Autonomic and vascular responses to reduced limb perfusion

Joseph C. Daley, III,1 Mazhar H. Khan,2 Cynthia S. Hogeman,2 and Lawrence I. Sinoway2,3

Divisions of 1Pulmonary, Allergy, and Critical Care and 2Cardiology, The Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, Hershey 17033; and 3Lebanon Veterans Affairs Medical Center, Lebanon, Pennsylvania 17042

Submitted 17 April 2002; accepted in final form 23 June 2003

DURING EXERCISE THE SYMPATHETIC nervous system is activated. This activation results in increased heart rate (HR), mean arterial blood pressure (MAP) and peripheral vasoconstriction (2, 13–15, 19–21, 26, 30). Two neural systems may contribute to this process: central command and the muscle reflex. Central command is a feedforward central neural process in which a motoneuron recruitment is thought to parallel sympathetic nervous system engagement (9). Activation of the muscle reflex results from stimulation of metabolite and mechanically sensitive afferents within contracting skeletal muscle (2, 18). Whether engagement of these systems acts to increase or decrease flow to exercising muscle is unclear. This issue is complicated because reflex increases in blood pressure would tend to raise muscle flow and reflex vasoconstriction within exercising muscle would act to reduce flow delivery.

In this study, the effects of graded muscle ischemia on sympathetic responses were examined. We measured HR, MAP, muscle sympathetic nerve activity (MSNA), limb metabolites, and limb flow velocity. The results of these studies suggest that flow limitation evokes a reflex increase in perfusion pressure that restores blood flow to exercising muscle. At high levels of ischemia, this increase in perfusion pressure is no longer flow restorative. We speculate that this is due to vasoconstriction within the exercising ischemic limb.

METHODS

Subjects. Seven healthy volunteers (24 ± 2 [SE] yr old; 4 men, 3 women] participated in the study. The subjects were normotensive, nonsmokers, nonobese, and not taking any medications. Each subject gave written, informed consent, and all procedures used in the study had prior approval of the Institutional Review Board of The Milton S. Hershey Medical Center (MSHMC) of the Pennsylvania State University. Each subject performed two exercise protocols on each arm.

Blood flow. Brachial artery mean blood velocity (MBV) was measured continuously and collected on-line at 100 Hz with a 4-MHz continuous-wave Doppler probe (model 500M, Multigon Industries, Yonkers, NY). Doppler signal strength was optimized by using both visual and auditory feedback of shift frequencies.

Brachial artery diameter. Brachial artery diameter was measured continuously in four subjects with a pulsed-wave Doppler probe (range of 5–12 MHz; System 5000, Advanced Technology Laboratories, Bothell, WA). To obtain time course analysis of MBV and MAP, three- to five-beat averages of blood velocity were obtained at baseline and at 10-s intervals during exercise.

Exercise paradigm. Subjects reported to the Hershey Medical Center General Clinical Research Center (GCRC) and received a prestudy history and physical examination. The protocols consisted of a 5-min baseline period, followed by 6

Address for reprint requests and other correspondence: L. I. Sinoway, Div. of Cardiology, MC H047, The Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, P.O. Box 850, Hershey, PA 17033 (E-mail: lsinoway@psu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
of rhythmic handgrip exercise. Arrows, venous blood sampling. In
voluntary contraction (MVC) was assessed in each arm sepa-
min of exercise and 5 min of recovery (Fig. 1). Maximal
–
1494 MUSCLE REFLEX AND OXYGEN UPTAKE
vides direct recordings of sympathetic nerve traf
fi
ure from MAP (29). The
mion was calculated by subtracting external arm tank pres-
nometer (Dinamap, Critikon, Tampa, FL). Perfusion pres-
resting BP was obtained with an automated sphygmoma-
napres, Ohmeda, Madison, WI) on the nonexercising hand.
ecardiography. Systemic blood pressure (BP) was mea-
adjustments.
flow velocity were also examined every 10 s. At
ow conditions.
Protocol 2, the positive pressure trial, was a variant of the ischemic
eurographic technique pro-
designed to evaluate re
response to graded reductions in muscle perfusion pressure. The
the usual fashion, using MBV as a surrogate for flow in the
equation mVo2 = Q × (SaO2 – SvO2)-hemoglobin (in
tal external positive tank pressure resulted in higher
sonal between pressure and positive pressure. A
Bonferroni adjustment was made to all simple effects com-
points between ambient pressure and positive pressure. A
Bri
fl
Arm tank was a specially constructed, sealed wooden
cols with each arm and the study sequence was randomized.
exercise, and during recovery. To eliminate arm dominance
illustrated schematically in Fig. 1. Venous blood samples
performed both protocols with each arm and the study sequence was randomized.
The arm tank was a specially constructed, sealed wooden
tank with an air pressure regulator and continuous pressure
cx increases in MAP and MSNA in
in the ambient pressure
ompression. Arrows, venous blood sampling. min of exercise and 5 min of recovery (Fig. 1). Maximal
untary contraction (MVC) was assessed in each arm sepa-
min of exercise and 5 min of recovery (Fig. 1). Maximal
mmsHg above ambient barometric pressure was achieved.
levels of positive pressure, the perfusion pressure and flow velocity were also examined every 10 s. At
each level of positive pressure, the initial values for perfusion
pressure and velocity were subtracted from the last two to
determine time course of perfusion pressure and flow velocity
adjustments.
HR and blood pressure. HR was monitored via three-lead
electrocardiography. Systemic blood pressure (BP) was mea-
sured continuously by using photoplethysmography (Fi-
napres, Ohmeda, Madison, WI) on the nonexercising hand.
Resting BP was obtained with an automated sphygmoma-
nometer (Dinamap, Critikon, Tampa, FL). Perfusion pres-
sure was calculated by subtracting external arm tank press
ure from MAP (29).
Microneurography. The microneurographic technique pro-
vides direct recordings of sympathetic nerve traffic directed
to blood vessels in skeletal muscle. The method, as used in
our laboratory, has been described previously (3, 24, 27).
Briefly, multunit recordings of postganglionic MSNA were
obtained from the peroneal nerve with an insulated 200-μm
diameter tungsten electrode tapered to an uninsulated 1- to
5-μm tip. The microelectrode was inserted percutaneously
into the peroneal nerve posterior to the head of the fibula,
with a reference electrode inserted subcutaneously 1–3 cm
from the active electrode. The nerve activity was amplified,
band-pass filtered (700–2,000 Hz), rectified, and then inte-
grated to obtain a mean voltage neurogram. We counted the
number of bursts and expressed the data in this report as
bursts per minute.

Blood samples. Venous blood was obtained via a 20-gauge
intravenous catheter placed in a retrograde fashion in the
deep antecubital vein of the exercising forearm. Plasma was
obtained immediately by centrifuge of the specimen for 30 s
at 3,000 rpm (model 5415C, Eppendorf, Hamburg, Germany).
Analysis of the samples for metabolites was conducted with
Radioneter model ABL 510 and EML 610 analyzers (Copen-
hagen NV, Denmark) in the Core Laboratory of the MSHMC
GCRC. Arterial oxyhemoglobin saturation was assumed to be
95% for the purpose of calculations. Oxygen extraction was
estimated by the difference between arterial and venous
oxyhemoglobin saturation. Oxygen uptake was calculated in
the usual fashion, using MBV as a surrogate for flow in the


RESULTS
In four of the seven subjects, brachial artery diam-
ter was measured (Table 1). No paradigm or between-
group effects on arterial diameter were noted. Given
that mean blood flow (MBF) can be expressed as
MBF = MBV·π·(brachial artery diameter/2)² and that
arterial diameter (or radius) did not change with fore-
arm pressure, we used MBV as a surrogate for MBF.
The data are presented in Table 1 and Figs. 2–7.
Ischemic exercise raised the perceived level of effort
(Fig. 2). Rhythmic handgrip exercise during incremen-
tal external positive tank pressure resulted in higher
plasma lactate (P < 0.02) and higher hydrogen ion
concentrations at +40 and +50 mmHg (Fig. 3).

Hemodynamic responses. In the ambient pressure
trial, MSNA, MAP, MBV, and the ratio of MBV to
perfusion pressure rose gradually as a function of
exercise duration. Of note, application of increment-
tal external positive pressure resulted in a greater
rate of rise of MAP, reduced MBV and increased
MSNA vs. ambient pressure (Fig. 4). Compared with
freely perfused handgrip, positive pressure aug-
mented the MAP and MSNA response at +40 mmHg
and +50 mmHg (Fig. 4). Despite the effects of positive pressure on flow velocity, oxygen consumption was maintained (Fig. 5).

Ten-second analyses of MBV and perfusion pressure during the ischemic paradigm are shown in Fig. 6. Positive forearm pressure led to a prompt reduction in flow at each workload. At each level of positive pressure except the last, flow was restored by the rise in perfusion pressure (Fig. 7).

**DISCUSSION**

Application of external positive pressure to the forearm reduced perfusion to the exercising muscle compared with perfusion during the ambient pressure condition. This led to greater plasma lactate concentration and increased hydrogen ion concentration (at +40 and +50 mmHg; Fig. 3). Progressive ischemia led to greater MAP and MSNA responses (Fig. 4) than were seen

---

**Table 1. Forearm response to ambient pressure and positive pressure exercise**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>E5</th>
<th>E6</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSNA, bursts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>13.3 ± 2.0</td>
<td>13.1 ± 2.6</td>
<td>14.1 ± 1.7</td>
<td>16.4 ± 1.7</td>
<td>16.1 ± 1.7</td>
<td>17.1 ± 1.7</td>
<td>14.9 ± 1.4</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>13.0 ± 1.7</td>
<td>12.6 ± 1.5</td>
<td>14.0 ± 1.0</td>
<td>16.8 ± 1.4</td>
<td>19.4 ± 2.3</td>
<td>24.4 ± 2.5*</td>
<td>26.5 ± 3.1*</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>MAP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>89.5 ± 3.1</td>
<td>97.1 ± 2.7</td>
<td>101.4 ± 2.8</td>
<td>104.1 ± 5.0</td>
<td>105.7 ± 3.3</td>
<td>107.5 ± 3.4</td>
<td>109.9 ± 3.2</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>90.5 ± 3.4</td>
<td>97.5 ± 3.0</td>
<td>100.2 ± 3.5</td>
<td>106.5 ± 3.5</td>
<td>110.4 ± 3.9</td>
<td>117.1 ± 3.9</td>
<td>129.1 ± 4.4*</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>MBV, cm/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>5.5 ± 0.9</td>
<td>19.3 ± 1.9</td>
<td>22.8 ± 1.7</td>
<td>25.9 ± 2.2</td>
<td>27.1 ± 1.6</td>
<td>28.4 ± 1.3</td>
<td>29.8 ± 1.8</td>
<td>Condition P &lt; 0.044</td>
</tr>
<tr>
<td>PP</td>
<td>7.6 ± 0.9</td>
<td>23.0 ± 2.1</td>
<td>23.9 ± 2.1</td>
<td>22.6 ± 1.5</td>
<td>20.6 ± 1.8*</td>
<td>18.4 ± 1.8*</td>
<td>19.5 ± 2.2*</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Conductance,</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml/min mmHg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>0.065 ± 0.013</td>
<td>0.205 ± 0.024</td>
<td>0.230 ± 0.021</td>
<td>0.251 ± 0.022</td>
<td>0.258 ± 0.015</td>
<td>0.266 ± 0.010</td>
<td>0.273 ± 0.016</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>0.072 ± 0.010</td>
<td>0.237 ± 0.022</td>
<td>0.270 ± 0.025</td>
<td>0.266 ± 0.202</td>
<td>0.262 ± 0.021</td>
<td>0.246 ± 0.025</td>
<td>0.258 ± 0.037</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Heart rate,</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>63.8 ± 1.9</td>
<td>73.0 ± 1.9</td>
<td>70.4 ± 2.2</td>
<td>73.1 ± 2.0</td>
<td>71.9 ± 2.5</td>
<td>72.6 ± 2.3</td>
<td>73.9 ± 2.3</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>63.1 ± 2.0</td>
<td>70.9 ± 1.7</td>
<td>70.0 ± 1.9</td>
<td>72.8 ± 2.1</td>
<td>75.1 ± 1.8</td>
<td>75.0 ± 2.6</td>
<td>76.2 ± 3.6</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Lactate, mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>0.85 ± 0.07</td>
<td>1.41 ± 0.15</td>
<td>1.83 ± 0.16</td>
<td>2.03 ± 0.13</td>
<td>2.12 ± 0.13</td>
<td>2.17 ± 0.15</td>
<td>2.17 ± 0.015</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>0.82 ± 0.04</td>
<td>1.15 ± 0.08</td>
<td>1.69 ± 0.11</td>
<td>2.05 ± 0.15</td>
<td>2.45 ± 0.18</td>
<td>2.78 ± 0.26*</td>
<td>3.17 ± 0.33*</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>H^+ , nmol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>2.16 ± 0.02</td>
<td>2.29 ± 0.04</td>
<td>2.40 ± 0.04</td>
<td>2.45 ± 0.03</td>
<td>2.49 ± 0.04</td>
<td>2.49 ± 0.04</td>
<td>2.47 ± 0.04</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>2.14 ± 0.01</td>
<td>2.18 ± 0.01</td>
<td>2.33 ± 0.01</td>
<td>2.44 ± 0.03</td>
<td>2.55 ± 0.04</td>
<td>2.62 ± 0.06*</td>
<td>2.72 ± 0.07*</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>O_2 extraction, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>29.7 ± 4.3</td>
<td>48.0 ± 2.3</td>
<td>49.3 ± 2.0</td>
<td>51.3 ± 2.1</td>
<td>50.0 ± 1.7</td>
<td>50.4 ± 2.2</td>
<td>49.0 ± 2.5</td>
<td>Condition P &lt; 0.037</td>
</tr>
<tr>
<td>PP</td>
<td>21.6 ± 2.2</td>
<td>43.7 ± 3.0</td>
<td>52.7 ± 2.5</td>
<td>58.8 ± 2.5</td>
<td>62.4 ± 2.7*</td>
<td>61.9 ± 3.1*</td>
<td>59.1 ± 3.9*</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>mV_{O_2}, au</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>2.1 ± 0.3</td>
<td>14.4 ± 2.0</td>
<td>17.8 ± 1.9</td>
<td>22.3 ± 1.9</td>
<td>22.4 ± 1.9</td>
<td>23.8 ± 1.8</td>
<td>23.4 ± 2.2</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>2.4 ± 0.3</td>
<td>16.0 ± 2.2</td>
<td>20.8 ± 2.9</td>
<td>21.8 ± 2.2</td>
<td>23.4 ± 2.8</td>
<td>21.0 ± 2.8</td>
<td>20.5 ± 3.2</td>
<td>Paradigm P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Brachial artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diameter, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>0.437 ± 0.049</td>
<td>0.430 ± 0.032</td>
<td>0.442 ± 0.032</td>
<td>0.439 ± 0.032</td>
<td>0.451 ± 0.031</td>
<td>0.448 ± 0.026</td>
<td>0.451 ± 0.025</td>
<td>Condition NS</td>
</tr>
<tr>
<td>PP</td>
<td>0.429 ± 0.040</td>
<td>0.434 ± 0.042</td>
<td>0.436 ± 0.042</td>
<td>0.442 ± 0.035</td>
<td>0.444 ± 0.037</td>
<td>0.449 ± 0.037</td>
<td>0.447 ± 0.037</td>
<td>Paradigm NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 14 measurements for each variable except for brachial artery diameter (n = 4). Data are for baseline and six 1-min increments of exercise (E1–E6). Positive pressure was applied during the trial (PP) as described in the text. MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; MBV, mean blood velocity; conductance, MBV/MAP; mV_{O_2}, muscle oxygen uptake; au, arbitrary units; AP, ambient pressure trial; NS, not significant. *Significantly different from PP, P < 0.05 (by 2-way repeated-measures ANOVA).

---

![Fig. 2. Borg data for perceived level of effort obtained during the 2 paradigms. Values are means ± SE. NS, not significant. *P < 0.05 (by Dunnett’s test).](https://www.jap.org)
during freely perfused handgrip. Of note, MAP and MSNA were greater during +40 and +50 mmHg. Analysis of flow velocity and perfusion pressure at each level of positive pressure demonstrated that the initial fall in flow velocity was restored by the rise in perfusion pressure at all levels of positive pressure except the last (Figs. 6 and 7). Thus, at the greatest level of positive pressure (+50 mmHg) where MSNA was clearly augmented, perfusion pressure similar to that seen at +10 to +40 mmHg did not restore flow to exercising muscle. These findings raise two key questions: 1) what is the mechanism for flow restoration at the lower levels of positive pressure? and 2) why does flow not rise with the increase in perfusion pressure seen at +50 mmHg?

With regard to the first question, it is unlikely that this effect is due to engagement of the metaboreflex at +10 to +30 mmHg. There was little increase in hydrogen ion or lactate concentration, and MSNA was augmented only at +40 mmHg. On the basis of prior literature, if the metaboreflex were engaged at the lower levels of positive pressure, we would have anticipated a rise in MSNA and/or some increase in markers of muscle ischemia (5, 28).

Could the increased perfusion pressure have been due to stimulation of the muscle mechanoreflex? This is clearly possible, because it has shown that mechanoreflex mediated autonomic responses are seen early in exercise and can be enhanced by ischemia (1, 16). However, if mechanoreceptor engagement were responsible for the restoration of flow at the lower workloads, we would have expected a rise in MSNA before the +40-mmHg level of positive pressure (12).

Is it possible that muscle ischemia enhances central command? Ischemic exercise is clearly perceived as more fatiguing than nonischemic work (Fig. 2), and it has been suggested that central command is linked to α-motoneuron activity and the perceived level of effort. It is known that muscle afferent feedback facilitates motoneuron activity (10) and that postexercise ischemia prevents motoneuron firing rate from returning to baseline values (4). Moreover, human studies suggest that central command evokes increases in HR and BP and far less impressive increases in MSNA (31, 32). In fact, it has been suggested that central command can act to decrease peripheral sympathetic constrictor outflow (25) and to evoke an increase in flow to skeletal muscle (33). Thus we postulate that ischemia augments α-motoneurons and autonomic responses in a parallel fashion through a mechanism that involves central locomotor regions of the brain. We further speculate that these systems act to raise muscle perfusion to exercising muscle.

Fig. 3. Δ Hydrogen ion concentration (A; ΔH⁺) and Δ plasma lactate (B; Δlactate) during ambient and positive pressure conditions. Δ Data represent the change from baseline. Values are means ± SE. *P < 0.05 (by Dunnett’s test). Level of positive pressure of E2–E6 as in Fig. 1.

Fig. 4. Δ Muscle sympathetic nerve activity (ΔBursts; A), Δ mean arterial blood pressure (ΔMAP; B), mean blood velocity (ΔMBV; C), and Δ conductance (D) in ambient and positive pressure conditions. Δ Data represent the change from baseline. Values are means ± SE. Conductance = flow velocity ÷ (MAP − tank pressure) and is expressed in arbitrary units. *P < 0.05 (by Dunnett’s test). Level of positive pressure at E2–E6 as in Fig. 1.
With regard to the second question, we believe that flow was not restored at /H11001 50 mmHg because the muscle reflex was engaged and thus sympathetic constriction was present within the exercising muscle. This hypothesis is based on the fact that at /H11001 50 mmHg, MSNA was clearly augmented. An increase in MSNA in the absence of a fall in BP should be associated with limb vasoconstriction in nonexercising muscle (22). We acknowledge that we have no data to demonstrate that MSNA and more importantly norepinephrine at the neurovascular junction is the same in exercising and nonexercising muscle. However, on the basis of the limited data currently present, we suspect that MSNA is likely to be similar in exercising and nonexercising muscle (6).

In conclusion, external positive pressure leads to flow reduction to muscle. At +10 to +40 mmHg, external pressure a rise in perfusion pressure acts to restore flow to muscle. At +50 mmHg, flow was no longer restored by the rise in perfusion pressure. We postulate that at +10 to +40 mmHg, autonomic adjustments do not include vasoconstriction within the active muscle. At +50 mmHg, we postulate that autonomic adjustments include an increase in sympathetic drive to muscle.

The authors greatly appreciate the technical support of Kristen Gray, Tania Mohammed, and Nikki DiVittore, the statistical support of Allen Kunselman, and the superb secretarial support of Jennifer Stoner in preparation of this manuscript.
REFERENCES


