Differences in Tail Vascular Bed Reactivity in Rats with and without Heart Failure following Myocardial Infarction

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ABSTRACT

Myocardial infarction (MI) was induced in rats by coronary ligation to compare changes in vascular reactivity from animals that developed heart failure (InfHF) with those that did not (Inf). Infarct size was similar in both groups. In vitro preparations of tail vascular bed were used to investigate the vascular responses to acetylcholine, sodium nitroprusside, and phenylephrine. Acetylcholine-induced relaxation was impaired in the Inf group (53 ± 2%, n = 6) when compared with Sham (80 ± 2%, n = 6, P < 0.05). The maximal response (E_max) to phenylephrine increased in the Inf group (423 ± 10 mm Hg, n = 9, P < 0.01) and decreased in InfHF (279 ± 10 mm Hg, n = 7, P < 0.05) when compared with Sham (319 ± 11 mm Hg, n = 8). Regardless of endothelial integrity, E_max to phenylephrine increased in the Inf, nitro-L-arginine methyl ester, and indomethacin groups. An increased release of a prostanoid vasodilator was detected in the Inf group. Differently, the InfHF group presented a reduction of the E_max to phenylephrine and an increment of nitric oxide release. This study demonstrates that MI without heart failure impairs endothelium-dependent relaxation and increases the reactivity to phenylephrine. This increase seems to involve a muscular component. The endothelium participates with an increased release of a vasodilator prostanoid, possibly to compensate the increased smooth muscle response. When heart failure follows MI, the reactivity to phenylephrine decreases, possibly due to an increased nitric oxide release.

Coronary artery ligation in rats has been used as a model of chronic left ventricular failure that closely mimics human condition (Hodsman et al., 1988). Indeed, compromised cardiac function (De Felice et al., 1989; Solomon et al., 1999) and increased peripheral resistance (Drexler and Lu, 1992; Schrier and Abraham, 1999) are found in this model. Systemic vasoconstriction can result from many compensatory mechanisms, including activation of the renin-angiotensin system, activation of the sympathetic nervous system, and alterations in the synthesis of local vascular factors (Zelis and Flaim, 1982; Gschwend et al., 2003).

Much has been reported regarding endothelial dysfunction in postinfarction myocardial failure (Teerlink et al., 1993, 1994; Didion et al., 1997; Bauersachs et al., 1999; Indik et al., 2001; Gschwend et al., 2003), but there is no consensus regarding vascular function in heart failure due to heterogeneity of alterations in vascular reactivity. These alterations may depend on the duration of the disease and the type of artery studied (Stassen et al., 1997a). Despite much research on vascular reactivity in postinfarction heart failure, little is known concerning vascular reactivity in a chronic phase of myocardial infarction (MI), when heart failure is absent.

The purpose of this study was, in the first place, to show that there are two animal models with similar infarction areas produced by coronary ligation: chronic MI without heart failure and with heart failure and, secondly, to demonstrate that these two models have different mechanisms involved in the control of vascular tone.

Materials and Methods

Myocardial Infarction Model. Male Wistar rats (120; 220–240 g) were housed with free access to food and water. Ninety of these rats were randomly selected to undergo MI and 30 to undergo sham surgery. MI was induced by ligation of the left coronary artery as described previously (Pfeffer et al., 1979). The anesthesia was induced by halothane. After recovery, the animals were kept in collective cages at the animal facility.

ABBREVIATIONS: MI, myocardial infarction; bw, body weight; RV, right ventricle; LVEDP, left ventricle end diastolic pressure; InfHF, myocardial infarction with heart failure; Inf, myocardial infarction without heart failure; LV, left ventricle; LVSP, left ventricle systolic pressure; +dp/dt, positive rate of pressure development; −dp/dt, negative rate of pressure development; MPP, mean perfusion pressure; ACh, acetylcholine; SNP, sodium nitroprusside; CHAPS, 3-[N-(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; L-NAME, nitro-L-arginine methyl ester; ANOVA, analysis of variance.
From the total number of 90 rats that were MI operated, 27 rats died early after the operation (<24 h). Sixty-three of the 90 rats (70%) survived the entire 4-week period and were included for analyses. All the 30 animals survived the sham surgery procedure. The experimental procedures were performed in accordance with the National Institutes of Health guidelines.

Heart failure was considered when three criteria were met: lung/body weight (bw) ratio greater than lung/bw sham, approximately 2-fold (Francis et al., 2001), right ventricle (RV) hypertrophy (RV/bw) proportional to lung/bw (Davidoff et al., 2004), and the left ventricle end diastolic pressure (LVEDP) greater than 15 mm Hg (Teerlink et al., 1993). Thirty of the 63 MI rats met the criteria for inclusion into the InfHF group, and the remaining 33 were used in the Inf without heart failure group.

The presence of infarction was confirmed when the animals were sacrificed. Hypertrophy was evaluated by the ratios of left ventricle (LV) and RV to bw. Tetrazolium chloride was used to discriminate between viable and nonviable myocardium. The infarct area was calculated as described previously (Mill et al., 1990).

**Hemodynamic Measurements and LV Function.** Rats were anesthetized with chloral hydrate (0.3 g/kg i.p.). The measurements performed were: systolic and diastolic blood pressure, left ventricular systolic pressure (LVSP), LVEDP, heart rate, and positive (+dP/dt) and negative (−dP/dt) rates of pressure development.

**In Vitro Preparation of Rat Tail Vascular Bed.** Isolated tail vascular bed preparations were obtained from rats 4 weeks after MI and anesthetized with sodium pentobarbital (60 mg/kg i.p.), as previously described (França et al., 1997). Briefly, after anesthesia, the tail vascular bed preparations were obtained from rats 4 weeks after MI and anesthetized with sodium pentobarbital (60 mg/kg i.p.). The vascular bed was perfused with Krebs’ solution (36°C, 95% O₂, and 5% CO₂) at a constant flow of 2.5 ml/min. Results from the MI rats (n = 63), 45% (n = 30) developed HF. Infarct size did not differ between Inf and InfHF groups. The RV/bw and lung/bw ratios were increased in the InfHF group when compared with Inf and Sham (n = 30) groups. Body weight did not differ among groups (Table 1). There were no correlations between infarct size and RV/bw and lung/bw ratios (r = 0.08, P > 0.05).

The Inf group (n = 33) presented changes in all measured hemodynamic variables. Systolic, diastolic, and mean aortic blood pressures were increased. Heart rate increased about 20% compared with Sham rats. In contrast, there were no changes in the variables for left ventricular function: LVSP, +dP/dt, −dP/dt, and LVEDP (Table 1). In InfHF rats, LV function was affected, with a reduction of the LVSP, systolic dP/dt, and diastolic dP/dt, and elevation of

**Results**

**Rat Characteristics.** From the MI rats (n = 63), 45% (n = 30) developed HF. Infarct size did not differ between Inf and InfHF groups. The RV/bw and lung/bw ratios were increased in the InfHF group when compared with Inf and Sham (n = 30) groups. Body weight did not differ among groups (Table 1). There were no correlations between infarct size and RV/bw and lung/bw ratios (r = 0.08, P > 0.05).

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the LVEDP. Arterial systolic blood pressure in rats with heart failure was reduced (Table 1).

**Vascular Reactivity.** No differences were found in the basal perfusion pressures in preparations obtained from all groups. The respective values for the Sham (n = 24), Inf (n = 27), and InfHF (n = 24) groups were 86 ± 3, 85 ± 3, and 78 ± 3 mm Hg. The constrictor response evoked by KCl was similar among groups (Sham, 182.5 ± 8; Inf, 202.3 ± 10.7; InfHF, 197.3 ± 7.7 mm Hg).

Table 2 summarizes the results of the curve fitting to obtain the parameters for maximal response and sensitivity for the vasodilator actions of ACh and SNP.

Figure 1 shows the dose-response curves for endothelium-dependent relaxation produced by ACh. Ach induced a dose-dependent relaxation that was attenuated in the Inf rats (Table 2). The $E_{\text{max}}$ to SNP was not altered, whereas the sensitivity of the InfHF group was increased compared with the Inf group (Table 2).

Dose-response curves for phenylephrine were constructed in three different series of experiments in which the effects of endothelial damage, L-NAME, or indomethacin were determined. The rank order of the $E_{\text{max}}$ values was Inf > Sham > InfHF and each was significantly different from the others ($P < 0.01$). There were no significant differences in the potency for phenylephrine (Table 3).

Figure 2 shows the dose curves for the constrictor actions of phenylephrine on the tail artery bed generated in the presence of an intact endothelium, where the $E_{\text{max}}$ value was increased in preparations obtained from the Inf group (Table 3). In the InfHF group, $E_{\text{max}}$ value for phenylephrine was reduced. Following endothelial damage (Fig. 2), the sensitivity and $E_{\text{max}}$ values for phenylephrine were increased in all three groups without changing the rank order found in the presence of the endothelium (Table 3).

The effects of L-NAME (Table 3) were similar to those of endothelial damage in that the sensitivity and $E_{\text{max}}$ value for phenylephrine increased in each group. The increase in the $E_{\text{max}}$ for phenylephrine in the InfHF group was relatively larger than that in the other groups. Namely, in the presence of L-NAME, the $E_{\text{max}}$ value was not significantly different from that of the Sham group in the presence of L-NAME. The differences of area under the curves from Sham, Inf, and InfHF were, respectively, 32.3 ± 1.87, 37.5 ± 3.85, and 42.2 ± 2.14% ($P < 0.05$, InfHF versus Sham). This suggests that in relation to control, there is a higher basal release of nitric oxide in tail artery preparations following the development of postinfarction heart failure.

**TABLE 2**

Dose-response parameters for the actions of ACh and SNP on the isolated perfused tail artery preparation with intact endothelium

<table>
<thead>
<tr>
<th>Agonist/Treatment</th>
<th>n</th>
<th>$E_{\text{max}}$</th>
<th>pED$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>Ach</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>80 ± 2</td>
<td>8.64 ± 0.08*</td>
</tr>
<tr>
<td>Inf</td>
<td>6</td>
<td>83 ± 2*</td>
<td>7.92 ± 0.14*</td>
</tr>
<tr>
<td>InfHF</td>
<td>6</td>
<td>81 ± 2</td>
<td>8.30 ± 0.01</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>82 ± 2</td>
<td>7.84 ± 0.15</td>
</tr>
<tr>
<td>Inf</td>
<td>6</td>
<td>83 ± 2</td>
<td>7.56 ± 0.30</td>
</tr>
<tr>
<td>InfHF</td>
<td>6</td>
<td>84 ± 3</td>
<td>8.57 ± 0.18†</td>
</tr>
</tbody>
</table>

* One-way ANOVA: $P < 0.05$ compared with Sham.
† One-way ANOVA: $P < 0.05$ compared with Inf. $n$ is the number of animals.

The putative role of prostanoids in modulating vascular responses was investigated by determining the effects of indomethacin (10$^{-5}$ M) on dose-response curves for phenylephrine. When compared with its respective group control value, the $E_{\text{max}}$ for phenylephrine increased in the Inf group and remained unchanged in the other two groups in the presence of indomethacin (see Table 3). The maximal responses for phenylephrine prior to indomethacin showed the same rank order as the control values for those experiments described in Table 3. This effect of indomethacin suggests that the modulation of the response to phenylephrine by a vasodilator prostanoid is greater in the Inf group than in other groups. In addition, the difference observed in the $E_{\text{max}}$ between the Sham and InfHF groups before indomethacin

![Image](https://example.com/image.png)

**Fig. 1.** Dose-response curves for the endothelial-dependent relaxation produced by acetylcholine in tail vascular bed from Inf (n = 6) and InfHF (n = 6) rats 4 weeks after MI, compared with Sham (n = 6). **, $P < 0.01$ Inf versus Sham.

**TABLE 3**

Effects of endothelial damage, treatment with L-NAME, or INDO on the $E_{\text{max}}$ and pED$_{50}$ values for vasoconstrictor actions of phenylephrine

<table>
<thead>
<tr>
<th>Treatment/Group</th>
<th>n</th>
<th>$E_{\text{max}}$</th>
<th>pED$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mm Hg</td>
<td></td>
</tr>
<tr>
<td>Intact endothelium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>319 ± 11</td>
<td>2.23 ± 0.007</td>
</tr>
<tr>
<td>Inf</td>
<td>9</td>
<td>423 ± 10*</td>
<td>2.13 ± 0.005*</td>
</tr>
<tr>
<td>InfHF</td>
<td>7</td>
<td>279 ± 10*</td>
<td>2.40 ± 0.03*</td>
</tr>
<tr>
<td>Damaged endothelium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>425 ± 16*</td>
<td>3.1 ± 0.003</td>
</tr>
<tr>
<td>Inf</td>
<td>9</td>
<td>504 ± 25*</td>
<td>3.13 ± 0.003*</td>
</tr>
<tr>
<td>InfHF</td>
<td>7</td>
<td>383 ± 23*</td>
<td>3 ± 0.01*</td>
</tr>
<tr>
<td>L-NAME control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>334 ± 8</td>
<td>2.21 ± 0.01</td>
</tr>
<tr>
<td>Inf</td>
<td>7</td>
<td>429 ± 7*</td>
<td>2.13 ± 0.006*</td>
</tr>
<tr>
<td>InfHF</td>
<td>6</td>
<td>278 ± 6*</td>
<td>2.38 ± 0.009*</td>
</tr>
<tr>
<td>L-NAME perfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>387 ± 10*</td>
<td>2.67 ± 0.007</td>
</tr>
<tr>
<td>Inf</td>
<td>7</td>
<td>504 ± 11*</td>
<td>2.70 ± 0.003</td>
</tr>
<tr>
<td>InfHF</td>
<td>6</td>
<td>422 ± 30*</td>
<td>2.65 ± 0.009</td>
</tr>
<tr>
<td>INDO control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham;*</td>
<td>6</td>
<td>325 ± 9</td>
<td>2.36 ± 0.01</td>
</tr>
<tr>
<td>Inf</td>
<td>6</td>
<td>396 ± 14*</td>
<td>2.22 ± 0.003*</td>
</tr>
<tr>
<td>InfHF</td>
<td>6</td>
<td>281 ± 8*</td>
<td>2.35 ± 0.01*</td>
</tr>
<tr>
<td>INDO perfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>335 ± 16</td>
<td>2.41 ± 0.01</td>
</tr>
<tr>
<td>Inf</td>
<td>6</td>
<td>490 ± 11*</td>
<td>2.05 ± 0.02*</td>
</tr>
<tr>
<td>InfHF</td>
<td>6</td>
<td>318 ± 14*</td>
<td>2.38 ± 0.02*</td>
</tr>
</tbody>
</table>

* Two-way ANOVA: $P < 0.01$ versus Sham.
† Two-way ANOVA: $P < 0.05$ versus Sham.
‡ $P < 0.01$ versus Inf.
§ $P < 0.01$ versus L-NAME.
¶ $P < 0.05$ with its control. $n$ is the number of animals.
perfusion was abolished, suggesting a little modulation of the response to phenylephrine by a vasodilator prostanooid in this group. The effects of indomethacin on the dose-response curves for phenylephrine are shown in Table 3.

Discussion

The primary finding in this study is the fact that, despite a similar infarct size, 4 weeks after coronary ligation, we found animals with and without heart failure. The heart failure rats presented a depressed LV function, which is consistent with heart failure (De Felice et al., 1989; Francis et al., 2001). Additionally, this study demonstrated that there is no correlation between infarct size and the development of heart failure, although there are studies demonstrating a rapid development of heart failure after large infarcts in rats (Norman and Coers, 1960; Anversa et al., 1984; Novaes et al., 1996).

As to vascular function, we observed that the changes in tail vascular bed reactivity differed between animals that developed heart failure from those that did not.

In agreement with other studies (Baggia et al., 1997; Bauersachs et al., 1999, 2002; Ceiler et al., 1999; Schäfer et al., 2003), we found no impairment of the endothelium-independent relaxation produced by SNP following infarction. However, we found impairment in the endothelium-dependent relaxation mediated by AC N in the tail vascular beds in Inf rats. Impairments in endothelium-dependent relaxation have also been reported in patients with cardiovascular disease and chronic heart failure (Belardinelli, 2001; Annuk et al., 2003; Linke et al., 2003). Studies in different vessels using the rat infarction model for heart failure show either reductions or no changes in endothelial dependent relaxation, at different times after infarction (Teerlink et al., 1993; Baggia et al., 1997; Bauersachs et al., 1999, 2002; Ceiler et al., 1999; Indik et al., 2001; Annuk et al., 2003). Possibly, the endothelial dysfunction observed in heart failure may depend on its stage and the type of vessels studied (Fang and Marwick, 2002).

In human subjects with heart failure and in the rat postinfarction model for heart failure (Stassen et al., 1997a,b; Fang and Marwick, 2002; Annuk et al., 2003) reductions in the contractile activity of α-adrenoceptor agonists have been reported. This reduced contractile activity might be due to a down-regulation of α-adrenoceptors because of high and prolonged sympathetic stimulation during heart failure. Although we did not evaluate the neurohumoral activation, the InfHF rats presented hyporeactivity to the α-agonist phenylephrine. In contrast with a down-regulation of α-adrenoceptors found in rat mesenteric arteries after MI, Feng et al. (1996) and Stassen et al. (1997b) found no changes in the density of α1-adrenoceptors in those vascular beds that displayed a decreased responsiveness to phenylephrine. However, absence of changes in the density of α1-adrenoceptors does not rule out changes in the receptor signaling pathway that may lead to a reduction in responsiveness. In addition, the decrease in reactivity to phenylephrine is paralleled by an increased nitric oxide basal release, which can contribute to this hyporeactivity.

The increase in responsiveness to phenylephrine seen in the Inf group is paralleled by a decrease in responsiveness to acetylcholine, which suggests that the reduced endothelium-dependent relaxation is a common factor.

These changes in phenylephrine responsiveness occurred in the absence of any changes in the contractile response to KCl, suggesting that there is no defect in the contractile apparatus per se in the tail arterial bed.

The effects of endothelial damage and L-NAME were similar in that both treatments increased the $E_{\text{max}}$ and $pED_{50}$ values for phenylephrine in all groups. Endothelial damage decreased the difference between the Sham and InfHF groups and further accentuated the increase in the contractile actions of phenylephrine in the Inf group. This suggests an involvement of a muscular component on the increased response to phenylephrine in the Inf group.

When the contribution of nitric oxide to the negative modulation of contractile agents was evaluated, an increased nitric oxide basal release was observed in the InfHF group. The nitric oxide basal production is reported as having increased (Habib et al., 1994), decreased (Annuk et al., 2003), or unaltered (Drexler and Lu, 1992) in experimental models for postinfarction heart failure. The results obtained in the present study suggest that an increase in the basal production and the availability of nitric oxide contributed to the decreased responsiveness to phenylephrine in the tail artery bed in animals with heart failure.

Additionally, our findings suggest that the actions of another endothelial-derived negative modulator of contractility were enhanced in the Inf group. The contribution of a vasodilator prostanooid was examined by the inhibition of cyclooxygenase by indomethacin. In the presence of indomethacin only, the $E_{\text{max}}$ to phenylephrine in the Inf group was significantly altered. There was an increase in the $E_{\text{max}}$ in this group that was accompanied by a change in the sensitivity to phenylephrine. These results suggest that a vasodilator prostanooid plays a modulatory role in the tail bed vascular contractility in the Inf group. This is likely to be a compensatory adaptation in response to the increased contractility observed in the Inf group.

In summary, this study demonstrated that despite similar extension of infarct, two animal models can be found after coronary ligation: one chronic MI without heart failure and another with heart failure. Comparing these models, we found differences in the vascular reactivity of the tail vascular bed. Interestingly, the vascular responses to phenylephrine and acetylcholine differed between the groups. Compro-
mised cardiac function in rats with heart failure was related to a hyperreactivity to phenylephrine, to an increased nitric oxide basal release, and to a normal endothelium-dependent relaxation. In the MI rats without heart failure, the cardiac function was preserved, and there were an impaired endothelium-dependent relaxation and an increased responsiveness to phenylephrine regardless of the endothelial integrity. This hyperreactivity occurred despite a normal nitric oxide basal release and despite an increased production of a vasodilator prostaglandin. Moreover, our results suggest that this hyperreactivity was due, in part, to nonendothelial changes.

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References


