Dynamic exercise attenuates sympathetic responsiveness of canine vascular smooth muscle

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Ruble, Stephen B., Zoran Valic, John B. Buckwalter, and Philip S. Clifford. Dynamic exercise attenuates sympathetic responsiveness of canine vascular smooth muscle. J Appl Physiol 89: 2294–2299, 2000.—The phenomenon of reduced responsiveness of the skeletal muscle arterial vasculature to sympathetic activation during exercise (sympatholysis) remains controversial. The purpose of this study was to examine the vascular effects of sympathoactivation in dynamically exercising skeletal muscle. Mongrel dogs (19–24 kg) were instrumented chronically with transit-time ultrasonic flow probes on the external iliac arteries. After pretreatment with atropine (0.2 mg/kg), an intravenous bolus (4 μg/kg) of a nicotinic ganglion stimulant [1,1-dimethyl-4-phenylpiperazinium iodide (DMPP)] was given at rest and during treadmill exercise at graded intensities. Administration of DMPP was associated with prompt reductions in iliac blood flow and increases in arterial pressure under all conditions. There were significant reductions (P < 0.05) in iliac vascular conductance of 58 ± 4 (SE), 48 ± 3, 36 ± 5, and 16 ± 3% at rest, 3 miles/h and 0% grade, 6 miles/h and 0% grade, and 6 miles/h and 15% grade, respectively. These data demonstrate that activation of postganglionic sympathetic nerves with DMPP caused vasoconstriction in the skeletal muscle vasculature at rest and during exercise. Additionally, the magnitude of vasoconstriction was inversely related to exercise intensity. These results support the concept of exercise sympatholysis.

vascular conductance; sympatholysis; blood flow; sympathetic nervous system; adrenergic

THE MECHANISMS THAT GOVERN skeletal muscle blood flow during exercise are not well understood. At the onset of dynamic exercise, there are increases in oxygen consumption and blood flow in active skeletal muscle. This is accompanied by the redistribution of cardiac output away from inactive tissues to working muscles and is generally attributed to changes in sympathetic nervous system activity (6). There are two lines of evidence that indicate augmented sympathetic activity to the skeletal muscle vasculature during exercise: 1) directly measured muscle sympathetic nerve activity is elevated (8, 11, 13) and 2) norepinephrine spillover from exercising muscle is increased (29). Despite the apparent increase in sympathetic nerve activity to skeletal muscle vasculature, blood flow and vascular conductance increase. A partial explanation is that the local vasodilator factors override sympathetic vasoconstriction (15). However, metabolic by-products associated with muscle contraction may also alter norepinephrine release, reuptake, and receptor binding (37), making sympathetic activation less effective during muscle contractions. Indeed, a number of studies have reported attenuated sympathetic vasoconstrictor responses in skeletal muscle vasculature associated with contractions (3–5, 12, 14, 25, 27, 28, 32, 36). Several experimental approaches have been employed to elicit sympathetic vasoconstrictor responses during muscle contraction, including direct nerve stimulation (4, 16, 27, 28, 32), baroreflex activation of sympathetic outflow (24, 26), and exogenous sympathomimetic agonists (2–5, 12, 14, 25, 32). The use of exogenous α₁- and α₂-adrenergic receptor agonists provides limited information because this approach does not answer the question of vascular responsiveness to release of endogenous neurotransmitter. No previous studies have examined vascular reactivity to pharmacologically induced release of endogenous neurotransmitter during dynamic exercise.

The purpose of this study was to examine the changes in skeletal muscle vascular conductance elicited by release of endogenous neurotransmitter during graded exercise. Specifically, we examined the relationship between increasing exercise intensity and vasoconstriction due to pharmacological sympathoactivation. We hypothesized that sympathoactivation would produce less vasoconstriction with increasing exercise intensities.

METHODS AND PROCEDURES

All experimental procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the American Physiological Society’s Guiding Principles in the Care and Use of Animals. Eight mongrel dogs (19–24 kg) were selected for their willingness to run on a motorized treadmill and were chronically instrumented using sterile surgical procedures. Anesthesia was induced with thiopental sodium (25 mg/kg; Gensia Pharmaceuticals, Irvine, CA). After intubation with a cuffed en-
dotracheal tube, a surgical level of anesthesia was main-
tained with 1.5% halothane (Halocarbon Laboratories, River
Edge, NJ) and 98.5% oxygen. Postoperatively, animals were
given an analgesic for pain management (buprenorphine
hydrochloride, 0.3 mg; Reckitt and Coleman, Kingston-upon-
Hull, UK) and treated with antibiotics for 10 days (cefazolin
sodium, 500 mg twice a day; Apothecon, Princeton, NJ).
Carotid arteries were externalized and placed in neck skin
tubes for percutaneous cannulation and measurement of
arterial blood pressure. After a 1-wk recovery period, the
second surgery was performed to instrument the dogs with
ultrasonic transit-time flow probes (4 mm, Transonic Sys-
tems, Ithaca, NY). Probes were placed around both external
iliac arteries to measure hindlimb blood flow, and cables were
tunneled under the skin to the back. The dogs were given a
2-wk recovery period from the flow probe implantation before
any experiments were conducted.

All experiments were performed in a laboratory in which
the temperature was maintained below 20°C. A 20-gauge
intravascular catheter (Insyte, Becton-Dickinson, Sandy,
UT) was inserted retrogradely into the lumen of the carotid
artery and attached to a solid-state pressure transducer
(Ohmeda, Madison, WI). The flow probe cables were con-
ected to the flowmeter. Experiments were performed at rest
in a sling and under three conditions of exercise on a motor-
ized treadmill (Quinton Instruments, Seattle, WA): 3.0
miles/h, 6.0 miles/h, and 6.0 miles/h with a 15% grade. These
workloads represent mild, moderate, and heavy exercise in-
tensities, respectively. A catheter placed in the cephalic vein
(Insyte, Becton-Dickinson, Sandy, UT) was used to deliver a
bolus (4 μg/kg) of the nicotinic ganglion stimulant 1-1-di-
methyl-4-phenylpiperazinium iodide (DMPP; Sigma Chemi-
cal, St. Louis, MO), which stimulates the release of endoge-
nous neurotransmitter from the sympathetic nerve terminal.
This dose was selected from preliminary experiments show-
ing it elicited a physiologically relevant degree of vasocon-
striction. Because DMPP stimulates nicotinic receptors in
autonomic ganglia, it activates both sympathetic and para-
sympathetic nerves. Therefore, all dogs were pretreated with
atropine (0.2 mg/kg) to allow examination of the sympathetic
effects alone.

Our experience has shown that 3 min of exercise are
enough to produce steady-state blood flows, so DMPP infu-
sions were performed during the fourth minute of exercise
and the preceding 10 s were used as a baseline for compari-
on. Resting infusions were always performed before any
exercise bouts. All workloads were performed in random
order on a single day, with dogs resting between exercise
bouts until blood flow returned to resting values (at least 15
min).

Arterial blood pressure and external iliac blood flow were
recorded at 100 Hz directly to a computer (Apple 8500 Power
personal computer) using a MacLab system (ADInstruments,
Castle Hill, Australia). Data were analyzed off-line using the
MacLab software to calculate the peak or nadir responses for
mean arterial pressure, heart rate, iliac blood flow, and iliac
vascular conductance (iliac blood flow/mean arterial pres-
sure) for comparison to baseline values. Values obtained in
both limbs were averaged to obtain a single value for each
dog.

An α level of P < 0.05 was used to establish significance.
Statistical analyses of baseline heart rate, mean arterial
blood pressure, blood flow, and iliac vascular conductance
were performed with two-way repeated-measures analyses of
variance. The absolute changes and percent changes in iliac
blood flow and iliac vascular conductance were analyzed with
one-way analyses of variance. Where significant F ratios
were found, a Tukey’s post hoc test was performed. All data
are expressed as means ± SE.

RESULTS

A bolus intravenous infusion of DMPP (4 μg/kg)
produced vasoconstriction in both hindlimbs at rest
and during exercise. Figure 1 is an original tracing
from an individual dog at rest. The tracing shows that
DMPP caused substantial reductions in iliac vascular
conductance.
Exercise attenuates the vasoconstrictor response to cise intensity. These results demonstrate that dynamic neurotransmitter was inversely related to exercise intensity. The magnitude of vasoconstriction, as represented by the percent decrease in iliac blood flow, was progressively attenuated as exercise intensity increased. Values are means ± SE. Dogs were pretreated with atropine (0.2 mg/kg iv). DMPP, 1-1-dimethyl-4-phenylpiperazinium iodide. *P < 0.05 compared with rest. †P < 0.01 compared with rest.

Table 1 displays baseline hemodynamic measurements before infusion of DMPP but after administration of atropine. As expected, there were significant increases in iliac blood flow (P < 0.01) and iliac vascular conductance (P < 0.01) from rest to exercise. Infusion of DMPP caused significant (P < 0.01) increases in blood pressure at rest (49 ± 9 mmHg) and during exercise at all workloads (39 ± 8 mmHg for 3.0 miles/h; 42 ± 7 mmHg for 6.0 miles/h; 27 ± 10 mmHg for 6.0 miles/h and 15% grade). Heart rate also increased (P < 0.01) after DMPP infusion at rest and the two lowest workloads (24 ± 6 beats/min at rest; 31 ± 3 beats/min at 3.0 miles/h; 18 ± 3 beats/min at 6.0 miles/h) but not at 6.0 miles/h and 15% grade (8 ± 3 beats/min). The lack of a significant effect on heart rate at the highest workload may be because the baseline heart rate of 273 ± 6.5 beats/min was close to maximum (22).

Figure 2 depicts the nadir in iliac blood flow in response to DMPP infusion. Figure 2A shows that there were reductions in blood flow at rest and at all three workloads. When the reductions in iliac blood flow data were expressed as a percent change (Fig. 2B), there was a significant (P < 0.01) intensity-related attenuation in the response to DMPP from rest to exercise.

The nadir in iliac vascular conductance after DMPP is presented in Figure 3. As shown in Figure 3A, iliac vascular conductance decreased in a manner similar to blood flow. The reduction in iliac vascular conductance as a percentage of the baseline (Fig. 3B) was attenuated from rest to exercise (P < 0.01) in an exercise intensity-dependent manner. Thus the magnitude of vasoconstriction, as represented by the percent decrease in vascular conductance (see DISCUSSION below), was progressively attenuated as exercise intensity increased.

DISCUSSION

The purpose of this study was to examine the effect of release of endogenous neurotransmitter on iliac vascular conductance during dynamic exercise. The important new findings of this study are 1) intravenous administration of DMPP to conscious, dynamically exercising dogs caused vasoconstriction in skeletal muscle vasculature, and 2) the magnitude of vasoconstriction caused by DMPP-stimulated release of endogenous neurotransmitter was inversely related to exercise intensity. These results demonstrate that dynamic exercise attenuates the vasoconstrictor response to sympathoactivation, thus supporting the existence of exercise sympatholysis.

Several techniques have been used to examine the consequences of sympathetic activation on vasomotor response during exercise. These include direct nerve stimulation (4, 16, 27, 28, 32, 36), baroreflex activation of sympathetic nerve activity (24, 26), and infusion of α-adrenergic agonists (2, 3, 5, 12, 14, 25, 28). Many of these studies were performed in anesthetized animals, which because of the nonphysiological nature of electrically stimulated muscle contraction and confounding cardiovascular effects of anesthesia provide limited information. A number of studies performed in conscious animals and humans have addressed this topic (2, 3, 12, 14, 24, 25), and all but one (24) used infusions of α-adrenergic agonists. However, the use of exogenous α1- and α2-adrenergic-receptor agonists does not answer the question of vascular responsiveness to endogenous neurotransmitter. The use of a ganglionic

![Fig. 2. Peak change in iliac blood flow (A) and percent change in iliac blood flow (B). Percent change in iliac blood flow decreased as exercise intensity increased. Values are means ± SE. 3, 3 miles/h and 0% grade; 6, 6 miles/h and 0% grade; 6/15%, 6 miles/h and 15% grade. *P < 0.05 compared with rest. †P < 0.01 compared with rest.](http://jap.physiology.org/Downloadedfrom)
sympathoactivation: aptic modulation of the vasoconstrictor response to norepinephrine infusion, suggesting an additional effect of presynaptic modulation during exercise.

The mechanism underlying sympatholysis is unclear. It is well established that sympathetic efferent nerve activity rises in an exercise intensity-dependent manner (8, 13), yet it appears that the functional effect of this increase in nerve traffic is antagonized such that the net result is reduced sympathetic vasoconstriction with increases in exercise intensity. Exogenous administration of norepinephrine during muscle contraction has been associated with reduced vascular responsiveness, suggesting a postsynaptic mechanism (4). In the same study, however, there was a larger contraction-related reduction in vascular responsiveness to direct sympathetic nerve stimulation than with norepinephrine infusion, suggesting an additional effect of presynaptic modulation. Presynaptic modulation could be mediated by metabolites released during muscle contractions. For example, adenosine has been shown to inhibit norepinephrine release from nerve endings (30, 38).

There are three potential mechanisms for postsynaptic modulation of the vasoconstrictor response to sympathoactivation: 1) metabolites, 2) nitric oxide, and 3) temperature. Metabolic effects may be mediated by factors such as acidosis (19, 21, 31), hypoxia (19, 31), and ischemia (20), which preferentially inhibit \( \alpha_2 \) but not \( \alpha_1 \)-adrenergic vasoconstriction. Metabolic activation of ATP-sensitive potassium channels has also been implicated in the attenuation of \( \alpha_2 \)-adrenergic vasoconstriction (1, 33). Another line of evidence suggests that nitric oxide released during exercise diminishes the magnitude of vascular response to \( \alpha \)-adrenergic agonists (25, 34) or sympathetic nerve stimulation (35). Inhibition of nitric oxide synthase partially restored sympathetic vasoconstriction in contracting limbs (25, 35), and mice with deficiencies in neuronal nitric oxide synthase or endothelial nitric oxide synthase did not exhibit sympatholysis (34). Finally, temperature influences the response to vasoconstrictor agents (7, 9, 10, 18). Specifically, there is evidence that \( \alpha_2 \)-receptors become less responsive as muscle temperature increases (7). Because heat production is a consequence of skeletal muscle contraction, a temperature-related decrease in responsiveness of \( \alpha_2 \)-receptors could explain the progressive reduction in response to DMPP infusions as exercise intensity increased.

The experimental approach used in this paper offers several advantages. By using conscious, dynamically exercising animals, higher exercise intensities can be achieved than in an anesthetized model. In addition, there are no confounding cardiovascular effects of anesthesia. The experimental approach used in previous publications from this laboratory (2, 3) has been to use intra-arterial infusion of \( \alpha \)-adrenergic agonists into the exercising limb. One limitation to this approach is the necessity of adjusting for changes in arterial blood flow to avoid dilution of the drug. In the present study, systemic administration of the ganglionic agonist precluded the need to adjust the dose for changes in blood flow. In addition, the use of a ganglionic agonist (DMPP) released the neurotransmitter at the abluminal surface of the vessels, whereas exogenous agonists are administered intraluminally. Therefore, the use of DMPP should more closely mimic a true physiological response. One limitation to the present study is the lack of evidence that DMPP has the same effect (i.e., norepinephrine release) during exercise that it has at rest. It is theoretically possible that an impairment in ganglionic neurotransmission during exercise would manifest itself in a reduced response to DMPP. However, it is well established that postganglionic sympathetic nerve activity increases from rest to exercise and continues to rise with increasing intensity (8). Thus it is unlikely that exercise impairs ganglionic neurotransmission or diminishes responsiveness to DMPP. Measurement of norepinephrine spillover would be desirable but is precluded by the brevity of the response to intravenous infusion of DMPP, which makes it impractical to accurately time the sample withdrawals for measurement of arterial and venous catecholamines.

Hansen et al. (12) indicated that the contradiction in previous studies investigating sympatholysis is probably a function of physiological (nature and intensity of muscle contraction, fiber type of muscle) and technical (model and methods used to measure and quantify
vasomotor responses) factors. Interpretation and expression of the data in previous papers has led to some disagreement (23, 24). Rowlands and Donald (28) noted that, when expressing changes in vascular tone from baseline, percent change is more appropriate than absolute change. Because conductance has a linear relationship with flow, it is a more appropriate measure of vessel radius than resistance (17). Moreover, a given percent change in vascular conductance will always reflect a given percent change in radius of the vessel. In this study, expression of the data as a percent change in vascular conductance is important precisely because of changing baselines from rest to different exercise intensities.

The data from this study show that activation of postganglionic sympathetic nerves with DMPP caused vasoconstriction in the skeletal muscle vasculature at rest and during exercise. The vasoconstrictor response was attenuated during dynamic exercise compared with rest and was inversely related to exercise intensity. Thus our results support the concept of exercise sympatholysis.

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REFERENCES


