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Coronary Endothelial Dysfunction Precedes Heart Failure and Reduction of Coronary Reserve in Awake Dogs

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M. Knecht, D. Burkhoff, G.-H. Yi, S. Popilskis, S. Homma, M. Packer and J. Wang. Coronary Endothelial Dysfunction Precedes Heart Failure and Reduction of Coronary Reserve in Awake Dogs. *Journal of Molecular and Cellular Cardiology* (1997) **29**, 217–227. Endothelial dysfunction in coronary circulation is well documented in heart failure (HF). However, whether this dysfunction is a consequence of heart failure or precedes the development of HF remains unknown. To determine endothelium-dependent regulation in the remote coronary vasculature in a canine coronary microembolization-induced HF model, seven dogs were chronically instrumented for measurement of systemic hemodynamics, for selective coronary microembolization via an implanted coronary catheter and for measurement of coronary blood flow in the non-embolized coronary artery. Microembolizations were performed daily until hemodynamic and echocardiographic measurements showed HF. The responses of coronary blood flow to acetylcholine (0.25, 0.5, 5, 10 μg/kg), nitroglycerine (0.2, 0.8, 5, 25 μg/kg), adenosine (0.25, 0.5, 2, 5 μmol/kg) and brief coronary occlusions (5, 10, 15, 20, 30 s) were examined. Although no signs of HF developed and the responses of coronary blood flow to nitroglycerine, adenosine and occlusions were not altered, the response to acetylcholine was selectively reduced after 1 week of embolization (275 000 $\pm$ 55 000 microspheres). Resting coronary flow increased from 21.3 $\pm$ 1.4 ml/min in control state to 27.7 $\pm$ 3.5 ml/min (P<0.001). As HF developed, characterized by an elevated left ventricular end-diastolic pressure (6.4 $\pm$ 1.6 *v* 16 $\pm$ 1.6 mmHg, P<0.001), a decreased area ejection fraction (54 $\pm$ 5 *v* 36 $\pm$ 5%, P<0.05) and a reduced β-adrenergic response to isoproterenol, the responses of coronary blood flow to acetylcholine, nitroglycerine, adenosine and occlusions were consistently depressed. Resting coronary blood flow was decreased to 15.4 $\pm$ 2.7 ml/min (P<0.01). Our results indicate, that there is a selectively impaired endothelium-mediated dilator capacity of the resistance coronary vasculature before the development of HF and a reduction of the coronary flow reserve.

Key Words: Heart Failure; Nitric oxide; Acetylcholine; Coronary microembolization; Coronary blood reserve.

Introduction

The endothelium regulates vascular tone of conduit and resistance coronary vessels by varying synthesis and release of endothelium-derived relaxing factors (EDRFs) (Marshall and Kontos, 1990). Nitric oxide (NO), which has been identified as one of these substances (Palmer *et al.*, 1987), is released continuously by the endothelium and regulates vascular tone. Shear stress and a variety of receptor-mediated agonists (such as acetylcholine) can increase NO release and cause vascular dilation (Furchgott, 1983; Vanhoutte *et al.*, 1986; Bassenge and Busse, 1988). Importantly, alterations of NO production and release occurring in certain disease states have been implicated as mechanisms underlying associated hemodynamic abnormalities. For example, endothelial dysfunction occurs in

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experimental and clinical heart failure (Kaiser *et al.*, 1989; Katz *et al.*, 1992; Wang *et al.*, 1994), resulting in attenuated endothelium/NO-dependent vasodilation. However, previous studies of endothelial function have been performed in either normal subjects or after establishment of the heart failure state (Katz *et al.*, 1992; Drexler *et al.*, 1992). Thus, whether endothelial dysfunction occurs as a consequence of heart failure or precedes and possibly contributes to development of heart failure remains unknown.

We recently described a canine model in which heart failure is created gradually by selective microembolization of the dominant coronary artery (Knecht *et al.*, 1995). This model offers the opportunity to evaluate endothelial function and flow reserve before, during and after the development of heart failure in the remote non-embolized coronary artery. The strength of this strategy is that the evolution of vascular changes due to heart failure can be studied independent of direct endothelial damage by the microspheres and outside the region of direct ischemic insult. The purpose of this study, therefore, was: (1) to determine whether and how resting coronary blood flow in a non-embolized artery is altered during the development of heart failure; (2) to determine the time course of endothelial dysfunction relative to the time course of the development of heart failure; and (3) to determine the relative time course of change in coronary reserve. The results indicate that endothelial dysfunction precedes development of overt heart failure during a period of time when baseline coronary flow is significantly greater than normal. Furthermore, it is not until development of heart failure that both resting coronary flow and coronary vascular reserve are depressed. The implications of these findings are discussed within the context of possible mechanisms of progression of contractile dysfunction in heart failure.

Materials and Methods

Twelve mongrel dogs (23–29 kg) were chronically instrumented for repeated microembolization and coronary flow measurements. Two of the animals were excluded because of malfunctioning instrumentation and three animals died unexpectedly shortly after microsphere infusion prior to study in the heart failure state. Thus, the study is based on results from seven chronically instrumented dogs.

Surgical preparation

Briefly, animals were anesthetized (inhaled isoflurane 1–2%) and mechanically ventilated. A thoracotomy was performed in the left fifth intercostal space under sterile conditions. Tygon catheters (i.d.: 0.04–0.05 in., o.d.: 0.7–0.09 in., Cardiovascular Instr. Corp., Boston, MA, USA) were placed in the descending thoracic aorta, the apex of the left ventricle and the left atrium. A custom-made silicon catheter was implanted into the proximal part of the dominant coronary artery and the other coronary artery was instrumented with a flow-cuff transducer (Epoxy cuff type, pulsed 20 MHz Doppler probes, 3.5–4.5 mm diameter, Baylor College of Medicine, Houston, TX, USA) and a custom-made hydraulic occluder. Among seven successfully studied dogs the left anterior descending coronary artery was dominant in six dogs while the left circumflex coronary artery was dominant in one dog. The catheters and wires were run subcutaneously to the back of the dog, the chest was closed in layers and a chest tube was inserted to reduce the pneumothorax. Antibiotics were given after surgery as necessary. Dogs were allowed to recover fully from surgery and trained to lie quietly on a laboratory table.

Protocol

Assessment of systemic and ventricular hemodynamics and coronary vascular properties were made on three separate occasions: before coronary embolization in a baseline control state, early in the development of heart failure and after establishment of moderate heart failure. All hemodynamic measurements were made with the dog lying on its right side in a conscious state after acclimation to the laboratory environment for at least 30 min. The previously-implanted fluid-filled Tygon catheters were connected to Statham transducers (Statham Instruments, Inc., Rahway, NJ, USA) to measure aortic pressure (MAP), left ventricular pressure (LVP) and left atrial pressure (LAP). The LVP signal was electronically differentiated (Differentiator Signal Conditioner, Gould Electronics, East Rutherford, NJ, USA) to measure left ventricular dP/dt (LV dP/dt). The data were recorded on an 8-channel Gould recorder (Gould, 3800). Mean values were derived for aortic pressure, LAP and coronary blood flow (CBF). In order to investigate the β_1-adrenergic receptor regulation of inotropic state, isoproterenol (Elkins-Sinn, Cherry Hill, NJ, USA) was given as intravenous bolus injections at doses of 0.1 and 0.5 μg/kg and the maximum changes of LV dP/dt, HR and MAP were measured.

To assess various aspects of coronary vascular function, multiple intravenous bolus injections of acetylcholine (0.25, 0.5, 5, 10 μg/kg, Sigma, St. Louis, MO, USA), nitroglycerine (0.2, 0.8, 5, 25 μg/kg, Parke Davis, Morris Plains, NJ, USA) and adenosine (0.25, 0.5, 2, 5 μmol/kg, Sigma, St. Louis, MO, USA) as well as multiple transient coronary occlusions (5, 10, 15, 20, 30 s) were performed. In each experiment these interventions were performed in a random order. Enough time was provided between interventions to allow resting CBF and the other hemodynamic parameters to return to the control level. The peak responses of CBF, MAP, heart rate, LAP, LVP and LV dP/d*t* to each intervention were recorded.

Heart failure was created by daily microsphere injections (Spheriglass, 90 μm mean diameter) through the previously implanted coronary catheter until left ventricular end-diastolic pressure (LVEDP) increased to at least 15 mmHg and heart rate increased to at least 120 b/min. Further details concerning the development of heart failure are provided in Results. Transthoracic echocardiography was performed in a conscious state prior to every experiment in five animals. Mean area ejection fraction (AEF, %), mean end-systolic area (ESA, mm^2) and mean end-diastolic area (EDA, mm^2) were calculated by averaging at least five steady-state cardiac cycles.

After completion of the final hemodynamic experimental recordings, animals were killed, and the heart was excised and weighed.

This study was approved by the Institutional Animal Care and Use Committee, College of Physicians & Surgeons of Columbia University and animals were cared for in accordance with the *Guiding Principles for the Use and Care of Laboratory Animals* (NIH Publication No 82-23, 1985).

Statistical analysis

Data were expressed as mean($\pm$S.D.). The responses of CBF to the interventions were expressed as changes from baseline. Significant differences compared to control were determined using a one-way analysis of variance (ANOVA), followed by the Duncan multiple range test. Statistical significance was determined at a value of $P<0.05$.

Results

Repeated microembolization leads to heart failure

Table 1 summarizes the effects of microembolization on systemic hemodynamics under control conditions and at two different time points after commencing coronary microembolizations. After 6 ± 1 days of microembolization and a total dose of $275\,000\pm55\,000$ microspheres (range 200 000–350 000) there were no significant changes in any hemodynamic parameter measured. After 18 ± 9 days of embolization with a total dose of $735\,000\pm125\,000$ microspheres (range 700 000–900 000) LVP, MAP and LV dP/d*t* were significantly decreased, whereas LVEDP, LAP and HR were significantly increased. The AEF was significantly decreased and, in parallel, ESA and EDA were significantly increased after the full dose of emboli were given.

The peak change of LV dP/d*t* in response to intravenous injections of isoproterenol was not changed after the first week of embolization, but it was depressed significantly after a mean of 18 days of microsphere injections (Table 1).

No animals developed clinical symptoms of heart failure after the first week of embolization. All dogs had pulmonary edema, protein wasting and dyspnea after development of heart failure and ascites was observed in three of seven dogs.

Left ventricular mass averaged 87.7 ± 13.3 g with a corresponding LV–body weight ratio of $3.7\pm0.46\%$. Both of these values are greater than corresponding values from 25 normal dogs (75 ± 13.5 and 3.09 ± 0.5, respectively, $P<0.05$) obtained from historical controls (Wang *et al.*, 1994). In contrast, neither RV mass nor RV mass–body weight ratio were significantly different from normal animals (RV mass: 48.6 ± 7.5 g *v* 45 ± 9.5 g; RV mass/body weight and $2.07\pm0.38\%$ *v* $1.82\pm0.5\%$, respectively).

Remote coronary blood flow at rest

Resting CBF measured in the remote (non-embolized) coronary artery, determined at the beginning of each experiment is shown in Figure 1. Compared to control, CBF in the remote artery increased significantly immediately after the first embolization and remained elevated for at least 1 h (21.3 ± 1.6 control *v* 35.1 ± 4.2 ml/min after 1 h, $P=0.0001$). There was no significant change in any global hemodynamic parameter during this period. After one week of daily coronary embolization the flow was still increased significantly (27.9 ± 3.2 ml/min, $P=0.002$), whereas after 2–3 weeks and with the development of heart failure CBF was decreased significantly (15.3 ± 3.0 ml/min, $P=0.005$).

Table 1 Cardiac and systemic hemodynamics ($n=7$), echocardiographic parameters ($n=5$) and responses to isoproterenol ($n=7$) at various times after commencing microembolization

	Control	6 ± 1 days	18 ± 9 days
LVP (mmHg)	132.6 ± 14.3	119.3 ± 5.9	106.4 ± 7.2*
LVEDP (mmHg)	6.4 ± 1.6	7.9 ± 0.5	16 ± 1.6**
MAP (mmHg)	100 ± 9.8	93.8 ± 11	84.5 ± 7.9*
LAP (mmHg)	5.8 ± 1.3	7.4 ± 0.2	11.2 ± 1.2**
HR (b/min)	88 ± 12	102 ± 22	136 ± 9.5**
LVdP/*dt* (mmHg/s)	3414 ± 794	2955 ± 610	2147 ± 421.5*
AEF (%)	53.6 ± 5.5	42.6 ± 3.8	36.5 ± 5*
ESA (cm^2)	5.33 ± 2.07	8.27 ± 2.77	11.25 ± 2.79*
EDA (cm^2)	11.04 ± 2.8	14.22 ± 4.16	17.62 ± 3.44*
ΔLVdP/*dt* (%)			
0.1 μg/kg Isoproterenol	82.9 ± 22.4	63.2 ± 41.8	38.1 ± 16*
0.5 μg/kg Isoproterenol	139.3 ± 38.2	120.7 ± 79.7	61.1 ± 23.7*

All values are mean $\pm$ S.D., LVP = left ventricular pressure, LVEDP = LV end-diastolic pressure, MAP = mean arterial pressure, LAP = left atrial pressure, HR = heart rate, AEF = area ejection fraction, ESA = end-systolic area, EDA = end-diastolic area (* $P<0.05$, ** $P<0.01$ *v* control).

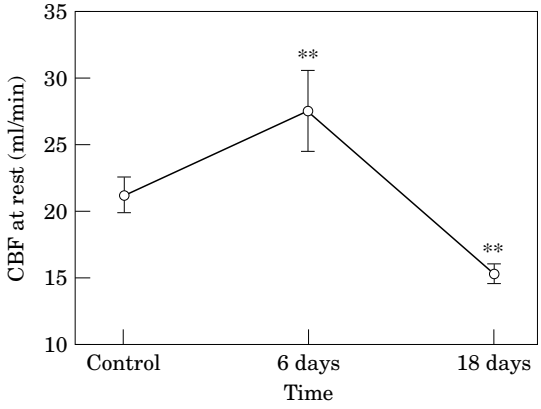


Figure 1 Remote testing coronary blood flow (CBF) at control state, 6 ± 1 days and 18 ± 9 days after the first coronary embolization. Compared to control, resting CBF was significantly increased after 6 days ($P<0.01$) and significantly decreased after 18 days ($P<0.01$).

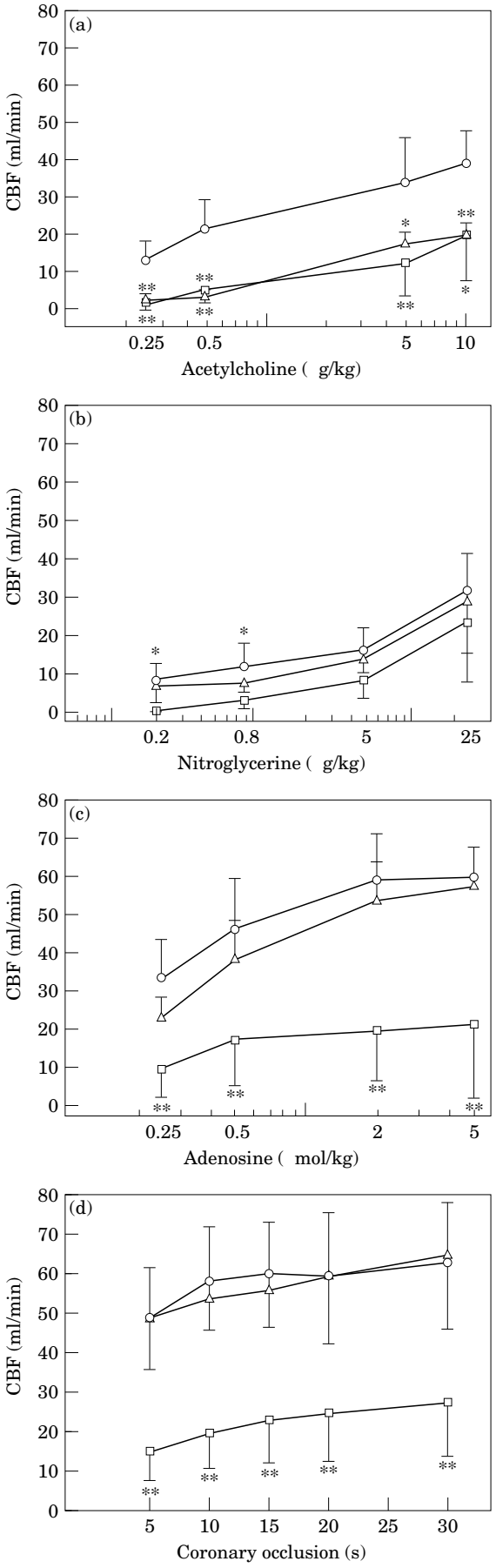
Response of remote coronary blood flow to acetylcholine, nitroglycerine, adenosine, and release of a brief coronary occlusion

The changes in coronary blood flow in response to different doses of acetylcholine, an endothelium-dependent vasodilator, are shown in Figure 2(a). These responses were markedly attenuated after 1 week of embolization, a time point at which there were no changes in systemic hemodynamic or echocardiographic parameters (Table 1). This attenuation of endothelial function persisted after the onset of heart failure and the curves did not differ between the 1 week and the 2–3 week experiments.

There was no significant change in the CBF response to nitroglycerine, an endothelium-independent vasodilator, after 1 week of embolization. With the onset of heart failure after 2–3 weeks there was a trend for the response to nitroglycerine to be attenuated but this reached statistical significance only at the two lower doses studied [Fig. 2(b)].

To assess coronary reserve, the vascular responses to adenosine and release of a brief coronary artery occlusion were investigated. The plateau of the dose–response curve to adenosine was reached with the dose of 2 μmol/kg, which reflects the near maximal vasodilator reserve of the resistance vasculature [Fig. 2(c)]. No changes were observed in this response after 1 week of microembolization. After 2–3 weeks of microembolization the peak responses of CBF were significantly depressed for all doses of adenosine, indicating a depressed coronary reserve after development of heart failure. Figure 2(d) shows the CBF response to brief periods of coronary occlusions of 5, 10, 15, 20 and 30 s at control state, after 1 week and after 2–3 weeks of coronary microembolization. Compared to control, no alterations were observed after 1 week. With the development of heart failure after 2–3 weeks there was a significantly reduced reactive hyperemic response to coronary occlusions.

To determine whether altered systemic hemodynamic responses to these interventions contribute to altered CBF responses during development of heart failure, Table 2 summarizes the effects of these interventions at the highest dose (acetylcholine, nitroglycerin and adenosine) or longest time length (coronary occlusion) on systemic hemodynamics on the control day and at two different time points after coronary embolization. The data show that hemodynamic responses to these interventions were comparable between control day, after 1 week and



after 2–3 weeks of coronary embolization, except the responses of LVdP/dt to nitroglycerine and adenosine. Therefore, altered physiology of coronary circulation cannot be explained by alterations of systemic hemodynamic responses after commencing coronary embolization.

Assessment of absolute and relative changes in CBF

In the analysis presented above vascular properties of the coronary circulation were assessed by determining absolute changes in blood flow in response to various interventions. However, different conclusions could be arrived at if other indices of CBF are examined. Thus, in order to provide an additional assessment and overview of changes in vascular properties of coronary resistance vessels observed during the development of heart failure, the data were also analyzed using different indexes of CBF: absolute values and percent changes from baseline (Fig. 3).

These indexes of changes in CBF are summarized for three of the conditions examined in this study: resting blood flow (CBF_{rest}, ▲), blood flow after the highest dose of acetycholine (10 μg/kg, ○) which provides the maximum flow achievable by endothelium dependent mechanisms (CBF_{endo}), and blood flow following a 30-s occlusion (■) which provides the maximum coronary dilation achievable by any means (CBF_{max}). (a) shows the absolute coronary flow changes 1 week and 2–3 weeks after the first embolization. After 1 week, CBF_{max} was not changed, CBF_{endo} was decreased and CBF_{rest} was increased. After 2–3 weeks and the development of heart failure, CBF_{max}, CBF_{endo} and CBF_{rest} were all attenuated significantly.

When CBF was expressed as coronary flow changes from baseline (b), CBF_{max} remained unchanged whereas the reduction in CBF_{endo} was more pronounced after 1 week of embolization. After the

Figure 2 Responses of coronary blood flow (CBF) to intravenous bolus injections of acetylcholine (a), nitroglycerine (b), adenosine (c) and to release of a brief coronary occlusion (d) at different time point during development of heart failure. After 1 week of daily embolization without heart failure the responses of CBF to all doses of acetylcholine were significantly reduced (* $P<0.05$, ** $P<0.01$ *v* control), despite no changes in responses to nitroglycerine, adenosine or release of a brief coronary occlusion. In heart failure after 2–3 weeks the responses to all interventions except two higher doses of nitroglycerine-induced CBF responses were significantly reduced. (–○–), control; (–△–), 1 week; (–□–), 2–3 weeks.

Table 2 Responses of systemic hemodynamics to acetylcholine (Ach), nitroglycerine (NTG), adenosine (ADO) and release of a brief coronary artery occlusion (OCC) before and after coronary embolization ($n=7$)

	Control		6 ± 1 days		18 ± 9 days	
	Baseline	Response	Baseline	Response	Baseline	Response
Left ventricular systolic pressure (mmHg)						
Ach, 10 μg/kg	132 ± 3.3	109 ± 3.6*	112 ± 5.5	91 ± 6.8*	106 ± 6	84 ± 5.3*
NTG, 25 μg/kg	130 ± 5.1	90 ± 6.3*	110 ± 5.5	79 ± 3.6*	109 ± 5.8	76 ± 5.2*
ADO, 5 μM/kg	138 ± 3.3	112 ± 3*	112 ± 6.9	91 ± 9.8*	108 ± 7.6	85 ± 6.4*
OCC, 30 s	130 ± 5.1	124 ± 4.4	121 ± 5.9	110 ± 8.8	115 ± 5.2	110 ± 10
Left ventricular dP/*dt* (mmHg/s)						
Ach, 10 μg/kg	3378 ± 232	4048 ± 304*	2754 ± 258	3368 ± 403*	2203 ± 291	2703 ± 328*
NTG, 25 μg/kg	3246 ± 225	3821 ± 353*	2581 ± 237	2864 ± 399*,**	2260 ± 233	2584 ± 495*,**
ADO, 5 μM/kg	3176 ± 192	4309 ± 380*	2590 ± 257	3023 ± 368**	2275 ± 367	2638 ± 377**
OCC, 30 s	3021 ± 168	2746 ± 123*	2954 ± 249	2383 ± 285*	2243 ± 274	1700 ± 268*
Mean arterial pressure (mmHg)						
Ach, 10 μg/kg	98 ± 4.8	58 ± 2.4*	90 ± 4.6	50 ± 1.9*	88 ± 3.1	57 ± 2.9*
NTG, 25 μg/kg	99 ± 6	52 ± 4.7*	90 ± 4.4	47 ± 2.8*	88 ± 2.5	55 ± 3*
ADO, 5 μM/kg	100 ± 5.2	65 ± 6.2*	90 ± 4.7	53 ± 7.6*	87 ± 3.2	56 ± 5.1*
OCC, 30 s	94 ± 3.8	92 ± 4.7	94 ± 3.9	87 ± 5.5	88 ± 2.3	74 ± 7.9
Heart rate (bt/min)						
Ach, 10 μg/kg	91 ± 12	162 ± 13*	108 ± 14	171 ± 12*	117 ± 4	172 ± 16*
NTG, 25 μg/kg	86 ± 6	161 ± 13*	104 ± 16	138 ± 4**	119 ± 4	174 ± 15*
ADO, 5 μM/kg	81 ± 4	143 ± 14*	109 ± 13	134 ± 14*,**	118 ± 2	143 ± 13*,**
OCC, 30 s	75 ± 8	75 ± 12	123 ± 14	149 ± 17	132 ± 7	140 ± 11

All values are mean $\pm$ s.d. * $P<0.05$ from baseline, ** $P<0.05$ control.

development of heart failure both CBF_{max} and CBF_{endo} were depressed.

When CBF was expressed as a percentage of baseline flow (c) CBF_{max} was still unchanged after 1 week and CBF_{endo} remained depressed at this time point. The percentage change of CBF_{max} was persistently depressed after 2–3 weeks. However, in view of a decreased CBF_{rest} at this time point, CBF_{endo} was not changed significantly in this form of presentation.

These data therefore reveal, that even when expressed in terms of absolute or relative flow changes, endothelial function was attenuated after 1 week of embolization. After the development of heart failure the maximal achievable flow was depressed as assessed by any parameter and endothelial function remained depressed when CBF was expressed in terms of absolute flow values and in terms of absolute changes in flow.

Discussion

Current understanding of vascular properties in heart failure is largely based on information obtained after the establishment of advanced heart failure. Much less is known about the time course of change of vascular properties relative to the time course of systemic hemodynamic deterioration. The model of ischemic heart failure used in the present study allowed for examination of these relations in a coronary vascular bed that was adjacent to a region of ischemic damage. Thus, changes in vascular properties in this remote vascular bed can be attributed to the combined effects of recruitment of collaterals into the ischemic region and to the effects of heart failure state *per se* (discussed further below). The present study revealed three major findings pertaining to vascular physiology in this remote coronary artery. First, in the early stage of ischemic myocardial damage prior to systemic signs of heart failure, baseline coronary flow in the non-embolized artery was significantly elevated but the vasodilator response to acetylcholine was markedly blunted. Once signs of heart failure were evident, baseline coronary flow decreased significantly and the acetylcholine responsiveness remained equally blunted. Second, maximal coronary flow reserve (assessed by adenosine and the hyperemic response) was normal in the non-embolized artery until there were systemic signs of heart failure, at which point it decreased substantially. Finally, the vasodilator response to nitroglycerine of the non-embolized artery was not altered early in the course of heart failure, but was depressed mildly after the establishment of heart failure.

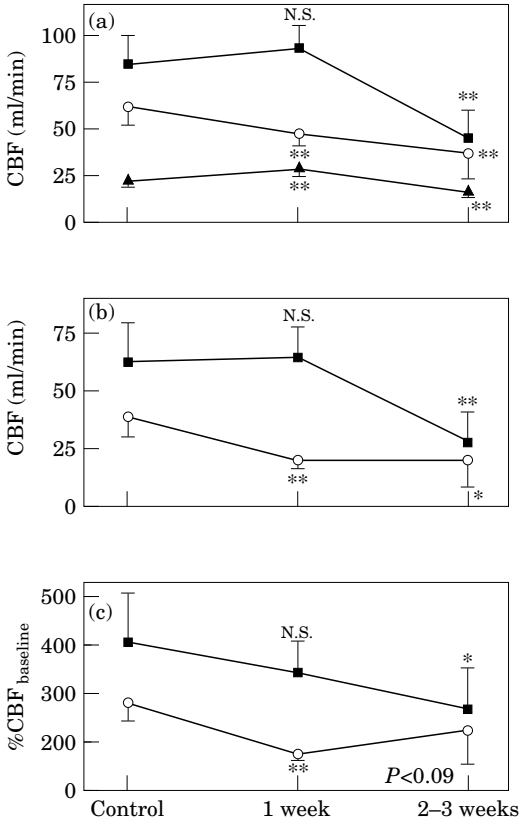


Figure 3 This figure shows absolute and relative coronary blood flow values and changes for resting coronary blood flow (▲), after the longest duration of coronary occlusion (30 s, ■) and after the highest dose of acetylcholine (10 μg/kg, ○). (a) provides the absolute flow changes, (b) shows the delta flow changes and in (c) the percentage flow changes from baseline are shown before, 1 week and 2–3 weeks of coronary microembolization. ** $P<0.01$ from control; * $P<0.05$ from control.

Resting coronary blood flow during the development of heart failure

Remote resting coronary flow increased significantly immediately following the first injection of microspheres and was still elevated significantly after 1 week of daily coronary embolizations. With the development of heart failure however, resting flow decreased significantly. The initial increase of blood flow after the first microspheres injection as well as the sustained elevated flow after 1 week of coronary embolization may reflect compensatory mechanisms. One possible explanation is that by opening and through the creation of new collaterals, the flow increased in an attempt to normalize oxygen supply to the ischemic myocardium. With ongoing daily embolizations this compensatory mechanism was, by design, determined to fail. After the injected microspheres reached a critical number, resting coronary flow diminished, we hypothesize, due to embolization of these collateral vessels, and this coincided with the progression of heart failure.

Previous studies have also revealed an increase in coronary flow in vessels remote to an embolized artery. In an acute, isolated, isovolumic canine heart preparation Monroe *et al.* (1971) studied coronary flow changes after repeated microembolizations of the whole coronary vascular bed. In agreement with our finding, they observed an initial increase in coronary flow after embolization with small plastic microspheres (mean diameter 9.95 μm), which then decreased after a critical number of injected spheres accumulated in the coronary bed. Also, in that study, the enhanced coronary flow after embolization was observed up to 1 hour, and decreased subsequently when the end-diastolic pressure started to increase. Herzberg *et al.* (1966) found in an acute open-chest canine model, that after infusion of microspheres (75–150 μm diameter) there was an increase in coronary flow in the non-embolized vessel when the coronary flow in the embolized artery was reduced to very low levels. Hori *et al.* (1986) investigated acute flow changes in the left anterior descending coronary artery after single and multiple embolizations in open-chest dogs. These investigators observed an initial increase in coronary flow in the embolized vessel with the smallest extent of embolization and were able to show a partial attenuation of this increase after the administration of theophylline. They therefore concluded that adenosine played a role in this hyperemic response but also suggested that other vasoactive substances released by the ischemic area may play a role in the response. Our finding of a chronically elevated coronary flow in the non-embolized artery may also be related to the release of adenosine and other vasoactive substances.

It is noteworthy that data related to coronary flow in the remote coronary artery are only available for acute states. Thus, our results of remote coronary flow changes after weeks of daily coronary embolization in a conscious animal model may help to better elucidate the relationship between resting coronary flow and systemic and ventricular hemodynamics in a chronic states.

Endothelial function during the development of heart failure

Endothelial dysfunction in heart failure has been described previously for several peripheral vascular

beds (Kaiser *et al.*, 1989; Ontkean *et al.*, 1991; Drexler and Lu, 1992) as well as for the coronary circulation (Wang *et al.*, 1994). Wang *et al.* (1994) reported a depressed coronary flow response to acetylcholine, nitroglycerine and coronary occlusion after establishment of severe heart failure by rapid ventricular pacing in dogs. Treasure *et al.* (1990) described an impaired coronary flow response to acetylcholine in patients with dilated cardiomyopathy (mean EF 28%, no coronary artery diseases) compared to controls. These two reports are in agreement with our finding of an attenuated flow response to acetylcholine after the development of heart failure induced by microembolization.

Our findings of a selectively impaired endothelium-dependent flow response to acetylcholine prior to the development of heart failure at a time when resting coronary flow is increased, appears paradoxical. It is well described that an increase in flow leads to an increase in shear stress on the endothelial surface (Pohl *et al.*, 1986; Lamontagne *et al.*, 1992). It has also been described that this increase in shear stress, possibly via stretch-activation of ion channels, is responsible for the release of EDRF (Lansman *et al.*, 1987; Davies, 1989) which then lead to vasodilation (Rubanyi *et al.*, 1986; Treasure *et al.*, 1990). Recently it was demonstrated that chronic exercise, which provides a physiological stimulus for increases in coronary blood flow and shear stress, increased coronary vascular nitric oxide production (Wang *et al.*, 1994). Accordingly, we did not expect to find a significantly decreased vasodilator response to acetylcholine in a state of significantly increased resting coronary flow. However, this finding could potentially be explained by alteration in parasympathetic innervation at this time point, which in turn may lead to a decreased muscarinic receptor density on the vascular endothelium that could explain reduced responsiveness to acetylcholine. In fact, it was demonstrated for a sarcolemmal preparation of overload heart failure in dogs, that muscarinic receptor density was decreased (Vatner *et al.*, 1988). Furthermore, it was described in an experimental model of heart failure in the guinea pig, that parasympathetic innervation as assessed by choline acetyl transferase levels was reduced (Roskoski *et al.*, 1975). Because our model is a myocardial ischemia and infarction-induced heart failure model, it is also possible that this early endothelial dysfunction might be due to the release of substances from the adjacent ischemic myocardium (e.g. free oxygen radicals) which in turn may alter the metabolism and effects of nitric oxide itself (Omar *et al.*, 1991; Mügge *et al.*, 1992). In ischemic heart failure the attenuated coronary blood flow response to acetylcholine might be a response to myocardial infarction and represent an early stage of heart failure.

In contrast to our finding of early endothelial dysfunction before the development of heart failure, Teerlink *et al.* (1993) found in an *in vitro* study no changes in the acetylcholine-mediated vasodilation of aortic rings in rats 1 week after coronary ligation, a time point at which hemodynamic insufficiency was already markedly developed. A decreased endothelial-dependent relaxation in the systemic circulation was evident only at 4–16 weeks after coronary ligation and it was suggested that the observed endothelial dysfunction in heart failure is not merely the direct result of hemodynamic insufficiency. These authors concluded that endothelial dysfunction plays a minor role in early heart failure. However, it is difficult to compare the results of that study directly to our results because, in contrast to our investigation, a different heart failure model was used and *in vitro* measurements were used to assess endothelial function of the systemic and not the coronary circulation. The results of the present report lead to the opposite conclusion, that endothelial dysfunction precedes hemodynamic insufficiency in the coronary bed and indeed may play a role in the early stages of heart failure. Whether our results pertain to other vascular beds, however, has not been determined.

Drexler suggested a reduced stimulated release of NO in response to acetylcholine and an enhanced basal release of NO in heart failure (Drexler *et al.*, 1994), based on the observations of: (1) a blunted flow response to acetylcholine; and (2) the decrease of blood flow induced by NO inhibitor was exaggerated in patients with heart failure (Drexler *et al.*, 1992). Our results in the coronary vasculature show that endothelial dysfunction occurs at a time point when resting coronary flow is increased. An attenuated stimulated release of nitric oxide is demonstrated by the reduced response to acetylcholine, whereas evidence for an increased basal release of nitric oxide is provided indirectly by the increased coronary flow at this time point. Thus a dissociation may already occur before the establishment of heart failure.

The reduced increase of coronary flow in response to nitroglycerine demonstrates that this regulation of coronary blood flow is impaired in our model of heart failure. This observation is consistent with findings in pacing-induced heart failure (Wang *et al.*, 1994). In contrast to our finding, it was described in a rat model of myocardial infarction and heart failure that the vasodilator effect of nitroglycerine on hindquarter resistance vessels were similar for

heart failure and normal rats (Drexler and Lu, 1992). Clinical observations pertaining to peripheral vascular endothelium-independent blood flow response to nitroglycerine in patients with congestive heart failure are also contradictory. Katz *et al.* (1992) demonstrated impaired vasodilation after nitroglycerine administration in the forearm circulation in heart failure patients. Others describe a similar peripheral blood flow responses to nitroglycerine in the forearm circulation of patients with congestive heart failure and normal subjects (Hirooka *et al.*, 1990; Drexler *et al.*, 1992).

Coronary dilator capacity during the development of heart failure

The significantly blunted coronary flow response to adenosine and blunted hyperemic response after the development of heart failure reflects a reduced maximal vasodilator capacity of the coronary microvasculature. This is consistent with observations made in clinical and experimental heart failure. For example, studies in humans showed a reduced coronary vasodilator capacity in response to isoproterenol (Horwitz *et al.*, 1974) or dipyridamole in dilated cardiomyopathy (Opherk *et al.*, 1983; Nitenberg *et al.*, 1985; Cannon *et al.*, 1987) and in human coronary artery disease (Uren *et al.*, 1993).

Reduced coronary blood flow reserve has also been shown in hearts with LV hypertrophy (Wangler *et al.*, 1982), perhaps due to the proposed mechanisms such as histological changes in the coronary microcirculation (Tomanek *et al.*, 1986). This could be the case in the present study because dogs developed LV hypertrophy after heart failure. Another possible mechanism for reduced coronary blood flow responses after heart failure is altered resting hemodynamics, reduced inotropic and chronotropic responses (Klocke, 1987; Klocke *et al.*, 1987; Farhi *et al.*, 1989; Shannon *et al.*, 1993) to interventions used in our study. For instance, Shannon *et al.* (1993) showed a partial restoration of coronary flow response to adenosine by normalizing the increased LV end-diastolic pressure in dogs with pacing-induced heart failure. But, it is unlikely that alterations in reflex (inotropic and chronotropic responses) and cardiac function are the limiting factors for changes in coronary blood flow and reserve after heart failure in our study, because during and after brief coronary artery occlusion, there is no significant reflex inotropic or chronotropic response. Therefore, the reduced coronary dilator capacity after heart failure not only resulted from coronary endothelial dysfunction but also other mechanisms. Nevertheless, these possibilities do not disparage our conclusion of an early coronary endothelial dysfunction during development of heart failure because a selective depression of coronary blood flow response to acetylcholine occurred before the appearance of abnormal hemodynamics, LV hypertrophy or onset of heart failure.

Summary and conclusions

The present report describes changes in vascular properties of the remote coronary circulation during the development of heart failure. However, as noted on several occasions, the underlying mechanisms for the observed changes have not been elucidated. Insights into these might be obtained by blocking the synthesis of NO using, for example, L-NMMA which selectively blocks the formulation of NO from L-arginine (Moncada *et al.*, 1989; Moncada and Higgs, 1993). However, as pointed out above, much controversy exists over how endothelial properties and coronary vascular reserve change in heart failure. Furthermore, no data has previously been presented dealing with the time couse of change in coronary physiology relative to that of the development of heart failure. Accordingly, it was the intent of this initial study to carefully describe and analyze the physiological changes in coronary vascular properties in this microembolization model during the development of heart failure. In this regard, an interesting phenomenon has been identified: there is selective endothelial dysfunction of the coronary vascular bed which occurs as an early pathophysiological alteration during the development of heart failure. The link between this endothelial dysfunction and the progression of heart failure as well as the underlying mechanisms of this phenomenon remain to be defined.

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