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Noradrenaline-induced increases in calcium and tension in skeletal muscle conductance and resistance arteries from rats with post-infarction heart failure

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Abstract

We tested the hypothesis that arterial reactivity to noradrenaline is augmented in congestive heart failure (CHF), which could contribute to the deleterious changes in peripheral vascular resistance and compliance in this condition. From male Wistar rats with post-infarction CHF and sham-operated rats, skeletal muscle conductance and resistance arteries (mean lumen diameters: 514 and 186 μm) were isolated and mounted on wire myographs, and wall tension was recorded in response to cumulative application of acetylcholine and noradrenaline to the vessel segments. In a subset of experiments, wall tension and cytosolic free calcium ion concentration $[Ca^{2+}]_i$ were recorded simultaneously during noradrenaline application, using wire myography and the FURA-2 technique. No significant differences were found in the arterial baseline levels of $[Ca^{2+}]_i$ or tension between CHF and sham rats. In the resistance arteries of CHF rats, the noradrenaline-induced increases in $[Ca^{2+}]_i$ were significantly enhanced ($P=0.003$). Despite the augmented $[Ca^{2+}]_i$ levels, the tension responses to noradrenaline were unaltered in these arteries. In the conductance arteries, there were no significant differences in noradrenaline-induced $[Ca^{2+}]_i$ or tension responses between CHF and control rats. CHF did not alter vascular morphology or change vascular relaxations to acetylcholine in either type of artery. In conclusion, these results do not support the contention that arterial reactivity to noradrenaline is augmented in the skeletal muscle vascular bed in CHF. On the contrary, the unchanged contractile responsiveness in the resistance arteries despite the enhanced levels of $[Ca^{2+}]_i$ during noradrenaline application suggests that the contractile function of these vessels is compromised in CHF. Neither vascular remodeling, endothelial dysfunction nor changes in baseline vascular tone could be demonstrated in the skeletal muscle vascular bed of this animal model of heart failure.

Keywords: Noradrenaline; Acetylcholine; Cytosolic calcium; Artery; Heart failure; Infarction

1. Introduction

Congestive heart failure (CHF) is a syndrome characterised by compromised myocardial function and reduced cardiac output. Compensatory activation of neurohumoral systems including the renin–angiotensin–aldosterone, the vasopressin and the sympathetic nervous systems (Cohn et al., 1984) aims at maintaining blood perfusion of vital organs. However, the increased peripheral vascular resistance, which is one of the effects of this neurohormonal activation, is detrimental in the chronic state of CHF.

Resistance arteries (approximately 50–250 μm in diameter) are responsible for the major part of precapillary vascular resistance, since up to 50% or more of the peripheral resistance appears to lie proximal to vessels with diameters of 100 μm. In contrast, the larger conductance arteries (>500 μm in diameter) account for only a small part of the peripheral vascular

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resistance (Mulvany and Aalkjaer, 1990). However, the passive and active properties of both types of arteries have an important influence on left ventricular function: decreased compliance of conductance arteries and increased tone of resistance vessels increase left ventricular outflow resistance and reduce stroke volume, and more so when the contractile function of the myocardium is depressed (Maruyama et al., 1993). Structural and functional factors, which reduce arterial compliance and increase peripheral vascular resistance, have a negative influence on the heart failure state and disease progression (Cohn et al., 1984; Mitchell et al., 2001; Zelis and Flaim, 1982). The impact of CHF on vascular resistance and blood flow is specific to each organ or vascular bed, both in patients (Leithe et al., 1984; Zelis et al., 1975) and animal models (Drexler et al., 1987; Musch and Terrell, 1992). Sympathetic vasomotor drive is mediated via release of noradrenaline from postganglionic nerve endings in the arterial wall. Active arterial constriction is based on contraction of vascular smooth muscle cells, circumferentially arranged in the arterial wall. The intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is a key determinator of vascular smooth muscle cell force production . Studies of mostly non-skeletal-muscle resistance or conductance arteries from patients and animal models (rat, dog) have indicated that the arterial contractile response to noradrenaline is altered in CHF, with the various vascular beds being differentially affected (Indolfi et al., 1994; Stassen et al., 1997a). However, the changes in arterial vascular smooth muscle cell $[Ca^{2+}]_i$ associated with excitation–contraction coupling during stimulation with noradrenaline have not been studied in this condition.

The endothelium plays an important role in the regulation of vascular tone, through release of relaxing and constrictor substances to the underlying vascular smooth muscle cells (Mombouli and Vanhoutte, 1999). Endothelium-derived relaxation factors include nitric oxide, prostaglandin I_2 and E_2 and endothelium-derived hyperpolarising factor. These factors are released in response to shear stress and stimulation with mediators such as acetylcholine, histamine and bradykinin. Endothelium-derived constrictor factors include prostaglandin $F_{2\alpha}$, endothelin-1 and thromboxane A_2. The influence of the endothelium on vascular tone is determined by the balance between these relaxing and constrictor substances.

In this study, we tested the hypothesis that the contractile response to noradrenaline is augmented in the skeletal muscle vascular bed in CHF. We also investigated the changes in arterial vascular smooth muscle cell $[Ca^{2+}]_i$ levels associated with noradrenaline-induced tension development. We used the post-infarction rat model of CHF, which is considered to be a useful animal model in the study of the pathophysiology in this condition (Pfeffer et al., 1979; Drexler et al., 1987), with sham-operated rats serving as controls. Responsiveness to noradrenaline and acetylcholine was measured in isolated femoral resistance and conductance arteries from the skeletal muscle vascular bed, using dual-wire isometric myography. In vessels from some of the animals, we used the combination of dual-wire isometric myography and the FURA-2 calcium fluorescence technique, enabling simultaneous real-time recordings of isometric tension development and $[Ca^{2+}]_i$ during noradrenaline application.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–350 g) with free access to standard rat chow and drinking water were randomised for either coronary artery ligation or sham surgery. The investigation was approved by the Danish Council for Animal Research, and conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Animal preparation

The rats were anaesthetised with a subcutaneous (s.c.) injection of Hypnorm® (fentanyl citrate and fluanisone, Janssen Animal Health) and Dormicum® (midazolam, Roche), containing fentanyl citrate (0.16 mg/kg), fluanisone (5.0 mg/kg) and midazolam (2.5 mg/kg). The animals were then intubated with a catheter (1.6–1.8 mm) and ventilated with 100% oxygen (70 strokes/min, tidal volume 4 ml). Through a left anterior thoracotomy, the left anterior descending coronary artery was ligated. In a modification of the original procedure (Fishbein et al., 1978), we then expanded the myocardial infarction laterally by placing another suture superficially through the myocardium from the primary suture to the basis of the left atrial appendage. The chest cavity was emptied of blood and air through a polyethylene tube after suturing of the thoracic wall. Control rats were subjected to the same procedure without ligation. The animals were postoperatively pain relieved with s.c. injections of buprenorphine (Temgesic®, Schering-Plough) 0.20 mg/kg in 10 ml isotonic saline solution, and ventilated with pure oxygen until strong, spontaneous respiration was re-established.

2.3. Haemodynamic measurements

Eight weeks after myocardial infarction, the animals were anaesthetised with isofluorane–N_2O. A micro-tip transducer (Millar®, model SPR-671, 1.4 F) coupled to a PowerLab® system was inserted through the right carotid artery into the thoracic aorta for measurements of arterial blood pressure and heart rate, and then advanced into the left ventricle for ECG-synchronised recordings of left ventricular end-diastolic pressure and maximum rates of isovolumetric pressure development ($+dP/dt$) and decay ($-dP/dt$). Only rats with left ventricular end-diastolic pressure > 20 mmHg were included in the study. For later collection of blood samples, a Tygon® catheter was inserted into the thoracic aorta, tunnelled subcutaneously to the back of the neck, filled with a glucose solution (50 g/l) containing heparin (250 i.e./ml) and plugged with nylon.

2.4. Plasma catecholamines

Plasma catecholamines were measured in the rats used in the FURA-2 experiments. One week after haemodynamic measurements, blood samples were drawn from the chronically implanted arterial catheter between 9 and 10 A.M. The nylon plug was removed from the catheter, and blood samples were drawn from the unrestrained, resting rats into chilled tubes containing gluthathione (6.5 mM) and EDTA (6.7 mM). The plasma was separated by centrifugation (1.000 ×*g*, 5 min., 4 °C), frozen in liquid nitrogen and stored at −80 °C for later determination of catecholamine concentrations by a single isotope radio enzyme method (Ben Jonathan and Porter, 1976).

2.5. Measurement of infarct size

After blood sampling, the rats were sacrificed by crushing of the cervical medulla and exsanguination. The left ventricle was fixed in a 6% formaline solution and cut in 1.5 mm thick transverse segments from the apex to the basis. From the midventricular segment containing the papillary muscles, a 10 μm slice was stained with a combined orcein–alcian and Van Gieson–Hansen dye for maximum contrast between the infarcted and non-infarcted areas of the left ventricle. Using computerised planometry (Image Compact® software, Matrix Vision Image) the percentage of the total endocardial circumference consisting transmurally of fibrotic tissue was determined (Nelissen-Vrancken et al., 1998).

2.6. Preparation and mounting of arteries

From the femoral artery, second order side branches (conductance arteries) and constant third order muscle side branches (resistance arteries) were isolated. The arteries were cleaned of connective tissue and placed in ice-cold physiological saline solution (PSS, composition: see below). Segments of the arteries (1.5–2.0 mm long) were mounted onto two stainless wires on a small vessel Mulvany–Halpern myograph (Danish Myo Technology A/S, Aarhus, Denmark) as previously described (Mulvany and Nyborg, 1980). In some of the resistance arteries the endothelium was removed, using a human scalp hair.

The vessel segments were suspended in an organ chamber containing PSS, and aerated with 5% CO_2 in 95% O_2 at a pH of 7.4 and a temperature of 37 °C. The myograph used for measurements of $[Ca^{2+}]_i$, had a quartz window inset in the bottom and the myograph was placed on the stage of an inverting fluorescence microscope (Leica DMIRB).

2.7. Baseline tensions and reference contractions

After mounting of the arterial segments, a 30-min equilibration period was allowed before vessel segments were normalised, i.e. stretched to an internal circumference L_1, equal to 90% of the circumference L_{100}, which the vessel would have if exposed to a transmural pressure of 100 mmHg (13.3 kPa). This was done to obtain standardised conditions for tension development (Mulvany and Halpern, 1977). The corresponding normalised internal vessel diameter, l_1, was calculated as L_1/π, assuming a circular lumen.

After normalisation, all vessels were challenged six times with PSS containing 125 mM K^+ (KPSS, composition: see below). The last contraction was used as a reference for the tension development during application of noradrenaline. Vessels were accepted only if the maximum active pressure (ΔP_{max}, calculated according to the Laplace relation: $\Delta P_{max} = 2 \times$ maximum tension development$/l_1$) exceeded 13.3 kPa (Mulvany and Halpern, 1977)).

2.8. Acetylcholine concentration–relaxation curves

Experimental Protocol 1: After the last contraction with KPSS, the vessels were thoroughly washed with PSS. The arteries were then precontracted with 3×10^{-6} M prostaglandin $F_{2\alpha}$. In preliminary experiments, this concentration of prostaglandin $F_{2\alpha}$ had been shown to elicit a contraction of approximately 65% of the reference contraction with KPSS in both types of arteries. At a stable level of contraction, cumulative concentration–relaxation curves were constructed by adding acetylcholine (10^{-10} to 3×10^{-5} M, 12 steps) to the organ bath. Only vessel segments with a maximum relaxation of >50% were included in the study, to ensure a fully intact endothelium. This criterion led to the exclusion of less than 3% of the arterial segments from CHF rats with endothelial function.

2.9. Noradrenaline concentration–tension curves

Experimental Protocol 2: Following acetylcholine challenges, vessel wall tension responses were measured during cumulative application of noradrenaline to the organ chamber (10^{-9} to 3×10^{-4} M, 12 steps). Before application of noradrenaline was commenced, the arteries were incubated for 25 min either with vehicle (Protocol 2a), with 4 μM propranolol to elucidate the effects of β-adrenergic activation by noradrenaline on the noradrenaline concentration–tension curves (Protocol 2b) or with 4 μM cocaine+4 μM normetanephrine to elucidate the effects of neuronal and non-neuronal uptake of noradrenaline on the noradrenaline concentration–tension curves (Protocol 2c). In the resistance arteries where the endothelium had been removed, noradrenaline reactivity was examined as according to Protocol 2a.

2.10. Measurements of $[Ca^{2+}]_i$ and tension during cumulative application of noradrenaline

Experimental Protocol 3: In this protocol, the arterial segments were contracted 6 times with KPSS (3 times before and 3 times after loading with FURA-2, see below), with the last contraction serving as reference. The presence of an intact endothelium in the vessel segments was ascertained by application of 10 μM acetylcholine (Furchgott and Zawadzki, 1980) at a stable level of precontraction with 10 μM

prostaglandin $F_{2\alpha}$. After a thorough washout in PSS, $[Ca^{2+}]_i$ and tension responses were measured simultaneously during cumulative application of noradrenaline to the organ chamber as described in Protocol 2.

2.11. FURA-2 loading and measurement of $[Ca^{2+}]_i$

The vessel segments were loaded with 10 μM FURA-2 acetoxymethyl ester (Fura-2 AM) for two consecutive periods of 45 min. A dual-excitation wavelength DeltaScan® (Photon Technology Inc., USA) was used for alternating excitation of the vessels at 340 and 380 nm. The emitted light was passed through filters (500–530 nm), detected by a photomultiplier and recorded by a Pentium computer using the Felix® software package (PTI). The sampling rate was set to 5 measurements per second.

$[Ca^{2+}]_i$ was estimated by applying the following equation: $[Ca^{2+}]_i = K_d \times \beta \times [(R - R_{min})/(R_{max} - R)]$, where K_d is the dissociation constant of the FURA-2–Ca^{2+} complex at 37 °C (assumed to be 224 nM) and β is the ratio between fluorescence at 380 nm under Ca^{2+}-free and Ca^{2+}-saturated conditions, respectively (Grynkiewicz et al., 1985). R is the ratio between background-subtracted emission signals (F_{340}-background)/(F_{380}-background). R_{min} and R_{max} are the ratios between background-subtracted emission signals under Ca^{2+}-free and Ca^{2+}-saturated conditions, respectively. R_{min} and R_{max} were determined in each vessel at the end of the experiment by adding 40 μM ionomycin in calcium-free buffer solution (composition: see below) and by using buffer-solution containing 5 mM Ca^{2+} (composition: see below), respectively (Jensen et al., 1992). Background fluorescence signals were obtained by quenching the calcium sensitive FURA-2 fluorescence with 20 mM Mn^{2+} at the end of each experiment (Roe et al., 1990; Jensen et al., 1992). The mean values of R_{max}, R_{min} and β were 2.81±0.02, 1.19±0.01 and 1.30±0.04 (n=20). No differences detected between experimental groups (two-factor ANOVA: all P>0.05).

2.12. Vascular morphology

Vascular morphology was measured in the vessels used for FURA-2 experiments. The arterial segments were fixed in a 6% formaline solution, embedded in paraffin and transversely cut into 2.5 μm thin ring segments. These slices were then stained with a combined orcein–alcian and Van Gieson–Hansen dye. Using computerised planometry (Image Compact® software, Matrix Vision Image), the media cross-sectional area was calculated by subtracting the area enclosed by the internal elastic layer from the area enclosed by the border between the external elastic layer and the media. Lumen cross-sectional area, assuming a circular lumen, was calculated as $\pi(l_1)^2$, where l_1 is the lumen diameter determined during normalisation. Media thickness could then be calculated as — $l_1/2$+the square root of ((media cross-sectional area/π+$(l_1/2)^2$) (Stassen et al., 1997a), assuming a circular lumen and a uniform media layer. The media-to-lumen ratio was calculated as the ratio between media cross-sectional area and the lumen cross-sectional area, and is given in per cent.

2.13. Drugs and solutions

PSS had the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.5, $MgSO_4$ 1.17, $NaHCO_3$ 25.0, KH_2PO_4 1.18, ethylene–diamine–tetraacetic acid (EDTA) 0.026, and glucose 5.5. KPSS, 125 mM K^+, was prepared by replacing NaCl with equimolar KCl.

The solutions for determination of R_{max} and R_{min} had the following composition (mM): 4-(2-hydroxyethyl)-1-pipirazineethan (HEPES) 5.0, KCl 125.0, $MgCl_2$ 1.17, and glucose 5.5, and then either 5 mM $CaCl_2 \cdot 2H_2O$ (for determination of R_{max}) or 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′,-tetraacetic acid (EGTA) (for determination of R_{min}) was added. Fura-2 AM was dissolved in a loading mixture of anhydrous dimethylsulphoxide (DSMO), pluronic F-127 and cremophor just before loading (Sheykhzade and Berg Nyborg, 2001).

Drugs used were: noradrenaline HCl, prostaglandin $F_{2\alpha}$ Tris salt, acetylcholine chloride, cocaine HCL, (±) propranolol HCL, DL-normetanephrine HCL, ionomycin, pluronic F-127, cremophor EL (Sigma-Aldrich, St. Louis, MO, USA) and Fura-2 AM (Molecular Probes, Leiden, The Netherlands). Stock solutions were kept at −20 °C and dilutions were made just prior to experimentations.

2.14. Data analysis

The mechanical response of the vessels to PSS and KPSS is expressed as active wall tension T (Newton per meter of vessel wall, N/m). The resting tension in PSS and noradrenaline-induced tension development are expressed as percentage of the steady-state reference response to KPSS. The tensions in calcium-free buffer and KPSS were designated to be 0% and 100%, respectively. Similarly, relaxation to acetylcholine is expressed as per cent reduction of the tension, where the precontraction level with prostaglandin $F_{2\alpha}$ was set to 100% and the tension in Ca^{2+}-free buffer was set to 0%.

The concentration–response curves were fitted to Hill's equation: $E/E_{max} = [A]^n/([A]^n + EC_{50}^n)$ by non-linear regression analysis, using the GraphPad Prism® 4.0 software (GraphPad Corp., San Diego, CA, USA). E/E_{max} is the relative vessel response to the agonist concentration; A is the molar concentration of the agonist. EC_{50} is the molar concentration of agonist required to elicit half maximum response, and n (the Hill's coefficient) is a fitting constant (Kenakin, 1997). Sensitivity to noradrenaline and Ca^{2+} is expressed in terms of pD_2 values, where $pD_2 = -\log(EC_{50})$.

Sigmastat Statistical Software® (SPSS Inc.) was used for all statistical evaluation of data. Single parameters from two experimental groups were compared by two-tailed Student's t-tests. Single parameters from all four experimental groups, i.e. conductance and resistance arteries from CHF and sham rats, were compared using two-factor analysis of variance (ANOVA) to ascertain the effects of CHF in both types of arteries. Bonferroni-corrected Student's t-tests were used for post hoc analysis between relevant groups, when a

Table 1
Organometric and haemodynamic characteristics

	Control rats	CHF rats	Statistics
	$n=24$	$n=19$	Student's t-test
Organometric measurements			
Body weight (BW) at infarction (g)	306±7	301±7	NS
Weight gain until experiments (g/day)	1.87±0.13	1.84±0.18	NS
Infarction size (% of left ventricle circumference)	–	50.1±1.9	–
Right ventricle weight (mg/g BW)	0.47±0.01	1.13±0.07	$P<0.001$
Lung weight (mg/g BW)	3.06±0.05	8.64 ± 0.50	$P<0.001$
Left ventricle weight (mg/g BW)	1.75±0.03	1.90±0.05	$P<0.01$
Haemodynamic measurements			
Left ventricular end-diastolic pressure (mmHg)	7.2±0.8	31.6±1.8	$P<0.001$
$+dP/dt$ (mmHg/ms)	12.0±0.6	6.4±0.3	$P<0.001$
$-dP/dt$ (mmHg/ms)	13.1±0.7	4.5±0.2	$P<0.001$
Mean arterial pressure (mmHg)	122±4	96±3	$P<0.001$
Heart rate (bpm)	414±7	385±8.4	$P<0.05$
Plasma catecholamines	$n=11$	$n=9$	
Plasma adrenaline (ng/ml)	0.41±0.08	0.82±0.38	NS
Plasma noradrenaline (ng/ml)	0.60±0.14	0.80±0.19	NS

$+dP/dt$ and $-dP/dt$, maximum rates of isovolumetric pressure development and decay. Results are expressed as means±S.E.M. NS: not significant ($P>0.05$).

significant interaction term was detected using two-factor ANOVA. In addition, whole concentration–response data sets were compared pairwise by two-factor repeated measures ANOVA, to detect overall differences in the concentration–response curves. All results are given as means±S.E.M., and results are considered statistically significant if $P<0.05$. NS is non-significant.

3. Results

3.1. CHF in the rats with myocardial infarctions (Table 1)

All CHF rats included in the study had large, dilated right and left ventricles, dilated atria and pleural effusion at gross visual examination. The CHF rats had large infarctions and developed right ventricular hypertrophy and increased lung-to-body weight ratios. Left ventricular-to-body weight ratios were also significantly increased in the CHF rats. Other indications of heart failure include increased left ventricular end-diastolic pressure, reduced $+dP/dt$ and $-dP/dt$, reduced mean blood pressure and heart rate. Although the mean plasma concentrations of adrenaline and noradrenaline were 100% and 33% higher in the CHF versus the sham rats, statistical significance was not achieved for these differences (Table 1).

3.2. Vascular morphology (Table 2)

The mean normalised lumen diameter for the all vessels included in the study were 186±4 μm in the resistance arteries and 514±8 μm in the conductance arteries, with no differences detected between arteries from CHF and sham rats (Student's t-tests, both $P>0.05$). Also, normalised lumen diameter, media muscle layer thickness, media-to-lumen ratio and media cross-sectional area of the conductance and resistance arteries used for simultaneous measurements of $[Ca^{2+}]_i$ and tension did not differ significantly between CHF and sham rats (Table 2).

3.3. Baseline tension in PSS and reference contractions with KPSS (Table 3)

Baseline vascular tone in PSS (in N/m and in % of KPSS) and active tension in KPSS (in N/m) were similar between CHF and sham rats in both types of arteries. Active tension in

Table 2
Vascular morphology data

	Resistance arteries		Conductance arteries		Statistics		
	CHF	Sham	CHF	Sham	Two-factor ANOVA		
	$n=4$	$n=5$	$n=5$	$n=6$	±CHF	Vessel type	Interact.
Lumen diameter (μm)	169±7	168±2	471±40	464±24	NS	$P<0.001$	NS
Media thickness (μm)	6.56±0.55	6.75±0.60	25.2±1.7	22.8±1.7	NS	$P<0.001$	NS
Media-to-lumen ratio (%)	16.2±1.5	17.7±1.5	22.9±2.2	21.1±2.3	NS	$P<0.05$	NS
Media cross-sectional area (μm^2)$\times 10^{-3}$	3.63±0.37	3.53±0.49	39.4±4.5	34.8±2.8	NS	$P<0.001$	NS

Results are expressed as means±S.E.M. NS: not significant ($P>0.05$).

Table 3
The effects of CHF and vessel type on reference parameters and acetylcholine concentration–relaxation curves

	Resistance arteries		Conductance arteries		Two-factor ANOVA		
	CHF	Sham	CHF	Sham	±CHF	Vessel type	Interact.
	$n=35$	$n=33$	$n=30$	$n=31$			
Reference parameters							
Passive tension in Ca^{2+}-free PSS (N/m)	0.51±0.04	0.51±0.03	1.36±0.05	1.28±0.05	NS	$P<0.001$	NS
Active tension in PSS (N/m)	0.06±0.02	0.07±0.03	0.06±0.02	0.03±0.01	NS	NS	NS
Active tension in PSS (% of KPSS)	2.20±0.24	2.59±0.62	1.38±0.20	1.12±0.18	NS	$P<0.005$	NS
Active tension in KPSS (N/m)	3.65±0.17	3.69±0.17	6.58±0.31	5.88±0.21	NS	$P<0.001$	NS
Acetylcholine concentration–relaxation curves							
Maximum relaxation (% of precontraction)	88.0±1.9	82.3±2.3	87.5±1.3	86.7±1.0	NS	NS	NS
pD_2 for log [acetylcholine]–relaxation curve	7.49±0.09	7.38±0.09	7.08±0.07	7.16±0.08	NS	$P<0.001$	NS

Results are expressed as means±S.E.M. NS: not significant ($P>0.05$).

KPSS (N/m) was significantly higher in conductance versus resistance arteries (Table 3).

3.4. Acetylcholine concentration–relaxation curves (Fig. 1, Table 3)

No differences in maximum relaxation were detected between the four experimental groups, and no differences in sensitivity to acetylcholine were detected between CHF and sham rats in either type of vessel. The sensitivity to acetylcholine was significantly higher in the resistance versus the conductance arteries, regardless of CHF (Fig. 1).

3.5. Noradrenaline concentration–tension curves (Fig. 2, Table 4)

Vessel wall tension responses to noradrenaline were similar between vessels from CHF and sham rats, both in terms of maximum active wall tension and sensitivity. When comparing resistance versus conductance arteries, the sensitivity to noradrenaline was significantly higher, and the maximum tension development in response to noradrenaline significantly lower in the resistance versus the conductance arteries (Fig. 2).

3.6. Effects of β-adrenoceptor blockade and inhibition of noradrenaline uptake

Incubation with 4 μM propranolol did not significantly change the maximum tension response to noradrenaline in resistance arteries (CHF: 95.8±3.5, $n=17$ vs. 89.7±3.8%, $n=9$ with propranolol; sham: 102.9±4.7, $n=15$ vs. 95.7±3.4%, $n=9$ with propranolol, two-factor ANOVA, $P>0.05$) or conductance arteries (CHF: 123±7.7, $n=14$ vs. 118.1±5.5%, $n=8$ with propranolol; sham: 130.7±4.2, $n=15$ vs. 139±5.0%, $n=8$ with propranolol, two-factor ANOVA: $P>0.05$). Contractile sensitivity (pD_2) to noradrenaline was also unaffected by propranolol, both in resistance arteries (CHF: 6.21±0.14, $n=17$ vs. 5.60±0.22, $n=9$ with propranolol; sham: 6.02±0.13, $n=15$ vs. 6.00±0.12, $n=9$ with propranolol, two-factor ANOVA: $P>0.05$) and conductance arteries (CHF: 5.30±0.13, $n=14$ vs. 5.59±0.23, $n=8$ with propranolol; sham: 5.40±0.05, $n=15$ vs. 5.53±0.09, $n=8$ with propranolol, two-factor ANOVA: $P>0.05$).

Following inhibition of neuronal and non-neuronal uptake of noradrenaline with 4 μM cocaine+4 μM normetanephrine, maximum tension responses to noradrenaline were not significantly different between CHF and sham-operated rats in

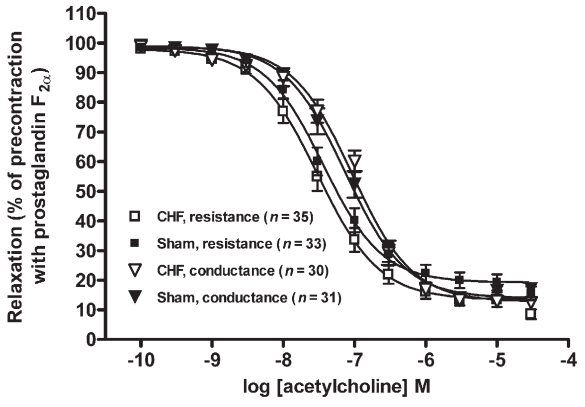

Fig. 1. Relationship between acetylcholine concentration and relaxation in resistance and conductance arteries from CHF rats and sham-operated rats. Results are given relative to precontraction levels with 3×10^{-6} M prostaglandin $F_{2\alpha}$, and are expressed as means±S.E.M.

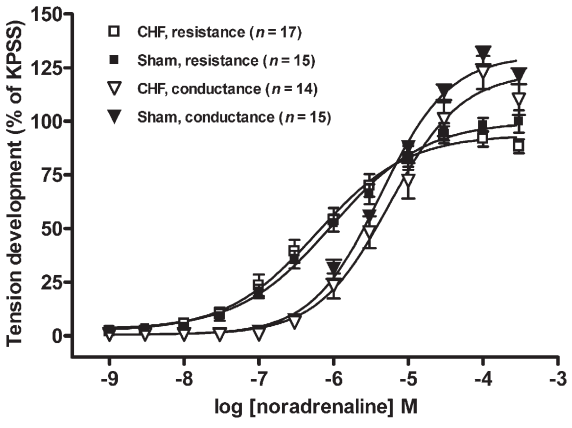

Fig. 2. Relationship between noradrenaline concentration and tension in resistance and conductance arteries from CHF rats and sham-operated rats. Tensions are given relative to a steady-state reference contraction induced by KPSS. Results are expressed as means±S.E.M.

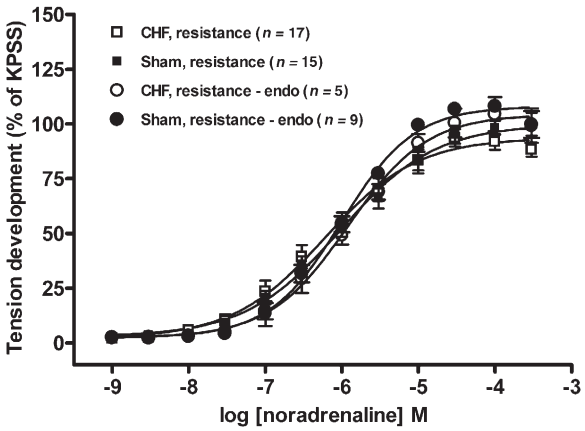

Fig. 3. Relationship between noradrenaline concentration and tension in endothelium-denuded versus endothelium-intact resistance arteries from CHF rats and sham-operated rats. Tensions are given relative to a steady-state reference contraction induced by KPSS. Results are expressed as means±S.E.M.

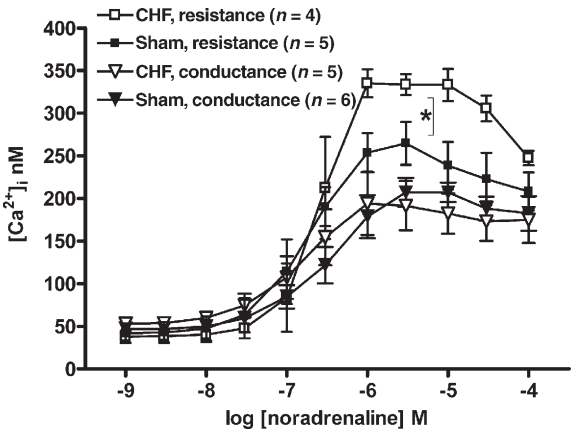

Fig. 4. Relationship between noradrenaline concentration and $[Ca^{2+}]_i$ in resistance and conductance arteries from CHF rats and sham-operated rats. The asterisk denotes significant statistical difference between CHF and sham in the resistance arteries, (two-factor repeated-measures ANOVA, $P=0.003$). Results are expressed as means±S.E.M.

resistance arteries (CHF: 90.4±3.1%, $n=9$ with uptake inhibitors; sham: 92.1±6.0%, $n=9$ with uptake inhibitors, two-factor ANOVA: $P>0.05$) or conductance arteries (CHF: 119.9±4.3%, $n=8$ with uptake inhibitors; sham: 132.9±4.2%, $n=8$ with uptake inhibitors, two-factor ANOVA: $P>0.05$). The contractile sensitivity (pD_2) to noradrenaline after noradrenaline uptake inhibition was similar between CHF and sham-operated rats in resistance arteries (CHF: 6.22±0.10, $n=9$ with uptake inhibitors; sham: 6.04±0.06, $n=9$ with uptake inhibitors, two-factor ANOVA: $P>0.05$) and conductance arteries (CHF: 5.86±0.19, $n=8$ with uptake inhibitors; sham: 6.08±0.10 $n=8$ with uptake inhibitors, two-factor ANOVA: $P>0.05$).

3.7. Effects of endothelial removal in resistance arteries (Fig. 3)

In the endothelium-denuded resistance arteries, successful removal of the endothelium was confirmed by <10% maximum relaxation during cumulative acetylcholine application. Baseline tension in PSS (CHF: 2.28±1.39%, $n=5$ without endothelium; sham 1.52±0.46%, $n=9$ without endothelium), maximum active wall tension (CHF: 105.5±6.9; sham 109.4±2.4%) and contractile sensitivity (pD_2) to noradrenaline (CHF: 5.92±0.06; sham 6.00±0.08) were unaffected by endothelium removal both in arteries from CHF and sham rats (two-factor ANOVAs, all $P>0.05$) (Fig. 3).

3.8. Baseline $[Ca^{2+}]_i$ in PSS and during depolarisation with KPSS

The $[Ca^{2+}]_i$ levels in PSS did not differ between CHF and control rats in resistance arteries (CHF: 38±7 nM; sham: 42±12 nM) or conductance arteries (CHF: 55±2 nM; sham: 48±6 nM). When the bathing medium was changed from PSS to KPSS, both $[Ca^{2+}]_i$ and tension rapidly increased to reach steady-state levels. Though there was a tendency towards augmented steady-state levels of $[Ca^{2+}]_i$ in the resistance arteries from CHF rats during depolarisation with KPSS, $[Ca^{2+}]_i$ levels were not significantly different between CHF and control rats in resistance arteries (CHF: 300±9 nM; sham: 247±30 nM) or conductance arteries (CHF: 220±27 nM; sham: 249±38 nM).

Table 4
The effects of CHF and vessel type on noradrenaline concentration–tension and noradrenaline concentration–$[Ca^{2+}]_i$ curves and the relationship between $[Ca^{2+}]_i$ and tension in vessels during cumulative application of noradrenaline

	Resistance arteries		Conductance arteries		Statistics		
					Two-factor ANOVA		
	CHF	Sham	CHF	Sham	±CHF	Vessel type	Interact.
Noradrenaline concentration–tension curves	$n=17$	$n=15$	$n=14$	$n=15$			
Max. active tension with noradrenaline (%)	95.8±3.5	102.9±4.7	123.0±7.7	130.7±4.2	NS	$P<0.001$	NS
pD_2 for log [noradrenaline]–tension curve	6.21±0.14	6.02±0.13	5.30±0.13	5.40±0.05	NS	$P<0.001$	NS
Noradrenaline-induced $[Ca^{2+}]_i$ levels	$n=4$	$n=5$	$n=5$	$n=6$			
Maximum rise in $[Ca^{2+}]_i$ with noradrenaline	307±21[a]	227±22	150±28	172±13	–	–	$P<0.05$
pD_2 for log [noradrenaline]–$[Ca^{2+}]_i$ curve	6.69±0.13	6.81±0.11	6.66±0.22	6.42±0.16	NS	NS	NS
pD_2 for log $[Ca^{2+}]_i$–tension curve	6.56±0.05[b]	6.73±0.06	6.83±0.04	6.81±0.03	–	–	$P<0.05$

Results are expressed as means±S.E.M. NS: not significant ($P>0.05$). [a,b]Post hoc Student's t-tests, resistance CHF significantly different from resistance sham, [a]$P=0.017$, [b]$P=0.023$.

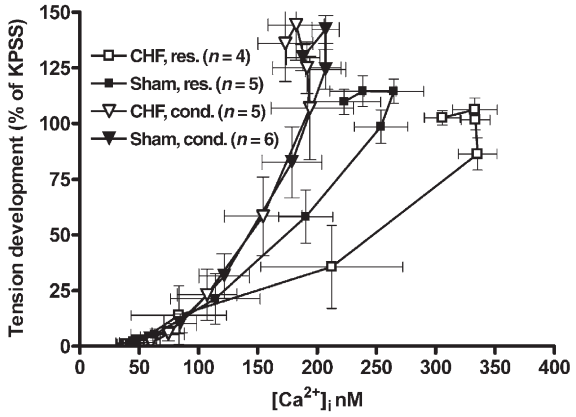

Fig. 5. Relationship between $[Ca^{2+}]_i$ and tension during cumulative application of noradrenaline in resistance and conductance arteries from CHF rats and sham-operated rats. Tensions are given as relative to a steady-state reference contraction induced by KPSS. Results are expressed as means±S.E.M.

3.9. $[Ca^{2+}]_i$ during cumulative application of noradrenaline: (Fig. 4, Table 4)

The pD_2 values for this relationship were not different between CHF and sham rats in either type of artery, or between resistance and conductance arteries (Table 4). However, a significant interaction term was detected by two-factor ANOVA in the maximum rise in $[Ca^{2+}]_i$ from baseline values during cumulative application of noradrenaline (Table 4), and post hoc *t*-tests showed that the maximum increase in $[Ca^{2+}]_i$ was significantly augmented in the resistance arteries from CHF versus sham rats (Table 4). Additionally, the *overall* $[Ca^{2+}]_i$ levels during cumulative application of noradrenaline were significantly augmented in the resistance arteries from CHF versus sham rats (two-factor repeated measures ANOVA, $P=0.003$), but this was not the case in the conductance arteries (two-factor repeated measures ANOVA, $P>0.05$). When comparing resistance versus conductance arteries, the *overall* $[Ca^{2+}]_i$ levels during cumulative application of noradrenaline were significantly higher in the resistance versus the conductance arteries, both in the arteries from CHF rats (two-factor repeated measures ANOVA, $P<0.001$) and in the arteries from sham rats (two-factor repeated measures ANOVA, $P<0.05$) (Fig. 4).

3.10. Relationship between $[Ca^{2+}]_i$ and tension during noradrenaline application: (Fig. 5, Table 4)

As a significant enhancement of overall $[Ca^{2+}]_i$ levels, but not tension levels, during noradrenaline application was detected in the resistance arteries from CHF versus sham rats, the relationship between $[Ca^{2+}]_i$ and tension during application of noradrenaline was examined in the four experimental groups. In Fig. 5, where tension is plotted as a function of $[Ca^{2+}]_i$, it can be visually appreciated that the tension level induced by a given level of $[Ca^{2+}]_i$ was generally lower in the resistance arteries from CHF rats versus sham rats. In accordance with this, the pD_2 values for the log $[Ca^{2+}]_i$–tension relationship during application of noradrenaline were significantly lower in the resistance arteries from CHF versus sham rats (Table 4).

4. Discussion

4.1. The rat model of CHF

The post-infarction rat is a widely used animal model of CHF with many similarities to the disease in man (Hasenfuss, 1998). Measurements of structural and haemodynamic characteristics of this rat model of heart failure confirmed severely impaired left ventricular function with backward failure, pulmonary congestion, right and left ventricular dilatation and remodeling. Though there was a tendency towards increased plasma catecholamine levels in the CHF rats, statistical significance was not achieved, probably due to the wide scatter compatible with the large fluctuations of these hormones in rats (McCarty et al., 1981). Additionally, plasma catecholamine levels are somewhat unreliable proxies for sympathetic activity, since these levels are influenced by catecholamine reuptake and metabolism as well as sympathetic activity (Goldstein et al., 2003; Patel et al., 2000). Other investigators have consistently reported elevated levels of circulating catecholamines (Ishigai et al., 1999; Mulder et al., 1997; Stassen et al., 1997b), and, employing more specific techniques, a high level of sympathetic activation in CHF rats, also in the skeletal muscle vascular bed (Patel et al., 2000). Specifically, the plasma levels of noradrenaline in CHF rats, including severe cases, correlate positively with the degree of left ventricular functional impairment (Ishigai et al., 1999).

Although vascular contractile sensitivity to stimulation with noradrenaline has been widely studied in post-infarction CHF rats, it remains unclear whether abnormalities in vascular smooth muscle cell calcium handling play a role in the genesis of enhanced vascular tone and augmented peripheral vascular resistance, since no studies have examined both vascular contractile force and $[Ca^{2+}]_i$ in animal models of heart failure. It also remains to be determined to which extent endothelial dysfunction and/or vascular remodeling contribute to the increase in vascular resistance in this condition.

In this study we examined vascular tone and $[Ca^{2+}]_i$ in resistance and conductance arteries from CHF and sham-operated rats. Contractile function of these vessels was measured by wire myography under baseline conditions and during noradrenaline stimulation, and corresponding alterations in $[Ca^{2+}]_i$ associated with the pharmaco-mechanical coupling were examined employing the fluorometric Ca^{2+} indicator FURA-2. The isometric myograph method, including the vascular normalisation procedure, is widely employed in identifying and quantifying changes in vasoconstrictor responsiveness to agents such as noradrenaline (Mulvany and Halpern, 1977; Mulvany and Aalkjaer, 1990).

4.2. Methodological considerations

Depolarisation with high-K^+ buffers (e.g. KPSS) in non-denervated vascular preparations may lead to release of

noradrenaline from sympathetic nerves, potentiating the contractile response due to activation of α-adrenergic receptors on the vascular smooth muscle cell surface (Fouda et al., 1991). In preliminary experiments using conductance and resistance vessels from CHF and sham-operated rats, the α-adrenergic receptor antagonist phentolamine (1 μM) was added to the organ bath at a stable level of contraction during the reference with KPSS. In none of the vessels examined did the tension level change in response to phentolamine, demonstrating that release of noradrenaline had no significant influence on vessel wall tension levels during reference contractions with KPSS. This indicates that the release of noradrenaline from the sympathetic nerves during KPSS depolarisation is small or even absent in these vessels due to sparse sympathetic innervation (Mulvany et al., 1982), and/or that the six-fold application of KPSS to the vessels had effectively depleted the sympathetic nerve endings of noradrenaline (Fouda et al., 1991).

In non-denervated vessels, the contractile response to noradrenaline may be attenuated by uptake of noradrenaline into sympathetic nerve endings (neuronal uptake) or into other cell types (non-neuronal uptake). In neither resistance nor conductance arteries did maximum wall tension development or sensitivity to noradrenaline differ between CHF and control rats after incubation with cocaine and normetanephrine. Our findings are in accordance with the demonstration by Mulvany et al. (1982), that rat skeletal muscle resistance arteries are sparsely innervated, and that incubation with cocaine does not change the concentration–tension curves in response to cumulative application of noradrenaline.

Noradrenaline is a β- as well as an α-adrenergic agonist. As in man, β-adrenoceptors are present in the skeletal muscle vascular bed of rats, where β-adrenergic stimulation leads to vasodilation (Mulder et al., 1997). Changes in the number or function of α- and β-adrenergic receptors or in their respective post-receptor signaling pathways due to CHF could therefore alter the balance between α- and β-adrenergic mediated vascular responses to noradrenaline. In the present study, incubation with propranolol did not affect maximum wall tension development or sensitivity to noradrenaline in the four experimental groups. In accordance with this, Mulder et al. (1997) found that concentration–relaxation curves for the β_2-adrenoceptor agonist salbutamol were similar in skeletal muscle resistance arteries from CHF and sham rats.

4.3. Vascular contractile reactivity to noradrenaline

Baseline $[Ca^{2+}]_i$ and wall tension levels were similar in CHF compared to sham-operated control rats in either type of artery. Baseline wall tension represents the spontaneous or myogenic tone of the vessels. We also detected no differences in contractile reactivity to noradrenaline between CHF and sham rats in either type of artery. Alterations in the contractile response to noradrenaline have been reported in various species and vascular beds: in dogs with heart failure due to ventricular pacing, an increased responsiveness to noradrenaline has been found in the dorsal pedal artery (Forster and Armstrong, 1990). Increased responses to noradrenaline have also been found in thoracic aorta in CHF rats (Teerlink et al., 1994), while unaffected responses to phenylephrine have been reported in main femoral arteries and femoral resistance arteries from CHF rats (Bergdahl et al., 1995; Mulder et al., 1997). In skeletal muscle arterioles from rats with small and moderate size myocardial infarctions, an exaggerated response to noradrenaline has been demonstrated even in the absence of changes in mean arterial pressure and heart rate (Thomas et al., 1998). In one study, investigators found a general hyperreactivity to constrictor agonists in coronary septal arteries, a general hyporeactivity in mesenteric resistance arteries and no changes in responsiveness in aorta from CHF rats (Stassen et al., 1997a). In other studies using isolated mesenteric resistance arteries from CHF rats, attenuated responses to adrenoceptor agonists have also been demonstrated (Stassen et al., 1997a,b). Data from patients with CHF are sparse. Unaltered forearm vascular responses to infusion of the α_1 agonist phenylephrine into the brachial artery have been reported in patients with CHF (Indolfi et al., 1994). The reactivity to noradrenaline of subcutaneous small arteries from patients with mild CHF (NYHA class II-III) has been investigated in a few studies: in two of these studies, neither reactivity to noradrenaline nor vascular morphology was changed by CHF (Stephens et al., 1998; Hillier et al., 1999). In a third study, attenuated maximum contraction to noradrenaline was found in subcutaneous arteries, also from patients with mild CHF (Angus et al., 1993). Thus, considerable disparity exists between experimental results. Interpretation and comparison of the results from these studies require caution due to different methodological approaches, different species, varying degree and duration of heart failure and possible intercurrent medical conditions (e.g. atherosclerosis).

4.4. $[Ca^{2+}]_i$ levels in response to noradrenaline

The arterial vascular smooth muscle cell $[Ca^{2+}]_i$ levels during α-adrenergic stimulation have not previously been measured in CHF. In a subset of experiments, $[Ca^{2+}]_i$ and tension were measured simultaneously during cumulative application of noradrenaline. In these experiments, $[Ca^{2+}]_i$ levels during stimulation with noradrenaline were significantly augmented in the resistance arteries from CHF rats. Ca^{2+} is a key mediator in the vascular α-adrenergic excitation–contraction coupling. Binding of catecholamines to adrenoceptors on the surface of smooth muscle cells activates a signaling cascade, leading to graded depolarisation of the cell membrane (Nilsson, 1998). $[Ca^{2+}]_i$ levels increase due to activation of voltage-operated and/or receptor-activated Ca^{2+}-channels in the cell membrane and through inositol triphosphate-mediated release of Ca^{2+}, mainly from the sarcoplasmic reticulum (Karaki et al., 1997). The augmented $[Ca^{2+}]_i$ levels observed in the noradrenaline-activated resistance arteries from CHF rats in the present study could be due to changes in the mechanisms regulating membrane potential or Ca^{2+} influx in response to noradrenaline-induced membrane depolarisation. Bergdahl et al. (2001) found that depolarisation with high-K^+ buffer induced similar contractile responses in mesenteric resistance arteries from

CHF rats and sham-operated rats, as also demonstrated in the conductance and resistance arteries investigated in present study. In the study by Bergdahl et al. (2001), the L-type calcium-channel blocker nifedipine induced a stronger inhibition of the contractile response to depolarisation with K^+ in the arteries from CHF rats compared with control rats. In the present study, a tendency towards higher $[Ca^{2+}]_i$ levels was observed during depolarisation with KPSS in resistance arteries from CHF rats versus sham-operated rats. Our data and those from the study by Bergdahl et al. (2001) suggest that CHF is accompanied by alterations in Ca^{2+} influx through voltage-dependent and/or receptor operated calcium channels in the resistance arteries. Also the production of inositol triphosphate is regulated by membrane potential, and changes in the regulation of the membrane potential could therefore affect release of Ca^{2+} from intracellular stores (Nilsson, 1998). We did not examine vascular smooth muscle cell membrane potential, Ca^{2+} influx through Ca^{2+} channels or Ca^{2+} release from intracellular stores. Therefore it remains to be elucidated, which particular mechanism(s) are involved in the changes in Ca^{2+} handling in the resistance arteries from CHF rats.

4.5. Relation between $[Ca^{2+}]_i$ and vessel wall tension

During cumulative application of noradrenaline, vessel wall tension levels were similar in resistance arteries from CHF and sham-operated rats, despite augmented $[Ca^{2+}]_i$ levels in these vessels. Considering the well-known relationship between Ca^{2+} and force, how can our observation of unchanged contractile force in the face of increased $[Ca^{2+}]_i$ be explained? The simplest explanation for this would be a decrease in the sensitivity of the contractile machinery to $[Ca^{2+}]_i$, a phenomenon termed *Ca^{2+} desensitisation* (Somlyo and Somlyo, 2003). This is further supported by the significantly decreased pD_2 values for the log $[Ca^{2+}]_i$–tension relationship detected in these arteries. Very few studies have addressed the possibility of altered arterial calcium sensitivity in CHF, and results are diverging, suggesting both increased (Bergdahl et al., 2001; Sato et al., 1998) and decreased (Stassen et al., 1997b) Ca^{2+} sensitivity in this condition. However, in none of these studies were $[Ca^{2+}]_i$ actually measured, and one of the studies (Sato et al., 1998) was performed on permeabilised arterial preparations, in which the intracellular environment may be perturbed. The possible existence of altered arterial Ca^{2+} sensitivity in CHF is as yet not fully elucidated, and further studies are required.

Other mechanisms could be responsible for the alterations in the $[Ca^{2+}]_i$–tension relationship in the resistance arteries from CHF rats. Changes in arterial morphology due to CHF could decrease contractile capacity; according to the Laplace equation, the pressure against which a vessel is able to contract at a given level of activation is proportional to the media-to-lumen ratio of the vessel (Mulvany, 1999). However, vessel morphology was unchanged by CHF in the present study. Other investigators have also found no indications of arterial remodeling in conductance and resistance arteries from various vasculatures of post-infarction CHF rats, including the skeletal muscle vascular bed (Mulder et al., 1996, 1997; Stassen et al., 1997a,b). Matrix remodeling in the media muscle layer, e.g. by increased de novo synthesis of connective fibres or changes in vascular smooth muscle cell composition and ultrastructure could also affect vascular contractility. We did not examine these factors. However, Mulder et al. found no changes in elastin or collagen density in mesenteric resistance arteries and skeletal muscle resistance arteries from CHF rats up to a year after myocardial infarction (Mulder et al., 1996, 1997).

4.6. Endothelial function

The endothelium plays an important role in tempering contractions induced by receptor-dependent agents (Xavier et al., 2004), and endothelial dysfunction has been linked to the increased vascular resistance in CHF (Endemann and Schiffrin, 2004). In the present study, no changes in acetylcholine responsiveness were detected in CHF rats versus sham-operated rats in either type of artery. Additionally, removal of the endothelium did not affect baseline tension or noradrenaline concentration–vessel wall tension curves in resistance arteries from neither CHF nor sham-operated rats. In summary, we did not detect any changes in endothelial function in the arteries examined in the present study.

Previous studies in CHF rats have demonstrated significantly attenuated vasodilatory responses to acetylcholine in skeletal muscle resistance arteries and arterioles (Didion and Mayhan, 1997; Mulder et al., 1997), and when using a whole-limb approach (Drexler and Lu, 1992). As in the present study, an apparent lack of endothelial dysfunction or only moderately affected endothelial function has been reported in large conductance arteries, including the femoral main arteries and the aorta of CHF rats (Brandes et al., 1998; Teerlink et al., 1993). These findings suggest that endothelial dysfunction is more prominent in smaller versus larger arteries (Mulder et al., 1997), and it is possible that endothelial dysfunction may have been present downstream to the arteries investigated in the present study, e.g. in the arterioles.

In conclusion, we have found no evidence of increased arterial contractile responsiveness to noradrenaline in neither resistance nor conductance arteries from rats with heart failure. Also, we found no evidence of endothelial dysfunction, increased basal tone or vascular remodeling in the rats with CHF. However, the arterial $[Ca^{2+}]_i$ levels during activation with noradrenaline were significantly augmented in resistance arteries from rats with post-infarction heart failure. The unaltered wall tension response to noradrenaline of these arteries, despite the increased $[Ca^{2+}]_i$ levels, may represent a physiologically appropriate compensatory response to the sympathoexcitation in CHF, aimed at counteracting the otherwise detrimental increase in vascular resistance.

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