Neuropeptide Y1 receptor vasoconstriction in exercising canine skeletal muscles

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Buckwalter, John B., Jason J. Hamann, and Philip S. Clifford. Neuropeptide Y1 receptor vasoconstriction in exercising canine skeletal muscles. J Appl Physiol 99: 2115–2120, 2005. First published August 11, 2005; doi:10.1152/japplphysiol.00427.2005.—Existing evidence suggests that neuropeptide Y (NPY) acts as a neurotransmitter in vascular smooth muscle and is coreleased with norepinephrine from sympathetic nerves. We hypothesized that release of NPY stimulates NPY Y1 receptors in the skeletal muscle vasculature to produce vasoconstriction during dynamic exercise. Eleven mongrel dogs were instrumented chronically with flow probes on the external iliac arteries of both hindlimbs and a catheter in one femoral artery. In resting dogs (n = 4), a 2.5-mg bolus of BIBP-3226 (NPY Y1 antagonist) infused into the femoral artery increased external iliac conductance by 150 ± 82% (1.80 ± 0.44 to 3.50 ± 0.14 ml·min⁻¹·mmHg⁻¹; P < 0.05). A 10-mg bolus of BIBP-3226 infused into the femoral artery in dogs (n = 7) exercising on a treadmill at a moderate intensity (6 miles/h) increased external iliac conductance by 28 ± 6% (6.00 ± 0.49 to 7.64 ± 0.61 ml·min⁻¹·mmHg⁻¹; P < 0.05), whereas the solvent vehicle did not (5.74 ± 0.51 to 5.98 ± 0.43 ml·min⁻¹·mmHg⁻¹; P > 0.05). During exercise, BIBP-3226 abolished the reduction in conductance produced by infusions of the NPY Y1 agonist [Leu²⁶Pro³³]NPY (−19 ± 3 vs. 0.5 ± 1%). Infusions of BIBP-3226 (n = 7) after α-adrenergic receptor antagonism with prazosin and rauwolscine also increased external iliac conductance (6.82 ± 0.43 to 8.22 ± 0.48 ml·min⁻¹·mmHg⁻¹; P < 0.05). These data support the hypothesis that NPY Y1 receptors produce vasoconstriction in exercising skeletal muscle. Furthermore, the NPY Y1 receptor-mediated tone appears to be independent of α-adrenergic receptor-mediated vasoconstriction.

THE AUTONOMIC NERVOUS SYSTEM plays an important role in cardiovascular regulation during exercise. The absence of such control leads to hypotension and inhibits sustained dynamic exercise (5, 26). During exercise, sympathetic vasoconstriction maintains adequate arterial pressure and redistributes cardiac output away from inactive tissue toward exercising skeletal muscle. To accomplish the redistribution of cardiac output, there are increases in sympathetic nerve activity to inactive tissues such as the kidney (30) and nonexercising skeletal muscle (12). Interestingly, sympathetic nerve activity to exercising skeletal muscle also increases (11, 12, 14). The increase in sympathetic nerve activity to exercising skeletal muscle does not appear to produce skeletal muscle vasodilation (5, 9), but instead restrains skeletal muscle hyperemia (4, 8, 13, 31), which is important for the regulation of arterial blood pressure during exercise (2, 37). Traditionally, sympathetic vasoconstriction of the skeletal muscle vasculature has been characterized as the release of norepinephrine from the sympathetic nerve terminal, which stimulates α-adrenergic receptors to contract vascular smooth muscle. However, there is strong evidence that a number of other neurotransmitters are coreleased along with norepinephrine from sympathetic nerves (15, 17, 23, 34). Neuropeptide Y (NPY) is a peptide comprised of 36 amino acids that acts as a sympathetic neurotransmitter in both humans and animals, producing vasoconstriction in resistance vessels through the stimulation of NPY Y1 receptors on vascular smooth muscle (42). Although NPY has been shown to constrict the arterial vasculature of resting skeletal muscle in a number of species including the dog (16, 23, 28, 32), it is unknown whether NPY produces vasoconstriction in skeletal muscle during exercise. Interestingly, there is evidence for a substantial increase in plasma concentrations of NPY during dynamic exercise (22, 33), suggesting that the increase in sympathetic nerve activity associated with exercise results in an increased release of NPY from the sympathetic nerve terminal.

The purpose of this study was to examine the effect of NPY Y1 receptor antagonism on skeletal muscle vascular tone in conscious dogs during exercise. We hypothesized that NPY Y1 receptors mediate vasoconstriction in exercising skeletal muscle that is independent of α-adrenergic vasoconstriction. We expected NPY Y1 receptor blockade would interrupt tonic vasoconstriction in exercising skeletal muscle, increasing muscle blood flow and muscle vascular conductance. Furthermore, in the presence of α-adrenergic receptor antagonism, we expected NPY Y1 receptor blockade would result in vasodilatation and NPY Y1 receptor stimulation would produce vasoconstriction.

METHODS

The experimental procedures described below were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the American Physiological Society’s Guiding Principles in the Care and Use of Animals. Mongrel dogs (n = 11, 18–22 kg) were chosen for their willingness to run on a motorized treadmill with minimal training. The dogs were chronically instrumented in a series of three sterile surgeries. The first surgical procedure placed the carotid arteries in skin tubes on the neck so that they could be cannulated percutaneously to measure arterial blood pressure (27, 29). During the second surgery, the dogs were instrumented with flow probes (4-mm ultrasonic transit-time flow probes, Transonic Systems, Ithaca, NY) around the external iliac arteries to measure skeletal
muscle blood flow in each hindlimb independently. The cables were then tunneled under the skin to the back. In the final surgery, a heparinized catheter (0.045 in. OD, 0.015 in. ID, 60 cm long; Data Science International, St. Paul, MN) was implanted in one hindlimb. This catheter was inserted through a side branch into the femoral artery distal to the flow probes and tunneled to the back of the dog. After recovery, this catheter allowed for the infusion of drugs into the arterial vasculature of one hindlimb. For all surgical procedures, anesthesia was induced with thiopental sodium (15–30 mg/kg; Genxia Pharmaceuticals, Irvine, CA). After intubation with a cuffed endotracheal tube, a surgical level of anesthesia was maintained through mechanical ventilation with 1.5% halothane (Halocarbon Laboratories, River Edge, NJ) and 98.5% oxygen. Antibiotics (cefazolin sodium, Apothecon, Princeton, NJ) and analgesic drugs (buprenorphine hydrochloride, 0.3 mg; Reckitt and Coleman, Kingston-upon-Hull, UK) were given postoperatively. To maintain patency, the femoral catheter was flushed daily with saline and filled with a heparin solution (100 IU heparin/ml in 50% dextrose solution). The dogs were given at least 2 days to recover from the final surgery before any experiments were performed. On completion of the experiments, the animals were euthanized (100 mg/kg pentobarbital sodium iv and saturated KCl iv), and the flow probes were retrieved.

All experiments were performed in a laboratory in which the temperature was maintained below 20°C. On the day of the experiment, the dog was brought to the laboratory, and a 20-gauge intravascular catheter (Insyte, Becton Dickinson, Sandy, UT) was inserted retrogradely into the lumen of the carotid artery. The carotid catheter was attached to a solid-state pressure transducer (Ohmeda, Madison, WI), and the flow probes were connected to a transit-time flowmeter (Transonic Systems).

To confirm the existence of tonic NPY Y1 receptor tone in resting skeletal muscle (16, 23, 28, 32), a selective NPY Y1 receptor antagonist (BIBP-3226, Bachem, King of Prussia, PA) was infused while the animals rested quietly. This antagonist was chosen for its selectivity for the NPY Y1 receptor (25) and for its ability to be dissolved roughly proportional to the dose given during exercise. This proportioning results in a similar effective concentration of the antagonist at different magnitudes of external iliac blood flow. To test the salient hypothesis of this study, that NPY Y1 receptors mediate vasoconstriction in the arterial vasculature of exercising skeletal muscle, a bolus intra-arterial infusion of BIBP-3226 (10 mg) was given while the dogs (n = 7) exercised at a moderate workload of 6 miles/h (9.7 km/h). Control experiments using the solvent vehicle (1 ml/min) were performed in separate experiments. In the presence of prazosin and rauwolscine (1 and 10 ml/min after BIBP-3226 (n = 7, P < 0.05) (Fig. 2). This was achieved in the absence of changes in vascular conductance (Fig. 2), systemic blood pressure and right and left external iliac blood flow during all experiments. Data were analyzed offline using the MacLab software to calculate mean arterial pressure, heart rate, iliac blood flow, and iliac vascular conductance (blood flow/mean arterial pressure). Vascular conductance was calculated rather than vascular resistance because conductance better reflects vascular tone when the experimental manipulation causes a change primarily in flow and not pressure (19). Baseline measurements were averaged over 30 s before antagonist and agonist infusion. After the infusion of BIBP-3226, all variables were averaged over 1-s intervals (100 consecutive data points), and the lowest 1-s average for vascular conductance was chosen as the peak response. In addition, steady-state values representing a 5 s (500 consecutive data points) average were taken 30 s after the end of the BIBP-3226 infusion. After all agonist (norepinephrine and [Leu31,Pro34]NPY) infusions, all variables were averaged over 1-s intervals (100 consecutive data points), and the nadir 1-s average for vascular conductance was chosen as the peak response.

An α level of P < 0.05 was used to establish statistical significance during all analysis. Statistical analyses of the data were performed with a repeated-measures analysis of variance. Where significant F-ratios were found, a Tukey’s post hoc test was performed. All data are expressed as means ± SE.

RESULTS

At rest (n = 4), intra-arterial infusion of BIBP-3226 produced increases in experimental limb blood flow and conductance. Steady state blood flow increased from 202 ± 55 to 377 ± 31 ml/min (P < 0.05). Vascular conductance increased by 150 ± 82% from 1.80 ± 0.44 to 3.50 ± 0.14 ml·min⁻¹·mmHg⁻¹ (P < 0.05).

Figure 1 is an original tracing from one experiment showing the effect of intra-arterial infusion of BIBP-3226. Intra-arterial infusion of BIBP-3226 interrupted tonic NPY Y1 receptor-mediated vasoconstriction in exercising skeletal muscle, resulting in substantial increases in experimental limb blood flow. In the seven dogs, intra-arterial infusion of selective NPY Y1 receptor antagonist BIBP-3226 during exercise produced peak increases in experimental limb conductance of 28 ± 6% (P < 0.05) (Fig. 2). This was achieved in the absence of changes in systemic hemodynamics or contralateral limb blood flow (Table 1). Intra-arterial infusion of the solvent vehicle for BIBP-3226 did not produce any significant (P > 0.05) changes in experimental limb vascular conductance (Fig. 2), systemic hemodynamics, or control limb blood flow (Table 1). The dose of BIBP-3226 produced effective blockade of NPY Y1 receptors as demonstrated by the fact that the selective NPY Y1 receptor agonist [Leu31,Pro34]NPY decreased vascular conductance by 1.17 ± 0.23 (or 19 ± 3%) ml·min⁻¹·mmHg⁻¹ and blood flow by 117 ± 24 ml/min under control conditions and by 0.06 ± 0.09 (or 0.5 ± 1%) ml·min⁻¹·mmHg⁻¹ and blood flow by 11 ± 10 ml/min after BIBP-3226 (n = 7, P < 0.05).

To examine the independence of NPY Y1 receptor vasoconstriction from α-adrenergic vasoconstriction in exercising skeletal muscle, intra-arterial infusions of BIBP-3226 were re-
peated in the presence of α-adrenergic receptor antagonism. 
α-Adrenergic receptor blockade evoked significant ($P < 0.05$) increases in experimental limb vascular conductance (Fig. 3). Intra-arterial infusion of BIBP-3226 after α-adrenergic blockade elicited additional significant ($P < 0.05$) increases in experimental limb vascular conductance (Fig. 3), indicating tonic NPY Y1 receptor vasoconstriction that is independent of α-adrenergic receptor tone. Further evidence in support of this is provided in Fig. 4. Intra-arterial infusions of norepinephrine or [Leu31,Pro34]NPY under control conditions produced significant ($P < 0.05$) vasoconstriction. However, after α-adrenergic receptor blockade, the response to norepinephrine was abolished ($P > 0.05$), whereas the vasoconstrictor response to NPY Y1 receptor stimulation with [Leu31,Pro34]NPY was preserved.

**DISCUSSION**

The salient finding of the present study is that NPY Y1 receptors in the arterial vasculature of skeletal muscle restrain blood flow to exercising skeletal muscle. Furthermore, it appears that tonic NPY Y1 receptor-mediated vasoconstriction of
active skeletal muscle is independent of α-adrenergic receptor constrictor tone. These data suggest a functional role for NPY in the regulation of skeletal muscle vascular tone during exercise. To our knowledge, these data are the first to demonstrate tonic vasoconstriction in the vasculature of exercising skeletal muscle that is mediated by NPY Y1 receptors.

A body of literature exists that indicates α-adrenergic receptors tonically restrain blood flow to exercising skeletal muscle even during intense exercise (4, 8, 31). Traditional dogma characterizes sympathetic vasoconstriction as the release of norepinephrine from sympathetic nerve terminals to stimulate α-adrenergic receptors on vascular smooth muscle. However, in recent years, evidence has emerged that other neurotransmitters are coreleased along with norepinephrine from sympathetic nerves (15, 17, 23, 34), the best described of these sympathetic cotransmitters being ATP and NPY. Recently, our laboratory provided evidence of a role for purinergic P2X receptors in the regulation of skeletal muscle blood flow at rest and during exercise (6, 10). Furthermore, the fact that stimulation of NPY Y1 receptors elicits vasoconstriction in the cardiovascular system. The Y1 receptor has been proposed to be primarily situated postjunctionally on vascular smooth muscle, where it mediates vasoconstriction in small vessels. NPY Y1 receptor stimulation produces vasoconstriction at the arteriolar level but not in larger conduit vessels (34). The Y2 receptor is mainly prejunctional and inhibits the release of norepinephrine (42). Interestingly, α2-adrenergic receptors located prejunctionally on the sympathetic nerve terminal have been shown to reciprocate by inhibiting the release of NPY (21).

Although neuropeptide Y is present in the perivascular noradrenergic neurons innervating the vasculature of skeletal muscle (34), and has been shown to produce vasoconstriction

Table 1. Steady-state hemodynamic measurement before and after intra-arterial infusion of BIBP-3226 or the solvent vehicle

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>Control Limb Blood Flow, ml/min</th>
<th>Experimental Limb Blood Flow, ml/min</th>
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<td>Postinfusion</td>
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<td>BIBP-3226</td>
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<td>185±7</td>
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<td>Vehicle control</td>
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<td>175±4</td>
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<td>109±6</td>
</tr>
<tr>
<td>BIBP-3226 (with α-blockade)</td>
<td>197±7</td>
<td>216±9</td>
<td>115±4</td>
<td>113±4</td>
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Values means ± SE. HR, heart rate; MAP, mean arterial pressure. *Significantly different from preinfusion value, P < 0.05.

Fig. 4. Percent changes from baseline in iliac conductance resulting from intra-arterial infusion of [Leu31, Pro34]NPY Y (NPY Y1 agonist) or norepinephrine (NE) before (control) and after (α-blockade) α-adrenergic receptor antagonism with rauwolscine and prazosin. Values are means ± SE, n = 7. Intra-arterial infusion of [Leu31, Pro34]NPY into the experimental limb produced significant (P < 0.05) reductions in iliac vascular conductance in the absence and presence of α-adrenergic receptor blockade. The magnitude of constriction produced with [Leu31, Pro34]NPY was not significantly altered (P > 0.05) by α-receptor antagonism. In contrast, intra-arterial infusion of norepinephrine produced significant (P < 0.05) reductions in iliac vascular conductance that were abolished by α-adrenergic receptor blockade. After α-adrenergic receptor blockade, NE did not significantly (P > 0.05) reduce vascular conductance.
in resting skeletal muscle, we believe the present study is the first to demonstrate that NPY Y_1 receptors influence vascular tone in exercising skeletal muscle. Previously, it has been reported that there is a substantial increase in plasma concentration of NPY during dynamic exercise (22, 33). One might speculate that the increase in circulating NPY is likely the result of NPY spillover associated with the large increase in sympathetic nerve activity to exercising skeletal muscle. This seems reasonable considering the data from the present study showing that NPY Y_1 receptors are tonically active during exercise and previous data showing that NPY Y_1 receptor stimulation produces vasoconstriction in exercising skeletal muscle even during intense exercise (10). The demonstration of tonic NPY Y_1 receptor-mediated vasoconstriction in exercising skeletal muscle has important implications for the regulation of blood pressure during exercise. To meet the increase in oxygen demands during exercise, there is a redistribution of cardiac output away from nonexercising tissue toward exercising skeletal muscle. It has long been argued that because a greater proportion of cardiac output is directed toward active skeletal muscle as exercise intensity increases, sympathetic vasoconstriction of active skeletal muscle becomes increasingly important for the regulation of arterial blood pressure (10). This vasoconstriction has previously been shown to be mediated by α-adrenergic receptors (4, 8, 13, 31). Along with α-adrenergic receptors, it now appears that NPY Y_1 receptors may play a role in the regulation of systemic arterial pressure during exercise.

The present study also confirms the existence of tonic NPY Y_1-mediated vasoconstriction in resting skeletal muscle (16, 23, 28, 32). Recognizing that the data come from different groups of animals, the present results permit a comparison of the magnitude of tonic Y_1 receptor vasoconstriction during rest and exercise. Interruption of tonic Y_1-mediated vasoconstriction resulted in a 150% increase in vascular conductance at rest compared with a 28% increase during moderate-intensity exercise. These data do not allow any conclusion regarding the intensity-related effects, but they do show greater NPY Y_1 receptor-mediated vasoconstriction at rest compared with exercise. This pattern is similar to that observed for α-adrenergic and purinergic receptors in the skeletal muscle vasculature (4, 10). Previous work from our laboratory (3, 4, 39) and others (18, 35, 36, 40) demonstrates that sympathetic vasoconstriction is attenuated in exercising skeletal muscle. In addition, our laboratory recently reported that Y_1 receptor responsiveness is attenuated during exercise compared with rest and that this attenuation is related to exercise intensity (7). Taken together, it seems reasonable to predict that compared with rest tonic Y_1 receptor-mediated vasoconstriction is attenuated during exercise to a greater degree as exercise intensifies, but further studies will be needed to specifically test this hypothesis.

Pernow and colleagues (32) showed in resting canine skeletal muscle that NPY mediates vasoconstriction that is resistant to α-adrenergic receptor blockade. The present study extends those findings to exercising skeletal muscle. Furthermore, the stimulation of NPY Y_1 receptors produces the same vasoconstrictor effect in the presence of tonic α-adrenergic vasoconstriction as it does in the absence of α-adrenergic tone. Although it appears that NPY Y_1 receptors can mediate vasoconstriction independently of α-adrenergic receptors, these data do not exclude an interaction between α-adrenergic receptors and NPY. Indeed a number of studies have shown that the effects of α-adrenergic stimulation are potentiated in the presence of NPY (24, 38, 41). Interestingly, NPY can potentiate the effect of α-adrenergic stimulation at doses that have negligible vasoconstrictor effects by themselves (38, 41). This phenomenon appears to be restricted to blood vessels predominantly populated by α_1-adrenergic receptors (41). It is possible that increases in circulating levels of NPY during exercise may be important in the regulation of blood pressure during exercising by potentiating α-adrenergic receptor-mediated vasoconstriction.

The results from the present study reveal that NPY Y_1 receptor antagonism in exercising skeletal muscle produces an increase in blood flow and conductance. In addition, there appears to be NPY Y_1 receptor-mediated vasoconstriction that is independent of α-adrenergic receptor tone. The demonstration that the NPY Y_1 receptor regulates skeletal muscle vascular tone during exercise suggests that it may be involved in the regulation of systemic arterial pressure during exercise.

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GRANTS

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REFERENCES

2120 NEUROPEPTIDE Y AND SKELETAL MUSCLE VASCULAR TONE


