ELSEVIER

European Journal of Pharmacology 355 (1998) 167–174

ejp

Impaired endothelium-dependent relaxation in large, but not small arteries in rats after coronary ligation

David L. Prior *, Garry L.R. Jennings, Pamela Arnold, Xiao-Jun Du, Jaye P.F. Chin-Dusting

Alfred and Baker Medical Unit, Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia

Received 15 January 1998; revised 14 May 1998; accepted 3 July 1998

Abstract

Vascular responses were studied in both large and small arteries of rats following 8 weeks of heart failure produced by coronary ligation. Responses to noradrenaline, acetylcholine and sodium nitroprusside were studied in isolated thoracic aorta and mesenteric arteries. In the aorta, concentration–response curves for noradrenaline were similar between heart failure and sham animals and unaffected by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NOARG). Relaxation by acetylcholine was impaired in heart failure rats (EC_{50} -6.79 log M heart failure vs. -7.15 log M sham). In the presence of L-NOARG, relaxation by acetylcholine was completely abolished in rings from sham rats, whereas constriction was observed in rings from heart failure rats. Relaxation by sodium nitroprusside was not different between sham and heart failure rats. In mesenteric arteries, responses to noradrenaline, acetylcholine and sodium nitroprusside were not different between heart failure and sham rats. L-NOARG reduced the maximum response to acetylcholine in both heart failure (82% to 50%) and shams (89% to 49%) by a similar magnitude, with no effect on relaxation to sodium nitroprusside. These data suggest that acetylcholine-induced relaxation is impaired in the aorta, but not mesenteric arteries in rats with heart failure. The mechanism is not solely due to impaired nitric oxide release and may be due to acetylcholine-induced contraction.

Keywords: Heart failure; (Rat); Artery; Endothelium; Nitric oxide (NO); Acetylcholine

1. Introduction

Studies of arterial vasodilator therapy in heart failure showing improved survival have established the importance of the peripheral vasculature in chronic heart failure (Cohn et al., 1986; CONSENSUS Trial Study Group, 1987; SOLVD investigators, 1991). In addition to enhanced vasoconstrictor activity, deficiencies of vasodilation, particularly in arteries to skeletal muscle during exercise (LeJemtel et al., 1986), have been identified in heart failure. It has thus been proposed that both excessive vasoconstrictor activity and deficient vasodilator activity play major roles in both the progression of heart failure and in the poor exercise tolerance that characterises this condition (Katz, 1995).

The role of the endothelium, an important physiological regulator of vascular tone, in heart failure is currently an area of intense research. Impaired vasodilator responses to endothelium-dependent vasodilators such as acetylcholine have been demonstrated both in patients with heart failure (Treasure et al., 1990; Kubo et al., 1991; Katz et al., 1992, 1993; Angus et al., 1993; Inoue et al., 1994; Chin-Dusting et al., 1996) and in a number of animal models of heart failure (Kaiser et al., 1989; Ontkean et al., 1991; Teerlink et al., 1993; Buikema et al., 1993). In contrast, responses to endothelium-independent vasodilators appear to be preserved suggesting that the abnormality is not in the vascular smooth muscle.

Regional blood flow is largely determined by the state of the small resistance vessels whereas large conduit arteries usually play a ‘permissive’ role and dilate in response to increased shear stress to accommodate the increased volume of blood (Anderson and Mark, 1989). In view of the differing roles of conducting arteries and resistance vessels, it may be that the effect of heart failure on dilatation in blood vessels of different size differs in magnitude and mechanisms and in the relationship to disease severity. Endothelium-dependent vasodilator function has been studied in small vessels from a number of

* Corresponding author. Tel.: +61-3-9276-2071; Fax: +61-3-9276-2495.

0014-2999/98/$19.00
PII: S0014-2999(98)00507-X

vascular beds in humans (Treasure et al., 1990; Kubo et al., 1991; Angus et al., 1993; Katz et al., 1993) where it has been found to be diminished and in large (Kaiser et al., 1989; Teerlink et al., 1993) and small arteries (Drexler and Lu, 1992; Mulder et al., 1995) in animal models. The relationship between the changes in large and small vessels within the same animal has not been extensively studied to date.

This study examined the effect of congestive heart failure produced by coronary artery ligation in rats on vascular reactivity and in particular endothelium-dependent vasodilation in the thoracic aorta, a large conduit artery, and in small resistance arteries of the mesenteric circulation. We have also addressed the role of nitric oxide in changes produced by heart failure.

2. Materials and methods

2.1. Coronary artery ligation

Male Sprague–Dawley rats (255–376 g) were randomly selected for either coronary ligation to produce heart failure or a sham operation. The rats were anaesthetised with intraperitoneal methohexitone, pentobarbitone and atropine and a left thoracotomy was performed. The heart was exposed and a silk suture placed around the proximal left coronary artery. In rats randomised to heart failure, the suture was tied securely and in sham operations, the suture was pulled through. The thorax was closed and the rats allowed to recover. The rats were then maintained with free access to standard rat chow and water until experiments were performed at eight weeks following operation, when heart failure is well-developed.

2.2. Haemodynamic measurements

Haemodynamic measurements were made to confirm the presence and severity of heart failure in experimental animals. At termination, rats were anaesthetised with intraperitoneal pentobarbitone (60 mg/kg). A micromanometer tipped catheter (Millar Instruments, Houston, TX, USA) was introduced into the ascending thoracic aorta via the right carotid artery. The catheter was then advanced into the left ventricle and simultaneous recordings of left ventricular pressures and the rate of change in left ventricular pressure (LV dP/dt) were made on a linear chart recorder (Grass Instrument, Quincy, MA, USA). The catheter was then pulled into the ascending aorta, and systemic arterial pressure recorded. Heart rate was calculated from the blood pressure recording and mean arterial pressure calculated from the systolic and diastolic values.

2.3. Tissue preparation

After completion of haemodynamic measurements, the thoracic aorta was removed and placed in aerated physiological buffer solution (PBS). The mesentery and intestine supplied by the superior mesenteric artery was removed and also placed in aerated PBS. The heart and lungs were removed for weighing and determination of infarct size. The lungs were dissected free of the heart at the hilum, blotted dry and both lungs were weighed together. The heart was then dissected free of the great vessels and adjacent tissues. The atria were removed from the ventricles and the right ventricular free wall was dissected away from the left ventricle. After blotting, the ventricles and atria were weighed immediately. All infarcts were transmural and infarct size was determined using a technique described previously (Chien et al., 1988). The left ventricle was incised so that the tissue could be pressed flat. The outlines of the ventricle and the infarcted region for both the endocardial and epicardial surfaces were outlined on a clear plastic sheet which was photocopied to a sheet of paper. The area of the infarcted region relative to the left ventricular area was determined by the weight of the paper within each segment. Infarct size was expressed as a percentage of the left ventricular area and the mean of endocardial and epicardial values given.

Rats with infarct sizes less than 30% of the left ventricular area or without evidence of heart failure as determined by increased left ventricular end-diastolic pressure and increased lung weight were excluded from study.

The use of animals in this study was approved by the Animal Ethics Committee, Baker Medical Research Institute under the guidelines of the National Health and Medical Research Council of Australia.

2.4. Aortic studies

The thoracic aorta was carefully dissected free of adjacent tissues and cut into ring segments of 3 mm in length using a scalpel blade. Care was taken not to damage the endothelium lining the aorta. Each aortic ring was suspended in a 20-ml organ bath containing physiologic buffer solution (composition in mmol/l: NaCl 119, KCl 4.7, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$, 25, $CaCl_2$ 2.5, Glucose 11.0) maintained at 37.0°C and gassed with a mixture of 95% O_2–5% CO_2. The rings were connected to a force transducer (Grass Instrument) and isometric force was recorded on an Apple Macintosh SE using a MacLab data acquisition system (MacLab 8E, Apple Computer, Cupertina, CA). Each ring was stretched to a tension of 5 g at rest and again after an equilibration period of 20 min before pharmacological studies were performed.

Cumulative concentration–response curves were performed to noradrenaline in half log increments (1 nM to 3 μM) in all rings. After washing and allowing each ring to return to its resting tension, the rings were then contracted submaximally using noradrenaline and concentration–response curves performed to acetylcholine (1 nM to 10 μM). Paired rings were then randomly assigned so that one ring of each pair was treated with 30 μM N^G-nitro-L-

arginine (L-NOARG) for 30 min. Cumulative concentration–response curves were then performed to noradrenaline, acetylcholine and sodium nitroprusside (1 nM to 3 μM) in paired vessels with and without L-NOARG. No more than four concentration–response curves were performed on any individual ring.

2.5. Small artery experiments

Paired second or third-order arterial segments were dissected from the superior mesenteric bed and each segment was mounted as a ring preparation on two 40 μm stainless steel wires attached to a force transducer and a micrometer in a myograph (Scientific Concepts, Australia). The vessels were bathed with PBS, the composition of which has been described above. After an equilibration period of 30 min, the artery was stretched at 1 min intervals to determine the passive wall tension-internal circumference relationship (McPherson, 1992). The circumference (L) of the artery at a transmural pressure of 100 mmHg (L_{100}) was then calculated from the Laplace relationship, where $P = T/r$ (P is the transmural pressure, T is wall tension and r is the internal radius). The circumference at $0.9 \times L_{100}$ was determined and the vessel diameter was set at $(0.9 \times L_{100})/\pi$. For contraction, the known length-tension relationship of the vessel was used to calculate the transmural pressure (mmHg) at each agonist concentration in order to compensate for variability of mesenteric artery diameters and segment lengths between animals.

After this normalisation procedure, the arteries were exposed on two occasions to a K^+ buffer solution (KPSS) in which the Na^+ in normal PBS was replaced by K^+ (124 mM) to test the viability of the contractile apparatus in the arterial wall. Twenty minutes later, a full cumulative concentration–response curve was performed to noradrenaline (10 nM to 30 μM). After returning to resting tension, the arteries were submaximally contracted with noradrenaline to a steady level of contraction. A cumulative concentration–response curve to acetylcholine (1 nM to 10 μM) was performed. L-NOARG (30 μM) was then added to one randomly chosen vessel of each pair for 30 min and all subsequent concentration–response curves for that artery were performed in the presence of L-NOARG. Contraction concentration–response curves to noradrenaline and relaxation curves to acetylcholine and sodium nitroprusside (1 nM to 3 μM) were generated.

2.6. Data analysis

Where multiple rings from the same rat were subjected to the same drugs, a mean response was calculated for each drug concentration and this value was used as the response for that rat at that concentration. Relaxation responses to acetylcholine and sodium nitroprusside were expressed as a percentage change from the precontracted level of active force. All concentration–response curves were fitted to a logistic equation $E = MA^p/(A^p + K^p)$ where E is response as a percentage of the maximum response M, A is concentration, K is the concentration of agonist at 50% of maximum response (i.e., EC_{50}) and p is the slope parameter. Whole concentration–response curves were compared by two-way repeated measures analysis of variance (RM-ANOVA) with drug concentration and treatment as the effects. Where significant treatment effects were detected, log EC_{50} values and maximum responses (E_{max}) were compared by analysis of variance (ANOVA) followed by Student's unpaired t-test between groups and a paired t-test between paired vessels from the same rat. Sigmastat statistical software (Jandel Scientific, San Rafael, CA, USA) was used for statistical analysis. The null hypothesis was rejected at the 0.05 level. All values are expressed as mean ± S.E.M.

2.7. Drugs

The following drugs were used: acetylcholine hydrochloride (Sigma Chemical, St. Louis, MO, USA), L-noradrenaline (Sigma), N^G-nitro-L-arginine (Sigma), sodium nitroprusside (Sigma). L-NOARG was diluted in 0.1 M $NaHCO_3$ and all other drugs were diluted in PBS or distilled water. Sodium nitroprusside was dissolved in distilled water at a concentration of 10 mM and frozen in aliquots of 1 ml which were used for daily preparations of dilutions. All other drug solutions were prepared fresh daily and stored at 4°C. Concentrations expressed are the drug concentration present in the organ bath or myograph.

3. Results

3.1. Haemodynamic and baseline characteristics (Table 1)

Fifteen heart failure and 13 sham rats were included in the analysis. Haemodynamic data could not be obtained in two heart failure rats due to reduced tolerance to anaesthetic, but they were included in other experimental data since all other indicators such as heart and lung weight were of severe heart failure. Body weight was not significantly different between groups. Heart weight was 49% greater in the heart failure group with greater weight of the left ventricle, right ventricle and the atria compared to the control animals. Organised thrombus was present in the left atrium in four of the heart failure rats. Lung weight was significantly higher in the heart failure rats consistent with chronic elevation of left atrial pressure. Mean systemic arterial pressure was not significantly different between sham and heart failure rats. Left ventricular end-diastolic pressure was higher and the maximum rate of change of the LV dP/dt was significantly lower in the heart failure group when compared to shams. Mean infarct size in the heart failure group was 37.5 ± 2.2% of the left

Table 1
Cardiac and haemodynamic characteristics of heart failure and sham operated rats

	Sham[a] ($n=13$)	CHF[b] ($n=15$)
Body weight (g)	452 ± 9	456 ± 12
RV[c] weight (g)	0.23 ± 0.01	0.46 ± 0.04[i]
LV[d] weight (g)	0.86 ± 0.03	1.03 ± 0.04[h]
Atrial weight (g)	0.16 ± 0.01	0.37 ± 0.20[i]
Heart weight (g)	1.25 ± 0.04	1.86 ± 0.09[i]
Lung weight (g)	1.46 ± 0.05	2.95 ± 0.26[i]
MAP[e] (mmHg)	91.8 ± 4.7	101.4 ± 4.0
LVEDP[f] (mmHg)	0.5 ± 0.3	12.8 ± 1.4[i]
LV dP/dt_{max}[g] (mmHg/s)	7627 ± 336	5016.7 ± 187[i]
Infarct Size (percent of LV[d] area)	0	37.5 ± 2.2[i]

[a]Sham, sham-operated rats; [b]CHF, rats with heart failure; [c]RV, right ventricle; [d]LV, left ventricle; [e]MAP, mean arterial pressure; [f]LVEDP, left ventricular end-diastolic pressure; [g]LV dP/dt_{max}, maximum rate of change in LV systolic pressure over time. [h]$P < 0.005$, [i]$P < 0.0001$.

ventricular surface area. These data confirm the presence of heart failure in the infarcted rats. (Table 1)

3.2. Large artery responses

Contraction to noradrenaline was not different in the thoracic aortic segments from heart failure and sham operated rats (Table 2). There was a significant rightward shift in the relaxation concentration–response curve to acetylcholine after preconstriction with noradrenaline in the heart failure rats (RM-ANOVA; $P = 0.03$) (Fig. 1). The EC_{50} for acetylcholine was more than twofold greater in the heart failure rats (-6.79 ± 0.07 log M heart failure vs. -7.15 ± 0.13 log M shams; $P < 0.01$). Relaxation in response to sodium nitroprusside was not different between heart failure and sham animals.

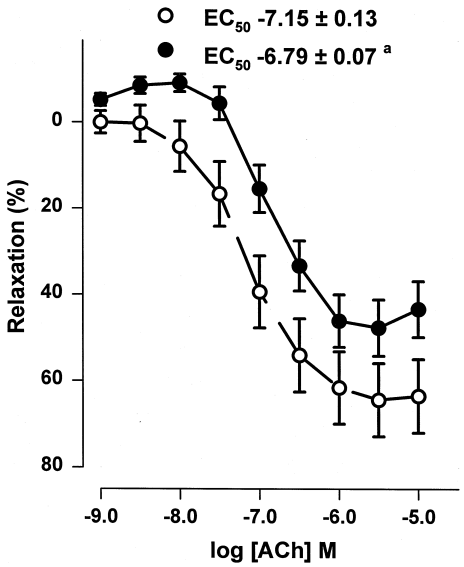

Fig. 1. Relaxation response to acetylcholine in aortic rings from sham (○) and heart failure (•) rats after precontraction with noradrenaline. Points shown are mean values with S.E.M. represented by the error bars. Relaxation to acetylcholine was impaired in the heart failure rats with a rightward shift in the relaxation curve. [a]$P = 0.01$ (sham vs. heart failure).

Table 2
Maximum response (E_{max}) and concentration producing 50% maximum response (EC_{50}) values for contraction to noradrenaline in aorta and mesenteric arteries from heart failure and sham rats in the absence and presence of 30 μM N^G-nitro-L-arginine (L-NOARG)

Group (n)	EC_{50} (log M)	E_{max}
Aorta		
Sham (9)	-7.33 ± 0.16	2.46 ± 0.22 g
Heart failure (15)	-7.47 ± 0.10	2.70 ± 0.26 g
Sham + L-NOARG (9)	-7.50 ± 0.17	2.96 ± 0.24 g
Heart failure + L-NOARG (15)	-7.57 ± 0.09	3.07 ± 0.26 g
ANOVA	NS	NS
Mesenteric artery		
Sham (11)	-5.72 ± 0.07	147 ± 13 mmHg
Heart failure (10)	-5.76 ± 0.06	150 ± 15 mmHg
Sham + L-NOARG (11)	-6.01 ± 0.09	142 ± 12 mmHg
Heart failure + L-NOARG (10)	-5.96 ± 0.08	174 ± 15 mmHg
ANOVA	NS	NS

The values shown are mean ± S.E.M. Two-way repeated measures analysis of variance showed no significant difference between the concentration–contraction curves for noradrenaline in heart failure and sham rats and no effect of addition of 30 μM L-NOARG in the aorta.
Two-way repeated measures analysis of variance showed that responses in mesenteric arteries to noradrenaline were not different between heart failure and sham rats and were unaffected by the addition of 30 μM L-NOARG.

Addition of the nitric oxide synthase inhibitor, L-NOARG, did not alter the concentration–contraction curves to noradrenaline in either the heart failure or the sham rats (Table 2). Relaxation responses to acetylcholine were abolished by L-NOARG in both groups (Fig. 2a). In the heart failure rats, further contraction of the precontracted aortic segments was seen in response to acetylcholine after nitric oxide synthase inhibition with L-NOARG so that even in the absence of nitric oxide production, a significant difference persisted between the heart failure and sham vessels (RM-ANOVA; $P = 0.03$). Relaxation responses to sodium nitroprusside were not affected by L-NOARG treatment (Fig. 2b) indicating normal smooth muscle response to nitric oxide.

3.3. Small artery responses

The diameters of the small mesenteric arteries from rats with heart failure at a tension equivalent to a perfusion pressure of 100 mmHg were comparable to those from the sham operated rats (416 ± 15 μm heart failure; 433 ± 16 μm sham). Contraction to KPSS was also not different between arteries from heart failure and sham rats (148 ± 11 mmHg; 142 ± 7 mmHg). Contraction responses to noradrenaline were not different in small arteries from heart failure and sham rats (Table 2). When relaxation responses to acetylcholine were examined (Fig. 3a), there were no

differences between heart failure and sham animals in the complete dose response curves by RM-ANOVA, in the maximum response ($84.3 \pm 5.28\%$; $90.9 \pm 2.26\%$) nor in the log EC_{50} values (-7.58 ± 0.08 log M, -7.69 ± 0.10 log M).

Nitric oxide synthase inhibition with 30 μM L-NOARG did not alter contraction to noradrenaline in arteries from sham and heart failure rats (Table 2). Nitric oxide synthase inhibition resulted in a reduction in the maximum relaxation produced by acetylcholine in sham vessels from $89.3 \pm 5.2\%$ to $49.3 \pm 9.78\%$ ($P = 0.002$) and in heart failure vessels by a similar amount from 81.6 ± 9.3 to $50.3 \pm 9.9\%$ ($P = 0.02$) (Fig. 3a). There was no change in the concentration producing 50% of the maximum relaxation and relaxation to acetylcholine was not different between heart failure and sham rats after L-NOARG treatment. Relaxation to sodium nitroprusside was identical in mesenteric vessels from heart failure and sham rats and was unaffected by the addition of L-NOARG (Fig. 3b).

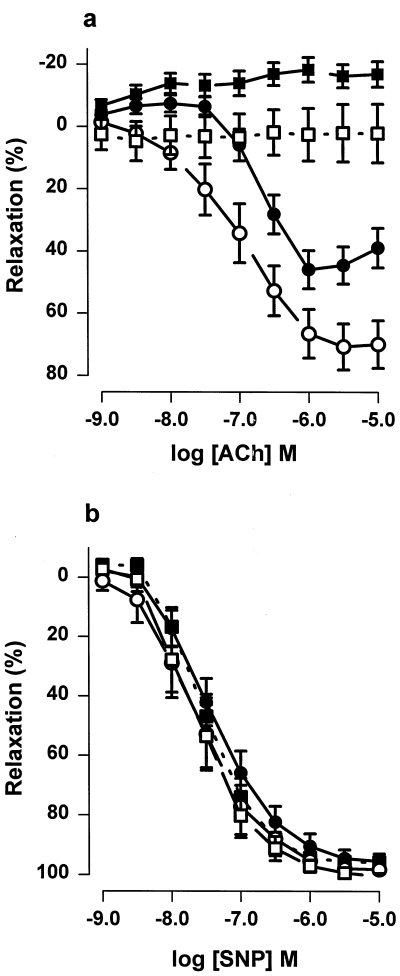

Fig. 2. The effect of 30 μM N^G-nitro-L-arginine (L-NOARG) on (a) relaxation to acetylcholine and (b) relaxation to sodium nitroprusside in paired aortic rings after precontraction with noradrenaline. Responses in sham operated rats in the absence (○) and after 30 min incubation with L-NOARG (□). Responses in rats with heart failure in the absence (•) and in the presence of L-NOARG (■). Points shown are mean values with S.E.M. represented by the error bars. L-NOARG completely abolished relaxation to acetylcholine in both sham and heart failure rats and a significant contraction was seen in heart failure ($P = 0.03$, sham vs. heart failure). Relaxation to sodium nitroprusside was not different between sham and heart failure rats and L-NOARG had no effect on relaxation in either group.

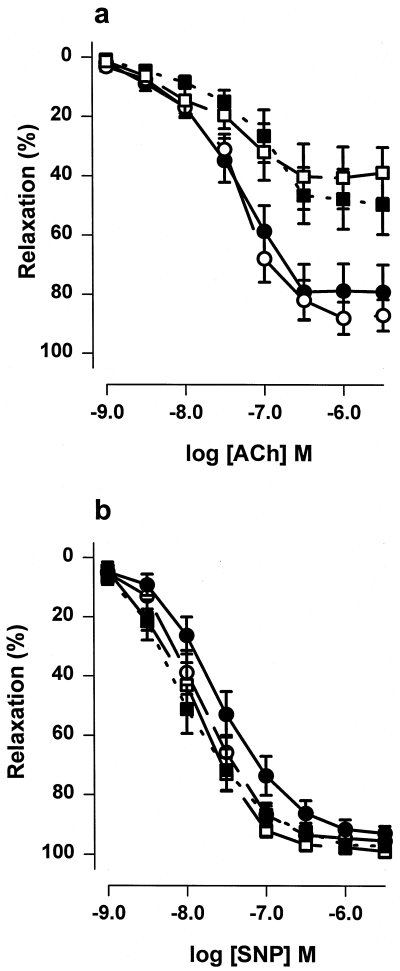

Fig. 3. Relaxation responses to (a) acetylcholine and (b) sodium nitroprusside in mesenteric vessels after precontraction with noradrenaline in sham operated rats (○,□) and rats with heart failure (•,■) in the absence (circles) and the presence (squares) of 30 μM N^G-nitro-L-arginine (L-NOARG). Points shown are mean values with S.E.M. represented by the error bars. Relaxation to acetylcholine was not different in rats with heart failure when compared to sham rats. L-NOARG reduced the maximum response to acetylcholine in both sham and heart failure rats, (two-way ANOVA, $P < 0.001$, with and without L-NOARG), but with no difference between the effect on sham and heart failure rats. Relaxation to sodium nitroprusside was not different between heart failure and sham rats and the addition of L-NOARG did not affect the relaxation response.

4. Discussion

We have demonstrated that heart failure differentially affects responses to the muscarinic agonist, acetylcholine, in a large systemic conduit vessel when compared to a small resistance artery. After 8 weeks of heart failure in rats, relaxation to acetylcholine was diminished in the

descending thoracic aorta, whereas relaxation to the same agonist was intact in the small mesenteric arteries. Relaxation to sodium nitroprusside, which acts directly on vascular smooth muscle through activation of soluble guanylyl cyclase, was normal in both the aorta and mesenteric arteries in heart failure. As vasodilation to acetylcholine in the rat aorta is endothelium-dependent, impaired relaxation to acetylcholine in the setting of normal relaxation to sodium nitroprusside thus suggests a defect localised to the endothelium. Responses to nitric oxide synthase inhibition in the large and small arteries were also different with complete abolition of relaxation in the aorta, but a reduction in maximal relaxation to acetylcholine of approximately 50% in the mesenteric arteries. This finding supports the hypothesis (Wu et al., 1993; Sudhir et al., 1994) that nitric oxide is the major endothelium-derived relaxing factor (EDRF) released by acetylcholine in large arteries whereas nitric oxide accounts for less than half of the response in the smaller vessels. It also demonstrates that there is no difference in non-nitric oxide dependent vasodilation in heart failure. Vasoconstriction in response to noradrenaline was unaffected by heart failure in both the large and small vessels.

The present results differ from those of Noll et al. (1994) who demonstrated enhanced responses to noradrenaline in the aorta after nitric oxide synthase inhibition while observing no difference between acetylcholine responses in both the aorta and mesenteric arteries of cardiomyopathic and control Syrian hamsters. It may be that the differences in the two studies relate to the different experimental heart failure models used or to a difference in the severity of heart failure observed. As no direct haemodynamic measurements were made by Noll et al., it is difficult to comment on the latter.

Although studies by Pfeffer et al. (1979) suggest that significant heart failure with elevation of cardiac filling pressures only occurs in infarcts involving more than 45% of the left ventricle, and the mean infarct size in our group was only 37.5%, we are confident that this group of rats had at least moderate heart failure given firstly, that infarct sizes were measured by the technique of Chien et al. (1988) who showed that significant features of heart failure occurred in rats with infarct sizes greater than 25% when measured by this technique and secondly, that markedly increased lung weight and right ventricular weight in this group of rats are consistent with chronically elevated left atrial and pulmonary artery pressures respectively, both features of significant heart failure.

Our findings agree with those of Baggia et al. (1997) who recently examined endothelium-dependent relaxation in the abdominal aorta, pulmonary artery and mesenteric vessels in the same model of heart failure used in our study in that acetylcholine responses are intact in the small resistance arteries, but differ in that these responses were impaired the thoracic aorta in our study. The question then arises as to why endothelium-dependent vasodilation was intact in the small mesenteric arteries in the setting of impaired vasodilation to acetylcholine in the aorta and further, to what extent these findings are applicable to other resistance arteries. Since abnormalities in resistance vessels associated with heart failure have been demonstrated in other vascular beds including human skin (Angus et al., 1993) and forearm resistance vessels (Kubo et al., 1991; Katz et al., 1992; Hirooka et al., 1992) as well as in the rat hindlimb (Drexler and Lu, 1992) and mesentery (Mulder et al., 1995) it may be that this finding is limited to this particular vessel bed at a particular point in time. In the study of Mulder et al., vascular function was studied after 12 months of heart failure whereas function was examined after only 8 weeks in the current study. That the development of abnormal endothelial function in large vessels in heart failure may be time dependent was explored and confirmed by Teerlink et al. (1993). Thus, a difference in the chronology of the development of endothelial dysfunction may exist between the aorta and the mesenteric arteries in heart failure such that the dysfunction develops more rapidly in the aorta, producing abnormalities after 8 weeks, but developing at a slower rate (such as at 12 months) in the small vessel.

Another possibility is that the mesenteric bed is protected or spared from the stimulus which leads to abnormal function early in the course of chronic heart failure. This would include either changes in regional haemodynamics or one of the many neurohormonal alterations observed with heart failure. Further, the major clinical feature of heart failure is impaired exercise tolerance and while abnormalities of vasodilator function in vessels supplying skeletal muscle have been demonstrated in heart failure (LeJemtel et al., 1986; Muller et al., 1992), the mesenteric bed is not involved in exercise tolerance. Calf blood flow, for example, is reduced to a greater degree than mesenteric blood flow in patients with heart failure (Muller et al., 1992). In a study comparing different models of heart failure (Buikema et al., 1993), it has been suggested that regional haemodynamics may be more important than systemic neurohormonal alterations in the pathogenesis of endothelial dysfunction in heart failure. If this is the case, different vascular beds could be expected to be heterogeneous in the degree of endothelial dysfunction that is seen with abnormalities being more prominent in vessels supplying skeletal muscle than those supplying the gut.

Some of the findings of the current study may allude to a possible mechanism for abnormal vasodilator function which has not previously been described in this model. Inhibition of nitric oxide synthase abolished all relaxation to acetylcholine in the aorta of both sham and heart failure animals and in the latter group, an increase in force was observed with acetylcholine. Constriction to acetylcholine in heart failure after nitric oxide synthase inhibition is consistent with either a direct smooth muscle constrictor action of acetylcholine or release of a vasoconstrictor substance. The rat aorta has not previously been shown to

constrict directly to acetylcholine (Eglen and Whiting, 1990) making the hypothesis of enhanced constriction unlikely. On the other hand, release of an abnormal cyclooxygenase-dependent constrictor from the endothelium has been postulated in canine (Kaiser et al., 1989) and human heart failure (Katz et al., 1993). Our findings are consistent with the hypothesis that acetylcholine causes release of a vasoconstrictor in rats with heart failure as well as nitric oxide thus reducing the overall magnitude of the nitric oxide vasodilator action. It is possible that the use of noradrenaline for preconstriction in our study facilitated this finding. Many other studies demonstrating impaired endothelium-dependent vasodilation in heart failure have used a prostaglandin such as PGF2α for preconstriction and/or have been performed in the presence of indomethacin (Ontkean et al., 1991; Noll et al., 1994; Baggia et al., 1997), possibly masking constriction due to vascular release of a prostanoid constrictor in heart failure by inhibiting its formation or by activating its effector pathway to such a degree that further constriction via this mechanism was not possible.

One limitation of this study is that the only endothelium-dependent vasodilator studied was acetylcholine and therefore relaxation mediated via muscarinic stimulation of the endothelium was the only pathway studied. Endothelium-dependent relaxation can be stimulated by a variety of agonists, which act via non-cholinergic mechanisms to produce endothelium-dependent relaxation. It is therefore possible that the effects of heart failure on non-cholinergic, endothelium-dependent vasodilation are different from those that we observed using acetylcholine and that the defect is specific to acetylcholine.

In summary, this study demonstrates that diminished acetylcholine-induced relaxation in the rat aorta is present 8 weeks after development of heart failure whereas the small mesenteric artery relaxes normally. This difference may be due a difference in the time-course of the development of vasodilator abnormalities between the large conduit vessel and the smaller resistance arteries or a difference in regional haemodynamics producing heterogeneous changes in this phase of heart failure. Further studies are required to confirm whether release of a vasoconstrictor substance in response to cholinergic stimuli in addition to a reduction in EDRF release is an important mechanism of impaired relaxation to acetylcholine in the rat aorta in heart failure.

Acknowledgements

Dr. David Prior is the recipient of a National Health and Medical Research Council Medical Postgraduate Research Scholarship and an Alfred Healthcare Foundation Postgraduate Research Scholarship. This work was supported by the National Health and Medical Research Council Institute Grant to the Baker Medical Research Institute.

References

Anderson, E.A., Mark, A.L., 1989. Flow-mediated and reflex changes in large peripheral artery tone in humans. Circulation 79, 93–100.

Angus, J.A., Ferrier, C.P., Sudhir, K., Kaye, D.M., Jennings, G.L., 1993. Impaired contraction and relaxation in skin resistance arteries from patients with congestive heart failure. Cardiovasc. Res. 27, 204–210.

Baggia, S., Perkins, K., Greenberg, B., 1997. Endothelium-dependent relaxation is not uniformly impaired in chronic heart failure. J. Cardiovasc. Pharmacol. 29, 389–396.

Buikema, H., van Gilst, W.H., van Veldhuisen, D.J., de Smet, B.J.G.L., Scholtens, E., Lie, K.I., Wessling, H., 1993. Endothelium dependent relaxation in two different models of chronic heart failure and the effect of ibopamine. Cardiovasc. Res. 27, 2118–2124.

Chien, Y.W., Barbee, R.W., MacPhee, A.A., Frohlich, E.D., Trippodo, N.C., 1988. Increased ANF secretion after volume expansion is preserved in rats with heart failure. Am. J. Physiol. 254, R185–R191.

Chin-Dusting, J.P., Kaye, D.M., Lefkovits, J., Wong, J., Bergin, P., Jennings, G.L., 1996. Dietary supplementation with L-arginine fails to restore endothelial function in forearm resistance arteries of patients with severe heart failure. J. Am. Coll. Cardiol. 27, 1207–1213.

Cohn, J.N., Archibald, D.G., Zeische, S. et al., 1986. Effect of vasodilator therapy on mortality in chronic congestive heart failure: results of a Veterans Administration cooperative study. N. Engl. J. Med. 314, 1547–1552.

CONSENSUS Trial Study Group, 1987. The consensus trial study group. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N. Engl. J. Med. 316, 1429–1435.

Drexler, H., Lu, W., 1992. Endothelial dysfunction of hindquarter resistance vessels in experimental heart failure. Am. J. Physiol. 262, H1640–H1645.

Eglen, R.M., Whiting, R.L., 1990. Heterogeneity of vascular muscarinic receptors. J. Auton. Pharmacol. 19, 233–245.

Hirooka, Y., Imaizumi, T., Harada, S., Masaki, H., Momohara, M., Tagawa, T., Takeshita, A., 1992. Endothelium-dependent forearm vasodilation to acetylcholine but not to substance P is impaired in patients with heart failure. J. Cardiovasc. Pharmacol. 20, S221–S225.

Inoue, T., Sakai, Y., Morooka, S., Hayashi, T., Takanagi, K., Yamaguchi, H., Kakoi, H., Takabatake, Y., 1994. Vasodilatory capacity of coronary resistance vessels in dilated cardiomyopathy. Am. Heart J. 127, 376–381.

Kaiser, L., Spickard, R.C., Olivier, N.B., 1989. Heart failure depresses endothelium-dependent responses in canine femoral artery. Am. J. Physiol. 256, H962–967.

Katz, S.D., 1995. The role of endothelium-derived vasoactive substances in the pathophysiology of exercise intolerance in patients with congestive heart failure. Prog. Cardiovasc. Dis. 38, 23–50.

Katz, S.D., Biasucci, L., Sabba, C., Strom, J.A., Jondeau, G., Galvao, M., Solomon, S., Nikolic, S.D., Forman, R., LeJemtel, T.H., 1992. Impaired endothelium-mediated vasodilation in the peripheral vasculature of patients with congestive heart failure. J. Am. Coll. Cardiol. 19, 918–925.

Katz, S.D., Schwarz, M., Yuen, J., LeJemtel, T.H., 1993. Impaired acetylcholine-mediated vasodilation in patients with congestive heart failure. Role of endothelium-derived vasodilating and vasoconstricting factors. Circulation 88, 55–61.

Kubo, S.H., Rector, T.S., Bank, A.J., Williams, R.E., Heifetz, S.M., 1991. Endothelium-dependent vasodilation is attenuated in patients with heart failure. Circulation 84, 1589–1596.

LeJemtel, T.H., Maskin, C.S., Lucido, D., Chadwick, B.J., 1986. Failure to augment maximal limb blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. Circulation 74, 245–251.

McPherson, G.A., 1992. Assessing vascular reactivity of arteries in the small vessel myograph. Clin. Exp. Pharm. Physiol. 19, 815–825.

Mulder, P., Devaux, B., Fertak, L.E., Compagnon, P., Richard, V.,

Henry, J.-P., Scalbert, E., Desché, P., Macé, B., Thuillez, C., 1995. Vascular and myocardial protective effects of converting enzyme inhibition in experimental heart failure. Am. J. Cardiol. 76, 28E–33E.

Muller, A.F., Batin, P., Evans, S., Hawkins, M., Cowley, A.J., 1992. Regional blood flow in chronic heart failure: the reason for the lack of correlation between patients' exercise tolerance and cardiac output?. Br. Heart J. 67, 478–481.

Noll, G., Tschudi, M.R., Novosel, D., Lüscher, T.F., 1994. Activity of the L-arginine/nitric oxide pathway and endothelin-1 in experimental heart failure. J. Cardiovasc. Pharmacol. 23, 916–921.

Ontkean, M., Gay, R., Greenberg, B., 1991. Diminished endothelium-derived relaxing factor activity in an experimental model of chronic heart failure. Circ. Res. 69, 1088–1096.

Pfeffer, M.A., Pfeffer, J.M., Fishbein, M.C., Fletcher, P.J., Spardaro, J., Kloner, R.A., Braunwald, E., 1979. Myocardial infarct size and ventricular function in rats. Circ. Res. 44, 503–512.

SOLVD investigators, 1991. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N. Engl. J. Med. 325, 293–302.

Sudhir, K., MacGregor, J.S., Amidon, T.M., Gupta, M., Yock, P.G., Chatterjee, K., 1994. Differential contribution of nitric oxide to regulation of vascular tone in coronary conductance and resistance arteries: intravascular ultrasound studies. Am. Heart J. 127, 858–865.

Teerlink, J.R., Clozel, M., Fischli, W., Clozel, J.-P., 1993. Temporal evolution of endothelial dysfunction in a rat model of heart failure. J. Am. Coll. Cardiol. 22, 615–620.

Treasure, C.B., Vita, J.A., Cox, D.A., Fish, R.D., Gordon, J.B., Mudge, G.H., Colucci, W.S., Sutton, M.G., Selwyn, A.P., Alexander, R.W., 1990. Endothelium-dependent dilation of the coronary microvasculature is impaired in dilated cardiomyopathy. Circulation 81, 772–779.

Wu, C.C., Chen, S.J., Yen, M.H., 1993. Different responses to acetylcholine in the presence of nitric oxide inhibitor in rat aortae and mesenteric arteries. Clin. Exp. Pharm. Physiol. 20, 405–412.