Vasoconstriction in active skeletal muscles: a potential role for P2X purinergic receptors?

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Buckwalter, John B., Jason J. Hamann, and Philip S. Clifford. Vasoconstriction in active skeletal muscles: a potential role for P2X purinergic receptors? J Appl Physiol 95: 953–959, 2003. First published May 23, 2003; 10.1152/japplphysiol.00173.2003.—There is evidence that ATP acts as a neurotransmitter in vascular smooth muscle and is coreleased with norepinephrine from sympathetic nerves. We hypothesized that P2X-receptor stimulation with the selective P2X-receptor agonist αβ-methylene ATP would produce vasoconstriction in resting and exercising skeletal muscle. Six mongrel dogs were instrumented chronically with flow probes on the external iliac arteries of both hindlimbs and a catheter in one femoral artery. The selective P2X agonist αβ-methylene ATP was infused as a bolus into the femoral artery catheter at rest and during mild, moderate, and heavy exercise. Intra-arterial infusions of αβ-methylene ATP elicited reductions in vascular conductance of 54 ± 5, 49 ± 8, 39 ± 8, and 30 ± 6% at rest, 3 miles/h, 6 miles/h, and 6 miles/h at a 10% grade, respectively. The agonist infusions did not affect blood flow in the contralateral iliac artery. To examine whether nitric oxide is responsible for the attenuated vasoconstrictor response to P2X stimulation, the infusions were repeated in the presence of NG-nitro-L-arginine methyl ester. After nitric oxide synthase blockade, intra-arterial infusions of αβ-methylene ATP elicited reductions in vascular conductance of 56 ± 7, 61 ± 8, 52 ± 9, and 40 ± 7% at rest, 3 miles/h, 6 miles/h, and 6 miles/h at a 10% grade, respectively. P2X-receptor responsiveness was attenuated during exercise compared with rest. Blockade of nitric oxide production did not affect the attenuation of P2X-receptor responsiveness during exercise. These data support the hypothesis that P2X purinergic receptors can produce vasoconstriction in exercising skeletal muscle.

blood flow; sympatholysis; autonomic nervous system; dogs; endothelial cells (11). P2X receptors on vascular smooth muscle cells are preferentially stimulated by ATP from sympathetic nerve endings (17). Although two recent studies (1, 19) showed that P2X-receptor stimulation produces vasoconstriction in the hindlimb of anesthetized cats and rats, it is unknown whether stimulation of P2X receptors in the arterial vasculature of skeletal muscle will produce vasoconstriction during exercise. In the past, the ability of the sympathetic nervous system to restrain blood flow in active skeletal muscle has been questioned (14, 24, 26). However, there is clear and convincing evidence that there is sympathetic restraint of skeletal muscle hyperemia during exercise (3, 5, 20, 34, 35, 43). Although the existence of sympathetic vasoconstriction in active skeletal muscle is generally accepted, whether the magnitude of this vasoconstriction is attenuated during exercise compared with rest remains an issue of debate. Indeed, it has been argued that, as exercise intensity increases, sympathetic vasoconstriction in active skeletal muscle also increases (34). An attenuation of vasoconstriction in the arterial vasculature of skeletal muscle during muscle contraction has been reported by a number of investigators (8, 22, 23, 36, 37, 42). This diminished vascular responsiveness to sympathetic stimulation during muscular contraction was termed “functional sympatholysis” by Remensnyder et al. (36). Recently, studies by Thomas and colleagues (12, 38, 40, 41) have provided evidence that the mechanism by which sympatholysis occurs is related to the production of nitric oxide.

The purpose of this study was to examine the effect of P2X-receptor stimulation on the skeletal muscle vasculature of conscious dogs at rest and during exercise. We hypothesized that P2X-receptor stimulation would elicit vasoconstriction in resting and exercising skeletal muscle. Furthermore, we hypothesized that P2X-receptor responsiveness would be attenuated from rest to exercise in an exercise intensity-dependent manner by the production of nitric oxide.

METHODS AND PROCEDURES

The experimental procedures described below were approved by the Institutional Animal Care and Use Committee.

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and conducted in accordance with the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Six mongrel dogs (20–23 kg) were selected for their willingness to run on a motorized treadmill. The animals were chronically instrumented in a series of sterile surgical procedures. 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 (31, 33). During the second surgery, all 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. The cables were then tunneled under the skin to the back of the dog. In the final surgery, a heparinized catheter (0.045 in. OD, 0.015-in. ID, 60-cm length, Data Science International, St. Paul, MN) was implanted. This catheter was inserted through a side branch into the femoral artery and tunneled to the back of the dog. After recovery, this catheter allowed infusion of drugs into the arterial vasculature of one hindlimb at rest and during exercise. For all surgical procedures, anesthesia was induced with thiopental sodium (15–30 mg/kg) and 1.5% halothane (Halocarbon Laboratories, River Edge, NJ) and 98.5% oxygen. Antibiotics (cefazolin sodium, Apothecan, Princeton, NJ) and analgesic drugs (buprenorphine hydrochloride, 0.3 mg; Reckitt and Coleman, Kingston-upon-Hull, UK) were given postoperatively. To maintain potency, 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.

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 (Inblue, Becton Dickinson, Deseret, 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 examine the effect of P2X-receptor stimulation on skeletal muscle vascular tone, α,β-methyleneadenosine 5′-triphosphate lithium salt (α,β-methylene ATP, Sigma, St. Louis, MO), a selective P2X agonist (11), was infused into one hindlimb at rest and during exercise. This agonist was chosen for its ability to be dissolved easily in aqueous solution and infused in a conscious, chronically instrumented dog without detrimental effects. We reasoned that, because hindlimb blood flow increases in an exercise intensity-dependent manner, administration of an identical amount of agonist at rest and exercise would result in a lower effective concentration of the drug during exercise. Therefore, as in previous investigations (6, 7), the dose of the agonist administered during exercise was increased from rest. The bolus infusion of α,β-methylene ATP given throughout this study was equal to 1 μg of α,β-methylene ATP per milliliter of external iliac blood flow. Infusions were performed at rest and during steady-state exercise while the dogs ran on the treadmill at three different intensities: a mild exercise intensity of 3 miles/h (4.8 km/h) at a 0% grade, moderate exercise intensity of 6 miles/h (9.7 km/h) at 10% grade, and a heavy exercise intensity of 6 miles/h (9.7 km/h) at 10% grade. Each exercise intensity was performed on a separate day. For each exercise intensity, there were two infusions of α,β-methylene ATP. The dog performed one bout of exercise at a given intensity during which α,β-methylene ATP was infused. After 10 min of rest, the bout of exercise was repeated. The data from the two infusions were averaged for determination of P2X-receptor responsiveness under each condition.

During preliminary studies to examine the reproducibility of repeated infusions of α,β-methylene ATP, it was determined that 10 min between the infusions of α,β-methylene ATP was sufficient time to avoid any tachyphylaxis.

To determine whether the production of nitric oxide was responsible for the attenuation of P2X-receptor responsiveness from rest to exercise, additional experiments were performed in all the animals. Nitric oxide production was inhibited with an intravenous infusion of Nω-nitro-l-arginine methyl ester (l-NAME, Sigma Chemical) at a dose of 15 mg/kg. Effective nitric oxide synthase blockade was inferred from a rise in resting mean arterial pressure of >15 mmHg. At least 10 min after administration of l-NAME, P2X-receptor responsiveness was determined with intra-arterial infusions of α,β-methylene ATP at rest and during exercise at 3 miles/h (4.8 km/h), 0% grade, 6 miles/h (9.7 km/h), 0% grade, and 6 miles/h (9.7 km/h), 10% grade. These data were collected in the same manner as the data without nitric oxide synthase inhibition (described above).

To demonstrate that α,β-methylene ATP selectively activated P2X receptors and not α-receptors, both norepinephrine and α,β-methylene ATP were given before and after α-adrenergic-receptor blockade. In two dogs, a bolus infusion of norepinephrine and α,β-methylene ATP was given while the dogs ran at 6 miles/h. After 10 min of rest, the dog ran again at 6 miles/h. During steady-state exercise, α-adrenergic-receptor blockade was accomplished with an infusion of prazosin (100 μg) and rauwolscine (1 mg). The doses of norepinephrine and α,β-methylene ATP were then repeated.

A computer (Apple G3 Power PC) using a Powerlab system (ADInstruments, Castle Hill, Australia) was used to record effects vascular tone when the experimental manipulation causes a change primarily in vascular conductance was calculated rather than vascular resistance. Statistical analyses of the data were performed. All data are expressed as means ± SE.

RESULTS

Intra-arterial infusion of α,β-methylene ATP produced a localized vasoconstriction in the experimental limb without corresponding changes in blood flow or conductance in the contralateral limb. Figure 1 is an original tracing from one experiment in which the
adrenergic-receptor agonist norepinephrine and the selective P2X agonist \( \alpha,\beta\)-methylene ATP were infused during steady-state exercise at 6 miles/h. In response to the agonist infusions, there were substantial decreases in blood flow in the experimental limb without a corresponding alteration in blood flow to the contralateral limb. In two dogs, the infusion of norepinephrine resulted in an average reduction in conductance of 21%. The infusion of \( \alpha,\beta\)-methylene ATP caused an average reduction in conductance of 25%. To demonstrate that vasoconstriction to \( \alpha,\beta\)-methylene ATP was independent of \( \alpha\)-adrenergic receptors, the intra-arterial infusions of both norepinephrine and \( \alpha,\beta\)-methylene ATP were repeated after \( \alpha\)-adrenergic-receptor blockade. It can be seen in Fig. 2 that \( \alpha\)-adrenergic blockade abolished the vasoconstrictor effects of norepinephrine, but not the vasoconstrictor response to \( \alpha,\beta\)-methylene ATP. In two dogs, the infusion of norepinephrine resulted in an average reduction in conductance of 2%, whereas the infusion of \( \alpha,\beta\)-methylene ATP reduced conductance by 37%. These data support the premise that the vasoconstrictor effects of

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**Fig. 1.** Original record from an individual dog exercising on the treadmill at 6 miles/h. Arrows indicate intra-arterial infusion of a nonselective \( \alpha\)-adrenergic agonist (norepinephrine (NE)) or a selective P2X-receptor agonist (\( \alpha,\beta\)-methylene ATP (mATP)) into the femoral artery of the experimental limb. Both infusions produced immediate reductions in iliac blood flow. Note that there were no changes in blood pressure or blood flow in the control limb to either infusion.

**Fig. 2.** An original record from an individual dog exercising on the treadmill at 6 miles/h (same dog as in Fig. 1) after \( \alpha\)-adrenergic-receptor blockade. Arrows indicate intra-arterial infusion of a nonselective \( \alpha\)-adrenergic agonist (NE) or a selective P2X-receptor agonist (mATP) into the femoral artery of the experimental limb. After \( \alpha\)-adrenergic receptor blockade, only the infusion of mATP produced a substantial reduction in iliac blood flow, indicating vasoconstriction independent of \( \alpha\)-adrenergic receptors.
Table 1. Baseline hemodynamic measurements at each workload, before intra-arterial infusion of α,β-methylene ATP, with and without L-NAME treatment

<table>
<thead>
<tr>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>Baseline Blood Flow, ml/min</th>
<th>Baseline Conductance, ml/min−1 mmHg−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No L-NAME</td>
<td>89 ± 10</td>
<td>94 ± 6</td>
<td>140 ± 28</td>
</tr>
<tr>
<td>L-NAME</td>
<td>55 ± 5</td>
<td>116 ± 3</td>
<td>89 ± 17</td>
</tr>
<tr>
<td>3 miles/h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No L-NAME</td>
<td>141 ± 8</td>
<td>103 ± 4</td>
<td>410 ± 47</td>
</tr>
<tr>
<td>L-NAME</td>
<td>123 ± 7</td>
<td>138 ± 6</td>
<td>341 ± 38</td>
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<tr>
<td>6 miles/h</td>
<td></td>
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</tr>
<tr>
<td>No L-NAME</td>
<td>183 ± 10</td>
<td>114 ± 6</td>
<td>588 ± 43</td>
</tr>
<tr>
<td>L-NAME</td>
<td>187 ± 16</td>
<td>129 ± 6</td>
<td>564 ± 45</td>
</tr>
<tr>
<td>6 miles/h at 10% grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No L-NAME</td>
<td>229 ± 7</td>
<td>129 ± 5</td>
<td>869 ± 68</td>
</tr>
<tr>
<td>L-NAME</td>
<td>213 ± 10</td>
<td>132 ± 5</td>
<td>812 ± 40</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; MAP, mean arterial pressure; L-NAME, Nω-nitro-L-arginine methyl ester. There was an increase in HR, blood flow, and conductance as exercise intensity increased, P < 0.001. After L-NAME infusion, MAP was significantly elevated, P < 0.001.

α,β-methylene ATP are independent of α-adrenergic receptors.

Table 1 gives the baseline hemodynamic measurements before intra-arterial infusion of α,β-methylene ATP. As expected, there were significant (P < 0.05) exercise intensity-dependent increases in hindlimb blood flow, mean arterial pressure, and heart rate from rest to exercise. The α,β-methylene ATP infusions significantly (P < 0.05) reduced experimental limb blood flow and conductance (Figs. 3 and 4, respectively). However, at the time of maximal vasoconstriction in the experimental limb, there were no significant (P > 0.05) changes from baseline in heart rate, mean arterial pressure, or blood flow in the contralateral limb.

The absolute changes in experimental limb blood flow with intra-arterial infusion of α,β-methylene ATP at rest and during exercise are shown in Fig. 3. However, vascular responsiveness is best described in vivo by using the percent change in vascular conductance from baseline (4). Figure 4 depicts the percent changes in iliac conductance with intra-arterial infusion of α,β-methylene ATP at rest and during exercise. There was a significant effect of exercise intensity on the percent changes in iliac conductance (P = 0.003). The attenuation of P2X-receptor responsiveness from rest to exercise.
ation of vascular responsiveness from rest to exercise was more pronounced as exercise intensity increased.

To examine whether nitric oxide production during exercise was responsible for the reduced vascular responsiveness, α,β-methylene ATP infusions were repeated after nitric oxide synthase inhibition with L-NAME. L-NAME administration reduced baseline experimental limb blood flow and elevated baseline mean arterial pressure \((P < 0.05; \text{Table } 1)\). However, the α,β-methylene ATP infusions still produced significant \((P < 0.05)\) reductions in experimental limb blood flow and conductance (Figs. 3 and 4, respectively). As in the previous series, at the time of maximal vasoconstriction in the experimental limb, there were no significant \((P > 0.05)\) changes from baseline in heart rate, mean arterial pressure, or blood flow in the contralateral limb. The absolute changes in experimental limb blood flow with intra-arterial infusion of α,β-methylene ATP at rest and during exercise with L-NAME are presented in Fig. 3. Figure 4 depicts the percent changes in iliac conductance with intra-arterial infusion of α,β-methylene ATP at rest and during exercise in the presence of L-NAME. Intra-arterial infusion of α,β-methylene ATP produced more vasoconstriction compared with the control condition \((P < 0.05)\). Although L-NAME enhanced the vasoconstrictor effects of α,β-methylene ATP, vascular responsiveness was still attenuated during exercise \((P < 0.05)\). However, there was not a statistically significant interaction \((P = 0.18)\) between exercise intensity and the L-NAME condition for the percent change in conductance. Thus the administration of L-NAME did not alter the degree to which exercise attenuated the responsiveness of P2X purinergic receptors to stimulation by α,β-methylene ATP.

**DISCUSSION**

There are three major new findings in this study. First, stimulation of P2X receptors in the arterial vasculature of skeletal muscle elicited vasoconstriction in both resting and active skeletal muscle. Second, the responsiveness of P2X receptors is attenuated from rest to exercise such that stimulation with the selective P2X agonist α,β-methylene ATP produced less vasoconstriction during heavy exercise compared with rest. Finally, the production of nitric oxide does not appear to be responsible for the reduction in P2X-receptor responsiveness during exercise. To our knowledge, this is the first demonstration in conscious animals that stimulation of P2X receptors produces vasoconstriction in resting and exercising skeletal muscle.

There is compelling evidence that ATP acts as a neurotransmitter in vascular smooth muscle and is coreleased with norepinephrine from sympathetic nerves (9, 18, 21, 29). Burnstock and Kennedy (11) proposed the subdivision of P2 purinergic receptors into P2X, which mediate vasoconstriction, and P2Y, which mediate vasodilation. P2X receptors on vascular smooth muscle cells are preferentially stimulated by ATP from sympathetic nerve endings (17). P2Y receptors are found predominantly on endothelial cells, but there is limited evidence for this receptor subtype on vascular smooth muscle (12). It should be stated that ADP is a weak agonist for the P2X receptor (100-fold less potent than ATP) and that UTP is inactive (2). Several studies have shown stimulation of neurotransmitter release caused vascular smooth muscle contractions in the presence of adrenergic blockade (10, 21, 32, 44). These nonadrenergic contractions can be abolished with desensitization of P2X purinoceptors (10), suggesting that the vasoconstriction is mediated by ATP or a related purine nucleotide. Two recent studies have shown that P2X-receptor stimulation will produce vasoconstriction in the hindlimb of anesthetized animals. Bivalacqua et al. (1) produced vasoconstriction in the isolated hindlimb of anesthetized cats with localized infusions of α,β-methylene ATP. In anesthetized rats, Johnson et al. (19) provided evidence that P2X receptors contribute to sympathetic vasoconstriction in skeletal muscle. Electrical stimulation of the sympathetic nerves innervating the hindlimb of the rat produced vasoconstriction that was attenuated with P2X-receptor blockade. Although our laboratory (3, 5, 15) and others (34) have previously demonstrated sympathetic restraint of blood flow to exercising skeletal muscle mediated by α-adrenergic receptors, it is unknown whether P2X receptors mediate vasoconstriction in exercising skeletal muscle. Data from the present study, coupled with those provided by Johnson et al. (19), might lead one to speculate that tonic sympathetic restraint of blood flow to exercising skeletal muscle is mediated by P2X receptors in addition to α-adrenergic receptors. Future investigation is needed to determine whether P2X receptors mediate tonic vascular tone in exercising skeletal muscle.

A previous study from our laboratory showed exogenous activation of both α1- and α2-adrenergic receptors produced vasoconstriction during exercise (7). However, compared with rest, exercise intensity differentially attenuated the responsiveness of α1 - and α2-adrenergic receptors in the arterial vasculature of exercising skeletal muscle. The vasoconstriction to a selective α1-adrenergic-receptor agonist was only attenuated during heavy exercise, whereas the vasoconstriction to a selective α2-adrenergic-receptor agonist was attenuated during mild exercise and further attenuated by subsequent increases in exercise intensity. The results of the present study suggest that P2X receptors are more similar to α1-adrenergic receptors in their pattern of attenuation in that, compared with rest, a mild bout of exercise did not alter the responsiveness of P2X receptors. The present study is the first to demonstrate functional sympatholysis involving a postsynaptic vascular receptor other than an α-adrenergic receptor.

Although the exact mechanism responsible for exercise sympatholysis remains to be definitively determined, alterations in the chemical environment of the vascular smooth muscle have been shown to change α-adrenergic-receptor responsiveness. Postsynaptic α2-adrenergic-receptor-mediated vasoconstriction appears to be readily attenuated by modest reductions in
vascular smooth muscle and mediate functional symp-
skeletal muscle is another localized factor that has
factors. The production of nitric oxide in exercising
soconstriction with P2X-receptor stimulation after L-
the data do not support this hypothesis. Although
receptor responsiveness during exercise. Surprisingly,
least partially responsible for the attenuation in P2X-
synthase abolishes the attenuation of sym-
find similar results. Children with Duchenne muscu-
lar dystrophy, which results in a loss of neuronal nitric
in contracting rat limbs. In addition, mice with genetic
deficiencies in neuronal nitric oxide synthase do not
be responsible for the reduction in P2X-receptor re-
sponsiveness during exercise compared with rest.
Nonetheless, the present study is the first to demon-
strate that stimulation of P2X purinergic receptors in
the arterial vasculature of exercising skeletal muscle
produces vasoconstriction. These results raise the pos-
sibility that the P2X purinergic receptor may regulate
skeletal muscle vascular tone during exercise.

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DISCLOSURES
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