Interstitial norepinephrine concentrations in skeletal muscle of ischemic heart failure

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The causes of exercise limitation in heart failure (HF) are poorly understood. However, it appears that reduced blood flow to active muscle may contribute to this response (14, 23, 29, 30). The causes of blood flow limitation may be due more to exaggerated sympathetic activation than to reductions in the intrinsic ability of the peripheral vasculature to dilate (23, 24). When the sympathetic nervous system is engaged, norepinephrine (NE) is released from the neurovascular junction, evoking vasoconstriction within a given vascular bed (15, 22). However, the determinants of the neurovascular concentrations of NE in skeletal muscle during exercise in congestive HF have received little attention. In this investigation, we utilized the microdialysis method to measure interstitial NE concentration in the hindlimb muscle after electrical stimulation of the lumbar sympathetic nerve in rats with myocardial infarction (MI) and in control animals. Interstitial NE was used as a surrogate of neurovascular NE. We hypothesized that interstitial NE concentration for a given level of electrical stimulation of the sympathetic nerves would be augmented in HF.

Furthermore, since NE uptake-1 represents the major pathway by which released NE is metabolized and interstitial NE concentration is regulated (11), we hypothesized that NE uptake-1 would be impaired in the skeletal muscle of MI rats. To test this hypothesis, an NE uptake-1 inhibitor, desipramine, was infused into the arterial blood supply of the hindlimb muscle in rats with MI and in control animals.

METHODS

All procedures were approved by the Animal Care Committee of this institution.

Coronary Artery Ligation

Male Sprague-Dawley rats (150–180 g body wt) were anesthetized by inhalation of isoflurane-oxygen (2–5% isoflurane in 100% oxygen), intubated, and artificially ventilated. A left thoracotomy between the fourth and fifth ribs exposed the left ventricular (LV) wall. The left coronary artery was ligated. Experiments were performed 8–10 wk after coronary artery ligation. Age- and body weight-matched rats that did not undergo the surgery served as controls.

A transthoracic echocardiogram was performed 1–2 wk before the experiments. The rats were anesthetized by inhalation of isoflurane-oxygen. The transducer was positioned on the left anterior chest, and LV dimensions were measured (Table 1). The fractional shortening (FS) was determined by echocardiographic measurements. On the basis of FS data, the animals were considered control with FS > 40% (n = 14), mild HF with 30% < FS < 40% (n = 6), and severe HF with FS < 30% (n = 18).

Experimental Preparation

The rats were anesthetized by inhalation of isoflurane-oxygen. An endotracheal tube was inserted into the trachea and attached to a ventilator. Polyethylene catheters were inserted into the common carotid artery and external jugular vein for measurement of arterial blood pressure and for drug administration, respectively. The lumbar sympathetic trunk at L3–L5 was carefully dissected from other connective tissues. A piece of laboratory film was placed under the isolated nerves. A bipolar electrode was then placed between the nerves and the film to deliver electrical stimulation and embedded in a silicone gel. Once the gel hardened, silicone rubber was fixed to the surrounding tissue with a glue containing α-cyanoacrylate. Lumbar sympathetic nerve activity at L3–L5 mainly reflects the component of the sympathetic nerve activity at L3–L5 mainly reflects the component of

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Interstitial NE concentration before, during, and after electrical stimulation of lumbar sympathetic nerves in experimental and control legs

Table 1. General and echocardiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 14)</th>
<th>Small MI (n = 6)</th>
<th>Large MI (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>449±18</td>
<td>455±10</td>
<td>458±14</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>1.28±0.05</td>
<td>1.49±0.05</td>
<td>1.84±0.06‡</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>3.1±0.05</td>
<td>4.5±0.7</td>
<td>10.7±1.1†</td>
</tr>
<tr>
<td>LVDD, cm</td>
<td>0.82±0.02</td>
<td>0.86±0.02</td>
<td>1.23±0.03‡</td>
</tr>
<tr>
<td>LVSD, cm</td>
<td>0.40±0.02</td>
<td>0.55±0.02</td>
<td>1.02±0.04‡</td>
</tr>
<tr>
<td>FS, %</td>
<td>51±2</td>
<td>36±1*</td>
<td>17±1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. MI, myocardial infarction; LVEDP, left ventricular (LV) end-diastolic pressure; LVDD and LVSD, LV diastolic and systolic dimension; FS, fractional shortening. *P < 0.05 vs. control. †P < 0.05 vs. control and small MI.

nerve activity that regulates the vasculatures of the hindlimb muscles (9). The femoral artery and arterial collaterals were isolated. A catheter was inserted into the popliteal artery through the femoral artery for the injection of drugs into the arterial blood supply of the hindlimb muscles. The animals were ventilated, and tidal CO₂ (9). The femoral artery and arterial collaterals were isolated. A nerve activity that regulates the vasculatures of the hindlimb muscles

Table 2. Interstitial NE concentration before, during, and after electrical stimulation of lumbar sympathetic nerves in experimental and control legs

<table>
<thead>
<tr>
<th></th>
<th>Stimulated Muscle</th>
<th>Control Muscle</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Peak response</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Hz</td>
<td>3.01±0.72</td>
<td>3.50±0.88</td>
</tr>
<tr>
<td>2 Hz</td>
<td>2.74±0.68</td>
<td>3.27±0.78</td>
</tr>
<tr>
<td>4 Hz</td>
<td>2.70±0.41</td>
<td>3.35±0.53</td>
</tr>
<tr>
<td>Small MI (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Hz</td>
<td>2.64±0.43</td>
<td>3.15±0.41</td>
</tr>
<tr>
<td>2 Hz</td>
<td>2.59±0.59</td>
<td>3.30±0.72</td>
</tr>
<tr>
<td>4 Hz</td>
<td>2.70±0.62</td>
<td>3.69±0.79*</td>
</tr>
<tr>
<td>Large MI (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Hz</td>
<td>2.85±0.71</td>
<td>3.74±0.83</td>
</tr>
<tr>
<td>2 Hz</td>
<td>3.03±0.78</td>
<td>4.39±1.08*</td>
</tr>
<tr>
<td>4 Hz</td>
<td>2.93±0.80</td>
<td>4.85±1.40*</td>
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</tbody>
</table>

Values are means ± SE expressed in nM; n, number of rats. Sympathetic nerve stimulation raised muscle interstitial norepinephrine (NE) concentration in stimulated, but not control, leg. *P < 0.05 vs. Before and After.

The concentrations of dialysate NE in each of the dialysis probes were measured using HPLC methods. Because the dialysate samples were ultraclean, purification was not required before analysis.

**Experimental Protocols**

After the microdialysis probes were inserted, a 60-min equilibration period was allowed. During this period, resting dialysate samples were collected every 20 min. The experimental intervention was then performed. Samples were collected during each 10-min intervention. A 60-min rest period was allowed between each intervention. Dialysate was collected before, during, and after each intervention. Baseline muscle tension was adjusted to and maintained at 0.1 kg during experiments to minimize the difference in the tension-induced NE response among experimental groups.

**Study 1: electrical stimulation of lumbar sympathetic nerves in large-MI, small-MI, and control rats.** After control data were collected from large-MI (n = 10), small-MI (n = 6), and control (n = 8) rats, the lumbar sympathetic nerve was electrically stimulated at 1, 2, and 4 Hz (2.5 V). Each level of stimulation was sustained for 10 min, and experimental data were collected for 20 min after stimulation was completed.

**Study 2: arterial injection of desipramine in large-MI and control rats.** Because there was no difference in interstitial NE during sympathetic nerve stimulation between control and small-MI rats, we collected experimental data from control (n = 6) and large-MI (n = 8) rats. After control data were obtained, 0.001, 0.01, 0.1, and 1.0 μM desipramine (dissolved in Ringer solution and obtained from Sigma Chemical) was infused into the blood supply of the hindlimb muscles. Recovery data were collected for 20 min after desipramine infusion. The total volume of infused solution containing desipramine was 0.1 ml, and the duration of each infusion was 2 min.

**Examination of LV End-Diastolic Pressure**

On completion of each experiment, a 2-Fr micro-Millar pressure transducer catheter (Millar Instruments) was inserted into the right carotid artery and threaded into the LV for measurement of LV end-diastolic pressure (EDP).

**Data Acquisition and Analyses**

Mean arterial pressure (MAP) and basal muscle tension were recorded on a personal computer that used PowerLab software. Control values were determined by analysis of ≥30 s of the data immediately before the interventions. The peak change of each variable was determined by the peak response from control.
Experimental data were analyzed with one-way repeated-measures ANOVA. Tukey’s post hoc analyses were utilized to determine differences between groups, as appropriate. A linear regression analysis was used to show a relationship between NE rise induced by the sympathetic stimulation and FS. Values are means ± SE. For all analyses, differences were considered significant at \( P < 0.05 \). All statistical analyses were performed using SPSS for Windows (version 13.0).

**RESULTS**

Interstitial NE stabilized 40 min after probe insertion, as previously reported (16). There were no significant differences in baseline values for muscle interstitial NE before the experiment in the control leg and in the experimental leg among healthy control (\( n = 14 \)), and small-MI (\( n = 6 \)), and large-MI (\( n = 18 \)) rats.

**NE Responses to Electrical Stimulation of Lumbar Sympathetic Nerves**

Basal values for dialysate NE and responses to electrical stimulation of the lumbar sympathetic nerves in both legs of control, small-MI, and large-MI rats are shown in Table 2. Activation of the sympathetic nervous system raised muscle interstitial NE concentration in the stimulated leg, but not in the control leg. This suggests that the elevated interstitial NE concentration was not due to a systemic activation of the autonomic nervous system. Interstitial NE concentration rose as a fraction of stimulus frequency. Importantly, the rise in interstitial NE for a given level of stimulation was greater in large-MI rats than in small-MI and control rats (Fig. 1). Moreover, the magnitude of the NE response was linearly linked to the FS (Fig. 2). FS is a measurement of LV function. Baseline values for MAP before 1-, 2-, and 4-Hz stimulation and the peak response evoked by lumbar stimulations are shown in Table 3.

**Effect of NE Uptake-1 Pathway on Interstitial NE Concentration**

Figure 3 shows the effect of inhibition of NE uptake-1 on the interstitial NE response of both lower limbs of control and large-MI rats. Desipramine (0.001–1.0 \( \mu \)M) injected into the blood supply of the hindlimb muscles increased interstitial NE concentration in a dose-related fashion in the ipsilateral leg (Fig. 3), but not in the contralateral control lower limb. In control animals, 0.001, 0.01, 0.1, and 1.0 \( \mu \)M desipramine increased interstitial NE by 1.06 ± 2.89, 1.17 ± 1.67, 0.48 ± 2.75, and 1.01 ± 2.56% in control muscle and 7.76 ± 3.62, 9.87 ± 2.83, and 23.32 ± 3.91% (\( P < 0.05 \) vs. control muscle) in injected muscle, respectively. This suggests that the effect of desipramine was localized to the infused limb. The desipramine-induced increase in interstitial NE was less in MI than in healthy control rats (Fig. 3). Desipramine at 1.0 \( \mu \)M elevated interstitial NE concentration by 24% in controls and by only 3% in MI animals (\( P < 0.05 \) vs. controls). Blood pressure was unchanged by the desipramine infusions. MAP before and after desipramine infusion is shown in Table 3.

**Fig. 1.** Percent increases of interstitial norepinephrine (NE) concentration during electrical stimulation of lumbar sympathetic nerves in stimulated leg in control rats (\( n = 8 \)) and rats with small (\( n = 6 \)) and large (\( n = 10 \)) myocardial infarction (MI). Interstitial NE was elevated with stimulation frequencies. Stimulation-induced rise in interstitial NE was greater in large-MI than in small-MI and control rats. *\( P < 0.05 \) vs. control. †\( P < 0.05 \) vs. control and small-MI. #\( P < 0.05 \) vs. 1 Hz.

**Fig. 2.** Regression analysis showing an inverse linear relationship between NE rise induced by the sympathetic stimulation and fractional shortening, an index of left ventricular function.
with physiological stress. This impairment in metabolism in HF was local. The greater rise in NE with sympathetic stimulation in HF was largely due to an impairment in NE uptake-1, since the increase in interstitial NE concentration induced by infusion of a given dose of desipramine into the arterial supply of the skeletal muscles was less in HF rats than in control animals. Interestingly, the magnitude of the rise in interstitial NE was linked to the magnitude of LV impairment. Thus we speculate that the impairment of peripheral sympathetic nerve NE uptake-1 in HF is somehow linked to the severity of LV function impairment. Furthermore, we believe that this effect, coupled with the high sympathetic tone during exercise, contributes to the heightened sympathetic vasoconstriction and blood flow limitation to skeletal muscle in HF (24). This decline in skeletal muscle blood flow likely contributes to the reduced exercise capacity in HF (14, 23, 29, 30).

Sympathetic nerve stimulation leads to the release of the vesicular contents into the synapse. The released NE can then bind to postsynaptic receptors, evoking physiological responses. NE can be 1) taken up by a variety of tissues in a “low-affinity” process termed NE uptake-2, 2) released into the circulation, a process termed spillover, and 3) taken up by the neuron in a sequestration process (NE uptake-1) (5, 7). NE uptake-1 represents the major pathway by which locally released NE is metabolized (11). NE spillover has been used as an index of NE release. However, this method has a number of limitations (2, 4, 10, 20). In this study, we have utilized microdialysis methods to measure dialysate concentrations of NE. Interstitial NE is thought to more closely reflect the NE concentration at the neurovascular junction than plasma NE or indexes of NE spillover (8).

Because collection of plasma NE samples could significantly affect baseline blood pressure and reduce blood volume in rats, we did not determine the concentrations of plasma NE together with muscle interstitial NE in the present experiment. However, previous studies have shown that, with elevated sympathetic nerve activity, plasma NE is increased (15), and the response parallels muscle interstitial NE (8). Those data suggest that NE responses may also occur in other organs in addition to active muscle.

**DISCUSSION**

A known hallmark of HF is increased sympathetic nerve activity. This induces abnormal cardiovascular regulation during exercise, including low cardiac outputs for a given level of physical exertion, as well as elevated sympathetic tones to maintain adequate blood pressure (13). Specifically, renal vasoconstriction is enhanced, and the rise in active muscle blood flow is attenuated (18, 19, 23). The reduced blood supply to the kidney leads to excessive stimulation of renin secretion and inappropriate salt and water retention (1), whereas the reduced blood supply to the heart contributes to the heightened sympathetic vasoconstriction and blood flow limitation to skeletal muscle in HF (24). This decline in skeletal muscle blood flow likely contributes to the reduced exercise capacity in HF (14, 23, 29, 30).

Baseline MAP and peak responses induced by each stimulation and during desipramine infusion

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Control (n = 8)</th>
<th>Small MI (n = 6)</th>
<th>Large MI (n = 10)</th>
<th>Desipramine (0.001–1.0 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001 μM Desipramine</td>
</tr>
<tr>
<td>1 Hz</td>
<td>98 ± 12</td>
<td>94 ± 16</td>
<td>107 ± 13</td>
<td>104 ± 12</td>
</tr>
<tr>
<td>Peak response</td>
<td>106 ± 12</td>
<td>105 ± 14</td>
<td>118 ± 22</td>
<td>105 ± 14</td>
</tr>
<tr>
<td>2 Hz</td>
<td>92 ± 8</td>
<td>96 ± 12</td>
<td>98 ± 14</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>Peak response</td>
<td>102 ± 16</td>
<td>108 ± 20</td>
<td>110 ± 16</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>4 Hz</td>
<td>96 ± 12</td>
<td>101 ± 15</td>
<td>95 ± 18</td>
<td>92 ± 8</td>
</tr>
<tr>
<td>Peak response</td>
<td>108 ± 13</td>
<td>108 ± 10</td>
<td>110 ± 20</td>
<td>98 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mmHg; n, number of rats. MAP, mean arterial pressure. There are no significant differences in basal values and peak responses among the groups.
Electrical stimulation of the lumbar sympathetic nerves has been reported to increase blood pressure (6, 26). To minimize the effect of arterial baroreflex activation on interstitial NE responses, a 2.5-V electrical stimulation was applied on the lumbar sympathetic nerves in the present experiment. An increase in MAP with such stimulation was small, and there was no difference in the MAP response between large-MI animals and controls. This suggests that application of a stimulus of this intensity to the sympathetic nerves can increase the interstitial NE response but may be insufficient to significantly augment the blood pressure response in MI rats. Additionally, impaired cardiac output should be considered to affect the blood pressure response induced by the sympathetic nerve stimulation in HF in this experiment. Because there was no difference in the blood pressure response, the rise in interstitial NE was unlikely due to activation of the arterial baroreflex.

It is possible that increased interstitial NE concentration could have caused vasoconstriction and led to a reduction in blood flow. This action attenuates the clearance of metabolites from the interstitium and contributes to accumulation of interstitial NE. Prior reports suggest that NE concentrations in active muscle are not affected by blood flow (3, 12). In addition, a reduction in blood flow can increase the concentrations of metabolic vasodilators. For instance, vasodilators released by contracting skeletal muscle cells and/or red blood cells stimulate endothelial cell pathways and induce the hyperpolarization of vascular smooth muscle (21, 25, 27). The vasodilation caused by such nonneural mechanisms can counter the sympathetic vasoconstriction effects of NE in active muscles. These peripheral blood flow responses are necessary to maintain adequate blood pressure, as well as to ensure that muscle blood supply and metabolic demand can be matched.

In conclusion, these data demonstrate that the interstitial NE response to sympathetic nerve activation is augmented in MI animals compared with a healthy control group. Reduced NE uptake-1 may play an important role in determining NE concentration in muscle sympathetic nerve terminals. These results suggest that this mechanism contributes to the exaggerated NE level at the neurovascular junction in HF, which, in turn, may alter muscle blood flow and exercise capacity in HF.

ACKNOWLEDGMENTS

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GRANTS

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