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/mmHg; 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/mmHg; P < 0.05), whereas the solvent vehicle did not (5.74 ± 0.51 to 5.98 ± 0.43 ml/min/mmHg; P > 0.05). During exercise, BIBP-3226 abolished the reduction in conductance produced by infusions of the NPY Y₁ agonist [Leu³¹,Pro³⁴]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/mmHg; 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.

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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; Genesia 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 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 intra-vascular 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 in aqueous solution. BIBP-3226 was dissolved in bacteriostatic water at a concentration of 1 mg/ml and administered as a bolus infusion of 2.5 mg. Given the lower external iliac blood flow at rest, this dose was roughly proportional to the dose given during exercise. This proportional dosing 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 was given while the dog ran on the treadmill at a moderate workload (6 miles/h (9.7 km/h)). All of the experiments examining the vasoconstrictor effect of norepinephrine and [Leu31, Pro34]NPY in the presence of α-adrenergic receptor antagonism occurred on a separate day from those experiments examining the effect of BIBP-3226 after α-adrenergic receptor antagonism.

A computer (Apple G3 Power PC) using a Powerlab system (ADInstruments, Castle Hill, Australia) was used to record (at 100 Hz) arterial 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 highest 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 antagonist [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 Y$_1$ 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 [Leu$_{31}$,Pro$_{34}$]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 Y$_1$ receptor stimulation with [Leu$_{31}$,Pro$_{34}$]NPY was preserved.

**DISCUSSION**

The salient finding of the present study is that NPY Y$_1$ receptors in the arterial vasculature of skeletal muscle restrain blood flow to exercising skeletal muscle. Furthermore, it appears that tonic NPY Y$_1$ 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. In recent years, evidence has emerged that other neurotransmitters are coreleased along with norepinephrine from sympathetic nerve terminals to stimulate α-adrenergic receptors on vascular smooth muscle. However, Y1 receptors appear to play the most prominent roles in the cardiovascular system. The Y1 receptor has been proposed as the release of norepinephrine from sympathetic nerve terminals to stimulate α-adrenergic receptors elicits vasoconstriction in the arterial vasculature of skeletal muscle that was independent of α-adrenergic receptor vasoconstriction.

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

<table>
<thead>
<tr>
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<th>Preinfusion</th>
<th>Postinfusion</th>
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<tr>
<td></td>
<td>HR, beats/min</td>
<td>MAP, mmHg</td>
</tr>
<tr>
<td>BIBP-3226</td>
<td>182 ± 7</td>
<td>185 ± 7</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>183 ± 5</td>
<td>175 ± 4</td>
</tr>
<tr>
<td>BIBP-3226 (with α-blockade)</td>
<td>197 ± 7</td>
<td>216 ± 9</td>
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Values means ± SE. HR, heart rate; MAP, mean arterial pressure. *Significantly different from preinfusion value, P < 0.05.

However, NPY is unique among the three peptides in the fact that it is strictly localized in neurons (1). Postganglionic sympathetic nerves have been shown to contain vesicles containing NPY (20). In contrast to norepinephrine, which is found in both small and large vesicles in sympathetic neurons, NPY is stored only in large dense-cored vesicles (21). The release of these large dense-cored vesicles containing NPY appears to be dependent on high-frequency stimulation (20). Once released, NPY can stimulate a number of different subtypes of NPY receptors. Although different subtypes of Y receptors exist, the Y1 and Y2 receptors appear to play the most prominent roles 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.

![Fig. 3](image-url)  
*Fig. 3. Experimental limb conductance during exercise at 6 miles/h before and after intra-arterial infusion of BIBP-3226 in the presence of α-adrenergic receptor blockade. Values are means ± SE; n = 7 dogs. Experimental limb iliac conductance was significantly (P < 0.05) higher after intra-arterial infusion of the α-adrenergic receptor antagonist (rauwolscine + prazosin) compared with baseline. Intra-arterial infusion of BIBP-3226 caused an additional increase (P < 0.05) in iliac vascular conductance in the experimental limb. Infusion of BIBP-3226 interrupted tonic NPY Y1 receptor-mediated vasoconstriction in the arterial vasculature of skeletal muscle that was independent of α-adrenergic receptor vasoconstriction.*

![Fig. 4](image-url)  
*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 Y1 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 Y1 receptors are tonically active during exercise and previous data showing that NPY Y1 receptor stimulation produces vasoconstriction in exercising skeletal muscle even during intense exercise (10). The demonstration of tonic NPY Y1 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 Y1 receptors may play a role in the regulation of systemic arterial pressure during exercise.

The present study also confirms the existence of tonic NPY Y1-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 Y1 receptor vasoconstriction during rest and exercise. Interruption of tonic Y1-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 Y1 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 Y1 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 Y1 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 Y1 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 Y1 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 α1-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 Y1 receptor antagonism in exercising skeletal muscle produces an increase in blood flow and conductance. In addition, there appears to be NPY Y1 receptor-mediated vasoconstriction that is independent of α-adrenergic receptor tone. The demonstration that the NPY Y1 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


