocytes was measured and defined as the capillary-to-myocyte distance (μm, total area analyzed for each wall: 0.06 mm^2). The same images were used to quantify the area of perivascular connective tissue and the cross sectional cardiomyocyte area (μm^2). In addition, Masson Goldner staining was performed to examine connective tissue. The area of connective tissue was measured and calculated as a percentage per field of view. Histological findings obtained in pig hearts were confirmed in sections from normoperfused rabbit hearts (n = 8).

Data analysis and statistics

Hemodynamic data were recorded on an 8-channel recorder (Gould MK 200A, Cleveland, OH, USA), simultaneously digitized at 200 Hz and directly stored to the hard disk of a personal computer. Systemic hemodynamic parameters were recorded and digitized over a 20 s period during each microspheres injection (approximately 33 consecutive beats over at least two complete respiratory cycles) using CORDAT II software [45]. Hemodynamic parameters analyzed were heart rate, LV peak and end-diastolic pressure, the maximum of the first derivative of left ventricular pressure (dP/dt_{max}), mean coronary arterial pressure (CAP) and mean coronary inflow (CBF). Calculation of all hemodynamic parameters was done on a beat-to-beat basis, and data were then averaged.

All data are reported as mean values ± SEM. A p-value less than 0.05 was accepted as indicating a significant difference in mean values. Systemic hemodynamic data were subjected to a two-way analysis of variance (ANOVA) for repeated measures, accounting for the two groups of pigs and the time course of the experiment.

Regional myocardial blood flow data of each group were also subjected to a two-way analysis ANOVA for repeated measures, accounting for viable and infarcted tissue samples and blood flow under control conditions and at 5 min of the index ischemia. Regional blood flow data for a given myocardial region were additionally subjected to a two-way ANOVA for repeated measures, accounting for the two groups of pigs and the time course of the experiment.

When significant differences were detected, individual mean values were compared using Tukey's post-hoc tests.

The area at risk and infarct size as a percent of the area at risk of the 2 groups of pigs were analyzed by unpaired t-test. Linear regression analyses between infarct size and subendocardial blood flow at 5 min of ischemia were performed and compared by analysis of covariance (ANCOVA).

Results

Systemic hemodynamics are shown in Table 1. There were no significant differences in systemic hemodynamics between the two groups of pigs during the time course of the experiment. Heart rate remained unchanged throughout the protocol in both groups. LV peak pressure, dP/dtmax, CAP and CBF were significantly decreased at 5 and 85 min of the sustained ischemia, while LV end-diastolic pressure increased.

Table 1 Systemic hemodynamics

		Baseline	5 min IP	5 min ischemia	85 min ischemia
HR	Group 1	100 ± 9		101 ± 9	102 ± 8
	Group 2	99 ± 9	103 ± 19	100 ± 11	105 ± 18
LVPP	Group 1	94 ± 7		82 ± 10*	81 ± 12*
	Group 2	94 ± 11	82 ± 11*	82 ± 7*	78 ± 11*
LVedP	Group 1	6 ± 3		14 ± 4*	12 ± 5*
	Group 2	6 ± 3	12 ± 4*	13 ± 3*	13 ± 5*
dP/dt_{max}	Group 1	1365 ± 325		1082 ± 290*	1087 ± 247*
	Group 2	1296 ± 260	1008 ± 178*	1027 ± 160*	1004 ± 179*
CAP	Group 1	118 ± 10		30 ± 3*	30 ± 5*
	Group 2	113 ± 22	29 ± 4*	30 ± 4*	30 ± 4*
CBF	Group 1	42.4 ± 16.2		6.5 ± 2.5*	6.6 ± 2.6*
	Group 2	40.3 ± 12.4	6.3 ± 2.8*	6.4 ± 2.8*	6.5 ± 2.8*

Group 1 90 min ischemia (n = 24); *Group 2* Ischemic preconditioning + 90 min ischemia (n = 24); *HR* heart rate (beats/min); *LVPP* left ventricular peak pressure (mmHg); *LVedP (mmHg)* left ventricular end-diastolic pressure; dP/dt_{max} maximum of the first derivative of left ventricular pressure (mmHg/s); *CAP* coronary arterial pressure (mmHg); *CBF* coronary blood flow (ml/min). *p < 0.05 vs Baseline

Table 2 Regional myocardial blood flow

	Group 1		Group 2	
	Baseline	5 min ischemia	Baseline	5 min ischemia
LVAFW	0.93 ± 0.35	0.05 ± 0.03*	1.06 ± 0.31	0.05 ± 0.03*
$LVAFW_{INF}$	1.05 ± 0.49[1]	0.04 ± 0.03*	1.28 ± 0.46[1]	0.06 ± 0.03*
LVS	0.80 ± 0.26[2]	0.05 ± 0.03*	0.90 ± 0.30[2]	0.05 ± 0.03*
LVS_{INF}	0.84 ± 0.31[3]	0.05 ± 0.03*	0.94 ± 0.31[3]	0.06 ± 0.03*

Group 1 90 min ischemia (n = 24); *Group 2* Ischemic preconditioning + 90 min ischemia (n = 24); *LVAFW* blood flow in the left ventricular anterior free wall (ml/min/g); *LVAFWINF* blood flow in infarcted parts of the LVAFW (ml/min/g); *LVS* blood flow in the left ventricular septum (ml/min/g); *LVSINF* blood flow in infarcted parts of the LVS (ml/min/g)
*p < 0.05 vs Baseline; [1]p<0.05 vs LVAFW; [2]p < 0.05 vs LVAFW; [3]p < 0.05 vs $LVAFW_{INF}$

In the LV anterior free wall, baseline myocardial blood flows of endocardial and epicardial layers were similar (ratio: 1.05 ± 0.41) but baseline myocardial blood flow to subsequently infarcted tissue was higher than blood flow to viable tissue pieces in both groups of pigs (Table 2). At 5 min ischemia, blood flow of viable and infarcted tissue was reduced to similar absolute values. Baseline myocardial blood flow in the septum did not significantly differ between the subendocardial layer towards the LV and the subendocardial layer towards the right ventricle (ratio: 1.12 ± 0.67), but it was significantly lower than that in the LV anterior free wall in both groups of pigs. In the LV septum, baseline blood flow to subsequently infarcted tissue was not different from blood flow to viable tissue pieces, again with no differences between groups. At 5 min ischemia, blood flow of viable and infarcted LV septum was reduced to similar absolute values, similar also to that measured in the LV anterior free wall. Since baseline blood flow was higher in the LV anterior free wall than in the LV septum, relative blood flows during ischemia were lower in the LV anterior free wall (5.0 ± 2.0 % and 5.0 ± 3.1 % of baseline in groups 1 and 2, respectively) than in the LV septum (6.3 ± 2.1 % and 6.6 ± 3.1 % of baseline in groups 1 and 2, respectively, both p < 0.05 vs. LV anterior free wall).

The area at risk – perfused by the LAD – was similar in both groups of pigs (Fig. 1). Also the distribution of LV anterior free wall and LV septum perfused by the LAD was similar between groups. Infarct size was smaller in the LV anterior free wall than in the LV septum following ischemia/reperfusion in non-preconditioned hearts, while it was similar in both LV areas in preconditioned hearts (Fig. 2). The linear relationship between regional myocardial blood flow at 5 min ischemia and infarct size obtained in the LV septum (y = –368.5 · × +43.3, n = 24, r = 0.56) was significantly shifted upwards compared to that obtained in the LV anterior free wall (y = –172.3 · × +24.4, n = 24, r = 0.47) (Fig. 3a). No significant correlation between ischemic regional myocardial blood flow and infarct size was found in preconditioned hearts (Fig. 3b).

In a subgroup of pigs, the cardiomyocyte cross-sectional area was slightly increased in the LV septum (272

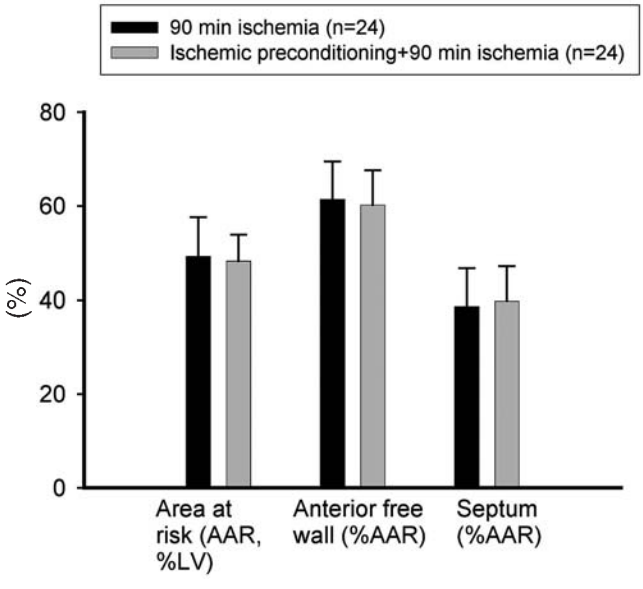

Fig. 1 The area at risk – perfused by the LAD – was similar in both groups of pigs Also the distribution of LV anterior free wall and LV septum perfused by the LAD was similar between groups

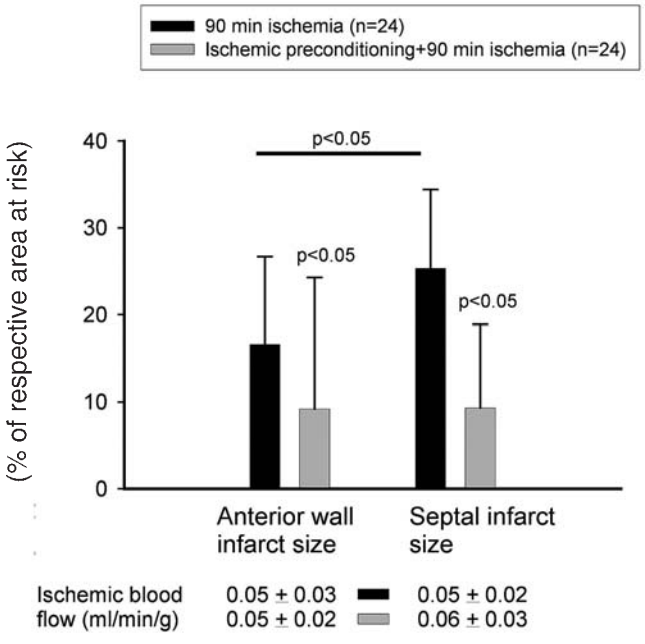

Fig. 2 Infarct size was smaller in the LV anterior free wall than in the LV septum following ischemia/reperfusion in non-preconditioned hearts, while it was similar in both LV areas in preconditioned hearts

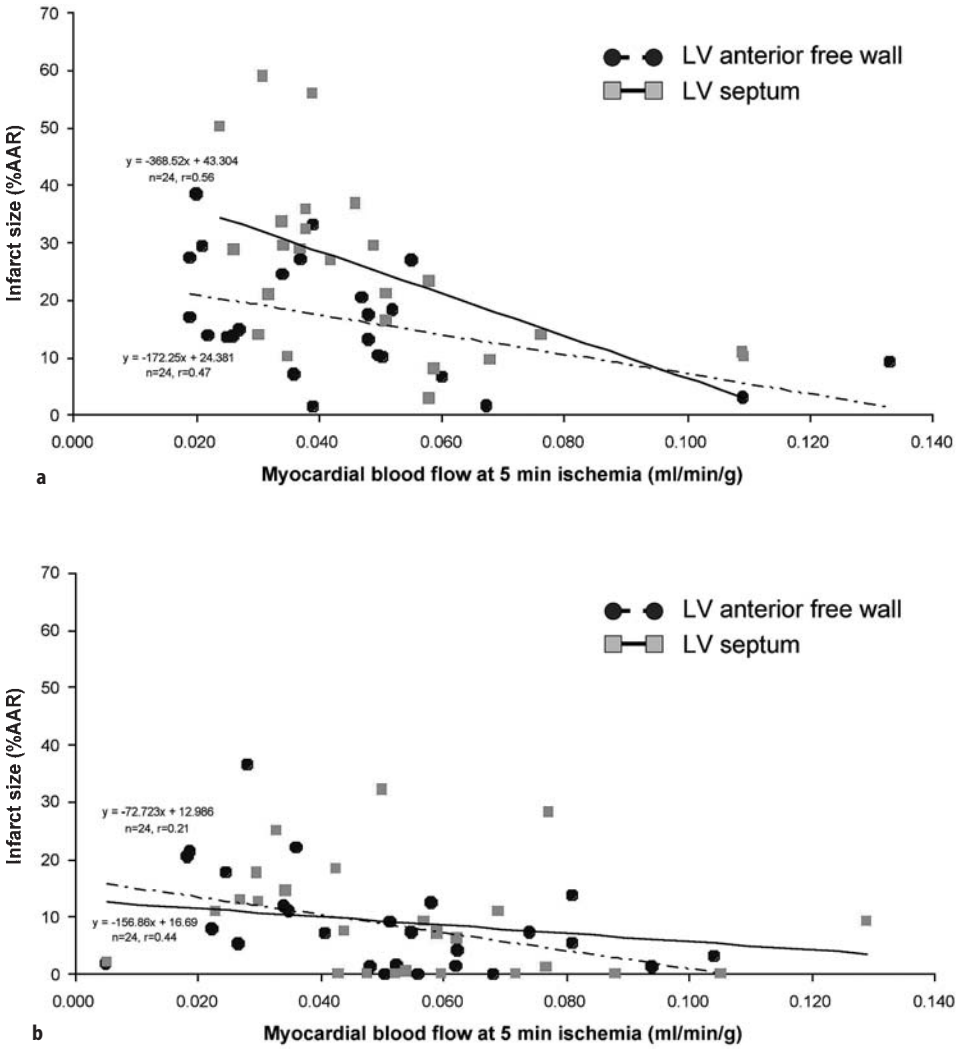

Fig. 3 Relationship between ischemic myocardial blood flow and infarct size following ischemia/reperfusion in non-preconditioned (**a**) and preconditioned (**b**) hearts. Infarct sizes of the left ventricular anterior free wall (LVAFW) and the LV septum are expressed as percentage of the respective area at risk. While infarct size for a given ischemic myocardial blood flow is higher in the LV septum than in the LVAFW of non-preconditoned hearts (**a**), it is similar in preconditioned hearts (**b**)

± 20 μm²) compared to the LV anterior free wall (254 ± 11 mm², p = 0.05). Also total myocardial connective tissue (Figs. 4 and 5), the amount of perivascular connective tissue (Fig. 6) and the capillary-to-myocyte distance (Fig. 7) were increased in the LV septum compared to the LV anterior free wall. These histological findings were confirmed in healthy hearts from rabbits (Figs. 5–7).

Discussion

In pigs, regional differences in infarct development and protection from it by ischemic preconditioning exists in the LAD perfusion territory, which is independent of ischemic blood flow but apparently related to pre-existing structural differences.

Critique of methods

The present experiments were performed in pigs since infarct development in this species, due to the sparsity of the innate collateral circulation, most closely resembles that observed in man [32].

Since collateral flow is small in pigs [32], complete occlusion of the proximal left anterior descending coronary artery results in extensive infarction of the left ventricle and subsequent pump failure. Therefore in the present study, the left anterior descending coronary artery perfusion territory was hypoperfused at low, but maintained flow, resulting in a large area at risk (49% of the LV mass on the average), but a small infarct size when expressed as a percent of the area at risk (16 – 25%). However, infarct size expressed as a percent of the total LV mass in the present study averaged 7–12% and was thus comparable to that of previous studies using pigs

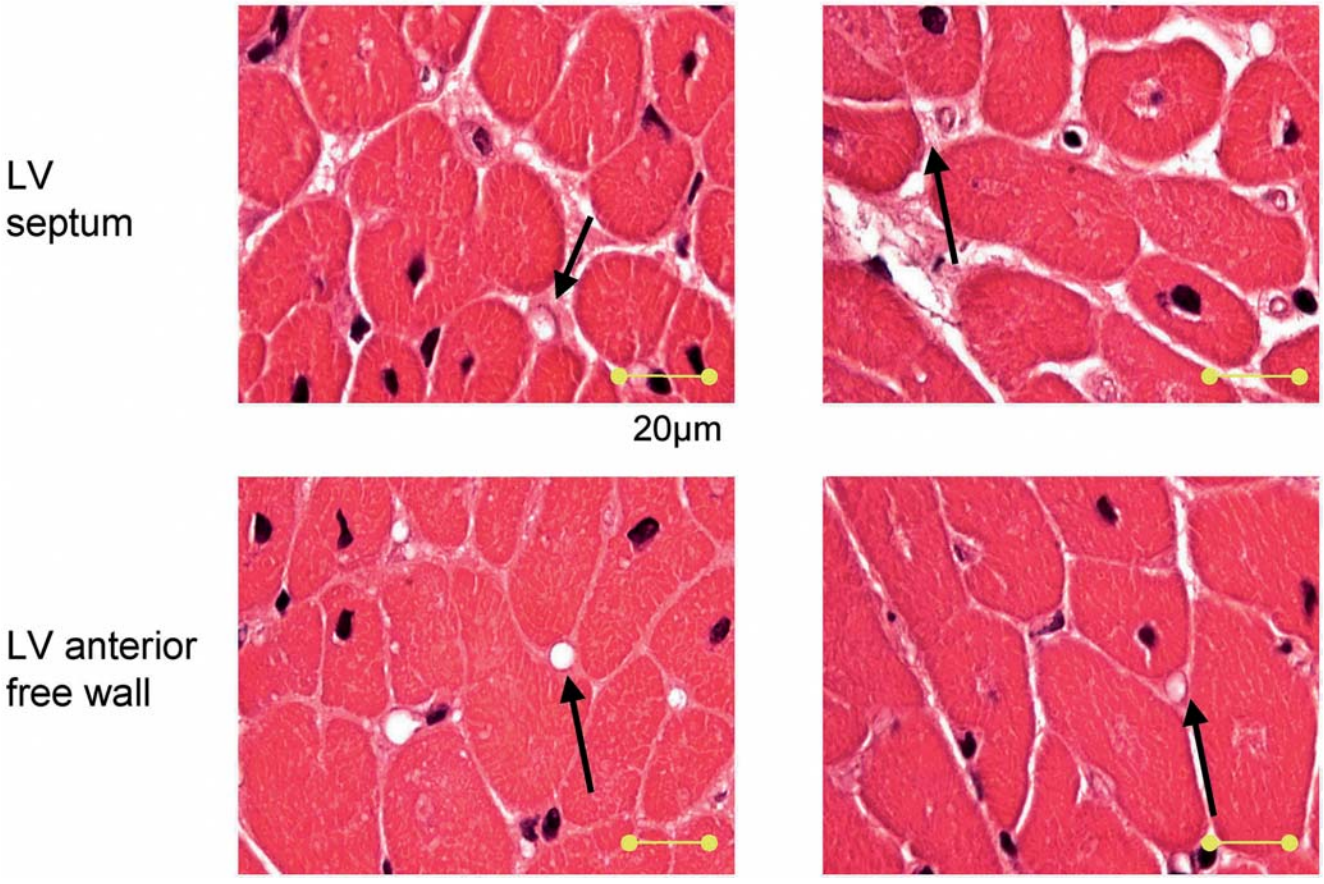

Fig. 4 Tissue sections from the LV septum (upper two graphs) and the LV anterior free wall (lower two graphs). The connective tissue (arrows) is increased in the LV septum compared to the LV anterior free wall

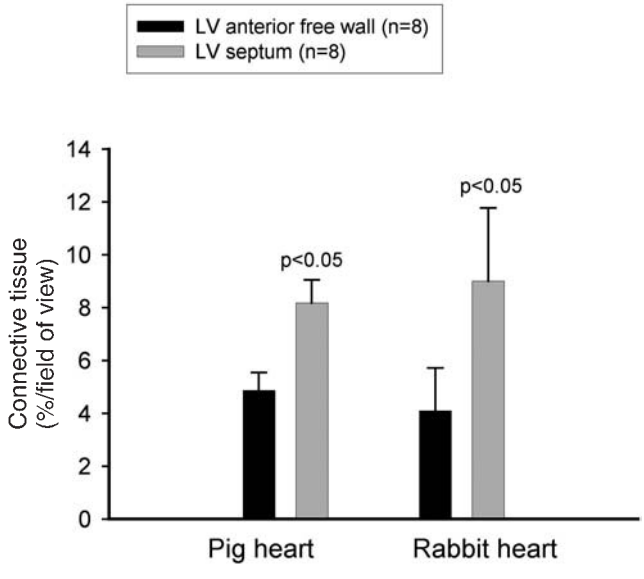

Fig. 5 Total myocardial connective tissue was increased in the LV septum compared to the LV anterior free wall in pig and rabbit hearts

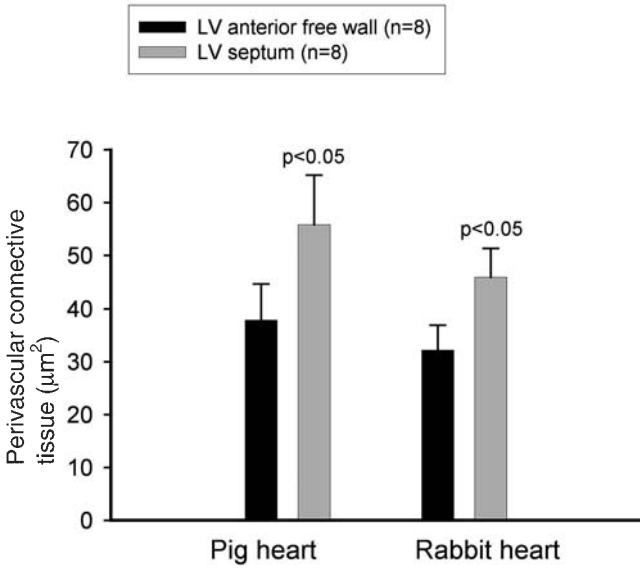

Fig. 6 Perivascular connective tissue was increased in the LV septum compared to the LV anterior free wall in pig and rabbit hearts

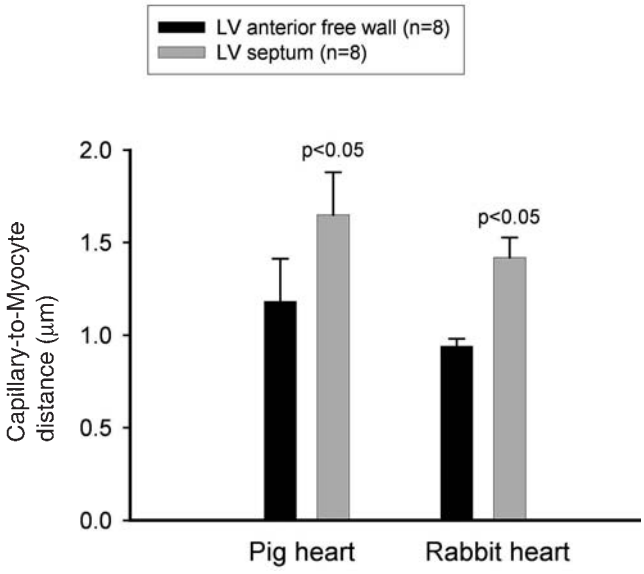

Fig. 7 The capillary-to-myocyte distance was increased in the LV septum compared to the LV anterior free wall in pig and rabbit hearts

with a total occlusion of only one distal left anterior descending coronary artery branch [33].

Blood flow

While no difference in blood flow between the LV septum and the LV anterior free wall exists in dogs [52], blood flow distribution within the LAD perfusion territory under baseline conditions differs between the LV septum and the LV anterior free wall in humans [12]. The reduced blood flow in the LV septum compared to the LV anterior free wall in the present study could have resulted from an increased extravascular compression [51] and/or LV fibrosis; the latter explanation, however, appears to be of little importance for the present findings on infarction, since the pronounced difference in blood flow seen under baseline conditions was lost during ischemia. In agreement with previous results, myocardial tissue in the LV anterior free wall undergoing infarction had higher baseline blood flow than tissue which remained viable [17]. However, this was not observed in the LV septum, for reasons unknown.

Blood flow and ischemic preconditioning

In pig hearts, ischemic preconditioning increases the number of infarcted tissue pieces (y-axis) with lower blood flow values (x-axis) compared to non-preconditioned hearts [21]. Similarly, the ischemic myocardial blood flow (x-axis) below which irreversible tissue damage develops (y-axis) is shifted leftwards in preconditioned pig hearts [28]. These results imply that downregulation of energy expenditure (or less energy wastage) occurs in preconditioned hearts and plays a role in its cardioprotective effects [27]. Therefore, at similar ischemic blood flow – as seen in the present study – irreversible tissue damage in preconditioned myocardium is less than in non-preconditioned myocardium. Whether or not the blood flow threshold for the development of irreversible tissue is similar for the LV septum and the LV anterior free wall is unknown at present.

Myocardial morphology

The cardiomyocyte cross-sectional area and the extent of myocardial connective tissue were greater in the LV septum than in the LV anterior free wall, and increased connective tissue was also measured around the myocardial blood vessels. These findings were not related to the animal species or the experimental setup, since they were also observed in intact rabbit hearts. Therefore, the general assumption of a homogenous distribution of cardiomyocyte size and collagen network within the healthy LV is challenged by the present study. In human hearts, differences in the extent of fibrosis between the LV septum and the LV anterior free wall have been found in hypertrophied [47], but not in healthy hearts [48]. However, significant differences in myocardial fiber diameter between the LV septum and the LV anterior free wall have been reported [20], with fiber diameter being greater in the LV anterior free wall than in the LV septum. The increase in the diffusion distance for oxygen (approximately 8%), as estimated from the increased capillary-to-myocyte distance and the increased cardiomyocyte cross sectional area, in the presence of a comparable ischemic blood flow could potentially contribute to the regional differences in infarct development in the LV septum vs. anterior free wall, although its exact contribution remains to be established.

In addition, differences in myocardial energy demand – which were measured previously within small regions of the left ventricle [13] – could explain the regional differences in infarct development. Since the myocardium consumes most of the oxygen to generate contractile function [30] and maintain wall stress independent of shortening [11], regional myocardial function must be measured to assess differences in myocardial energy demand between the LV septum and the LV anterior free wall. Indeed, wall thickening is greater in the LV septum than in the LV anterior free wall in humans under baseline conditions [9], but wall stress appears to be similar [3]. More importantly, in pigs regional myocardial function (fractional area) during ischemia is decreased to similar absolute values in the LV septum and the LV anterior free wall [24], and this decrease occurs more rapidly in the LV septum [25].

Thus at present, although differences in regional myocardial function cannot completely be ruled out, a major contribution to the observed regional differences in infarct development does not appear likely. Also, the established determinants of regional myocardial blood flow such as driving pressure, diastolic duration, number and diameter of blood vessels and their vasomotor tone do not predict the regional differences in infarction.

Ischemic preconditioning's protection

The close inverse relationship between ischemic regional myocardial blood flow and infarct size obtained in non-preconditioned hearts is lost in preconditioned hearts [26], indicating that the protection obtained by ischemic preconditioning is independent from ischemic energy supply. Similarly in the present study, although infarct size was greater in the LV septum than in the LV anterior free wall in non-preconditioned hearts, it no longer differed in preconditioned hearts, again supporting the above notion. Both the above [26] and the present study imply that a certain maximum of cardioprotection by ischemic preconditioning exists; i.e. some irreversible tissue damage remains even in preconditioned hearts. The reasons for the independence of infarct development from energy supply in preconditioned hearts remain to be elucidated.

In conclusion, regional differences in infarct development and protection from it by ischemic preconditioning exists in the LAD perfusion territory of pigs, which is independent of ischemic blood flow but apparently related to pre-existing structural differences.

References

1. Aker S, Belosjorow S, Konietzka I, Duschin A, Martin C, Heusch G, Schulz R (2003) The serum but not the myocardial TNFα concentration is increased in pacing-induced heart failure in rabbits. Am J Physiol Regul Integ Comp Physiol 285: R463–R469
2. Balaban RS, Arai A (2001) Function, metabolic, and flow heterogeneity of the heart. The view is getting better. Circ Res 88:265–267
3. Balzer P, Furber A, Delépine S, Rouleau F, Lethimonnier F, Morel O, Tadéi A, Jallet P, Geslin P, Le Jeune J-J (1999) Regional assessment of wall curvature and wall stress in left ventricle with magnetic resonance imaging. Am J Physiol 277 (46):H901–H910
4. Bassenge E, Heusch G (1990) Endothelial and neuro-humoral control of coronary blood flow in health and disease. Rev Physiol Biochem Pharmacol 116:77–165
5. Bassingthwaighte JB, Beard DA, Li Z (2001) The mechanical and metabolic basis of myocardial blood flow heterogeneity. Basic Res Cardiol 96:582–594
6. Bassingthwaighte JB, Malone MA, Moffett TC, King RB, Little SE, Link JM, Krohn KA (1987) Validity of microsphere depositions for regional myocardial flows. Am J Physiol Heart Circ Physiol 253:H184–H193
7. Bishop SP, White FC, Bloor CM (1976) Regional myocardial blood flow during acute myocardial infarction in the conscious dog. Circ Res 38:429–438
8. Boerth RC, Covell JW, Pool PE, Ross Jr J (1969) Increased myocardial oxygen consumption and contractile state associated with increased heart rate in dogs. Circ Res 24:725–734
9. Bogaert J, Rademakers FE (2001) Regional nonuniformity of normal adult human left ventricle. Am J Physiol Heart Circ Physiol 280:H610–H620
10. Buckberg GD, Luck JC, Payne DB, Hoffman JIE, Archie JP, Fixler DE (1971) Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol 31:598–604
11. Buffington CW, Strum DP, Watanabe S (1994) Regional oxygen consumption persists in dyskinetic canine myocardium. J Cardiovasc Pharmacol 24:37–44
12. Chareonthaitawee P, Kaufmann PA, Rimoldi O, Camici PG (2001) Heterogeneity of resting and hyperemic myocardial blood flow in healthy humans. Cardiovasc Res 50:151–161
13. Decking UKM, Skwirba S, Zimmermann MF, Preckel B, Thämer V, Deussen A, Schrader J (2001) Spatial heterogeneity of energy turnover in the heart. Pflügers Arch – Eur J Physiol 441:663–673
14. Deussen A, Lauer T, Loncar R, Kropp J (2001) Heterogeneity of metabolic parameters in the left ventricular myocardium and its relation to local blood flow. Basic Res Cardiol 96:564–574
15. Fishbein MC, Meerbaum S, Rit J, Lando U, Kanmatsuse K, Mercier JC, Corday E, Ganz W (1981) Early phase acute myocardial infarct size quantification: validation of the triphenyl tatrazolium chloride tissue enzyme staining technique. Am Heart J 101:593–600
16. Franzen D, Conway RS, Zhang H, Sonnenblick EH, Eng C (1988) Spatial heterogeneity of local blood flow and metabolic content in dog hearts. Am J Physiol Heart Circ Physiol 254: H344–H353
17. Ghaleh B, Shen Y-T, Vatner SF (1996) Spatial heterogeneity of myocardial blood flow presages salvage versus necrosis with coronary artery reperfusion in conscious baboons. Circulation 94:2210–2215
18. Guth BD, Schulz R, Heusch G (1991) Pressure-flow characteristics in the right and left ventricular perfusion territories of the right coronary artery in swine. Pflügers Arch 419:622–628
19. Guth BD, Schulz R, Thaulow E (1991) Interventricular redistribution of myocardial blood flow during metabolic vasodilation. Pflügers Arch 417:485–492
20. Hoshino T, Fujiwara H, Kawai C, Hamashima Y (1983) Myocardial fiber diameter and regional distribution in the ventricular wall of normal adult hearts, hypertensive hearts and hearts with hypertrophic cardiomyopathy. Circulation 67:1109–1116
21. Huang C-H, Kim S-J, Ghaleh B, Kudej RK, Shen Y-T, Bishop SP, Vatner SF (1999) An adenosine agonist and preconditioning shift the distribution of myocardial blood flow in conscious pigs. Am J Physiol 276:H368–H375

22. Laussmann T, Janosi RA, Fingas CD, Schlieper GR, Schlack W, Schrader J, Decking UKM (2002) Myocardial proteome analysis reveals reduced NOS inhibition and enhanced glycolytic capacity in areas of low local blood flow. Faseb J 16:628–630
23. Matsumoto T, Kajiya F (2001) Microheterogeneity of myocardial blood flow. Basic Res Cardiol 96:547–552
24. Mor-Avi V, Caiani EG, Collins KA, Korcarz CE, Bednarz JE, Lang RM (2001) Combined assessment of myocardial perfusion and regional left ventricular function by analysis of contrast-enhanced power modulation images. Circulation 104:352–357
25. Mor-Avi V, Collins KA, Korcarz CE, Shah M, Spencer KT, Lang RM (2001) Detection of regional temporal abnormalities in left ventricular function during acute myocardial ischemia. Am J Physiol Heart Circ Physiol 280:H1770–H1781
26. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–1136
27. Opie LH, Sack MN (2002) Metabolic plasticity and the promotion of cardiac protection in ischemia and ischemic preconditioning. J Mol Cell Cardiol 34:1077–1089
28. Post H, Schulz R, Vahlhaus C, Hüsing J, Hirche H, Gallagher KP, Heusch G (1998) Impact of resting and ischemic blood flow on infarct probability in ischemic preconditioning – a new approach to infarct size-blood flow data by logistic regression. J Mol Cell Cardiol 30:2719–2728
29. Rivas F, Cobb FR, Bache RJ, Greenfield Jr JC (1976) Relationship between blood flow to ischemic regions and extent of myocardial infarction. Serial measurement of blood flow to ischemic regions in dogs. Circ Res 38:439–447
30. Rooke GA, Feigl EO (1982) Work as a correlate of canine left ventricular oxygen consumption, and the problem of catecholamine oxygen wasting. Circ Res 50:273–286
31. Savage RM, Guth BD, White FC, Hagan AD, Bloor CM (1981) Correlation of regional myocardial blood flow and function with myocardial infarct size during acute myocardial ischemia in the conscious pig. Circulation 64:699–707
32. Schaper W, Görge G, Winkler B, Schaper J (1988) The collateral circulation of the heart. Prog Cardiovasc Dis 31:57–77
33. Schott RJ, Rohmann S, Braun ER, Schaper W (1990) Ischemic preconditioning reduces infarct size in swine myocardium. Circ Res 66:11331142
34. Schulz R, Aker S, Belosjorow S, Konietzka I, Rauen U, Heusch G (2003) Stress kinase phosphorylation is increased in pacing-induced heart failure in rabbits. Am J Physiol Heart Circ Physiol 285: H2084–H2090
35. Schulz R, Belosjorow S, Gres P, Jansen J, Michel MC, Heusch G (2002) p38 MAP kinase is a mediator of ischemic preconditioning in pigs. Cardiovasc Res 55:693–700
36. Schulz R, Gres P, Skyschally A, Duschin A, Belosjorow S, Konietzka I, Heusch G (2003) Ischemic preconditioning preserves connexin 43 phosphorylation during sustained ischemia in pig hearts in vivo. Faseb J 17:1355–1357
37. Schulz R, Miyazaki S, Miller M, Thaulow E, Heusch G, Ross Jr J Guth BD (1989) Consequences of regional inotropic stimulation of ischemic myocardium on regional myocardial blood flow and function in anesthetized swine. Circ Res 64:1116–1126
38. Schulz R, Post H, Vahlhaus C, Heusch G (1998) Ischemic preconditioning in pigs: A graded phenomenon. Its relation to adenosine and bradykinin. Circulation 98:1022–1029
39. Schulz R, Rose J, Heusch G (1994) Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine. Am J Physiol Heart Circ Physiol 267:H1341–H1352
40. Schulz R, Rose J, Martin C, Brodde OE, Heusch G (1993) Development of short-term myocardial hibernation: its limitation by the severity of ischemia and inotropic stimulation. Circulation 88:684–695
41. Schulz R, Rose J, Post H, Heusch G (1995) Involvement of endogenous adenosine in ischaemic preconditioning in swine. Pflügers Arch 430:273–282
42. Schwanke U, Deussen A, Heusch G, Schipke JD (2000) Heterogeneity of local myocardial flow and oxidative metabolism. Am J Physiol Heart Circ Physiol 279:H1029–H1035
43. Skyschally A, Schulz R, Gres P, Konietzka I, Martin C, Haude M, Erbel R, Heusch G (2004) Coronary microembolization does not induce acute preconditioning against infarction in pigs – the role of adenosine. Cardiovasc Res 63:313–322
44. Skyschally A, Schulz R, Gres P, Korth H-G, Heusch G (2003) Attenuation of ischemic preconditioning in pigs by scavenging of free oxyradicals with ascorbic acid. Am J Physiol Heart Circ Physiol 284:H698–H703
45. Skyschally A, Schulz R, Heusch G (1993) Cordat II: A new program for data acquisition and on-line calculation of hemodynamic and regional myocardial dimension parameters. Comput Biol Med 23:359–367
46. Sonntag M, Deussen A, Schultz J, Loncar R, Hort W, Schrader J (1996) Spatial heterogeneity of blood flow in the dog heart. I.Glucose uptake, free adenosine and oxidative/glycolytic enzyme activity. Pflügers Arch 432:439–450
47. Tanaka M, Fujiwara H, Onodera T, Wu D, Hamashima Y, Kawai C (1986) Quantitative analysis of myocardial fibrosis in normal, hypertensive hearts, and hypertrophic cardiomyopathy. Br Heart J 55:575–581
48. Unverferth DV, Baker PB, Swift SE, Chaffee R, Fetters JK, Uretsky BF, Thompson ME, Leier CV (1986) Extent of myocardial fibrosis and cellular hypertrophy in dilated cardiomyopathy. Am J Cardiol 57:816–825
49. Vahlhaus C, Schulz R, Post H, Onallah R, Heusch G (1996) No prevention of ischemic preconditioning by the protein kinase C inhibitor staurosporine in swine. Circ Res 79:407–414
50. Vahlhaus C, Schulz R, Post H, Rose J, Heusch G (1998) Prevention of ischemic preconditioning only by combined inhibition of protein kinase C and protein tyrosine kinase in pigs. J Mol Cell Cardiol 30:197–209
51. Vis MA, Bovendeerd PHM, Sipkema P, Westerhof N (1997) Effect of ventricular contraction, pressure, and wall stretch on vessels at different locations in the wall. Am J Physiol 41:H2963–H2975
52. Weiss HR, Neubauer JA, Lipp JA, Sinha AK (1978) Quantitative determination of regional oxygen consumption in the dog heart. Circ Res 42:394–401
53. Wieringa PA, Stassen HG, VanKan JJIM, Spaan JAE (1993) Oxygen diffusion in a network model of the myocardial microcirculation. Int J Microcirc Clin Exp 13:137–169