Carotid Chemoreceptor Modulation of Regional Blood Flow Distribution During Exercise in Health and Chronic Heart Failure

Michael K. Stickland, Jordan D. Miller, Curtis A. Smith, Jerome A. Dempsey

Abstract—Previous work has shown sensitization of carotid chemoreceptor (CC) responsiveness during exercise as well as in chronic heart failure (CHF). Accordingly, we hypothesized that the CCs contribute to the sympathetic restraint of skeletal muscle blood flow during exercise and CHF. We examined the effect of transient CC inhibition on total (ConT) and hindlimb (ConL) conductance, and blood pressure at rest and during exercise (2.5 miles per hour, 5% grade) in chronically instrumented dogs. Via a carotid arterial catheter, CCs were inhibited using dopamine (5 to 10 μg/kg) or hyperoxic lactated Ringer’s solution. Although vasodilation did not occur with CC inhibition in resting healthy dogs, CC inhibition during exercise caused an immediate vasodilatory response (increase in ConT and ConL and decrease in blood pressure). When comparing the peak ConL response from CC inhibition versus α-adrenergic blockade (phenolamine), we found that the CCs accounted for approximately one-third of the total sympathetic restraint during exercise. CHF was then induced by chronic rapid cardiac pacing and characterized by impaired cardiac function, enhanced chemosensitivity, and greater sympathetic restraint at rest and during exercise. In contrast to healthy dogs, CC inhibition in resting CHF dogs produced vasodilation, whereas a similar vasodilatory response was observed during exercise in CHF as compared with healthy dogs. The vasodilation following CC inhibition during exercise and in CHF was abolished with α-adrenergic blockade and was absent in healthy exercising animals after carotid body denervation. These results establish an important role for the CCs in cardiovascular control in the healthy animal during exercise and in the CHF animal both at rest and during exercise. (Circ Res. 2007;100:1371-1378.)

Key Words: chemosensitivity ▪ sympathetic nervous activity ▪ exercise ▪ chronic heart failure ▪ blood flow

During exercise, sympathetic vasoconstrictor activity increases, resulting in vasoconstriction in the gut and kidneys and will compete with local vasodilatory influences to constrain locomotor limb blood flow during exercise to maintain blood pressure. It is generally assumed that the increased sympathetic nervous activity (SNA) during exercise is caused by feedforward mechanisms such as central command and feedback from muscle metaboreceptors, muscle mechanoreceptors, and/or a resetting of systemic baroreceptors. We propose that the tonic sensory input from the carotid chemoreceptors (CCs) might also be an important source of exercise-induced sympathetic vasoconstrictor activity.

The CCs are traditionally thought to be the major oxygen sensors in the body, and their stimulation is assumed to cause a reflex-mediated increase in ventilation. However, CC stimulation also elicits significant increases in sympathetic vasoconstrictor outflow. Importantly, the CCs are sensitive to a variety of stimuli in addition to oxygen, including, metabolic acidosis, norepinephrine, potassium, glucose, and angiotensin II, all of which can change with exercise, causing increased CC activity. Indeed, previous work has revealed that peripheral chemosensitivity is enhanced with exercise. In humans, Forster et al11 have shown a greater ventilatory response to the CC stimulant doxapram during exercise as compared with rest, whereas hypoxia greatly potentiates the ventilatory and muscle SNA response to exercise. Furthermore, Bisceo and Purves14 demonstrated, in anesthetized animals, that passive exercise increased CC activity via feedback from the exercised limb, although subsequent studies failed to confirm this effect.15 No study to date has examined whether the enhanced chemosensitivity with exercise modulates cardiovascular function.

Chronic heart failure (CHF) is characterized by sympathoexcitation and enhanced peripheral chemosensitivity. Both the degree of resting sympathoexcitation as well as the magnitude of peripheral chemosensitivity are predictive of patient mortality. The enhanced CC sensitivity at rest contributes, at least in part, to the increased SNA in CHF, as inhibition of chemoreceptor activity with 100% inspired O2 has been shown to decrease renal SNA in CHF but not healthy animals. Importantly, the functional cardiovascular

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significance of enhanced CC sensitivity in CHF remains undefined.

Given these observations, we tested the following hypotheses: (1) that the CCs are sensitized during exercise in health, contributing to the sympathetic restraint of exercising muscle blood flow and that inhibiting the CCs would cause reflex-mediated vasodilation in the exercising locomotor limb; and (2) that the CCs are sensitized both at rest and during exercise in CHF, contributing to the sympathetic restraint of muscle blood flow and, similarly, that inhibiting the CCs would cause reflex-mediated vasodilation. We tested these hypotheses by using varied means to specifically inhibit the tonic CC activity that is normally present at rest and during exercise and then by determining the importance of this activity to sympathetically mediated regulation of blood flow distribution in health and CHF animals. Our findings demonstrate an important role for the CCs in the control of blood flow during exercise and in heart failure.

Materials and Methods

An expanded Materials and Methods section is available in the online data supplement at http://circres.ahajournals.org.

Chronic Instrumentation

All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin–Madison and conducted in accordance with the Guiding Principles in the Care and Use of Animals of the American Physiological Society. Six female mixed-breed hound dogs weighing between 19 and 23 kg were trained to lie quietly on a bed and run on a motorized treadmill (2.5 miles per hour, 5% grade). After training, 2 surgeries were required to instrument the dogs for study. A third surgery was later conducted on 2 dogs to denervate the right carotid body, which resulted in CC and carotid baroreceptor denervation. In 5 of the dogs, CHF was induced by rapid ventricular pacing at 210 bpm for 3 to 6 weeks. See the online data supplement for specific information regarding instrumentation and timeline of data collection.

Arterial Blood Gases

Arterial blood samples were taken from a chronically indwelling catheter both at rest and during exercise.

Peripheral Chemosensitivity

Peripheral chemosensitivity was evaluated by the resting ventilatory response to incremental, steady-state isocapnic hypoxia.

Close-Carotid Injections

During steady-state resting or exercise conditions, pharmacological agents were administered, using a constant infusion pump (0.75 mL/sec, to the right carotid body via the in-dwelling carotid artery catheter. Injections given to inhibit the CCs included the following: (1) dopamine hydrochloride (5 to 10 μg/kg); (2) hyperoxic lactated Ringer’s solution; and (3) serial injections of tropisetron (5 to 10 μg/kg) and dopamine (5 to 10 μg/kg). Importantly, dopamine has not been shown to sensitize the carotid baroreceptors and does not cross the blood–brain barrier. Thirty-second isovolumetric normoxic Ringer’s infusions were used as negative control injections to confirm that the cardiovascular response was not attributable to baroreceptor or thermal stimulation, or behavioral artifact. Injections were performed in random order, with a minimum of 5 minutes separating each injection. Reported data are of the mean individual response of 2 to 4 injections of each agent.

∞-Adrenergic Blockade

During steady-state resting or exercising conditions, phenotolamine (2 mg/kg, dissolved in sterile saline and diluted to a concentration of 10 mg/mL) was administered via the abdominal aortic catheter. See the online data supplement for additional information.

Data Analysis and Statistics

See the online data supplement for details.

Results

Resting Healthy Dog

A representative trace of the cardiovascular response to close-carotid injection of dopamine is shown in Figure 1, and group mean data are presented in Table 1. Infusions of normoxic Ringer’s solution did not show any consistent cardiovascular effect. Dopamine resulted in a significant bradycardia and subsequent reductions in cardiac output and hindlimb flow, which would be consistent with transient excitation of the CCs from dopamine. A delayed systemic vasodilatory response was observed starting ~30 seconds after injection, which was likely a systemic effect of dopamine, as vasodilation was also observed in the mesenteric artery. Injections of dopamine/tropisetron, as well as infusions of hyperoxic Ringer’s solution had no consistent cardiovascular effect.

Resting CHF Dog

Following 4 to 6 weeks of rapid cardiac pacing, the animals exhibited significant increases in resting end-diastolic and end-systolic cardiac left ventricular areas, as well as significant reductions in area ejection fraction (see Table 2, P<0.05 for all). Likewise, CHF animals exhibited significant reductions in cardiac output, hindlimb flow, and stroke volume (see Table 2, P<0.05 for all). CHF animals showed enhanced peripheral chemosensitivity, as demonstrated by a greater ventilatory response to steady-state isocapnic hypoxia (Δ minute ventilation/Δ end-tidal PO2; see Table 2 and Figure IV in the online data supplement), attributable solely to greater breathing frequency, despite lower eupneic end-tidal CO2 values (38 mm Hg Healthy versus 31 mm Hg CHF). Arterial PCO2 values were also lower in CHF dogs during room air breathing at rest and during exercise (see Table 2, P<0.05 for all).

See Figure 1 for a representative trace of the response to a close-carotid injection of dopamine in the resting CHF dog and Table 1 for grouped mean data of various injections. Infusions of normoxic Ringer’s solution did not show any cardiovascular effect. Within 20 seconds of infusion, dopamine caused a rapid vasodilatory response, as evidenced by increases in total and hindlimb conductance (P<0.05). The percentage increase in hindlimb flow and conductance was significantly greater (P<0.05) than the increase in cardiac output/total conductance. Close-carotid injections of tropisetron/dopamine in 3 dogs demonstrated a qualitatively similar response. In either case, the immediate vasodilatory response preceded a delayed secondary vasodilation in the mesenteric artery, which occurred ~30 seconds after injection.

Exercising Healthy Dog

A representative trace of the cardiovascular response to close-carotid injection of dopamine is shown in Figure 2. See
the online data supplement (supplemental Figure I) for representative traces of normoxic Ringer’s solution and hyperoxic Ringer’s solution responses. Group mean data are presented in Table 3. Infusions of normoxic Ringer’s solution did not show any cardiovascular effect. Close-carotid infusions of dopamine caused a rapid vasodilatory response, as evidenced by increases in cardiac output, total conductance, hindlimb flow, and hindlimb conductance and a decrease in

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Cardiovascular response to close-carotid injection of 10 µg/kg (~200 µg total) dopamine at rest in health (left) and CHF (right) in the same dog. The vertical line at 30 seconds denotes start of constant-infusion pump to deliver dopamine to the carotid body. Conductance was calculated using a moving 5-heart-beat average of hindlimb blood flow and arterial blood pressure. Note the immediate vasodilation that occurred in CHF. This vasodilation preceded the systemic effect of dopamine, which is demonstrated by increases in the mesenteric flow observed in both conditions.

### TABLE 1. Mean (±SE) Cardiovascular Responses to Close-Carotid Injections of Various Pharmacological Agents at Rest in Healthy and CHF Dogs

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th></th>
<th>Chronic Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Preinjection Steady State</td>
<td>Normoxic Ringer’s (% Change)</td>
<td>Dopamine (% Change)</td>
</tr>
<tr>
<td>Cardiac output, L·min⁻¹</td>
<td>4.08</td>
<td>0.2</td>
<td>−7.8*</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>42.1</td>
<td>0.4</td>
<td>0.0</td>
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<tr>
<td>Heart Rate, bpm</td>
<td>98</td>
<td>−1</td>
<td>−7.5*</td>
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<tr>
<td>Total conductance</td>
<td>49.22</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Hindlimb flow, L·min⁻¹</td>
<td>0.60</td>
<td>0.1</td>
<td>−7.9*</td>
</tr>
<tr>
<td>Hindlimb conductance</td>
<td>7.19</td>
<td>−1.2</td>
<td>−6.7</td>
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<tr>
<td>Mesenteric flow, mL·min⁻¹</td>
<td>23.0</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Mesenteric conductance</td>
<td>1.7</td>
<td>1.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>84</td>
<td>1.3</td>
<td>−1.2</td>
</tr>
</tbody>
</table>

Healthy, N=6; CHF, N=5. Tropisetron and dopamine injections in CHF dogs were not evaluated with inferential statistics. *P<0.05 vs normoxic Ringer’s solution.
TABLE 2. Mean (±SE) Cardiopulmonary Data at Rest and During Exercise in Healthy and CHF Dogs

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th></th>
<th>Chronic Heart Failure</th>
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<td></td>
<td>Rest</td>
<td>Exercise</td>
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<td>Exercise</td>
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<td>7.422</td>
<td>7.414</td>
<td>7.417</td>
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<tr>
<td>PO2, mm Hg</td>
<td>37.7</td>
<td>34.2</td>
<td>28.4</td>
<td>27.3</td>
</tr>
<tr>
<td>PCO2, mm Hg</td>
<td>1.8</td>
<td>2.0</td>
<td>1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>PO2, mm Hg</td>
<td>93.3</td>
<td>97.4</td>
<td>91.1</td>
<td>96.4</td>
</tr>
<tr>
<td>[HCO3−]</td>
<td>21.6</td>
<td>20.5</td>
<td>17.8</td>
<td>17.4</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>0.7</td>
<td>0.9</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>ΔVl/ΔP2O2, L min−1 mm Hg−1</td>
<td>0.4</td>
<td>0.2</td>
<td>0.16#</td>
<td>0.02</td>
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<tr>
<td>End-diastolic cardiac area, cm²</td>
<td>11.5</td>
<td>17.6#</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>End-systolic cardiac area, cm²</td>
<td>5.2</td>
<td>14.4#</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>54.7</td>
<td>18.1#</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Cardiac output, L min−1</td>
<td>4.08</td>
<td>6.27</td>
<td>3.17#</td>
<td>5.43#</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>42.1</td>
<td>43.8</td>
<td>30.5#</td>
<td>33.3#</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>98</td>
<td>144</td>
<td>104</td>
<td>163#</td>
</tr>
</tbody>
</table>

Vl indicates minute ventilation; P2O2, end-tidal O2. #P<0.05 vs healthy. For additional data, see the online data supplement.

blood pressure (P<0.05), which all occurred within 15 seconds of initiation of infusion. Similarly, close-carotid injections of tropisetron/dopamine as well as infusions of hyperoxic Ringer’s solution caused an immediate, significant increase in total conductance, hindlimb flow, and hindlimb conductance and a decrease in blood pressure (P<0.05). Similarly, close-carotid injections of tropisetron/dopamine and infusions of hyperoxic Ringer’s solution caused significant increases in hindlimb conductance and hindlimb flow. There were no significant differences in the peak hindlimb conductance response among dopamine, tropisetron/dopamine, and hyperoxic Ringer’s solution. In addition, the percentage increase in hindlimb flow and conductance was significantly greater (P<0.05) than the increase in cardiac output/total conductance.

α-Adrenergic Blockade
See the online data supplement for complete data. In all conditions, phentolamine administered via the abdominal aortic catheter resulted in a transient vasodilation. After the initial peak vasodilation, the cardiovascualar parameters obtained a steady state, which was below the initial peak, but above baseline (see supplemental Figure II). In the resting healthy dog, phentolamine resulted in a peak increase in hindlimb conductance of 50.3±5.4% (see supplemental Table I). Cardiac output, total conductance, and hindlimb flow also increased substantially, whereas blood pressure decreased slightly. In the CHF resting dog, α-blockade produced significantly greater increases in stroke volume, total conductance, hindlimb flow, and hindlimb conductance and reductions in blood pressure, as compared with the healthy dog. The exercising healthy dog demonstrated a greater response to α-blockade as compared with the healthy resting dog as evidenced by greater peak increases in heart rate, stroke volume, total conductance, and hindlimb conductance and reductions in blood pressure (see supplemental Table II). As compared with the healthy exercising dog, α-blockade produced significantly greater increases in stroke volume, cardiac output, hindlimb flow, and hindlimb conductance in the CHF exercising dog.

Adrenergic blockade abolished the vasodilatory response from close-carotid injections of dopamine, tropisetron/dopamine, and hyperoxic Ringer’s solution previously observed in the resting CHF; as well as the exercising healthy and CHF dogs (supplemental Figure II and supplemental Tables I and II).

Relative Contribution of the CCs to Sympathetic Vasoconstrictor Outflow
In the resting healthy animal, α-blockade resulted in a peak increase of 50% in hindlimb conductance, whereas CC inhibition had no consistent cardiovascular effect, indicating that the CCs do not contribute to resting sympathetic control. In CHF, α-blockade increased hindlimb conductance by 226%, whereas CC inhibition increased hindlimb conductance by 67%, signifying that the CCs are responsible for ≈30% of the sympathetic vasoconstrictor outflow in the resting CHF animal.

During exercise, α-blockade increased hindlimb conductance 127% in the healthy dog, and CC inhibition increased
hindlimb conductance by 42%, indicating that approximately one-third of the tonic sympathetic vasoconstrictor outflow during exercise in health is from the CCs (see Figure 3 for summary data). In CHF, α-blockade increased hindlimb conductance by 210%, whereas CC inhibition increased hindlimb conductance 40%, signifying that ≈20% of sympathetic vasoconstrictor outflow is from the CCs in CHF during exercise.

Figure 2. Cardiovascular response to close-carotid injection of 10 μg/kg (∼200 μg total) dopamine during exercise in health (left) and CHF (right) in the same dog. The vertical line at 30 seconds denotes start of constant-infusion pump to deliver dopamine to the carotid body. Conductance was calculated using a moving 5-heart-beat average of hindlimb blood flow and arterial blood pressure. Note the immediate vasodilation that occurred in both health and CHF. This vasodilation preceded the systemic effect of dopamine, which is demonstrated by increases in the mesenteric flow observed in both conditions.

TABLE 3. Mean (±SE) Cardiovascular Responses to Close-Carotid Injections of Various Pharmacologic Agents During Exercise in Health and CHF

<table>
<thead>
<tr>
<th></th>
<th>Healthy Close-Carotid Injection</th>
<th>CHF Close-Carotid Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Preinjection</td>
<td>Normoxic Ringer’s</td>
</tr>
<tr>
<td>Cardio output, L min⁻¹</td>
<td>6.27</td>
<td>−0.1</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>43.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>144</td>
<td>−1.5</td>
</tr>
<tr>
<td>Total conductance</td>
<td>70.73</td>
<td>−1.0</td>
</tr>
<tr>
<td>Hindlimb flow, L min⁻¹</td>
<td>1.54</td>
<td>−0.3</td>
</tr>
<tr>
<td>Hindlimb conductance</td>
<td>17.47</td>
<td>−1.2</td>
</tr>
<tr>
<td>Mesenteric flow, mL min⁻¹</td>
<td>19.4</td>
<td>−5.7</td>
</tr>
<tr>
<td>Mesenteric conductance</td>
<td>0.23</td>
<td>−0.1</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>90</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Health, N=6; CHF, N=5. *P<0.05 vs normoxic Ringer’s.
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Figure 3. Summary of mean data for dogs during mild exercise showing the peak change in hindlimb conductance following: total systemic α-adrenergic blockade (phenolamine) and close-carotid injections (CCI) of dopamine (5 to 10 μg/kg), hyperoxic Ringer’s solution, the 5-HT blocker tropisetron with dopamine (5 to 10 μg/kg), and normoxic Ringer’s solution. The hindlimb response to close-carotid injections of dopamine following carotid body denervation (CBX*) (N = 2) is also illustrated. The vasodilatory response to systemic α-adrenergic blockade was significantly greater compared with inhibiting the CCs with dopamine, hyperoxic Ringer’s, or 5-HT blockade and dopamine. No difference was found among peak response from dopamine, hyperoxic Ringer’s, or 5-HT blockade and dopamine; however, all were greater than the response observed with normoxic Ringer’s. Carotid body denervation abolished the vasodilatory response to close-carotid injections of dopamine.

Discussion

The CC is traditionally regarded as a significant sensor for control of breathing; however, our results establish an important role for the CC in cardiovascular control in the healthy animal during exercise and in the CHF animal both at rest and during exercise. Specifically, direct CC inhibition during mild-intensity exercise, with dopamine, tropisetron/dopamine, or hyperoxic Ringer’s solution caused an immediate, transient increase in hindlimb blood flow, hindlimb conductance, cardiac output, and total conductance and a drop in blood pressure. These findings indicate that the tonic activity of the CC actively restrains blood flow to the working muscle during exercise in healthy dogs. Importantly, the vasodilatory effect was not observed when the CCs were perfused with isovolumic normoxic Ringer’s solution, demonstrating that the increase in blood flow/conductance was not the result of the infusion, per se, stimulating the baroreceptors. Moreover, when the carotid body was denervated in 2 dogs, thereby removing both carotid chemo- and baroreceptors, the vasodilatory response from CC inhibition was abolished, ruling out a significant central effect of dopamine or hyperoxic Ringer’s solution on sympathetic vasoconstriction. In contrast to the resting healthy dog, reducing tonic CC activity at rest in CHF resulted in a transient vasodilatory response. Additionally, inhibiting the CCs during exercise in CHF also resulted in vasodilation. The vasodilatory response to CC inhibition during exercise and CHF was blocked with α-adrenergic blockade, indicating a reliance on an intact sympathetic nervous system. Importantly, the vasodilatory response following CC inhibition, both during exercise and CHF, occurred despite no obvious change in arterial blood gases or blood born chemical stimuli. Combined, our findings indicate that tonic CC activity is increased during exercise and in CHF, even without changes in circulating chemoreceptor stimuli, and that the carotid chemoreflex contributes to the sympathetic restraint of muscle blood flow during exercise and in CHF.

Methodological Considerations

We chose direct CC inhibition using close-carotid injections of various agents in the intact animal, as this was the most powerful technique to rapidly reduce CC activity. This method was preferable over carotid body denervation, as removal of both carotid bodies causes hypoventilation (increasing arterial Pco2 and reducing P02),30 ablation of carotid baroreceptor feedback, and secondary compensatory neuro-physiological changes that upregulate both central and aortic chemoreceptors.31 As mentioned previously, dopamine does not stimulate the carotid baroreceptors.27 Furthermore, isovolumic injections of normoxic Ringer’s showed no cardiovascular response and hyperoxic Ringer’s infusion caused vasodilation, both of which indicate that our response is secondary to CC inhibition, and not baroreceptor stimulation. Of note, the focus of our investigation was the CC; however, aortic chemoreceptors also contribute to cardiovascular control,32 although the present study did not allow for examination of their role in exercise or disease.

Ideally we would prefer to quantify the CC input on vascular control in the steady state. However, we chose transient CC inhibition to minimize the powerful influence of secondary compensatory mechanisms and/or the upregulation of redundant regulatory pathways, which have a time-dependent influence on the steady-state vascular response.33 A disadvantage of relying on transient effects is the increased variability inherent in brief, non–steady-state responses. However, we attempted to minimize this by averaging peak responses to several repeated trials of CC inhibition in the same animal and by using several means of inhibition.

Dopaminergic receptors are located throughout the body, and stimulation of these receptors can cause vasodilation.34 Indeed we regularly observed a delayed vasodilation in the mesenteric artery ≈30 seconds after close-carotid injections of dopamine or tropisetron/dopamine. Importantly, the rapid hindlimb and central vasodilatory response always preceded visceral vasodilation. Moreover, the rapid vasodilatory response was also observed with close-carotid infusions of hyperoxic Ringer’s solution, which (unlike dopamine) did not demonstrate any delayed systemic effect on recirculation. Finally, the vasodilatory response with dopamine was abolished following carotid body denervation. Combined, our results indicate that the vasodilatory response observed with dopamine or tropisetron/dopamine was the direct result of CC inhibition and not of a central effect or a secondary systemic response caused by drug recirculation.

CC Influence on Blood Flow Distribution

CC inhibition, during exercise as well as in CHF, caused a greater proportional increase in hindlimb conductance as compared with total conductance, whereas little effect was observed in the mesenteric vasculature. This finding is noteworthy, as this indicates the increase in hindlimb blood flow was attributable primarily to peripheral vasodilation and was not simply a direct effect of increasing cardiac output. Of
Note, Rutherford and Vatner also found a differential vascular response to CC stimulation via close-carotid injections of NaCN in the resting anesthetized dog. Specifically, considerably more vasoconstriction occurred with CC stimulation in the iliac artery (ie, blood flow to muscle) as compared with the mesenteric vascular bed. Consistent with these data, our findings of CC inhibition in the exercising and CHF dog show a greater effect on skeletal muscle conductance and blood flow as compared with the mesenteric vasculature. Our results in the exercising healthy animal demonstrating substantial vasodilation following α-adrenergic blockade also support previous evidence demonstrating substantial sympathetic restraint to skeletal muscle blood flow in health even during mild-intensity exercise. Based on our comparison of phentolamine versus CC effects on limb vascular conductance (Figure 3), we conclude that approximately one-third of the total sympathetic restraint during mild-intensity exercise in health is attributable to the tonic activity of the CC, with the remainder attributable to other influences such as central command, muscle mechanoreceptors, muscle metaboreceptors, and baroreceptors.3

**Carotid Chemoreception and CHF**

CHF is characterized by enhanced peripheral chemosensitivity,19,21 sympathoexcitation at rest,17,18 and an exaggerated sympathetic response to exercise.35,36 Our CHF dogs demonstrated enhanced chemosensitivity as compared with healthy dogs, as evidenced by a greater resting ventilatory response to incremental normocapnic hypoxia (ie, Δ minute ventilation/Δ end-tidal Po2; see Table 2 and supplemental Figure IV). Previous work has shown that the enhanced resting CC sensitivity in CHF contributes to the increased SNA, as chemoreceptor inhibition by breathing 100% O2 decreased renal SNA in CHF but not normal animals.20 That these increases in carotid chemosensitivity and SNA have important functional consequences in CHF is demonstrated by our observation that CC inhibition at rest in CHF caused reflex-mediated vasodilation and increases in regional blood flow. The abolition of these responses by α-adrenergic blockade strongly suggests that enhanced carotid chemosensitivity in CHF causes active sympathetic restraint of resting skeletal muscle blood flow. Consistent with previous work showing an exaggerated sympathetic response to exercise in CHF,35,36 we found greater sympathetic restraint of muscle blood flow during exercise in CHF as compared with health (see supplemental Table II). This increased sympathetic vasoconstrictor activity during exercise in CHF has been attributed to sensitization of the metaboreflex and/or mechanoreceptors from exercising locomotor37 and respiratory38 muscles. Our new finding is that CC tonic activity also contributes significantly to the enhanced sympathetic vasoconstrictor activity during exercise in CHF.

Despite enhanced sympathetic vasoconstrictor outflow in CHF during exercise, the vasodilatory response following CC inhibition in CHF was not greater than that observed in health, suggesting that the relative contribution of the CCs to exercise sympathetic outflow is actually lower in CHF. This may be explained by the muscle mechano- and/or metaboreflexes playing a greater role in the exercise SNA response in CHF.37 We emphasize that our attempts to compare sympathetic outflow between health and CHF are made somewhat difficult because we measured blood flow and not sympathetic nervous activity, and therefore our measurements do not allow differentiation between an elevated SNA versus enhanced α-receptor responsiveness39 in CHF.

**CC Sensitization**

Our results indicate increased sympathetic vasoconstrictor outflow during mild-intensity exercise secondary to increased CC responsiveness, despite a lack of obvious increase in circulating chemoreceptor stimuli within arterial blood (see Table 2), signifying an exercise-induced resetting or sensitizing of the CC. These findings are consistent with previous work in humans revealing the responsiveness of ventilation or muscle SNA to hypoxia or pharmacological CC stimuli are enhanced during mild- to moderate-intensity exercise.11–13 The exact mechanism(s) for the increased sensitivity during exercise is unclear but may include: CC sensitization via direct SNA afferent outflow to the carotid body,14,40,41 and/or a change in central integration of efferent CC signal secondary to increased cardiac sympathetic afferent outflow.42 In addition to these, a reduction of NO bioavailability and/or an increase in angiotensin II10,43 at the carotid body may explain the increased resting CC activity in CHF.

**Conclusion**

Our results establish an important functional cardiovascular consequence of the enhanced CC sensitivity during exercise and CHF. Specifically, the carotid chemoreflex can activate sympathetic outflow and vasoconstriction in exercising skeletal muscle in health and in heart failure, even in the absence of changes in blood gases or blood born chemical stimuli. Accordingly, the carotid chemoreflex should be considered in any integrative scheme of neural control of cardiovascular function during exercise and CHF. The mechanism(s) underlying the exercise-induced CC sensitization in the intact animal remains to be determined.

**Acknowledgments**

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**Disclosures**

None.

**References**


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Carotid chemoreceptor modulation of regional blood flow distribution during exercise in health and chronic heart failure.

Online Supplement

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METHODS

Chronic instrumentation

Similar to previous studies\(^1\)\(^2\) from our laboratory, the first surgery included the implantation of an ascending aortic flow probe (type 20A, 20mm, Transonic Systems Inc., Ithaca, NY, \(N = 6\)), a bipolar ventricular pacing lead (Medtronic, Minneapolis, MN, \(N = 5\)), costal diaphragmatic electromyographic leads (\(N = 6\)), terminal aortic flow probe (type 10A, 10mm, Transonics Systems Inc., \(N = 6\)), and mesenteric flow probe (type 1.5RB, 4mm, Transonics Systems Inc., \(N = 3\)). During this surgery an ovariohysterectomy was also performed in five dogs. The second surgery involved the implantation of a close-carotid sinus catheter, which was placed via the external carotid artery and passed retrograde such that the tip was just proximal to the bifurcation of the right common carotid artery. In two dogs, the left carotid chemoreceptor was also denervated by stripping the adventitia from all of the arteries within 2cm of the carotid sinus. In all dogs, a catheter was then placed in the abdominal aorta via cannulation of a small side branch of the femoral artery.

In two dogs, following data collection in the intact condition, a third surgery was performed whereby the right carotid body was denervated by stripping the adventitia from all of the arteries within 2cm of the sinus. Of note, this technique also results in baroreceptor denervation. Physiological chemoreceptor denervation was then confirmed by an absence of diaphragm EMG response to a close-carotid injection of 2\(\mu\)g/kg NaCN.

For all surgeries, anesthesia was induced using sodium pentothal (20 mg/kg), and a surgical plane of anesthesia was maintained using isoflurane (1 - 1.5 %) and mechanical ventilation. Strict sterile techniques were used during all surgical procedures, and appropriate antibiotics and analgesics were used postoperatively.

In five of the dogs, chronic heart failure was induced by rapid ventricular pacing at 210 bpm for 3-6 weeks. The animals were routinely exercised to maintain a constant level of training and familiarity with the laboratory. As detailed previously\(^2\), cardiac function was monitored by resting
echocardiography. Chronic heart failure was defined as a left ventricular ejection fraction < 30% with a considerably blunted cardiac output and stroke volume response to exercise (2.5mph/5% grade).

**Arterial Blood Gases.** Arterial blood samples were taken both at rest and during exercise. Blood-gas measurements were analyzed in duplicate on an automated gas analyzer (Radiometer ABL-505) validated daily with tonometered blood. Samples were corrected for body temperature and systematic error determined by tonometry data.

**Peripheral Chemosensitivity.** Peripheral chemosensitivity was evaluated by the ventilatory response to incremental, steady-state isocapnic hypoxia. At rest, dogs were fitted with a tight-fitting non-rebreathing muzzle mask. Ventilation was measured by a heated pneumotachograph, and end-tidal PO$_2$ and PCO$_2$ were measured using a mass spectrometer. Following determination of eupneic breathing, two five-minute step reductions in inspired PO$_2$ were conducted (target end-tidal PO$_2$ ~ 55 and 45 mmHg), while eupneic end-tidal PCO$_2$ was maintained. The slope of the change in minute ventilation / the change in end-tidal PO$_2$ was calculated for each dog.

**Close-carotid Injections.** During steady-state resting or exercise conditions, various pharmacological agents were administered, using a constant infusion pump (0.75ml/sec), to the right carotid body via the chronically in-dwelling carotid artery catheter. Dopamine hydrochloride (5-10μg/kg), an inhibitor of carotid chemoreceptor activity$^3$, was dissolved in sterile saline to a concentration of 1mg/ml, mixed with normoxic Ringer’s solution (PO$_2$ = ~130 mmHg, pH = 7.4), and infused over 5-10 seconds. Importantly, dopamine has not been shown to sensitize the carotid baroreceptors$^4$, and does not cross the blood-brain barrier$^5$. Bisgard et al. demonstrated that in some cases, inhibition of carotid discharge frequency from dopamine is preceded by a brief increase in carotid chemoreceptor discharge frequency$^3$ that can be eliminated by 5-HT blockade with Tropisetron (a 5-HT blocker) without affecting the later inhibition of carotid discharge frequency$^6$. In a subset of experiments, Tropisetron (5-10μg/kg) was dissolved in sterile saline and diluted down in normoxic Ringer’s solution, and serial injections of Tropisetron and dopamine were infused over 5-10 seconds with normoxic Ringer’s
solution. Close-carotid infusions of hyperoxic lactated Ringer’s were also administered, as it has been shown to suppress carotid chemoreceptor activity\textsuperscript{7}. For these experiments, standard Ringer’s solution was bubbled with oxygen until PO\textsubscript{2} approximated 730mmHg and was adjusted to physiological pH values (i.e. pH=7.4). The solution was delivered for 30 seconds at the same speed as the delivery of dopamine (~0.75ml/sec).

\textit{Alpha-adrenergic Blockade} During steady-state resting or exercising conditions, phentolamine (2mg/kg, dissolved in sterile saline and diluted to a concentration of 10mg/ml) was administered via the abdominal aortic catheter. Adrenergic blockade was confirmed by an absence of hindlimb vasoconstrictor response from a 25μg intra-arterial injection of phenylephrine\textsuperscript{8}. Following the attainment of a new, elevated steady-state, close-carotid injections were repeated in the exercising healthy and CHF dog, as well as the resting CHF dog, as described above. Alpha blockade was maintained with additional injections of phentolamine (0.5mg/kg every ten minutes), and confirmed at the end of each trial with a 25μg injection of phenylephrine.

\textit{Data analysis}

All signals were digitized and stored on the hard drive of a personal computer for subsequent analysis and on a polygraph (AstroMed K2G, West Warwick, RI). All blood flow, blood pressure and ventilatory data were analyzed on a beat-by-beat basis using custom analysis software developed in our laboratory. Total, hindlimb, and mesenteric conductance were calculated as: flow (ml per minute)/mean arterial pressure (mmHg). Group data for each variable are expressed as means ± SE.

Bisgard et al.\textsuperscript{3} have shown that close-carotid injections of dopamine inhibit CC discharge frequency for 10-15 seconds in the dog. Therefore, the peak 5-heart-beat cardiovascular response within 20 seconds of dopamine infusion was compared to baseline values obtained in the preceding 30 sec control condition. Likewise, the peak 5-heart-beat response within 20 seconds following the infusion of normoxic Ringer’s solution, hyperoxic Ringer’s solution or injection of tropisetron/dopamine was compared with the preceding 30 sec control condition. Our main findings were the same whether the
peak 3, 5 or 7 heart-beat change was used. The peak effect of phentolamine was similarly evaluated by comparing the peak five-heart-beat change relative to control. All peak change data are expressed as a % change from baseline.

Statistics. For all inferential analyses, the probability of type I error was set at 0.05. Within each workload (rest or exercise) steady-state data between each condition (health or CHF) were evaluated using a paired t-test. Within each workload and condition, the peak cardiovascular responses to the various substances infused via the close-carotid catheter were evaluated with one-way repeated measures ANOVA. Upon detection of an effect, paired t-test comparisons were made, and a Bonferroni correction factor was applied to maintain family-wise error rate at 0.05.
REFERENCES


Figure I. Cardiovascular response to close-carotid injection of hyperoxic Ringer's solution (left) or normoxic Ringer's solution (right) during exercise in health. Note vertical line at 30 sec denotes start of constant-infusion pump to deliver the various agents to the carotid body. Conductance was calculated using moving 5 heart-beat average of hindlimb blood flow and arterial blood pressure. Note the immediate vasodilation that occurred with hyperoxic Ringer's solution, but was absent with normoxic Ringer's solution.
Figure II. Cardiovascular response of an intra-arterial infusion of phentolamine into the femoral artery during exercise (left), and the cardiovascular response to a close-carotid injection of 10μg/kg (~200μg total) dopamine during exercise following alpha adrenergic blockade (right). Note: vertical line denotes delivery of pharmacologic agent. Conductance was calculated using moving 5 heart-beat average of hindlimb blood flow and arterial blood pressure. Note that an intra-arterial infusion of phentolamine into the femoral artery blocked all alpha adrenergic receptors, causing rapid vasodilation, and the attainment of an elevated steady state (left). The vasodilatory response typically observed with a close-carotid injection of dopamine (see Figure 2 of manuscript) was absent following adrenergic blockade (right).
Figure III. Cardiovascular response to close-carotid injection of 10μg/kg dopamine during exercise in health following surgical carotid body denervation. Note vertical line at 30sec denotes start of constant-infusion pump to deliver dopamine to the carotid body. Conductance was calculated using moving 5 heart-beat average of hindlimb blood flow and arterial blood pressure. Note the immediate vasodilation that was previously observed (see Figure 2 of manuscript) is now abolished following carotid body denervation.
Figure IV. Steady-state hypoxic ventilatory response in health and chronic heart failure (CHF).
Note: CHF = chronic heart failure, $V_E$ = minute ventilation, $P_{ETO_2}$ = end-tidal PO$_2$, $P_{ETCO_2}$ = end-tidal PCO$_2$. 

Healthy, $P_{ETCO_2} = 38$ mmHg
CHF, $P_{ETCO_2} = 31$ mmHg
**Table I.** Effect of systemic alpha-adrenergic blockade, and close-carotid injections following alpha blockade at rest in health (N=6) and chronic heart failure (N=5).

<table>
<thead>
<tr>
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<th>Health</th>
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<th>CHF</th>
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<tr>
<td></td>
<td>Pre-injection Steady State (Intact)</td>
<td>Peak Change Following Adrenergic Blockade (% Change)</td>
<td>Pre-injection Steady State (Intact)</td>
<td>Peak Change Following Adrenergic Blockade (% Change)</td>
<td>Close-carotid Dopamine following adrenergic blockade (% Change)</td>
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<td>Cardiac Output (L·Min⁻¹)</td>
<td>4.30</td>
<td>22.2</td>
<td>2.76</td>
<td>92.5</td>
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<td>0.32</td>
<td>3.4</td>
<td>0.33</td>
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<td>Stroke Volume (ml)</td>
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<td>3.1</td>
<td>27.9</td>
<td>49.7#</td>
<td>3.8</td>
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<td>1.4</td>
<td>1.6</td>
<td>2.0</td>
<td>12.6</td>
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<td>Heart Rate (bpm)</td>
<td>114</td>
<td>15.5</td>
<td>98</td>
<td>27</td>
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<td>7</td>
<td>2.6</td>
<td>6</td>
<td>5</td>
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<td>3.91</td>
<td>7.0</td>
<td>3.12</td>
<td>22.8</td>
<td>2.7</td>
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<td>Hindlimb Flow (L·Min⁻¹)</td>
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<td>5.7</td>
<td>0.08</td>
<td>22.7</td>
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<td>225.5#</td>
<td>-1.9</td>
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<td>5.4</td>
<td>0.87</td>
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<td>Mesenteric Flow (ml·Min⁻¹)</td>
<td>19.6</td>
<td>-23.9</td>
<td>16.2</td>
<td>-29.3</td>
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<td>0.1</td>
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<td>Blood Pressure (mmHg)</td>
<td>89</td>
<td>-4.9</td>
<td>85</td>
<td>-19.8#</td>
<td>-0.9</td>
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<tr>
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<td>3</td>
<td>2.9</td>
<td>5</td>
<td>2.5</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Note: # p<0.05 vs. healthy condition
Table II. Effect of systemic alpha-adrenergic blockade, and close-carotid injections following alpha blockade during exercise in health (N=6) and chronic heart failure (N=5).

<table>
<thead>
<tr>
<th></th>
<th>Health</th>
<th>Chronic Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardio Output (L·Min⁻¹)</td>
<td>6.69 24.9 3.2 2.9 4.70 74.5#</td>
<td>-1.2 1.4</td>
</tr>
<tr>
<td>Stroke Volume (ml)</td>
<td>46.1 -20.0* 10.0 1.4 30.8 36.3#</td>
<td>-0.2 0.1</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>146 34.3* -7.0 1.4 155 21</td>
<td>-1.1 1.3</td>
</tr>
<tr>
<td>Total Conductance</td>
<td>67.27 97.4* 5.5 -1.7 55.37 142.0</td>
<td>-3.3 -2.6</td>
</tr>
<tr>
<td>Hindlimb Flow (L·Min⁻¹)</td>
<td>1.69 43.5 -0.3 0.4 0.91 125.2#</td>
<td>1.3 0.8</td>
</tr>
<tr>
<td>Hindlimb Conductance</td>
<td>17.35 127.2* 1.9 -3.8 10.84 209.7#</td>
<td>-0.8 -3.1</td>
</tr>
<tr>
<td>Mesenteric Flow (ml·Min⁻¹)</td>
<td>20.5 -0.4 -6.1 0.5 13.5 3.2</td>
<td>-3.2 4.6</td>
</tr>
<tr>
<td>Mesenteric Conductance</td>
<td>0.20 0.3 0.0 -0.1 0.14 0.3</td>
<td>-0.1 0.0</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)</td>
<td>100 -36.5* -1.5 5.1 87 -26.1</td>
<td>2.4 4.4</td>
</tr>
</tbody>
</table>

Note: * p<0.05 vs. healthy resting condition, # p<0.05 vs. healthy exercising condition.