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Reduced responsiveness of rat mesenteric resistance artery smooth muscle to phenylephrine and calcium following myocardial infarction

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1 We evaluated responses of peripheral resistance arterial smooth muscle to α_1-adrenoceptor stimulation in a rat model of heart failure in relation to neurohumoral changes, wall structure, receptor density and cellular calcium handling.

2 Plasma samples and third order mesenteric artery side-branches were obtained from Wistar rats after induction of left ventricular infarction (MI) or sham surgery. Vessels were denuded of endothelium, sympathectomized, depleted of neuropeptides, and mounted in a myograph for recording of isometric force development in response to calcium, agonist and high potassium. Also, the morphology of these preparations was determined. Separate vessel segments were used in radioligand binding assays with [^{3}H]-prazosin.

3 At 1 week after MI, circulating plasma levels of adrenaline, angiotensin II, atrial natriuretic factor (ANF) and vasopressin were significantly elevated. At 5 weeks only a significant elevation of ANF persisted.

4 At 5 weeks after MI, the structure of the vessels and responsiveness to high potassium or Bay K 8466 (10^{-6} mol l^{-1}) were not modified. Yet, at this stage, sensitivity to phenylephrine was increased (pD_2: 6.24 ± 0.04 vs 5.98 ± 0.04 for controls) while maximal contractile responses to phenylephrine in the presence of 2.5 mmol l^{-1} calcium (2.26 ± 0.28 vs 3.53 ± 0.34 N m^{-1}) and the sensitivity to calcium in the presence of phenylephrine (pD_2: 2.81 ± 0.22 vs 3.74 ± 0.16) were reduced. Responses to the agonist in calcium-free solution and the calcium sensitivity in the presence of 125 mmol l^{-1} potassium or of phorbol myristate acetate (PMA, 10^{-6} mol l^{-1}) were not altered.

5 At 5 weeks after MI, the density of prazosin binding sites was not reduced (4.04 ± 1.40 vs 2.29 ± 0.21 fmol μg^{-1} DNA in controls).

6 In conclusion, myocardial infarction leads in the rat to a reduction of contractile responses of mesenteric resistance arterial smooth muscle to α_1-adrenoceptor stimulation. This seems to involve impaired agonist-stimulated calcium influx.

Keywords: Myocardial ischemia; adrenergic reactivity; calcium sensitivity; ligand binding; plasma analysis

Introduction

Postinfarction patients are particularly prone to develop heart failure. Though haemodynamic consequences of necrotizing injury to the myocardium may initially be compensated by hypertrophy of the remnant myocardium and by elevated peripheral resistance, decompensated heart failure may develop in the long run. Activation of the autonomic nervous system and various hormonal systems participate in the initial compensation.

It has been proposed that this chronic neurohumoral activation may be accompanied by adverse structural alterations and by tolerance of various effector organs. Though probably not the sole major determinant, the sympathetic nervous system participates in the pathophysiology of heart failure. (Cohn *et al*., 1984; Parmley, 1985; Leier *et al*., 1990; Swedberg *et al*., 1990). It also participates in the reduction of β-adrenoceptor mediated chronotropic and inotropic responses in experimental and human heart failure (Brodde, 1991). Though less well characterized, also in the vasculature adrenergic mechanisms may be altered during heart failure.

In a dog model of pacing-induced heart failure, contractile responses to α_1-adrenoceptor stimuli are decreased in coronary arteries (Main *et al*., 1991) and markedly increased in the dorsal pedal artery and saphenous vein (Forster *et al*., 1989; Forster & Armstrong, 1990). In the rat myocardial infarction model, responses of the thoracic aorta to α_1-adrenoceptor agonists are reduced at 1 week after the insult (Teerlink *et al*., 1994). Isolated subcutaneous resistance arteries obtained from patients with heart failure display a non-selective reduction of responses to both vasoconstrictor and vasodilator stimuli, including EDRF (Angus *et al*., 1993). Yet, forearm vascular responsiveness to α_1-adrenoceptor stimulation is not modified in heart failure patients (Indolfi *et al*., 1994). Besides interspecies and regional differences, differences in the duration of the pathology and effects of the drug treatment of the patients may have contributed to discrepancy between these findings. Most experimental animal studies were performed rather early after induction of heart failure and relatively little is known about later alterations that could be more relevant for decompensation. Although some information is available concerning vascular responses to α_1-adrenoceptor agonists, the density and affinity of the α_1-adrenoceptors and their relation to calcium handling have not yet been investigated.

The goal of the present experiments was to study responses to α_1-adrenoceptor activation and depolarization in the smooth muscle of a peripheral resistance artery of rats with a large myocardial infarction. The changes were evaluated with respect to the duration of the pathology, plasma levels of vasoactive agents, the structure of the vessels, the presence of the receptors and the mechanisms by which α_1-agonists trigger

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contraction, which include calcium influx, intracellular calcium release and protein kinase C activation (Mulvany & Aalkjaer, 1990; Boonen & De Mey, 1990a; 1991). To concentrate on changes in peripheral resistance artery smooth muscle, *in vitro* studies were performed in isolated mesenteric resistance arteries that had been denuded of endothelium, chemically sympathectomized and depleted of vasodilator neuropeptides before experimentation.

Methods

Animals

Male Wistar rats (approximately 300 g, Harlan-Winkelmann, Borchen, Germany) were used. They had free access to standard rat chow (Hope Farms, Woerden, The Netherlands) and drinking water. The experimental procedures were performed according to institutional guidelines and approved by the Ethics Committee for the Use of Experimental Animals of the Universiteit Maastricht.

Surgical procedures

Rats were randomly selected to undergo either coronary artery ligation (myocardial infraction group, MI, $n=30$) or sham surgery (sham group, Sham, $n=30$) (Pfeffer *et al*., 1979; Schoemaker *et al*., 1990). Briefly, animals were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and the trachea was intubated (PE-240) for respiration with room air (60 strokes min^{-1}, tidal volume 3 ml). The thorax was opened at the fourth left intercostal space and the heart was exteriorized by applying gentle pressure to the right side of the thorax. A silk ligature (6-0) was placed around the proximal left coronary artery and tied securely in the MI group while in the sham group a superficial suture was fixed in the epicardium of the left ventricle, near the left coronary artery. Then the heart was returned to its original position and the incision was closed. In the MI group, 4 animals died within 24 h after surgery; thereafter no further mortality was observed. In the sham group all animals survived surgery and no mortality was observed over the whole experimental period. Under ether anaesthesia some of the rats were equipped with a heparin-treated intra-aortic catheter (PE-10 connected to PE-50) that was advanced from a femoral artery, guided under the skin, exteriorized at the back of the head and closed with a metal plug.

Plasma analysis

At one and at five weeks after MI or Sham, arterial blood samples were obtained from undisturbed, freely moving conscious rats. Angiotensin II was determined by radioimmunoassay following extraction from plasma. Briefly, blood was rapidly drawn into chilled tubes containing EDTA, supplemented with 3.6 μmol ml^{-1} enalaprilate, 1.25 μmol ml^{-1} o-phenantroline, 2% ethanol and 2 mg ml^{-1} neomycin. The blood samples were centrifuged at 3000 *g* for 15 min and plasma was stored at $-20^{\circ}C$. Plasma samples were extracted with ethanol, and radioimmunoassays were performed with angiotensin II standard and rabbit antibody. Separation was performed with goat anti-rabbit secondary antibodies. Intra-assay and interassay variances were 4.6% and 7.7%, respectively. Rat plasma atrial natriuretic factor and vasopressin were quantified by radioimmunoassay with commercial kits (Nichols Institute Diagnostics BV., Wychen, The Netherlands). Catecholamines were determined by high performance liquid chromatography (h.p.l.c.) and fluorescent detection (van der Hoorn *et al*., 1989).

Isolation and preparation of arteries

Three or five weeks after surgery, rats were killed by cervical dislocation and exsanguination. The heart, lungs, and mesentery were rapidly removed and placed in Krebs-Ringer solution (KRB). From the mesentery, third order side-branches of the superior mesenteric artery (mesenteric resistance artery, diameter 200–250 μm, 1.5–2.0 mm long) were isolated. The endothelium was removed from the vessel segments as described by Osol *et al*. (1989). Sympathetic nerve endings in the arterial preparations were destroyed by incubating the arteries for 10 min in bicarbonate-free KRB solution containing 300 μg ml^{-1} 6-hydroxydopamine (Aprigliano & Hermsmeyer, 1977) and perivascular sensory motor nerves were depleted of vasodilator neuropeptides by 20 min incubation in KRB containing capsaicin (10^{-6} mol l^{-1}) (Wharton *et al*., 1986).

Recording of isometric force development

Resistance artery segments were mounted horizontally as ring segments between a Kistler-Morse DSC 6 force transducer (Seattle, U.S.A.) and a displacement device in a 10 ml organ chamber using stainless steel wires (diameter 40 μm) threaded through the lumen (Boonen & De Mey, 1990a; 1991; De Mey *et al*., 1991). During experimentation, the arteries were immersed in KRB that was maintained at $37^{\circ}C$ and aerated with 95% O_2-5%CO_2. To determine the optimal individual internal diameter of the preparations, an active length-tension protocol was used (De Mey & Brutsaert, 1984). Briefly, the vessel diameter was increased in steps of 40 μm at 5 min intervals and arteries were intermittently stimulated by replacing the bath solution with a high potassium solution (125 mmol l^{-1}, K-KRB). This procedure was carried out until a diameter was reached at which the acute response to the depolarizing stimulus was smaller than at a previous lower level of distension. For further experimentation, arteries were maintained at the diameter at which the maximal contractile response had been obtained. Resting wall tension at this diameter and corresponding transmural pressure did not differ significantly between MI and Sham at 3 and 5 weeks. The successful removal of the endothelium was confirmed by the absence of relaxing responses to acetylcholine (10^{-5} mol l^{-1}) after precontracting the arteries with phenylephrine (PE; 10^{-5} mol l^{-1}).

Contractile responses to K-KRB, PE (10^{-5} mol l^{-1}), phorbol myristate acetate (PMA, 10^{-6} mol l^{-1}) and the calcium channel activator Bay K 8644 (10^{-6} mol l^{-1}) were recorded in the presence of 2.5 mmol l^{-1} calcium. Furthermore, calcium concentration-response curves (10^{-5} to 10^{-2} mol l^{-1}) were constructed in the presence of these stimuli after the mesenteric resistance artery smooth muscle preparations had been exposed to calcium-free solution containing EGTA (3×10^{-4} mol l^{-1}) for 8–12 min during which they had been stimulated during 2 min with PE (10^{-5} mol l^{-1}) in order to deplete the intracellular agonist-sensitive calcium pool (Boonen & De Mey, 1990a). Also, concentration-response curves for PE (10^{-8}–3×10^{-5} mol l^{-1}) were constructed in the presence of 2.5 mmol l^{-1} calcium in order to determine the sensitivity for the α_1-adrenoceptor agonist.

Morphology

After *in vitro* experimentation, arteries were fixed overnight at their optimal diameter by replacing the organ bath solution with phosphate buffered (pH 7.4) formaldehyde (4%). Computer aided morphometric measurements were performed on 4 μm thick cross sections as previously described (De Mey *et al*., 1991). Hearts were fixed with phosphate buffered (pH 7.4) formaldehyde (4%) for determination of infarct size. Hearts were divided into slices (2.2 mm) and the third slice, starting from the apex of the heart, was used to determine infarct size as fraction of the left ventricle as described by Passier *et al*. (1995).

Ligand binding

Analysis of [^{3}H]-prazosin binding was performed essentially as described by Michel *et al*. (1993) but instead of microsomes,

intact arterial segments were used (Morel & Godfraind, 1989). Segments of third order mesenteric artery side branches (5–10 mm long) were incubated for 60 min at 37°C in 250 μl 50 mmol l^{-1} Tris-HCl, 5 mmol l^{-1} $MgCl_2$ (pH 7.4) containing 0.03 to 0.8 nmol l^{-1} [7-methoxy-^{3}H]-prazosin (79.2 Ci mmol^{-1}; NEN, Hertogenbosch, The Netherlands). Non-specific binding was determined in parallel incubations in the continuous presence of phentolamine (25 μmol l^{-1}).

After incubation, arterial segments were gently blotted and rinsed during vortexing for 30 s in 1 ml incubation buffer at 37°C. Subsequently the vessels were filtered over Whatman filters 5 times with 5 ml ice-cold incubation buffer. Arterial segments were then recovered from the filters and solubilized in 200 μl 1 N NaOH. To quantify the bound radioligand, 100 μl of the digested segment was dissolved in 5 ml scintillation liquid (Formula 989) and counted in a liquid scintillation counter. To the remaining 100 μl, 900 μl H_2O was added and from this sample both protein and DNA contents were determined as described by Bradford (1976) and by Labarca and Paigen (1980), with BSA and calf thymus DNA as internal standards, respectively.

Drugs and solutions

KRB had the following composition (in mmol l^{-1}): NaCl 118.5, KCl 4.7, $MgSO_4$ $7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and glucose 5.5. In high potassium solution (K-KRB, 125 mmol l^{-1} K^+), all NaCl was replaced by an equimolar concentration of KCl. BSA, calf thymus DNA, 6-hydroxydopamine-HCl, capsaicin, EGTA, PMA, and (-)-phenylephrine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Methyl,1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay K 8644) was kindly supplied by Dr S. Kazda (Bayer AG, Wuppertal, FRG). Stock solutions (10 mmol l^{-1}) were prepared at the day of use in bidistilled water except for PMA which was dissolved in 50% dimethylsulphoxide in water, and Bay K 8466 which was dissolved in 50% polyethylene glycol in absolute ethanol.

Table 1 Organ wet weights (ww) and infarct sizes in rats at 1, 3 and 5 weeks after myocardial infarction (MI) or sham surgery[#]

	Body weights (g)	*Ventricular ww* (g)	*Lung ww* (g)	*Infarct size* (%)
1 week				
Sham	312±4	0.99±0.03	1.45±0.04	0
MI	306±10	0.92±0.04	1.83±0.15*	41±4*
3 weeks				
Sham	361±8	1.15±0.05	1.51±0.17	0
MI	343±7	1.08±0.04	2.31±0.032*	37±3*
5 weeks				
Sham	380±6	0.99±0.03	1.20±0.03	0
MI	375±9	1.30±0.06*	2.99±0.21*	43±6*

[#]Infarct size is expressed as percentage of the left ventricular circumference. Data shown as means±s.e.mean ($n=6-9$). *The difference from Sham is statistically significant ($P<0.05$).

Data analysis

Contractile responses were expressed as increases in active wall tension (increases in force divided by twice the length of the vessel segment, N m^{-1}). pD_2 values were defined as the $-\log(EC_{50})$, in which EC_{50} values for agonist or calcium were determined by least square sigmoidal curve fitting on individual concentration-response curves (Graphpad Prism 1.00, San Diego, CA, U.S.A.). To calculate specific prazosin binding, non-specific binding was subtracted from total binding. Curve fitting by a one site binding model (Graphpad Prism 1.00, San Diego, CA, U.S.A.) was used to determine affinity (K_D) and density (B_{max}) of specific prazosin-binding sites for each individual saturation binding experiment. Findings are shown as means±s.e.mean. Data of sham-operated and MI animals were compared by Student's t test for unpaired observations. Differences with $P<0.05$ were considered statistically significant.

Results

General animal characteristics

After coronary artery ligation and sham surgery, body weight increased to the same extent (Table 1). At 5 weeks after MI, ventricular wet weight was significantly elevated (Table 1). Lung wet weight was significantly greater in MI than Sham from 1 week onwards. With time, this index of pulmonary oedema became more pronounced (Table 1). At 1, 3 and 5 weeks after coronary artery ligation, myocardial infarction exceeded 35% of the left ventricular wall (Table 1). In sham operated rats, no histological signs of infarction were noted. Also, no differences were observed in sham operated animals 5 weeks after surgery with respect to body weights, ventricular wet weights (ww) and lung wet weights when compared to aged matched unoperated control animals (control animals: body weight 385±7, ventricular ww: 1.01±0.03, lung ww: 1.28±0.01g, $n=5$).

One week after coronary artery ligation, circulating plasma levels of noradrenaline were not significantly altered while those of adrenaline, angiotensin II, atrial natriuretic factor and vasopressin were increased 2 to 3 fold (Table 2). At 5 weeks after MI, circulating levels of atrial natriuretic factor were still significantly increased but plasma concentrations of the other neurohumoral factors did not differ significantly from those in Sham (Table 2). The unexpected high level of angiotensin II in sham rats at this stage may have obscured a significant elevation of the peptide in MI.

Resistance artery structure

In Table 3, structural properties of mesenteric resistance arteries of MI and Sham rats are summarized. At neither 3 nor at 5 weeks, MI resulted in a significant alteration of media cross-

Table 2 Plasma concentrations of vasoactive agents in rats at 1 and 5 weeks after myocardial infarction (MI) or sham surgery[#]

	[NA] (pg ml^{-1})	*[A]* (pg ml^{-1})	*[AII]* (pg ml^{-1})	*[ANF]* (pg ml^{-1})	*[AVP]* (pg ml^{-1})
1 week					
Sham	182±40	56±7	38±5	42±3	7±2
MI	178±23	104±31*	106±23*	93±22*	23±8*
5 weeks					
Sham	170±31	66±20	72±12	36±2	6±2
MI	165±28	70±14	120±34	92±36*	6±2

Data are shown as means±s.e.mean ($n=6-9$). NA, noradrenaline; A, adrenaline; AII, angiotensin II; ANF, atrial natriuretic factor; AVP, vasopressin. *The difference from Sham is statistically significant ($P<0.05$).

Table 3 Structual properties of rat mesenteric resistance arteries at 3 and 5 weeks after myocardial infarction (MI) or sham surgery

	3 weeks		*5 weeks*	
	Sham	*MI*	*Sham*	*MI*
Media CSA (10^3 μm^2)	7.2±0.7	8.7±1.0	8.5±1.0	7.6±0.8
Lumen diameter (μm)	260±9	254±8	250±10	248±9
Media thickness (μm)	9.7±0.8	10.5±0.8	11.3±0.9	9.6±0.6
Media:radius (×100)	8.6±0.7	8.3±0.4	9.7±0.6	8.3±0.5
Protein:DNA (μg μg^{-1})	NA	NA	36±2	27±4*

Data are shown as means±s.e.mean ($n=10-12$). CSA, cross-sectional area; NA, not available. *The difference from Sham is statistically significant ($P<0.05$).

Table 4 Contractile responses to stimuli in the presence of calcium in rat mesenteric resistance artery smooth muscle at 3 and 5 weeks after myocardial infarction (MI) or sham surgery

	3 weeks		*5 weeks*	
Stimulus	*Sham*	*MI*	*Sham*	*MI*
K-KRB	3.20±0.17	3.10±0.22	3.32±0.23	3.21±0.17
PMA (10^{-6} mol l^{-1})	n.a.	n.a.	3.44±0.26	3.35±0.22
PE (10^{-5} mol l^{-1})	4.10±0.20	3.89±0.25	4.14±0.16	3.42±0.18*
K-KRB plus PE	4.80±0.19	4.65±0.27	4.72±0.39	4.23±0.20
Bay K 8644 (10^{-6} mol l^{-1})				
6 mmol l^{-1} K^+	NA	NA	0.76±0.25	0.98±0.29
12 mmol l^{-1} K^+	NA	NA	1.74±0.25	1.50±0.28
18 mmol l^{-1} K^+	NA	NA	2.42±0.20	1.89±0.32

Increases in wall tension (N m^{-1}) obtained in the presence of 2.5 mmol l^{-1} calcium are shown as means±s.e.mean ($n=6-16$). K-KRB, 125 mmol l^{-1} potassium; PE, phenylephrine; NA, not available. *The difference from Sham is statistically significant ($P<0.05$).

sectional area or of vessel lumen diameter. Consequently, media thickness and media to lumen ratio were not modified after MI either. It may be of interest that after 5 weeks, MI had resulted in a significant reduction of the protein to DNA ratio in the mesenteric small arteries (Table 3).

Resistance artery contractility

In mesenteric resistance arteries that had been acutely sympathectomized, depleted of neuropeptides, denuded of endothelium and stretched to optimal lumen diameter, responses to K-KRB, alone or in combination with PE, did not differ between MI and Sham at 3 or at 5 weeks (Table 4). Also, responses to 10^{-5} mol l^{-1} PE in the absence of extracellular calcium were not significantly different between both groups (0.70±0.17 vs 1.06±0.26 N m^{-1} for controls). Yet, responses to a single dose of PE (10^{-5} mol l^{-1}) were significantly reduced at 5 weeks after MI (Table 4). However, responses to the agonist did not differ between MI and Sham at 3 weeks (Table 4). In Figure 1 a cumulative dose-response curve is shown and illustrates that at 5 weeks after MI, maximal responses to PE were reduced despite significant elevation of the sensitivity to the agonist (pD_2 6.24±0.03 vs 5.98±0.04; $n=9$).

In contrast to the α_1-adrenoceptor agonist, contractile responses to the protein kinase C activator PMA (10^{-6} mol l^{-1}) and those to the calcium channel activator Bay K 8644 (10^{-6} mol l^{-1}) did not differ between Sham and MI at 5 weeks (Table 4).

Role of calcium

Within 2 min after removal of extracellular calcium, responses to K-KRB, PMA (10^{-6} mol l^{-1}) or Bay K 8466 (10^{-6} mol l^{-1}) were abolished but transient responses to PE (10^{-5} mol l^{-1}) persisted. The agonist-induced response in calcium-free solution (i) was increased in the presence of K-KRB and reduced by PMA, but (ii) did not differ between MI and Sham under either condition at 3 or at 5 weeks (data not shown).

After exposure to PE in calcium-free solution, vessels responded to calcium with concentration-dependent contractions in the presence of K-KRB, PMA (10^{-6} mol l^{-1}), PE (10^{-5} mol l^{-1}) and both K-KRB and PE (10^{-5} mol l^{-1}) (Figure 2). At 3 weeks, the sensitivity to extracellular calcium did not differ between vessels of MI and Sham in the presence

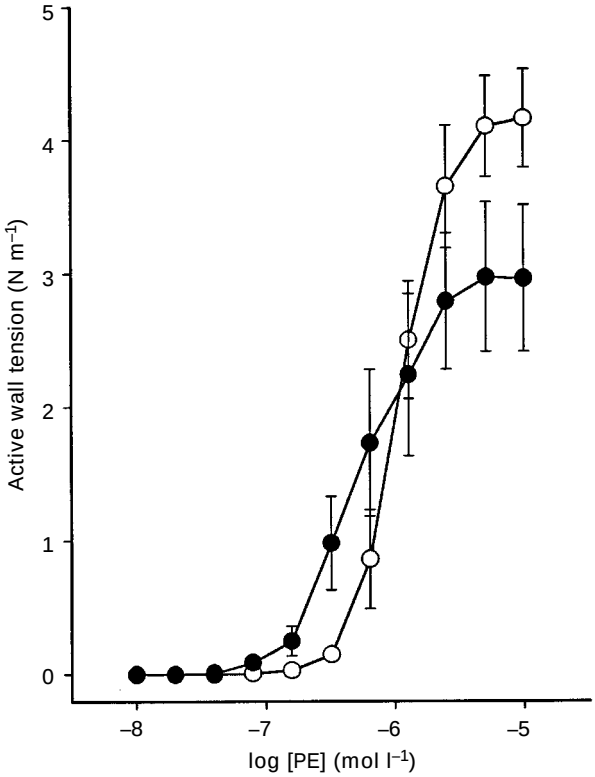

Figure 1 Cumulative concentration-response curves for phenylephrine (PE) in rat mesenteric resistance artery smooth muscle at 5 weeks after myocardial infarction (MI, ●) or sham surgery (Sham, ○). Data are expressed as increases in wall tension and are shown as mean ($n=9$); vertical lines indicate s.e.mean.

of high potassium and/or α_1-adrenoceptor agonist (Table 5). Also at 5 weeks after MI, calcium sensitivity in the presence of K-KRB, K-KRB plus PE (10^{-5} mol l^{-1}) or of PMA (10^{-6} mol l^{-1}) was not significantly modified (Table 5). Yet, at this stage the concentration of calcium that was required to induce 50% of the maximal response in the presence of PE was increased by almost 1 log unit compared to Sham (Figure 2, table 5). It is furthermore of interest that while calcium sensitivity was comparable during depolarization and agonist exposure for Sham, it was significantly lower in the presence of PE than in the presence of K-KRB at both 3 and 5 weeks after MI (Figure 2).

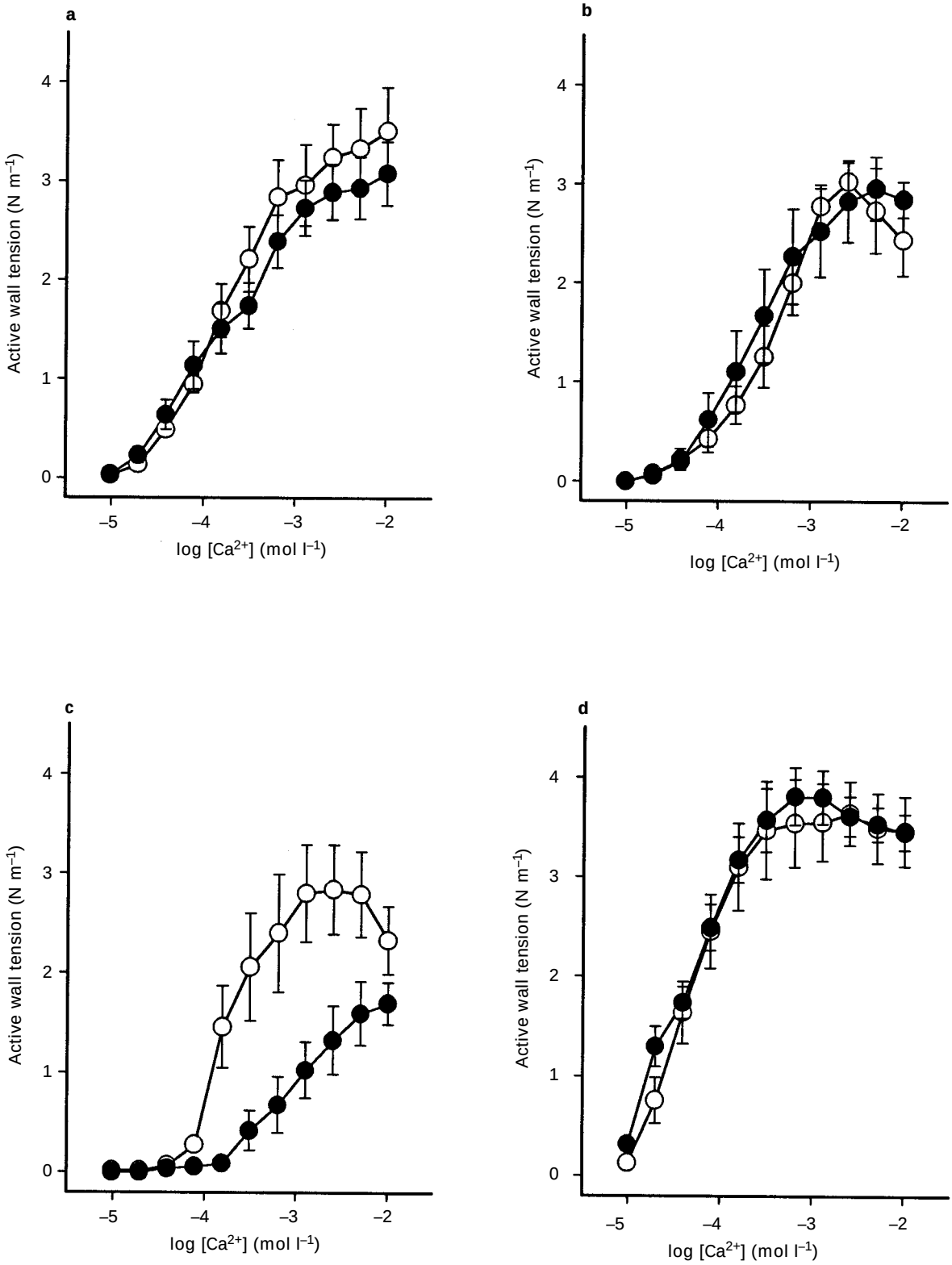

Figure 2 Cumulative concentration-response curves for calcium after exposure to calcium-free solution and depletion of the phenylephrine (PE)- sensitive intracellular calcium pool in rat mesenteric resistance artery smooth muscle at 5 weeks after myocardial infarction (MI, ●) or sham surgery (Sham, ○). Data are expressed as increases in wall tension and are shown as mean ($n=9$); vertical lines indicate s.e.mean. (a) In the presence of K-KRB; (b) in the presence of PMA (10^{-6} mol l^{-1}); (c) in the presence of PE (10^{-5} mol l^{-1}); (d) in the presence of both K-KRB and PE (10^{-5} mol l^{-1}).

α_1-Adrenoceptors

To evaluate whether reduced responses to PE in the presence of calcium and to calcium in the presence of PE were due to a reduction of α_1-adrenoceptor number, radioligand binding experiments were performed at 5 weeks after MI or sham operation. Findings with 0.035 to 0.8 nmol l^{-1} [^{3}H]-prazosin were expressed relative to the total protein content and total DNA content of the resistance artery preparations. The density of [^{3}H]-prazosin binding sites tended to be elevated after MI, irrespective whether density was expressed relative to protein content (B_{max}: 147 ± 42 vs 106 ± 15 fmol mg^{-1} protein) or to DNA content (B_{max}: 3.03 ± 1.40 vs 2.29 ± 0.21 fmol μg^{-1} DNA) (Figure 3). However, differences did not reach statistical significance. Also the affinity for the ligand was not significantly modified (K_D relative to protein: 0.32 ± 0.07 vs 0.35 ± 0.08 nM; K_D relative to DNA: 0.35 ± 0.11 vs 0.21 ± 0.03 nM).

Discussion

In a rat model of heart failure, contractile responses of mesenteric resistance arteries to calcium in the presence of PE were reduced after 5 weeks but not after 3 weeks. This does not seem to involve structural alterations, reduction of α_1-adrenoceptor density, impaired intracellular calcium release, or modified intracellular calcium sensitivity.

In the rat, infarction of the left ventricle leads to an increase in cardiac filling pressures (Thuillez *et al*., 1995), pulmonary oedema (Heeneman *et al*., 1995), a reduction of basal and maximally stimulated cardiac output (Nelissen-Vrancken *et al*., 1996), elevated total peripheral resistance (Pfeffer *et al*., 1985), and increased plasma levels of adrenaline, angiotensin II, vasopressin and ANF (Goldsmith *et al*., 1983; Hirsch *et al*., 1989). These have been considered signs in line with those observed in patients with heart failure. Clinical and experimental research of heart failure concentrates primarily on the myocardium and on the neurohumoral mechanisms that are engaged to maintain adequate perfusion. Observations of impaired exercise tolerance (Wilson & Mancini, 1993; Musch *et al*., 1986) and impaired contractile and relaxing reactivity in subcutaneous resistance vessels of patients with heart failure (Angus *et al*., 1993) suggest that the peripheral vasculature may be involved as well. This motiviated us to study the reactivity of isolated rat mesenteric resistance arteries following myocardial infarction. In order to concentrate on resistance arterial smooth muscle, the experiments were performed after acute removal or depletion of intravascular sources of vasoactive agents, such as endothelium, adrenergic nerves and sensory motor nerves.

Despite exposure to potential mitogens, such as adrenaline, angiotensin II, and vasopressin, mesenteric small artery structure was not modified at up to 5 weeks after myocardial infarction as has been previously shown (Heeneman *et al*., 1995). This could be due to (i) the limited and only transient elevation of these mediators (this study), (ii) the absence of pressure elevation (Schoemaker *et al*., 1991), and (iii) counteraction of hypertrophic effects by agents such as ANF which have been recognized to exert antimitogenic action in the rat arterial wall (Itoh *et al*., 1990). However, a significant reduction of the protein to DNA ratio was noted which is in line with the increased DNA to dry weight ratio observed by Heeneman *et al*. (1995). This suggests that while the mass of the vessel wall was not modified its composition was changed in this experimental pathological setting.

Up to 5 weeks after infarction, the contractile response of resistance artery smooth muscle to the combination of high

Table 5 Sensitivity to extracellular calcium during exposure to contractile stimuli in rat mesenteric resistance artery smooth muscle at 3 and 5 weeks after myocardial infarction (MI) or sham surgery

	3 weeks		*5 weeks*	
Stimulus	*Sham*	*MI*	*Sham*	*MI*
K-KRB	3.65 ± 0.12	4.06 ± 0.15	3.76 ± 0.09	3.87 ± 0.13
PMA (10^{-6} mol l^{-1})	NA	NA	3.47 ± 0.11	3.64 ± 0.03
PE (10^{-5} mol l^{-1})	3.61 ± 0.05	3.41 ± 0.06†	3.74 ± 0.16	2.81 ± 0.22†,*
K-KRB plus PE	4.67 ± 0.15†	4.76 ± 0.15†	4.46 ± 0.17†	4.68 ± 0.15†

The calcium concentrations required to induce 50% of the maximal response are expressed as $-\log$[mol l^{-1}] and presented as means $\pm$ s.e.mean ($n = 6-9$). K-KRB, 125 mmol l^{-1} potassium; PE, phenylephrine; NA, not available. †The difference from K-KRB is statistically significant ($P < 0.05$). *The difference from Sham is statistically significant ($P < 0.05$).

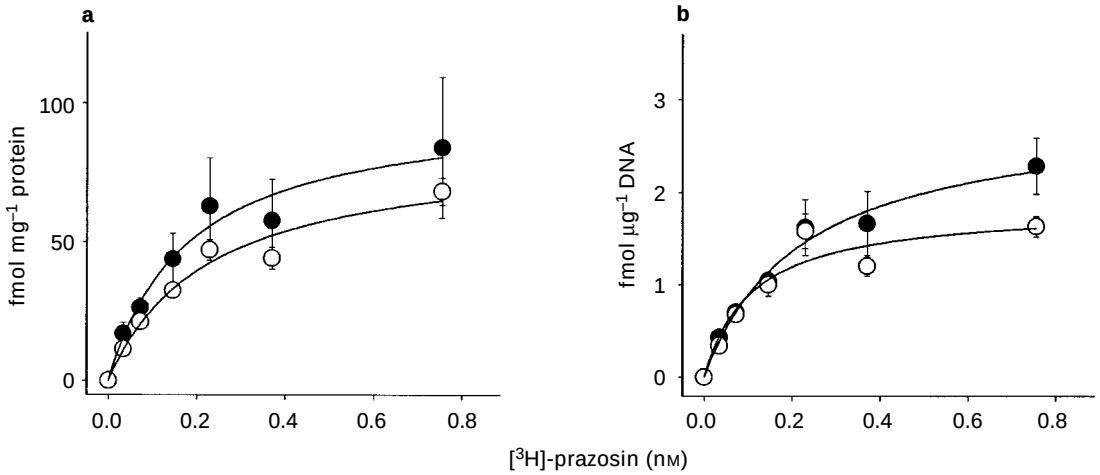

Figure 3 Saturation binding curves for [^{3}H]-prazosin in intact rat mesenteric resistance arteries at 5 weeks after myocardial infarction (●) or sham surgery (○). Observations were expressed relative to protein content (a) or DNA content (b) and are shown as mean ($n = 5-7$); vertical lines indicate s.e.mean. For affinities and densities, see text.

potassium, PE and extracellular calcium was not modified. Combined with an unaltered media mass this indicates that the contractility of the smooth muscle was not affected. The observation that responses to extracellular calcium in the presence of high potassium with and without PE and in the presence of a high concentration of PMA did not differ between Sham and MI at 3 and 5 weeks, indicates that the basal calcium sensitivity of the contractile apparatus and its hypersensitivity during protein kinase C stimulation were not modified either.

Unlike for high potassium, resistance artery maximal responses to PE in the presence of calcium and to calcium in the presence of PE were reduced after myocardial infarction. However, the capacity of PE to induce contraction in the absence of extracellular calcium and to increase the calcium sensitivity of depolarized preparations for extracellular calcium was not modified. Consequently, the observed alteration of resistance artery responsiveness seems to reside in processes leading to agonist-stimulated calcium influx and not in those involved in intracellular calcium release or sensitization of the contractile apparatus to calcium. We have previously suggested that protein kinase C activation and L-type voltage operated calcium channels participate in α_1-adrenoceptor stimulated calcium influx in rat mesenteric resistance arteries (Boonen & De Mey, 1990a; 1991). Yet, responses to phorbol ester and Bay K 8644, and their sensitivity to extracellular calcium were not altered after myocardial infarction. This suggests that the modification must be sought at a level proximal from protein kinase C and the calcium channels.

The density of high-affinity prazosin-binding sites was not reduced in mesenteric resistance arteries after myocardial infarction. This indicates that reduced receptor number is not responsible for the impaired responsiveness of peripheral vascular smooth muscle to α_1-agonist. Aspects that may be important to link these receptors to calcium influx include resting membrane potential and guanosine 5′-triphosphate (GTP)-binding regulatory proteins. Because contractile responses to Bay K 8644 and their amplification by small increases in extracellular potassium concentration were not modified after MI, a modification of resting membrane potential seems unlikely. Concerning the GTP-binding proteins, we have previously demonstrated that primarily pertussis toxin sensitive G-proteins are involved in rat mesenteric resistance artery responses to α_1-adrenoceptor stimulation, since contractile responses to PE and aluminium fluoride were readily abolished in these arteries after pretreatment with pertussis toxin (Boonen & De Mey, 1990b). However, in heart failure increased rather than decreased levels of pertussis toxin sensitive G-proteins (G_i) have been implicated in the modified β- and α_2-adrenoceptor responses of the myocardium (Neumann *et al*., 1988; Brodde, 1991) and have also been noted in erythrocytes (Kots *et al*., 1993). We observed a reduction of maximal responses to α_1-adrenoceptor stimulation, although the sensitivity to the agonist was increased and although the contractile effect of the agonist in the absence of extracellular calcium was not modified. It may be worth considering that this discrepancy finds its origin in the diversity of α_1-adrenoceptors. High-affinity prasozin binding sites consist of at least 3 different α_1-adrenoceptor subtypes. The α_{1A}- and α_{1B}-subtypes coexist in the mesenteric vasculature of intact rats (Piascik *et al*., 1994). More detailed pharmacological analyses and ligand binding studies will be required to verify whether changes in subtype composition develop with progression of heart failure.

It is well established that myocardial infarction leads to activation of various neurohumoral systems, including the sympathetic nervous system, and that chronically elevated levels of catecholamines can lead to homologous and heterologous desensitization of effector organs and to down regulation of their receptors (Rosenbaum *et al*., 1986; Hiremath *et al*., 1991; Boonen *et al*., 1993). However, in the present study no alterations of α_1-adrenoceptors were noted and reduced resistance artery reactivity became apparent only after plasma hormone-levels had largely normalized. Although only ANF, and possibly angiotensin II remained elevated at 5 weeks after infarction, this pathological condition is likely to involve various neurohumoral systems (Francis, 1988; Sigurdsson *et al*., 1993; Svanegaard *et al*., 1993) and it will be of interest to verify whether the changes we observed are restricted to α_1-agonists or involve stimuli such as angiotensin II, ANF and endothelin as well.

The mesenteric arterial bed participates in the control of peripheral vascular resistance and in the redistribution of blood flow during exercise. Altered mesenteric arterial smooth muscle function as noted in the present study, could compromize the compensatory elevation of resistance in heart failure and thus contribute to reduced exercise tolerance in heart failure. The mechanistic analysis of the suspected impairment of agonist-stimulated calcium influx may in the long run contribute to better pharmacotherapy of the peripheral vascular aspects of heart failure.

In summary, we observed that the responsiveness of mesenteric resistance artery smooth muscle to PE and calcium was progressively reduced after myocardial infarction in the rat. This was not due to a decrease in smooth muscle mass or altered α_1-adrenoceptor density but may involve reduced agonist-stimulated calcium influx.

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