Muscle interstitial ATP and norepinephrine concentrations in the human leg during exercise and ATP infusion

Stefan P. Mortensen,1 José González-Alonso,1 Jens-Jung Nielsen,2 Bengt Saltin,1 and Ylva Hellsten1,2

1The Copenhagen Muscle Research Centre, Rigshospitalet, Denmark, 2Institute of Exercise and Sports Sciences, University of Copenhagen, Copenhagen, Denmark

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ATP has been proposed to play multiple roles in local skeletal muscle blood flow regulation by inducing vasodilation and modulating sympathetic vasoconstrictor activity, but the mechanisms remain unclear. Here we evaluated the effects of arterial ATP infusion and exercise on leg muscle interstitial ATP and norepinephrine (NE) concentrations to gain insight into the interstitial and intravascular mechanisms by which ATP causes muscle vasodilation and sympatholysis. Leg hemodynamics and muscle interstitial nucleotide and NE concentrations were measured during 1) femoral arterial ATP infusion (0.42 ± 0.04 and 2.26 ± 0.52 μmol/min; mean ± SE) and 2) one-leg knee-extensor exercise (18 ± 0 and 37 ± 2 W) in 10 healthy men. Arterial ATP infusion and exercise increased leg blood flow (LBF) in the experimental leg from −0.3 l/min at baseline to 4.2 ± 0.3 and 4.6 ± 0.5 l/min, respectively, whereas it was reduced or unchanged in the control leg. During arterial ATP infusion, muscle interstitial ATP, ADP, AMP, and adenosine concentrations remained unchanged in both legs, but muscle interstitial NE increased from −5.9 nmol/l at baseline to 8.3 ± 1.2 and 8.7 ± 0.7 nmol/l in the experimental and control leg, respectively (P < 0.05), in parallel to a reduction in arterial pressure (P < 0.05). During exercise, however, interstitial ATP, ADP, AMP, and adenosine concentrations increased in the contracting muscle (P < 0.05), but not in inactive muscle, whereas interstitial NE concentrations increased similarly in both active and inactive muscles. These results suggest that the vasodilatory and sympathetic effects of intraluminal ATP are mainly mediated via endothelial purinergic receptors. Intraluminal ATP and muscle contractions appear to modulate sympathetic nerve activity by inhibiting the effect of NE rather than blunting its local concentration.

THE REGULATION of matching O2 delivery to the metabolic demand of contracting myocytes is thought to be brought about by an interaction between locally formed vasoactive substances and sympathetic vasoconstriction (2, 7, 11, 29). Intraluminal ATP has been proposed to contribute to the local regulation of skeletal muscle blood flow by inducing local vasodilation (9, 13) and modulating sympathetic vasoconstriction (24, 36, 37). ATP-sensitive P2 receptors are expressed in the endothelium (P2Y2 and P2X1) and smooth muscle cell (P2Y2) of human skeletal muscle (31), demonstrating the existence of receptors by which ATP could regulate local vasodilation. The mechanisms underlying the vascular effects of intraluminal ATP remain unclear. In animals, the endothelium has been described as an effective barrier for ATP (28), indicating that the vasodilatory and sympatholytic effect of ATP is mediated from the luminal side, but this has not been tested in humans in vivo. Furthermore, it remains unclear if intraluminal ATP exerts its sympatholytic effects by modulating local norepinephrine (NE) levels or by inhibiting the effect of NE.

During exercise, the sympathetic nervous system is engaged (a phenomenon widely known as exercise pressor reflex) (1, 21, 27, 43). Direct assessment of sympathetic nerve activity during exercise by peroneal microneurography suggests that this increase is targeted to both resting and contracting skeletal muscle (16, 33, 39, 44). In inactive tissues, the increase in sympathetic nerve activity causes vasoconstriction as evidenced by reductions in limb vascular conductance (3, 6, 38). In contracting muscle, however, evidence suggests that the effect of the increased sympathetic activity can be attenuated or even abolished, a phenomenon termed functional sympatholysis that is thought to allow adequate perfusion and O2 delivery to exercising muscle (15, 34, 45, 46). The mechanisms underlying functional sympatholysis during exercise are not well understood, but local modulation of NE levels in contracting muscle vs. inactive muscle may play a role. As microneurography cannot be performed in the dynamically contracting limb, this method does not provide insight into local activation and modulation of muscle sympathetic nerve activity (MSNA). Measurements of NE spillover by determination of NE in arterial and venous plasma may not reflect changes in interstitial NE concentrations. An alternative approach to determine if sympathetic activation and modulation are the same in active and inactive muscle is to measure NE levels directly in the interstitium.

Recent evidence suggests that interstitial ATP in contracting muscle contributes to the exercise pressor reflex by activating P2X receptors, which in turn stimulate and sensitize thin fiber muscle afferents (23, 26). In vivo data obtained in humans suggest that interstitial ATP concentrations increase in proportion to the exercise intensity (20) and that skeletal muscle contains ATP-sensitive P2X1 receptors in the muscle sarclemma (31). Data obtained in animal models suggest that interstitial ATP also stimulates local NE release (25) and NE modulation of muscle sympathetic nerve activity (MSNA). Measurements of NE spillover by determination of NE in arterial and venous plasma may not reflect changes in interstitial NE concentrations. An alternative approach to determine if sympathetic activation and modulation are the same in active and inactive muscle is to measure NE levels directly in the interstitium.

Accordingly, the aims of the present study were 1) to determine if intraluminal ATP can contribute to the increase in interstitial ATP and thereby act directly via smooth muscle P2 receptors and 2) to gain insight into the mechanisms by which intraluminal ATP and exercise modulate sympathetic nerve activation. To accomplish these aims we determined interstitial ATP and NE concentrations during arterial ATP infusion and one-leg knee-extensor exercise in healthy young men. We hypothesized that muscle interstitial ATP concentration does

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not increase during physiological intraluminal ATP infusion and that the sympatholytic effect of arterially infused ATP or exercise does not involve modulation of muscle interstitial NE levels.

METHODS

The subjects were informed of the risks and discomforts associated with the experiments before giving their informed, written consent to participate. The study was approved by the Ethics committee of Copenhagen and Frederiksberg (KF 01-013/96 and KF 11289201) and conducted in accordance with the guidelines of the Declaration of Helsinki.

Experimental protocol. Ten healthy male subjects with a mean (±SD) age of 25 ± 4 yr, body weight of 76 ± 7 kg, and height of 185 ± 8 cm participated in this study. Before the experimental day, the subjects completed two training sessions to get accustomed to the exercise model and determine peak workload. On the day of the experiment, the subjects arrived at the laboratory 1 h before the experiment after a light breakfast. Catheters were placed into the femoral artery and vein of the experimental leg and femoral artery of the nonexperimental leg under local anesthesia (Lidocain). Four microdialysis probes were inserted into the vastus lateralis muscle of the experimental leg, and two microdialysis probes were inserted into the vastus lateralis muscle of the control leg as previously described (20).

Following 30 min of rest, 10 min of one-leg knee-extensor exercise (20 W), and 20 min of rest, subjects completed 15 min of 1) femoral arterial ATP infusion (0.03 and 0.14 μmol·min⁻¹·kg leg mass⁻¹) and 2) one-leg knee-extensor exercise (18 ± 0 and 37 ± 1 W, i.e., 20 and 40% of peak work load). The order of the trials was randomized and separated by 45 min of supine rest.

Blood samples (1–5 ml) were drawn simultaneously from the femoral artery and vein at basal conditions, and during intra-arterial ATP infusion, and one-leg knee-extensor exercise (1.5, 4, and 10 min). Leg blood flow (LBF) was determined immediately following blood sample withdrawal. Microdialysate was collected for 15 min before each trial, during each trial, and during 15 min of recovery. The microdialysate probes were continuously perfused with a Ringer solution (Fresenius Kabi AB) with a high-precision syringe pump (CMA 102, Carnegie Medicine, Solna, Sweden) at a rate of 5 μl/min. A small amount (2.7 nM) of [2-3H]ATP (<0.1 μCi/ml) was added to the perfusate for the calculation of probe recovery. The purpose of determining probe recovery was to correct for differences in recovery occurring in the transition from rest to during exercise. After collection of samples, the microdialysate was weighed, and the actual flow rate was calculated to estimate any loss of fluid or abnormal decrease in perfusion rate. The relative loss for each probe was determined according to the internal reference method (22, 42). The molecular probe recovery (PR) was calculated as PR = (dpminfusate × dpmdialysate)/dpminfusate, where dpm denotes

Fig. 1. Leg hemodynamics before, during, and after exercise and arterial ATP infusion. Leg blood flow (top), mean arterial pressure (middle), and leg vascular conductance (bottom) before, during, and after one-leg knee-extensor exercise (left) and arterial ATP infusion (right) in the experimental (solid bars) and control leg (open bars) are shown. Data are means ± SE for 10 subjects. *Significantly different from control, P < 0.05.
disintegrations per minute. The \(^{14} \text{H}\) activity (in dpm) was measured on a liquid scintillation counter (Tri-Carb 2000; Copenhagen; Denmark) after addition of the infusate and dialysate (5 \(\mu l\) each) to 3.0 ml of Ultima Gold scintillation liquid (Packard Instruments, Groningen, The Netherlands). The relative loss (recovery) of ATP at rest, 18 W, 37 W, and during the recovery from exercise was 42 ± 2, 65 ± 5, 62 ± 3, and 51 ± 5%, respectively.

**Analytical procedures.** LBF was measured with ultrasound Doppler (CFM 800, Vingmed, Norway) (32) under basal conditions, and in the control leg whereas LBF in the experimental leg during arterial ATP infusion and exercise was measured by the constant-infusion thermotubulation method (2, 14). Heart rate was obtained from an electrocardiogram, while femoral arterial pressures were monitored with transducers positioned at the level of the heart (Pressure Monitoring Kit, Baxter). Blood gases and hemoglobin concentrations were measured using an ABL725 analyzer (Radiometer, Copenhagen, Denmark) and were corrected for temperature obtained in the femoral vein. Leg mass was calculated from whole body dual-energy X-ray absorptiometry scanning (Prodigy, General Electrics Medical Systems). Interstitial NE concentrations were determined with a radioimmunoassay (LDN, Nordhorn, Germany). ATP, ADP, AMP, and adenosine in perfusate and dialysate were analyzed with HPLC without prior treatment of samples (47).

**Statistical analysis.** A two-way repeated-measures ANOVA was performed to test significance within and between trials. Following a significant F-test, pairwise differences were identified using Tukey’s honestly significant difference (HSD) post hoc procedure. There was no difference in any variable between 1.5, 4, and 10 min during ATP infusion and exercise, and presented data are therefore means of the three measurements. The significance level was set at \(P < 0.05\), and data are means ± SE unless otherwise indicated.

**RESULTS**

**Leg and systemic variables during exercise and arterial ATP infusion.** ATP infusion and exercise increased LBF in the experimental leg from \(\sim 0.3\) l/min to 4.2 ± 0.3 l/min (2.26 \(\mu\)mol/min) and 4.6 ± 0.5 l/min (37 W), respectively (\(P < 0.05\)) (Fig. 1). ATP infusion lowered mean arterial pressure (MAP) from 96 ± 3 to 91 ± 3 mmHg (2.26 \(\mu\)mol/min), whereas exercise increased MAP from 101 ± 2 to 113 ± 2 mmHg (37 W) (\(P < 0.05\)). Arterial ATP infusion and exercise increased leg vascular conductance from 4 to 48 ± 5 and 40 ± 4 \(\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}\), respectively. Arterial O\(_2\) content remained unchanged during both trials and O\(_2\) delivery therefore increased in proportion to the increase in LBF. Leg arteriovenous oxygen difference \([\text{a-vO}_2\text{ difference}]\) decreased during ATP infusion (\(P < 0.05\)) such that leg oxygen consumption (\(\text{V}_{\text{O2}}\)) remained at baseline values, whereas exercise increased both leg (a-v)O\(_2\) and \(\text{V}_{\text{O2}}\) (\(P < 0.05\)). In the control leg, LBF and vascular conductance remained unchanged during exercise, but LBF was reduced compared with baseline values in the control leg during the high arterial ATP infusion rate (\(P < 0.05\)). Heart rate increased from 64 ± 4 to 74 ± 3 beats/min during ATP infusion (\(P < 0.05\)) and from 69 ± 2 to 93 ± 3 beats/min during exercise (\(P < 0.05\)).

**Table 1. Muscle interstitial nucleotides and adenosine during exercise and arterial ATP infusion**

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<thead>
<tr>
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<th>Muscle Interstitial Concentrations, (\mu)mol/l</th>
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<tr>
<td></td>
<td>ATP</td>
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<td><strong>Baseline</strong></td>
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<tr>
<td>ATP infusion</td>
<td></td>
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<tr>
<td>Experimental leg</td>
<td>0.20±0.00</td>
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<tr>
<td>Control leg</td>
<td>0.10±0.10</td>
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<tr>
<td>Exercise</td>
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<tr>
<td>Experimental leg</td>
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</tr>
<tr>
<td>Control leg</td>
<td>0.25±0.04</td>
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<tr>
<td>Mild hyperemia</td>
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<tr>
<td>ATP infusion</td>
<td>0.18±0.05</td>
</tr>
<tr>
<td>Experimental leg</td>
<td>0.14±0.05</td>
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<tr>
<td>Control leg</td>
<td>0.35±0.14</td>
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<tr>
<td>Exercise</td>
<td></td>
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<tr>
<td>Experimental leg</td>
<td>0.47±0.51*</td>
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<tr>
<td>Control leg</td>
<td>0.32±0.12</td>
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<tr>
<td>Recovery</td>
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<tr>
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<tr>
<td>Experimental leg</td>
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<tr>
<td>Control leg</td>
<td>0.32±0.14</td>
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<tr>
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<tr>
<td>Experimental leg</td>
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<tr>
<td>Control leg</td>
<td>0.46±0.21</td>
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Data are means ± SE for 10 subjects. *Significantly different from rest and control leg, \(P < 0.05\).

**DISCUSSION**

This study examined the role of extracellular ATP and exercise in the activation and modulation of sympathetic nerve activity. The main findings were 1) muscle interstitial nucleotide and adenosine concentrations did not change in response to arterially infused ATP, 2) NE concentrations increased to a similar extent in the muscle receiving arterially infused ATP and in the control muscle, 3) one-leg knee-extensor exercise increased muscle interstitial NE concentrations similarly in the exercising and control muscle, and 4) arterially inspired ATP concentrations increased in contracting muscle but remained unchanged in the inactive muscle. Collectively, these results suggest that the vascular effect of intraluminally infused ATP are mediated via endothelial receptors. Furthermore, low- and moderate-intensity exercise increases muscle interstitial NE in both contracting and inactive muscle, indicating that neither muscle contractions nor interstitial ATP appears to modulate local NE levels.

The present study demonstrates that arterial infusion of ATP, sufficient to increase LBF and vascular conductance \(\sim 15\)-fold, does not increase the concentrations of interstitial
ATP or any of its dephosphorylated breakdown products. Previous results show that P2 receptors capable of mediating the vascular effects of intraluminal ATP are present not only in the endothelium (P2Y2 and P2X1) but also in smooth muscle cells (P2Y2 and P2X1) and sarcolemma (P2X1) of human skeletal muscle (31). These observations suggest that intraluminal ATP may also act directly on smooth muscle cells. To examine this possibility, doses of ATP that induced pronounced vasodilation and increased arterial plasma ATP levels within the physiological range (estimated ~500 nmol/l) that have previously been reported (13, 30, 36) were infused arterially. We found no change in ATP and its degradation compounds ADP, AMP, and adenosine in the interstitial space, where smooth muscle cell P2 receptors are located. Because ATP is likely to be rapidly dephosphorylated in the artery, the actual gradient between the capillary and interstitium in the present study remains unknown. A study in the isolated dog muscle indicates that arterial ATP concentration of 10 μmol/l is needed to increase interstitial nucleotides (28), which is 5 times higher than has been reported during maximal leg exercise (13, 36). Regardless of the capillary ATP concentration, the present doses of ATP, inducing hyperemia similar to that observed during moderate-intensity exercise, clearly show that interstitial nucleotide and adenosine concentrations are not altered, suggesting that the vasodilatory (13) and sympatholytic (36) effects of intraluminal ATP are mediated via endothelial receptors. Because adenosine does not appear to play a role in ATP-induced vasodilation (31, 35), endothelial P2 receptors are likely to be the important mediators.

The present results indicate that MSNA activity is directed to both contracting and inactive muscle during dynamic exercise and that there appears to be no modulation of local NE levels in the contracting muscle. This study is the first to determine NE levels within contracting muscle, but our findings are supported by previous studies that have measured MSNA by peroneal microneurography and demonstrated that sympathetic activation is directed to both active and inactive limbs (16, 33, 44). The present data provide evidence that the functional sympatholysis during exercise does not occur prejunctionally, because local NE levels are similar in contracting and inactive muscle. In accordance, NE spillover, measured as arteriovenous plasma differences of NE, has previously been found to be similar in the exercising and inactive leg (41). These present results do not support the hypothesis that contracting muscle releases compounds that inhibit NE

Fig. 2. Interstitial ATP and norepinephrine concentrations during arterial ATP infusion and exercise. Muscle interstitial ATP (top) and norepinephrine concentrations (bottom) during one-leg knee extensor exercise (left) and arterial ATP infusion (right) in the experimental (solid bars) and control leg (open bars) are shown. Data are means ± SE for 8–10 subjects. *Significantly different from control, P < 0.05.

Fig. 3. Interstitial norepinephrine in contracting and inactive muscle. Regression analysis shows a linear relationship between interstitial norepinephrine rise in contracting and inactive muscle. Data are means ± SE for 8 subjects.
release and/or increase NE uptake (48, 49). There may have been little muscular activity in the control leg to assist in stabilizing the lower trunk, because vascular conductance did not decline \((P = 0.12)\) as may be expected during resting conditions with elevated MSNA, but this ought to be small as conductance did not increase either.

Similar to exercise, intraluminal ATP is capable of modulating sympathetic nerve activity (36). Here, we found that arterial ATP infusion increased muscle interstitial NE concentrations in both the experimental and control leg, which is in agreement with previous reports demonstrating that femoral arterial ATP infusion in resting humans increases MSNA (5, 36). Like exercise, we found evidence that ATP does not modulate sympathetic nerve activity prejunctionally, because muscle interstitial NE concentrations were strikingly similar in the leg receiving arterially infused ATP and the control leg. A likely explanation for the increased sympathetic nerve activity during arterial ATP infusion is a baroreceptor-mediated response to the lower MAP (5), because venous infusion of ATP does not appear to increase sympathetic nerve activity (12). Recent data have indicated that arterial injections of low and moderate, but not high, doses of ATP stimulate a local release of NE in rats (25). In this setting, NE levels would be expected to be elevated in contracting compared with resting muscle and thus not supported by the present findings. In the present study, NE was measured during ATP infusion, whereas it was measured after ATP infusion in the study by Li and coworkers (25), which, apart from species differences, may explain the discrepancy in findings. Collectively, the present data therefore indicate that modulation of sympathetic vasoconstriction during exercise and arterial ATP infusion does not occur via interference with prejunctional receptors.

Emerging evidence suggests that interstitial ATP contributes to the increase in sympathetic nerve activity by stimulating muscle afferents in the contracting muscle via P2X receptors (18, 23, 26). In support of this, we have recently shown that P2X1 receptors are present in human skeletal muscle sarcolemma (31). The present data from contracting muscle indicate that interstitial ATP may stimulate a central increase in MSNA via afferent fibers because both NE and ATP concentrations increased with exercise intensity in the contracting muscle. Extracellular ATP has also been suggested to play a role in modulating the contractile properties of skeletal muscle (40). The source of interstitial ATP remains unclear, but skeletal muscle may release ATP during contractions (8, 10), although this finding is not universal (19), and endothelial (4) and skeletal muscle cells (19) may release ATP in response to mechanical stress.

In conclusion, these results suggest that the vasodilatory and sympatholytic functions of intraluminal ATP are mediated via endothelial P2 receptors. Furthermore, both intraluminal ATP and low- and moderate-intensity exercise appear to modulate MSNA by inhibiting NE function rather than local NE levels. Interstitial ATP does not appear to modulate local NE levels but may contribute to the central increase in sympathetic nerve activity during muscle contractions.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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