Role of basal nitric oxide synthesis in vasoconstrictor hyporeactivity in the perfused rat hindlimb after myocardial infarction: effect of captopril

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Abstract

Objectives: The contribution of vascular changes to the development of heart failure is largely unknown. In the present study, we evaluated endothelial and vascular contractile function in the rat hindlimb vascular bed after myocardial infarction (MI), including the modulatory role of basal nitric oxide (NO) production and the effects of treatment with the angiotensin converting enzyme inhibitor captopril on vascular function. Methods: MI was induced in male Wistar rats by ligation of the left coronary artery. Acetylcholine-induced dilatations were assessed in the ex vivo perfused hindlimb at various time points. At 2 and 5 weeks post-MI, vascular contractile function in the perfused hindlimb was assessed from resistance changes induced by 35 mM and 125 mM potassium (K) and the maximum increase in resistance (ΔRmax, 125 mM K and 3 mg phenylephrine). Basal NO synthesis was blocked for 2 weeks with L-nitro-arginine methylester (L-NAME) in sham and MI rats and similar contractility experiments were performed. The effect of captopril treatment from 2 to 5 weeks post-MI on vasoconstrictor responses was also tested. Results: Acetylcholine-induced dilatations in the presence of 10 μM indomethacin were not different between sham and MI rats. Vasoconstrictor responses to K and ΔRmax were reduced at 2 weeks after MI. This reduction in vasoconstrictor ability was similar to that seen in L-NAME-treated sham rats, while chronic L-NAME treatment did not affect vasoconstrictor reactivity in MI rats. Similarly, L-NAME induced an increase in mean arterial pressure in sham rats, but not in MI rats. At 5 weeks after MI, vasoconstriction to 125 mM K and ΔRmax were still reduced in MI rats; this response was however partially restored after captopril treatment. Conclusion: The development of vascular contractile hyporeactivity in the rat hindlimb after MI may be due to reduced basal NO production. Delayed treatment with captopril improves peripheral vascular contractile function in this setting. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Heart failure; Myocardial infarction; Vasoconstriction; Endothelium-derived factors

1. Introduction

Abnormal responsiveness of the peripheral vasculature may contribute to the common symptoms of fatigue and reduced exercise tolerance in patients with heart failure. Reduced arterial dilator function may restrict blood flow to peripheral organs. Although clinical studies have demonstrated diminished endothelium-dependent relaxations [1,2], the basal production of the endothelium-dependent vasodilator, nitric oxide (NO) has been reported to be either increased or decreased [2,3]. In experimental animal models, acetylcholine (ACh)-induced responses have also generally been found to be reduced, but studies have largely centered on early time points and on large vessels [4,5]. Studies with inhibitors of NO synthase generally suggest that systemic and regional basal NO production is unaltered in experimental heart failure [6,7]. However as in vitro contractile hyperreactivity can be sometimes abolished by removal of the endothelium, evidence pointing towards decreased quantities of constitutively released endothelium-dependent dilators also exists [8,9]. While a shift towards exaggerated arterial constriction would impede blood flow to peripheral organs, reduced contractile function may result in inefficient cardiac output.
distribution. Angus et al. [10] demonstrated such a diminished contractile reactivity in isolated resistance arteries from patients with heart failure. In experimental heart failure, various authors have also demonstrated diminished vasoconstrictor capacity [9,11]. Teerlink et al. [9] demonstrated hyporeactivity to α-adrenergic stimuli and potassium in denuded thoracic aortas of rats at one week after myocardial infarction (MI). While Stassen et al. [11] found no alteration in rat thoracic aorta responsiveness five weeks after MI, they noted a non-selective hyporeactivity to vasoconstrictors in isolated mesenteric resistance arteries.

The aforementioned MI model in rats is an established model for studying the development and progression of heart failure [12–14]. In this model, acute hemodynamic adaptation deteriorates to overt heart failure, as characterized by increased left ventricular end-diastolic pressure, decreased cardiac index, and increased systemic vascular resistance [12,13]. The MI rat has been used to investigate mechanisms conferring the beneficial effects of angiotensin converting enzyme (ACE) inhibitors in heart failure treatment. ACE inhibitors have been shown to improve systemic hemodynamics, retard left ventricle hypertrophy and fibrosis, and prevent endothelial dysfunction [13–16]. While immediate administration of ACE inhibitors may impede the wound healing process in the infarcted heart, delayed administration of these drugs seems to retard the progression of heart failure [13,15].

For the present study, we hypothesized that vascular dysfunction may contribute to the pathology of heart failure. Accordingly, we examined vascular dilator and constrictor responses of a skeletal muscle vascular bed at physiological flows in the rat MI model. Minimal vascular resistance and endothelial function were examined at various time points after MI to assess whether dilator capacity changes with the development of heart failure. Furthermore, vascular contractile function was evaluated at 2 and 5 weeks post-MI. We chose these time frames to facilitate comparison with earlier studies from our laboratory [11,17]. As the hindlimb vascular bed demonstrated contractile dysfunction, we hypothesized that changes in NO levels may contribute to the development of vasoconstrictor hyporeactivity. Therefore, we assessed the effects of chronic NO synthase inhibition and late ACE inhibitor treatment with captopril on this abnormality.

2. Methods

2.1. Animals, surgery, and treatment

Male Wistar rats (280–320 g, Iffa Credo, Someren, The Netherlands) were housed under standard conditions (20°C, 12-h light/dark cycle) and given free access to standard chow (Hope Farms) and tap water. The experimental procedures were performed according to institutional guidelines and approved by the Ethical Committee for the Use of Experimental Animals of the Maastricht University (The Netherlands).

Rats were randomly selected to undergo myocardial infarction (MI) or sham surgery (sham). MI was induced by permanent ligation of the left coronary artery, as described previously [13]. Briefly, animals were anesthetized (pentobarbital; 60 mg/kg i.p.), intubated, and ventilated (room air; 60 strokes/min; tidal volume 3 ml). Following thoracotomy, the heart was exteriorized and a 6-0 silk suture looped around the proximal left coronary artery. After returning the heart to its normal position, the suture was securely ligated in the MI group. In sham rats, a superficial ligature was placed in the left ventricular wall near the left coronary artery and loosely tied. The thorax was closed, and the animals were allowed to recover for 1, 2, 3, 5, 13 weeks.

On the day of surgery, sham and MI rats were randomly selected and equipped with an osmotic mini-pump (2002, Alzet, Alza, Palo Alto, CA, USA) administering 25 mg/kg/day L-nitro-arginine methylester (L-NAME) s.c. for 2 weeks. In separate groups of sham and MI rats, captopril was administered at 12 mg/kg/day via osmotic minipumps (2ML2, Alzet) s.c. from days 21 to 35. Mini-pumps were implanted subcutaneously in the neck under ether anesthesia.

2.2. Measurement of blood pressure in conscious rats treated chronically with L-NAME

On treatment day 12, the rats were anesthetized with pentobarbital (60 mg/kg i.p.) and equipped with an arterial catheter (PE 50) implanted in the left carotid artery and aimed to terminate at the junction with the aortic arch. Saline-filled catheters were guided subcutaneously to the base of the neck, exteriorized, and sealed with a metal plug.

Mean arterial pressure (MAP) was measured in quietly resting rats over a period of approximately an hour on the morning of day 14 via a pressure transducer (CP-01, Century Technology, Inglewood, CA, USA) in conjunction with an on-line monitoring computer program (Hemodynamic Data Acquisition Systems, Instrumental Services, Universiteit Maastricht). Pressure was sampled at 10 Hz, averaged and recorded every 5 s.

2.3. Hindlimb perfusion

Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and subjected to hindlimb perfusion as described previously [18]. Briefly, after opening the abdomen, all side branches of the aorta and vena cava distal to the kidneys and proximal of the iliac bifurcation were ligated. Upon cannulation of the abdominal aorta, perfusion with an isotonic, oxygenated (95% O₂–5% CO₂) Krebs–Henseleit buffer via a wind kettle was initiated.
Flow was set to 7.5 ml/min for both sham and MI rats, since we and others noted no differences in resting skeletal muscle blood flow between groups [6,19]. Eluate was collected from a cannula implanted in the vena cava. The animal was killed (intracardiac injection of a saturated KCl solution) and the hindlimb flushed of all blood for approximately half an hour before starting experimentation. The aforementioned monitoring program was utilized to monitor pressure ($P$; as above) and flow ($F$; 1 mm diameter flow-through electromagnetic flow probe, Skalar, Delft, The Netherlands). Pressure and flow were sampled at 1000 Hz and resistance was calculated ($R = P/F$) on line. Drugs were administered proximal to a mixing chamber and experiments were conducted at 37°C.

2.4. Protocol

2.4.1. Minimal resistance ($R_{\text{min}}$) and endothelial function in untreated sham and MI rats

At 1, 3, 5, and 13 weeks after surgery, randomly-selected untreated sham and MI rats were chosen to undergo a hindlimb perfusion protocol to examine $R_{\text{min}}$ and endothelial function. For these experiments, the Krebs–Henseleit buffer was modified to include 0.1 μM indomethacin to inhibit cyclooxygenase-induced prostaglandin synthesis. After initiating hindlimb perfusion as described above, two subsequent bolus injections of 500 μg sodium nitroprusside (SNP) were injected to achieve $R_{\text{min}}$.

To assess endothelial function, the hindlimb vascular bed was constricted by a resistance of 2.7 mmHg·min/ml via a phenylephrine (Phe) infusion (≈7.5 μM) and a non-cumulative ACh dose–response curve (0.10 ng–10 μg) was generated. Thereafter, 150 μg l-nitro-arginine (L-NA), an NO synthase inhibitor, was injected. This dose was chosen from pilot experiments in which the ED$_{50}$ for the constrictor effect of L-NA was determined to be ≈60 μg [20]. After blocking NO synthase, 10 μg ACh was administered to assess the non-NO, non-prostaglandin component of the ACh-induced dilatation. Two subsequent 500-μg doses of SNP were injected to assess endothelium-independent vasodilator function.

2.4.2. $R_{\text{min}}$ and constrictor function in treated and untreated sham and MI rats

In separate groups of sham and MI animals at 2 and 5 weeks after surgery, $R_{\text{min}}$ was determined. An infusion of a potassium chloride solution (K$^+$) was then used to determine responses to 35 mM K$^+$ and 125 mM K$^+$. A 3-mg bolus injection of Phe was administered on top of the 125 mM K$^+$ response in order to determine the maximal increase in resistance ($\Delta R_{\text{max}}$). The dose of Phe is 100 times greater than the dose shown to induce a maximal α-adrenergic effect in a previous study [18].

2.5. Determination of myocardial infarction size

After experimentation, hearts were excised, the atria removed, and the ventricles cut into transverse slices of 1–2 mm, resulting in 5–6 slices. The mid-ventricular slice was fixed in 3% formaldehyde and embedded in paraffin. Whereafter transverse sections (4 μm) were stained according to the modified AZAN technique [21]. Infarct size was determined by planimetry and expressed in percentage of left ventricle circumference, calculated as the average of infarct sizes of endocardial and epicardial surfaces [21]. Only hearts with infarct sizes >21% were used in the MI groups, as smaller infarcts do not have detectable hemodynamic consequences in vivo [13].

2.6. Materials

The Krebs–Henseleit buffer had the following composition (mM): 111 NaCl, 5 KCl, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 1.25 CaCl$_2$, 11.1 glucose, and 40 g/l dextran-70, pH 7.4. All inorganic salts were purchased from Merck (Amsterdam, The Netherlands); dextran-70, captopril, indomethacin, phenylephrine, and L-NAME from Sigma (St. Louis, MO, USA); sodium nitroprusside from Janssen (Beerse, Belgium); L-NA from Research Biomedicals (Natick, MA, USA); acetylcholine from Ciba Vision Ophtha (Breda, The Netherlands).

2.7. Data analysis

All dilatations are expressed as percent of the preconstriction. The half-maximal effective dose (ED$_{50}$) and maximal response values for the ACh dose–response curve were obtained from non-linear regression analysis of individual dose–response curves according to the equation

$$\Delta R = \frac{\Delta R_{\text{max}} \cdot D^n}{ED_{50} + D^n}$$

where $\Delta R$ is the change in resistance, $\Delta R_{\text{max}}$ the maximal change in resistance, $D$ the dose of ACh, $n$ the Hill coefficient, and ED$_{50}$ the negative log of the dose inducing half maximal effect.

Minimal resistance, dilatation data and constrictor responses to L-NA for sham and MI rats at the different time points were compared by means of a Mann–Whitney U-test for unpaired observations. The contribution of the non-NO, non-prostaglandin component of ACh-induced dilatations was calculated as the response to 10 μg ACh after L-NA relative to that before L-NA.

Constrictions are expressed as absolute changes in resistance. The effect of MI surgery and L-NAME treatment on vascular function was assessed via the non-parametric Kruskal Wallis test. Similarly, the effect of MI surgery and captopril treatment on vascular function were also analyzed using the non-parametric Kruskal Wallis test.
All data are expressed as mean±S.E.M. Differences were considered statistically significantly at a value of \(P<0.05\).

3. Results

3.1. General characteristics

MI had no effect on body weights at 1, 3, 5, 13 weeks after surgery (Table 1). Heart-to-body weight ratios were significantly increased at 1, 5, and 13 weeks after MI.

3.2. \(R_{\text{min}}\) in untreated rats

Vascular tone in the buffer-perfused rat hindlimb was low, such that at near physiological flows of 7.5 ml/min, resistances of \(\approx 3.6\ \text{mmHg} \cdot \text{min/ml}\) were noted and two subsequent bolus injections of 500 \(\mu\text{g}\) sodium nitroprusside had little effect on resistance. Dilatations were in the order of a few percent and were not different between surgical groups at any time point (data not shown). \(R_{\text{min}}\) was not altered in the rat hindlimb at 1, 3, 5, 13 weeks after MI compared to sham rats (Table 1).

3.3. Endothelial function

ACh dilated the Phe-preconstricted hindlimb at all time points in a dose-dependent manner as shown at 13 weeks after surgery in Fig. 1. As shown in Table 2, neither the sensitivity, as indicated by the \(E_{50}\), nor the maximal dilator effect of ACh were altered after MI. Furthermore, the constriction induced by 150 \(\mu\text{g}\) l-NA was not different at any time point after surgery in MI, compared to sham rats. l-NA did not inhibit the dilatation to 10 \(\mu\text{g}\) ACh at any time point in either sham or MI rats. Maximal endothelium-independent vasodilator responses to sodium nitroprusside were approximately 90% of the preconstriction level and were not affected by the induction of MI (data not shown).

### Table 1

<table>
<thead>
<tr>
<th>Weeks post-op</th>
<th>Group</th>
<th>(n)</th>
<th>BW (g)</th>
<th>HW/BW</th>
<th>MI size (%)</th>
<th>(R_{\text{min}}) (mmHg·min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham</td>
<td>7</td>
<td>308±5</td>
<td>0.296±0.008</td>
<td>41±3</td>
<td>3.16±0.11</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>8</td>
<td>294±6</td>
<td>0.348±0.011*</td>
<td>46±3</td>
<td>3.33±0.13</td>
</tr>
<tr>
<td>2</td>
<td>Sham</td>
<td>10</td>
<td>274±7</td>
<td>0.274±0.017</td>
<td>41±4</td>
<td>3.42±0.13</td>
</tr>
<tr>
<td></td>
<td>Sham + l-NAME</td>
<td>7</td>
<td>277±5</td>
<td>0.314±0.012</td>
<td>41±4</td>
<td>3.44±0.15</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>10</td>
<td>304±8*</td>
<td>0.295±0.008</td>
<td>41±4</td>
<td>3.33±0.18</td>
</tr>
<tr>
<td></td>
<td>MI + l-NAME</td>
<td>7</td>
<td>263±8</td>
<td>0.298±0.024</td>
<td>41±5</td>
<td>3.37±0.14</td>
</tr>
<tr>
<td>3</td>
<td>Sham</td>
<td>8</td>
<td>354±4</td>
<td>0.297±0.007</td>
<td>41±5</td>
<td>3.36±0.15</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>8</td>
<td>358±9</td>
<td>0.312±0.014</td>
<td>41±5</td>
<td>3.14±0.10</td>
</tr>
<tr>
<td>5</td>
<td>Sham</td>
<td>18</td>
<td>393±8</td>
<td>0.271±0.006</td>
<td>41±5</td>
<td>3.43±0.11</td>
</tr>
<tr>
<td></td>
<td>Sham + cap</td>
<td>7</td>
<td>365±8*</td>
<td>0.271±0.007</td>
<td>41±5</td>
<td>3.27±0.20</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>19</td>
<td>387±10</td>
<td>0.312±0.012*</td>
<td>47±2</td>
<td>3.30±0.17</td>
</tr>
<tr>
<td></td>
<td>MI + cap</td>
<td>9</td>
<td>324±11*</td>
<td>0.281±0.017</td>
<td>43±3</td>
<td>3.10±0.10</td>
</tr>
<tr>
<td>13</td>
<td>Sham</td>
<td>17</td>
<td>447±11</td>
<td>0.261±0.006</td>
<td>40±3</td>
<td>3.24±0.15</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>12</td>
<td>456±7</td>
<td>0.330±0.012*</td>
<td>40±3</td>
<td>3.56±0.25</td>
</tr>
</tbody>
</table>

*l-NAME: 25 mg/kg/day s.c. from day 0 to 14. cap: 12 mg/kg/day captopril s.c. from days 21 to 35 post-operative; BW, body weight; HW, heart weight; \(R_{\text{min}}\), minimal resistance in the perfused hindlimb vasculature; mean±S.E.M.

\*\(P<0.05\) vs. untreated sham.

\*\(P<0.05\) vs. untreated MI day.
Table 2
Endothelial function in the presence of 10 μM indomethacin the perfused hindlimb of sham and myocardially infarcted (MI) rats

<table>
<thead>
<tr>
<th>Weeks post-op</th>
<th>Group</th>
<th>n</th>
<th>ACh ED$_{50}$ (ng)</th>
<th>ACh$_{\text{max}}$ (%)</th>
<th>ΔR$_{\text{NA}}$ (mmHg min/ml)</th>
<th>ACh$<em>{\text{post-L-NA}}$ / ACh$</em>{\text{pre-L-NA}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham</td>
<td>7</td>
<td>23 ± 1</td>
<td>77 ± 4</td>
<td>4.76 ± 0.72</td>
<td>1.10 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>8</td>
<td>14 ± 3</td>
<td>79 ± 3</td>
<td>5.63 ± 1.11</td>
<td>1.12 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>Sham</td>
<td>8</td>
<td>19 ± 10</td>
<td>82 ± 3</td>
<td>4.07 ± 0.33</td>
<td>1.05 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>8</td>
<td>25 ± 7</td>
<td>75 ± 4</td>
<td>4.90 ± 0.80</td>
<td>1.10 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>Sham</td>
<td>9</td>
<td>18 ± 6</td>
<td>72 ± 4</td>
<td>3.38 ± 0.86</td>
<td>1.02 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>11</td>
<td>22 ± 5</td>
<td>81 ± 2</td>
<td>4.34 ± 0.81</td>
<td>1.08 ± 0.01</td>
</tr>
<tr>
<td>13</td>
<td>Sham</td>
<td>12</td>
<td>21 ± 4</td>
<td>65 ± 4</td>
<td>4.65 ± 0.70</td>
<td>1.12 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>12</td>
<td>15 ± 3</td>
<td>72 ± 6</td>
<td>4.26 ± 0.46</td>
<td>1.06 ± 0.03</td>
</tr>
</tbody>
</table>

* ACh ED$_{50}$, half-maximal effective doses for acetylcholine calculated from dose–response curves; ACh$_{\text{max}}$, maximal relaxation calculated from dose–response curves; ΔR$_{\text{NA}}$, change in resistance induced by 150 μg l-nitro-arginine; ACh$_{\text{post-L-NA}}$ / ACh$_{\text{pre-L-NA}}$, non-NO component of ACh relaxation; mean ± S.E.M.

3.4. Vasoconstrictor function

Vascular contractile function in the perfused hindlimb was significantly reduced 2 weeks after MI. As shown in Fig. 2, the responses to 35 mM K$^+$ (4.1 ± 0.6 mmHg min/ml) and 125 mM K$^+$ (15.7 ± 1.0 mmHg min/ml), as well as ΔR$_{\text{max}}$ (22.2 ± 1.3 mmHg min/ml) were reduced in MI rats compared to sham rats (7.4 ± 0.7, 21.9 ± 0.9, and 27.7 ± 1.6 mmHg min/ml, respectively).

Unlike at 2 weeks after MI, the absolute change in resistance generated by 35 mM K$^+$ (Fig. 3) was not altered at 5 weeks after MI. Nonetheless, constrictor responses to 125 mM K$^+$ and maximal contractile ability were still significantly reduced (125 mM K$^+$: 15.7 ± 0.73 vs. 19.3 ± 0.9 mmHg min/ml; ΔR$_{\text{max}}$:21.7 ± 1.4 vs. 26.1 ± 1.3 mmHg min/ml; Fig. 3) in untreated MI rats compared to their sham counterparts.

3.5. Effect of l-NAME treatment

Body weight was significantly greater in untreated MI rats compared with the l-NAME treated MI rats and with untreated and l-NAME-treated sham rats at 2 weeks after surgery (Table 1). Treatment with l-NAME had no effect on heart-to-body weight ratios in either sham or MI rats. MI rats had lower blood pressures than their sham counterparts (109 ± 3 vs. 121 ± 3 mmHg). l-NAME induced hypertension in sham rats (164 ± 8 mmHg), and raised blood pressure significantly in MI rats (122 ± 5 mmHg). The blood pressure rise in MI rats was however less than that in sham rats in both absolute (13 vs. 43 mmHg) and relative terms (12 vs. 36%).

Two-week treatment with l-NAME did not alter R$_{\text{min}}$ in sham or MI rats (Table 1). Vasoconstrictor responses to potassium and ΔR$_{\text{max}}$ were reduced in l-NAME-treated
sham rats versus their untreated counterparts (35 mM K\(^+\): 4.3±0.5 mmHg·min/ml; 125 mM K\(^+\): 17.5±1.0 mmHg·min/ml; ΔP\(_{\text{max}}\): 20.4±1.5 mmHg·min/ml; Fig. 3). This reduction in vasoconstrictor ability was similar to that seen in untreated MI rats at 2 weeks after MI. In MI rats, 2-week administration of L-NAME had virtually no effect on vasoconstrictor function in MI rats: vasoconstriction to 35 mM K\(^+\) was significantly larger in treated (6.3±0.5 mmHg·min/ml; Fig. 2) than untreated MI but still slightly, though not significantly, reduced compared to untreated sham rats. Responses to 125 mM K\(^+\) and ΔP\(_{\text{max}}\) were not altered in L-NAME-treated MI compared to either untreated MI or L-NAME treated sham rats (125 mM K\(^+\): 17.5±1.0 mmHg·min/ml; ΔP\(_{\text{max}}\): 20.4±1.5 mmHg·min/ml; Fig. 2).

3.6. Effect of captopril treatment

Both sham and MI rats treated with captopril had lower body weights than their untreated counterparts (Table 1). This decrease in body weight was paralleled by a reduction in heart weight, as heart-to-body weight ratios were not changed. Captopril additionally reduced cardiac hypertrophy in MI rats since heart-to-body weight ratio in treated MI rats was not different from that in treated or untreated sham rats. Furthermore, treatment with captopril did not modify R\(_{\text{min}}\) (Table 1).

The response to 35 mM K\(^+\) was significantly increased in captopril-treated compared to untreated MI rats (6.4±0.6 vs. 4.1±0.3 mmHg·min/ml; Fig. 3). Additionally, captopril restored the constrictor response to 125 mM K\(^+\) (P<0.05 vs. untreated MI rats and P>0.1 vs. treated and untreated sham rats; Fig. 3). Treatment with captopril also slightly improved the maximal constrictor response in MI rats; ΔP\(_{\text{max}}\) generated in treated-MI rats (24.1±1.4 mmHg·min/ml) was not reduced compared to either treated (27.4±2.3 mmHg·min/ml) or untreated (26.1±1.3 mmHg·min/ml) sham rats.

4. Discussion

In the present study, we examined the dilator and constrictor function of a skeletal muscle vascular bed at physiological flows in the rat MI model. Acetylcholine-induced dilatations were not altered in the hindlimb vascular bed up to 3 months after myocardial infarction. Nonetheless, vasoconstrictor responses were reduced at both 2 and 5 weeks after MI. The reduction in vasoconstrictor ability at 2 weeks was similar to that seen in L-NAME-treated sham rats, while L-NAME had no effect on vasoconstrictor responsiveness in MI rats. Two-week treatment with the angiotensin-converting enzyme inhibitor captopril from week 3 to 5 significantly improved peripheral vascular constrictor function in MI rats.

Large left ventricular infarctions in the rat as seen in this study have been shown to result in hemodynamic alterations analogous to those in clinical heart failure. Acute cardiac depression with decreased cardiac output and stroke volume [22] is followed by a period of circulatory compensation which deteriorates into overt heart failure characterized by elevated left ventricular end diastolic pressures, increased total peripheral resistance, left ventricular hypertrophy, and pulmonary congestion [12–14].

4.1. Vasodilator function

Despite the classic features of heart failure which characterize the present model, evidence for a reduced stimulated endothelium-dependent dilator function, a recurrent symptom of clinical heart failure [1,2], was not found in the perfused hindlimb vascular bed in the present work. Nonetheless, several studies using the MI rat model have demonstrated depressed ACh-induced relaxation in isolated aortae [4,27–29]. Although these diminished responses were sometimes found to be endothelium-dependent [4,27,29], coincident decreases in endothelium-independent dilator responses were also found [28]. Since impaired non-specific vasodilatory capability has also been observed in human heart failure [1], care should be prescribed in the assessment of endothelium-dependent versus general vasodilator dysfunction.

In the femoral artery of dogs with pacing-induced heart failure, Kaiser et al. [5] demonstrated that diminished ACh-induced responses were normalized in the presence of indomethacin suggesting a role for prostaglandins in the reduced ACh-induced dilatations seen in that model. Increased plasma levels of vasoconstrictor prostaglandins have likewise been demonstrated in human heart failure [1]. Thus, these previous findings suggest that the inclusion...
of indomethacin in the perfusate in the present study may partially explain the discrepancy of our results with other studies and that the role of vasoconstrictor prostaglandins in heart failure warrants further attention.

The aforementioned studies strongly suggest that endothelial dilator capability is impaired in large vessels during heart failure; however, the situation in the resistance vasculature is less clear. Recent studies by Drexler and Lu [6] and Mulder et al. [16] support the development of endothelial dysfunction in resistance vascular beds after MI in rats. However, in line with the findings of the present study, Baggia et al. [30] reported endothelial dysfunction in large, but not in resistance-sized, vessels. While NO seems to be the primary endothelium-derived relaxing factor in large vessels, surmounting evidence points towards the increased contribution of endothelium-derived hyperpolarizing factor (EDHF) in relaxation of vascular contractile ability put forth in the present study is a phenomenon which has been previously observed [36,37]. This effect may be directly due to decreased NO levels of indirectly due to concomitant changes in other hormones, namely angiotensin II and endothelin. However, while chronic inhibition of NO synthase has been shown to increase the activity of the renin–angiotensin system [38], we have shown that chronic infusions of angiotensin II do not lead to decreased vascular smooth muscle function [39]. Likewise, while endothelin levels have been shown to be increased in this model of heart failure [40], no studies have demonstrated chronic negative effects of endothelin on vascular smooth muscle function. On the other hand, a recent study by Boerth et al. [41] suggests that NO contributes to the maintenance of a contractile phenotype in vascular smooth muscle cells via a cGMP-dependent kinase (PKG). Thus, it seems possible that a certain amount of NO is required for maintenance of normal smooth muscle function.

4.3. Vasoconstrictor function

Potassium-induced constrictions and maximal vasoconstrictor responses were found to be diminished at both 2 and 5 weeks after MI. This finding suggests that the noted vasoconstrictor dysfunction is not a receptor-specific phenomenon, but a general contractile defect. These results parallel work by Angus et al. [10] in skin resistance arteries from patients with heart failure and studies from our laboratory in isolated mesenteric resistance vessels of rats after MI [11,17]. All in all, the reduction in depolarization-induced and maximal vasoconstrictor responses seems to indicate generalized vasoconstrictor dysfunction in the hindlimb of the MI rat.

4.4. Reduced basal NO levels in heart failure

Several lines of evidence from the present findings suggest that basal NO levels may be reduced after MI in the rat and that this may contribute to the development of vascular contractile dysfunction in the hindlimb vascular bed. To begin, vasoconstriction upon acute NO synthase inhibition was not different in sham and MI rats; if anything the values were slightly but not significantly higher in MI rats. Undiminished constrictions to acute blockade of NO synthase in MI rats in light of the aforementioned general vasoconstrictor dysfunction suggest that NO synthase inhibition withdrew a larger fraction of the total basal NO production in the MI rats. Furthermore, chronic NO synthase inhibition increased blood pressure to a much lesser degree in MI rats. Lastly, 2-week administration of L-NAME reduced vascular contractile ability in sham rats analogous to the vasoconstrictive dysfunction seen in MI rats two weeks after surgery, whereas L-NAME treatment did not alter vasoconstrictor responses in MI rats. Thus, basal NO synthesis may be reduced after MI. Additional support for reduced NO production in heart failure arises from studies demonstrating decreased expression of NO synthase [32,33] and increased plasma concentrations of endogenous NO synthase inhibitors [34]. Furthermore, the availability of L-arginine, the substrate for NO synthase may be limiting since it has been demonstrated that L-arginine infusions improve endothelial function in heart failure [35].

The deleterious effect of reduced basal NO levels on vascular contractile ability put forth in the present study is a phenomenon which has been previously observed [36,37]. This effect may be directly due to decreased NO levels of indirectly due to concomitant changes in other hormones, namely angiotensin II and endothelin. However, while chronic inhibition of NO synthase has been shown to increase the activity of the renin–angiotensin system [38], we have shown that chronic infusions of angiotensin II do not lead to decreased vascular smooth muscle function [39]. Likewise, while endothelin levels have been shown to be increased in this model of heart failure [40], no studies have demonstrated chronic negative effects of endothelin on vascular smooth muscle function. On the other hand, a recent study by Boerth et al. [41] suggests that NO contributes to the maintenance of a contractile phenotype in vascular smooth muscle cells via a cGMP-dependent kinase (PKG). Thus, it seems possible that a certain amount of NO is required for maintenance of normal smooth muscle function.

4.5. Angiotensin converting enzyme inhibition in heart failure

The peripheral circulatory benefits of ACE inhibitors have been assumed to arise from their favorable effect on endothelium-dependent dilatation [8,16,42]. The present study suggests that, at least part of the peripheral effects of ACE inhibition post-MI may stem from the improvement of vasoconstrictor function via increased NO production. ACE inhibition augments NO levels through inhibition of bradykinin degradation [43]. Thus, administration of ACE inhibitors after MI in the rat may increase NO synthesis and thereby improve reduced vasoconstrictor responses. Indeed, Henrion et al. [37] recently reported that contractile hyporeactivity consequent to chronic NO synthase inhibition was prevented when an NO synthase and ACE inhibitor were administered concomitantly.

Okumura et al. [8] have presented data which suggest that chronic ACE inhibitor treatment can prevent enhanced vasoconstrictor reactivity in the rat after MI. At first
glance, these findings seem contradictory to the results of the present study. However, the contractile hyperreactivity noted in large vessels was endothelium-dependent and perhaps also due to a reduction in basal NO production [8]. The divergence with the results of the present study may be due to the unequivocal role of NO in large and small vessels as discussed above. Additionally, similar to the beneficial effects of ACE inhibition noted in the present study, chronic ACE inhibitor administration prevented the development of vasoconstrictor abnormalities in the Okumura study possibly by enhancing basal NO synthesis [8]. Thus, both studies suggest that ACE inhibitor treatment improves vasoconstrictor abnormalities by enhancing NO production.

4.6. Relevance of hypocontractility to the progression of heart failure

Although vasoconstrictor dysfunction seems incongruous with the elevated peripheral resistance in heart failure since reduced vasoconstriction should improve peripheral perfusion, it may actually contribute to a vicious circle. As cardiac function deteriorates, neurohormonal systems are activated to maintain systemic perfusion pressure. The development and progression of constrictor hypersensitivity will perpetuate the need for additional vasoconstrictor neurohormones to generate the necessary resistance. This continued upregulation of neurohormonal activity may hinder cardiac function.

From these results, we conclude that during the first 3 months after MI in the rat hindlimb vascular bed: (1) vascular network alterations having an effect on minimal resistance do not develop, (2) ACh-induced dilator responses are not reduced, (3) basal synthesis of NO may be reduced, (4) reduced arterial constrictor responses develop and (5) captopril treatment improves this dysfunction, possibly by enhancing basal NO production. As reduced vasoconstrictor responsiveness may perpetuate the progression of heart failure and has also been demonstrated in human heart failure [10], this finding may be of clinical relevance.

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References