

Circulating ATP-induced vasodilatation overrides sympathetic vasoconstrictor activity in human skeletal muscle

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Despite increases in muscle sympathetic vasoconstrictor activity, skeletal muscle blood flow and O₂ delivery increase during exercise in humans in proportion to the local metabolic demand, a phenomenon coupled to local reductions in the oxygenation state of haemoglobin and concomitant increases in circulating ATP. We tested the hypothesis that circulating ATP contributes to local blood flow and O₂ delivery regulation by both inducing vasodilatation and blunting the augmented sympathetic vasoconstrictor activity. In eight healthy subjects, we first measured leg blood flow (LBF) and mean arterial pressure (MAP) during three hyperaemic conditions: (1) intrafemoral artery adenosine infusion (vasodilator control), (2) intrafemoral artery ATP infusion (vasodilator), and (3) mild knee-extensor exercise (~20 W), and then compared the responses with the combined infusion of the vasoconstrictor drug tyramine, which evokes endogenous release of noradrenaline from sympathetic nerve endings. In all three hyperaemic conditions, LBF equally increased from $\sim 0.5 \pm 0.1$ l min⁻¹ at rest to $\sim 3.6 \pm 0.3$ l min⁻¹, with no change in MAP. Tyramine caused significant leg vasoconstriction during adenosine infusion (53 ± 5 and $56 \pm 5\%$ lower LBF and leg vascular conductance, respectively, $P < 0.05$), which was completely abolished by both ATP infusion and exercise. In six additional subjects resting in the sitting position, intrafemoral artery infusion of ATP increased LBF and leg vascular conductance 27 ± 3 -fold, despite concomitant increases in venous noradrenaline and muscle sympathetic nerve activity of 2.5 ± 0.2 - and 2.4 ± 0.1 -fold, respectively. Maximal ATP-induced vasodilatation at rest accounted for 78% of the peak LBF during maximal bicycling exercise. Our findings in humans demonstrate that circulating ATP is capable of regulating local skeletal muscle blood flow and O₂ delivery by causing substantial vasodilatation and negating the effects of increased sympathetic vasoconstrictor activity.

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In contracting skeletal muscle, blood flow is regulated to match O₂ delivery and O₂ demand in a variety of conditions including normoxia, hypoxia, CO-hypoxia, hyperoxia, anaemia, polycythaemia, heat stress and dehydration. At moderate and high exercise intensities, these circulatory responses are accompanied by an increase in muscle sympathetic nerve activity (MSNA), mainly due to activation of a reflex mechanism arising in the contracting muscle, where yet-to-be identified metabolic by-products of muscle contraction activate chemically sensitive afferent nerve endings in the muscle interstitium (the muscle metaboreflex) (Mitchell *et al.* 1983; Seals & Victor, 1991). This mechanism is believed to provide a signal to the brain of a mismatch between muscle

blood flow and metabolism and in turn evoke circulatory adjustments to minimize this mismatch. The increase in efferent MSNA is targeted both to resting and contracting skeletal muscle (Savard *et al.* 1987; Hansen *et al.* 1994). While sympathetic activation in resting muscle causes vasoconstriction and redistributes cardiac output to the contracting muscles, the functional consequences of sympathetic activation in contracting muscle has been difficult to define (for review see Rowell, 1991; Laughlin *et al.* 1996; Buckwalter *et al.* 2001; Hansen, 2002). There is abundant evidence in humans and animals that the sympathetic nervous system can control vascular conductance and blood flow in active muscle and thus help maintain arterial blood pressure during exercise

(Joyner *et al.* 1992; O'Leary *et al.* 1997; Buckwalter *et al.* 1997). However, there is also abundant evidence that this sympathetic control can be attenuated or even abolished in contracting muscle (Remensnyder *et al.* 1962; Thomas *et al.* 1994; Hansen *et al.* 1996; Ruble *et al.* 2002; Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003a; Wray *et al.* 2004; Fadel *et al.* 2004). This phenomenon, which was termed functional sympatholysis by Remensnyder *et al.* (1962), provides a mechanism to optimize blood flow and O₂ delivery to the contracting muscle despite sympathetic activation.

Contraction-induced modulation of sympathetic vasoconstriction has been hypothesized to involve metabolites released from the contracting skeletal muscle, which presumably modulate signal transduction pathways subservient to the activation of postjunctional α_1 and α_2 -adrenoreceptors located on the vascular smooth muscle (Nishigaki *et al.* 1991; Ohyanagi *et al.* 1992; Thomas *et al.* 1994; Hansen *et al.* 1996, 1999; Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003a; Wray *et al.* 2004). The muscle and interstitial metabolites that have been implicated include H⁺, P_i, K⁺, prostaglandins, adenosine and nitric oxide (NO) (McGillivray-Anderson & Faber, 1990, 1991; Nishigaki *et al.* 1991; Ohyanagi *et al.* 1992; Thomas *et al.* 1994; Tateishi & Faber, 1995; Thomas & Victor, 1998; Hansen *et al.* 2000). Although previous studies have provided strong experimental evidence in rats and humans supporting a role for NO produced by the contracting muscle (Thomas & Victor, 1998; Thomas *et al.* 1998; Chavoshan *et al.* 2002), this concept has recently been challenged (Rosenmeier *et al.* 2003b; Dinneno & Joyner, 2003; Buckwalter *et al.* 2004). While the precise mechanism(s) by which muscle contraction leads to attenuation of α -adrenergic vasoconstriction remains incompletely understood, there are data to suggest that contraction-induced reduction in tissue oxygenation (measured by near infrared spectroscopy) plays a primary role (Hansen *et al.* 2000). Moreover, decreased O₂ delivery relative to utilization (induced by ischaemia, hypoxic hypoxaemia and CO inhalation) can attenuate sympathetic vasoconstriction in resting muscle as well (Hansen *et al.* 2000; Hanada *et al.* 2003), suggesting that even in the absence of muscle contraction mechanisms are at work in the skeletal muscle microvasculature to preserve O₂ uptake under conditions of reduced arterial O₂ content and sympathetic activation.

We have now considered an alternative unifying mechanism for the attenuation of sympathetic vasoconstriction and regulation of muscle blood flow during contraction and during conditions with reductions in blood O₂ content. Over the last few years, the erythrocyte

has been hypothesized to function as an O₂ sensor, which contributes to the control of local blood flow and O₂ delivery by releasing ATP into the circulation in proportion to the offloading of O₂ from the haemoglobin molecule (Ellsworth *et al.* 1995; Dietrich *et al.* 2000; Jagger *et al.* 2001; González-Alonso *et al.* 2002; Ellsworth, 2004). The tight coupling between alterations in circulating plasma ATP and changes in the oxygenation state of haemoglobin with normoxia, hypoxia, hyperoxia and CO + normoxia in both exercising and non-exercising human limbs and *in vitro* vessel preparations perfused with red blood cells strongly supports this hypothesis (González-Alonso *et al.* 2002; Ellsworth, 2004). ATP is a potent vasodilator when infused in intact humans (Folkow, 1949; Rongen *et al.* 1994; González-Alonso *et al.* 2002) or when infused locally in *in vitro* vessel preparations (Ellsworth *et al.* 1995; McCullough *et al.* 1997; Ellsworth, 2004). Mechanistically, ATP can induce vasodilatation by binding to the purinergic P_{2y} receptors located on the vascular endothelial cells to release endothelium-derived hyperpolarization factors (EDHF), NO and/or prostaglandins, which diffuse to the vascular smooth muscle and result in vasodilatation (Ellsworth *et al.* 1995; Wihlborg *et al.* 2003). Whether ATP can attenuate sympathetic vasoconstriction in humans has not been studied. Clearly, a prerequisite for demonstrating a pivotal role of circulating ATP in the control of resting and exercising skeletal muscle blood flow and O₂ delivery is that ATP should be capable of both causing potent vasodilatation and abolishing α -adrenergic vasoconstriction.

Therefore, the primary aim of this investigation was to test the hypothesis that circulating ATP can override α -adrenergic vasoconstriction in human skeletal muscle and thus mimic the exercise-induced attenuation. A secondary aim was to test the hypothesis that circulating ATP can evoke large increases in leg blood flow that reach the maximal vasodilatory capacity of the exercising leg despite increasing sympathetic outflow. To test the first hypothesis, we compared the vasoconstrictor effects of tyramine in the leg during three matched hyperaemic conditions produced by low intensity knee-extensor exercise or ipsilateral intrafemoral artery infusion of ATP or adenosine in the resting leg (the latter used as a vasodilator control). In addition, both vasodilators were infused during exercise to further evaluate their vasodilatory capacity in contracting skeletal muscle in the presence and absence of tyramine. To test the second hypothesis, MSNA (peroneal microneurography), femoral venous noradrenaline and leg blood flow were first measured during incremental intrafemoral artery infusion of ATP at rest and then compared to the responses (with the exception of MSNA) during maximal bicycling

exercise. Additionally, blood gases were measured in each experimental condition to support the validity of the haemodynamic measures and investigate the effects of altered blood flow and O_2 delivery on resting and exercising skeletal muscle O_2 uptake.

Methods

Fourteen healthy recreationally active subjects participated in two studies. They had a mean (\pm s.d.) age of 25 ± 2 years, body weight of 72 ± 4 kg, and height of 179 ± 5 cm. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their informed written consent to participate. The studies conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki) and were approved by the Ethics Committee for Copenhagen and Frederiksberg communities.

In the first study, the eight subjects (7 males and 1 female) underwent three different protocols, separated by 20 min of rest, to determine: (1) the vasoconstrictor effects of the drug tyramine during adenosine-, ATP- and exercise-induced hyperaemia (protocol 1), (2) the vasodilatory effects of ATP and adenosine during exercise and tyramine infusion (protocol 2), and (3) the vasodilatory effects of ATP and adenosine during exercise without the presence of tyramine (protocol 3; Fig. 1). Two interventions were performed at rest and three during knee-extensor exercise at an intensity of ~ 20 W ($\sim 25\%$ of peak power), a model that allows the exercise to be confined to the quadriceps muscle (Andersen & Saltin, 1985). Before performing the three protocols, leg blood flows were determined during exercise. Resting leg blood flow was then increased in a stepwise manner by infusion of adenosine and ATP (Harvard pumps) until blood flow values matched those obtained during exercise. To do so, adenosine (Item Development AB, Stocksund, Sweden) dissolved in isotonic saline (1.25 mg ml^{-1}) was infused at a rate of $16 \mu\text{mol min}^{-1}$ whereas ATP (Sigma A7699) dissolved in isotonic saline (1 mg ml^{-1}) was infused at a rate of $1 \mu\text{mol min}^{-1}$. The aim during combined infusion of adenosine and tyramine was to evoke a vasoconstrictor response in the resting leg of $\sim 50\%$, without causing increases in arterial blood pressure, as previously documented in the forearm (Rosenmeier *et al.* 2003b). Tyramine (Sigma T-2879) dissolved in isotonic saline (0.52 mg ml^{-1}) was infused at a rate of $\sim 13.2 \mu\text{mol min}^{-1}$ through all the tyramine trials. In separate studies in the resting leg, tyramine infusion at this dose reduced blood flow by $44 \pm 3\%$, without altering mean arterial pressure.

A schematic representation of the protocols used in study 1 is depicted in Fig. 1. The subjects first received separate infusions of adenosine or ATP for 4 min followed by combined infusions of adenosine and tyramine or ATP and tyramine for an additional 4 min. These two protocols were followed by the three exercise interventions, each lasting 12 min. The independent effect of exercise was assessed during the first 4 min, followed by the infusion of tyramine from 4 to 8 min of exercise and the superimposition of the vasodilators adenosine and ATP during the last 4 min. In the last protocol, the combined infusion with ATP and adenosine, without the presence of tyramine, was performed in a similar manner.

In the second study ($n = 6$), ATP was infused in the femoral artery during stepwise 2 min infusion periods to determine the maximal vasodilatory capacity of the resting leg and its effect on MSNA (peroneal microneurography) while seated upright (Fig. 2). The

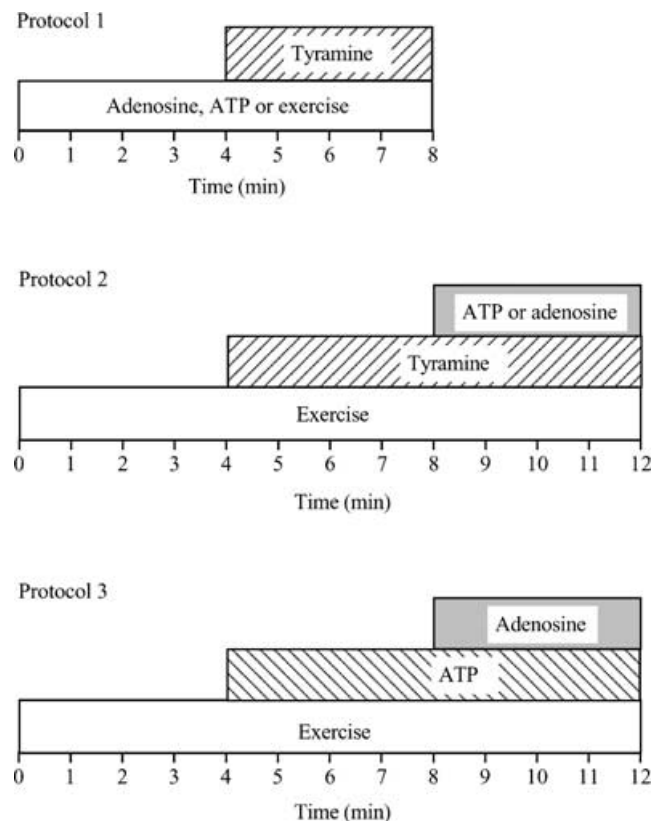


Figure 1. Sequence of the experimental protocols in the first study

This study aimed at assessing: (1) the effects of the vasoconstrictor drug tyramine under 3 matched hyperaemic conditions evoked by adenosine, ATP or exercise (protocol 1), (2) the vasodilatory capacity of adenosine and ATP during exercise and tyramine (protocol 2), and (3) the vasodilatory capacity of ATP and adenosine during exercise without the presence of tyramine (protocol 3).

infusion rates given were 1, 2, 4, 8, 16, 32 and $64 \mu\text{mol min}^{-1}$. Due to technical difficulties, micro-neurographic data at the higher infusion rates were only obtained in three subjects. Leg blood flow (LBF) was then measured during incremental upright bicycling exercise to exhaustion (76 ± 4 , 152 ± 9 , 228 ± 13 , 323 ± 20 and 361 ± 21 W; Lode ergometer) to compare the maximal vasodilatory capacities of the resting and maximally exercised leg (Fig. 2). During the ATP infusion and the exercise protocols, blood samples for ATP and catecholamine analyses were obtained following the LBF measurement, commencing after 1 min of infusion or exercise. LBF was measured again after blood withdrawal.

In both studies, the subjects reported to the laboratory at 8 a.m. or 1 p.m., following the ingestion of a light breakfast or lunch. Upon arrival, they rested in a supine position while three catheters were placed under local anaesthesia (1% lidocaine (lignocaine)) into the femoral

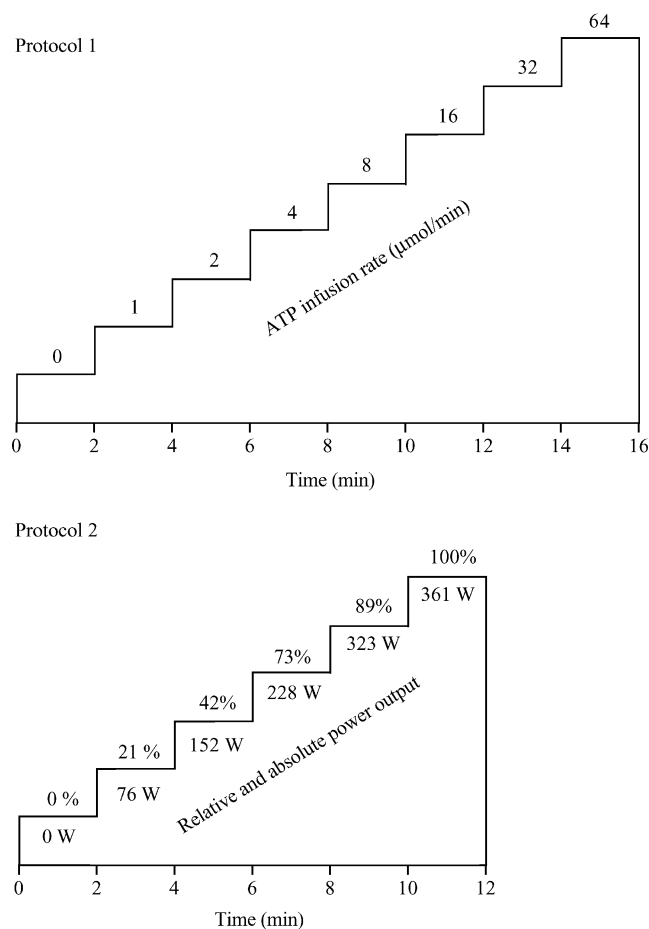


Figure 2. Sequence of the experimental protocols in the second study

This study aimed at assessing: (1) the maximal leg vasodilatory capacity during incremental intrafemoral artery infusion of ATP (protocol 1), and (2) the maximal vasodilatory capacity of the leg during normal maximal bicycle exercise (protocol 2).

artery and vein of the right leg and in the femoral artery of the left leg using the Seldinger technique. The femoral artery and vein catheters were positioned 1–2 cm distal from the inguinal ligament. A thermistor (Edslab probe 94–0.3–2.5F) to measure venous blood temperature was inserted through the femoral venous catheter orientated in the anterograde direction for the determination of femoral venous blood flow. Femoral venous blood flow (an index of leg blood flow (LBF)) was determined by the constant infusion thermodilution technique (Andersen & Saltin, 1985; González-Alonso *et al.* 2000). LBF represents the average of two measurements made 1–2 min after the start of exercise and 1–2 min after the start of infusion of ATP, adenosine or tyramine. Arterial blood pressure was continuously monitored by a pressure transducer (Pressure Monitoring Kit, Baxter) at the level of inguinal region and calculated by integration of the pressure curve. Heart rate was determined from an electrocardiogram. All data were continuously recorded using a Powerlab system (ADInstruments, Sydney, Australia).

ATP in plasma was determined with the luciferin-luciferase technique (Lundin, 2000) using a luminometer with two automatic injectors (ORION Microplate Luminometer, Berthold Detection System GmbH, Pforzheim, Germany). Blood samples (2.7 ml) for determination of plasma ATP were obtained using syringes containing EDTA (S-monovette, 2.7 ml KE; Sarstedt, Nümbrecht, Germany) and were centrifuged immediately for 30 s at 14000 r.p.m. (4°C ; Sigma 1–15 K, Osterode am Harz, Germany). Plasma was then pipetted into pre-chilled tubes, frozen down in dry ice and stored for later analysis. The duration of the whole procedure from blood withdrawal to plasma separation was ~ 90 s. Plasma ATP was measured at room temperature ($\sim 20^{\circ}\text{C}$) using a commercially available ATP Kit (ATP Kit SL 144–041; BioTherma AB, Dalarö, Sweden) with an internal ATP standard procedure. Samples were measured in duplicates. The coefficient of variation of 10 repeated resting plasma samples was 11%. Plasma haemoglobin was also analysed spectrophotometrically to determine if haemolysis had occurred during the handling of the samples. Samples showing an elevation in plasma haemoglobin were excluded from the analysis. Plasma noradrenaline and adrenaline concentrations were determined with high performance liquid chromatography with electrochemical detection (Hallman *et al.* 1978). Arterial and femoral haemoglobin concentration and O_2 saturation were determined spectrophotometrically (OSM-3 Hemoximeter, Radiometer). P_{O_2} was determined with the Astrup technique (ABL 5, Radiometer, Copenhagen, Denmark). Leg

vascular conductance was calculated as the quotient between LBF and mean arterial pressure. Leg O₂ delivery was calculated by multiplying LBF by arterial O₂ content. Leg O₂ uptake (Leg $\dot{V}O_2$) was calculated by multiplying the LBF by the difference in O₂ content between the femoral artery and vein (a-v O₂ difference).

Statistical analysis

A one-way repeated measures analysis of variance (ANOVA) was performed to test significance between and within treatments. Following a significant *F* test, pairwise differences were identified using Tukey's honestly

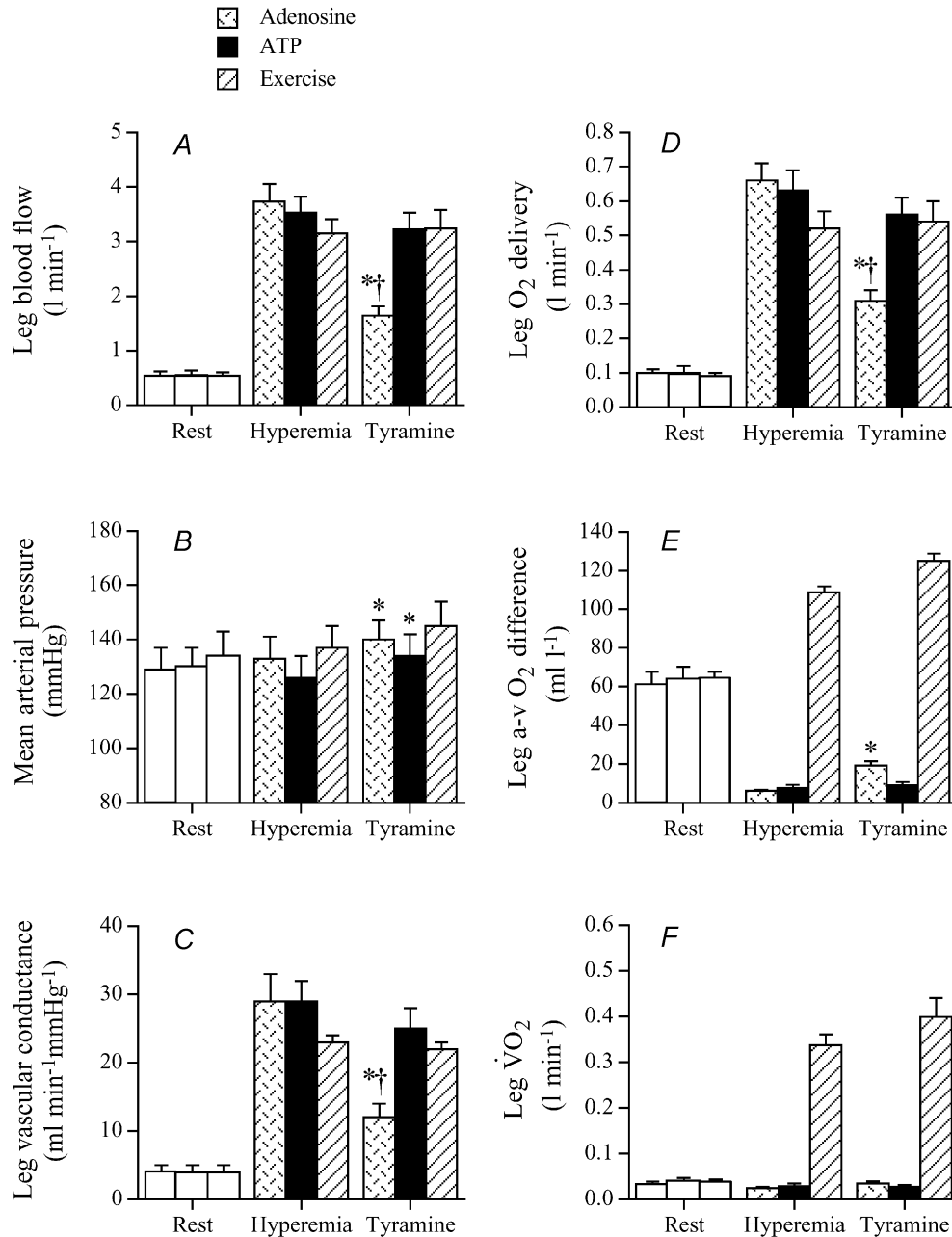


Figure 3. Leg haemodynamics and oxygenation during hyperaemia and superimposed α -adrenergic vasoconstrictor activity

Results were obtained at rest, during 3 different hyperaemic conditions (intrafemoral artery infusions of adenosine and ATP and mild knee-extensor exercise), and during the superimposition of the vasoconstrictor drug tyramine. A, leg blood flow; B, arterial blood pressure; C, leg vascular conductance; D, leg O₂ delivery; E, leg a-v O₂ difference; F, leg $\dot{V}O_2$. Data are means \pm s.e.m. for 8 subjects. *Significantly different from hyperaemia (*P* < 0.05). †Significantly different from combined ATP and tyramine and exercise and tyramine (*P* < 0.05).

significant difference (HSD) *post hoc* procedure. When appropriate, significant differences were also identified using Student's paired *t* tests. The significance level was set at $P < 0.05$. Data are presented as mean \pm S.E.M.

Results

Leg haemodynamic responses to tyramine infusion during hyperaemia

During pharmacologically induced vasodilatation, LBF increased to similar levels as during exercise hyperaemia ($\sim 3.6 \pm 0.3 \text{ l min}^{-1}$) from resting values of $\sim 0.5 \pm 0.1 \text{ l min}^{-1}$, while mean arterial pressure and

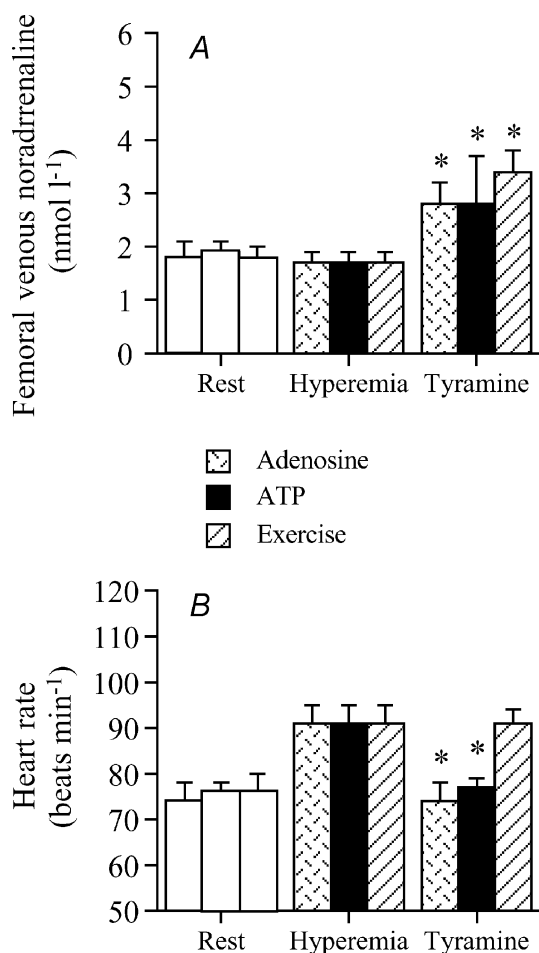


Figure 4. Circulating noradrenaline and heart rate during hyperaemia and superimposed α -adrenergic vasoconstrictor activity

Results were obtained at rest, during 3 different hyperaemic conditions (intrafemoral artery infusions of adenosine and ATP and mild knee-extensor exercise), and during the superimposition of the vasoconstrictor drug tyramine. A, femoral venous noradrenaline concentration; B, heart rate. Data are means \pm S.E.M. for 8 subjects.

*Significantly different from hyperaemia ($P < 0.05$).

arterial O_2 content remained unchanged. Hence, the elevations in leg vascular conductance and O_2 delivery during hyperaemia were proportional to the increases in LBF (Fig. 3). Tyramine infusion reduced LBF during adenosine infusion from 3.8 ± 0.3 to $1.7 \pm 0.2 \text{ l min}^{-1}$ and leg vascular conductance from 29 ± 4 to $12 \pm 4 \text{ ml min}^{-1} \text{ mmHg}^{-1}$ (both $P < 0.05$). In contrast during exercise and ATP infusion, tyramine did not alter either LBF ($P = 0.53$) or leg vascular conductance (Fig. 3). Reflecting the decline in LBF with combined infusion of tyramine and adenosine, leg a-v O_2 difference increased from $6.4 \pm 0.5 \text{ ml l}^{-1}$ with adenosine infusion to $20.1 \pm 2.2 \text{ ml l}^{-1}$ with combined adenosine and tyramine ($P < 0.05$; Fig. 3). Conversely, no change in leg a-v O_2 difference was observed with the addition of tyramine during ATP infusion (7.9 ± 1.7 and $9.5 \pm 1.6 \text{ ml l}^{-1}$, respectively; $P = \text{n.s.}$). However, both in the presence and absence of tyramine, leg \dot{V}_{O_2} was maintained at resting levels with adenosine and ATP infusions ($\sim 0.03 \text{ l min}^{-1}$) but increased ~ 10 -fold during exercise ($0.33 \pm 0.02 \text{ l min}^{-1}$) in association with almost a doubling in leg a-v O_2 difference from resting values (i.e. 109 ± 3 (exercise) versus $64 \pm 3 \text{ ml l}^{-1}$ (resting), respectively; Fig. 3). Femoral venous adrenaline was elevated from $\sim 1.7 \text{ nmol l}^{-1}$ during hyperaemia to $\sim 3.0 \text{ nmol l}^{-1}$ ($P < 0.05$) throughout all the tyramine infusions (Fig. 4), while noradrenaline (epinephrine) concentrations remained unchanged ($\sim 0.3 \text{ nmol l}^{-1}$). No significant changes in femoral venous and arterial ATP were observed during combined adenosine and tyramine infusion or during combined light exercise and tyramine infusion (0.7 – $1.0 \mu\text{mol l}^{-1}$). However, a trend for an elevation in plasma ATP was observed during combined ATP and tyramine infusion (from ~ 1.5 to $3.1 \mu\text{mol l}^{-1}$). During hyperaemia, heart rate was identical with adenosine, ATP and exercise ($91 \pm 4 \text{ beats min}^{-1}$) (Fig. 4).

Leg haemodynamic responses to combined tyramine, ATP and adenosine infusion during exercise

During the three exercise interventions, LBF increased to $\sim 3.4 \pm 0.3 \text{ l min}^{-1}$, whereas mean arterial pressure and arterial O_2 content remained unchanged compared to resting values. Therefore, leg vascular conductance and O_2 delivery increased in proportion to the elevation in LBF (Fig. 5). The superimposition of tyramine during exercise did not alter LBF or leg vascular conductance. However, the addition of ATP or adenosine during exercise and tyramine further increased LBF to $5.3 \pm 0.3 \text{ l min}^{-1}$ or $6.3 \pm 0.4 \text{ l min}^{-1}$, respectively ($P < 0.05$), whereas the

a-v O_2 difference declined from 125 to 129 (± 4) $ml l^{-1}$ during exercise + tyramine to $47 \pm 3 ml l^{-1}$ with addition of ATP and $60 \pm 3 ml l^{-1}$ with addition of adenosine. Thus, despite large differences in O_2 delivery among conditions, leg $\dot{V}O_2$ was maintained at $\sim 0.3 l min^{-1}$ during exercise by reciprocal changes in leg blood flow and a-v O_2 difference (Fig. 5). With combined tyramine and ATP or

adenosine infusions, femoral venous noradrenaline was significantly elevated ($2.9\text{--}4.7 nmol l^{-1}$; $P < 0.05$), being higher than values with ATP and adenosine infusion in the absence of tyramine ($1.7\text{--}2.4 nmol l^{-1}$; Fig. 6). Heart rate increased significantly when ATP and adenosine were infused during exercise but remained unchanged during tyramine infusion (Fig. 6).

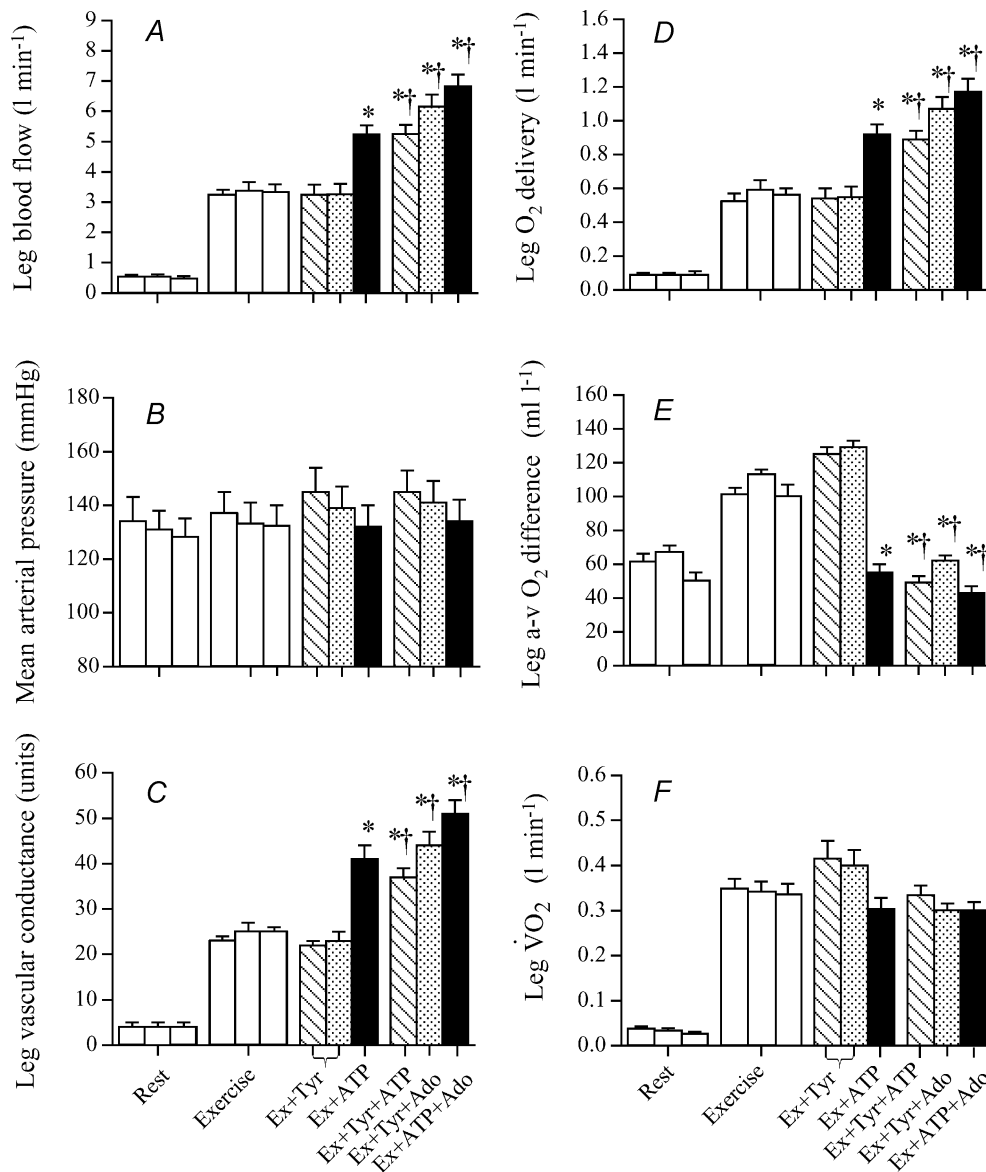


Figure 5. Leg haemodynamics and oxygenation during exercise with superimposed vasoconstrictor and vasodilator activities

Results were obtained at rest, during mild knee-extensor exercise (Ex), during the superimposition of the vasoconstrictor drug tyramine (Tyr) and during superimposition of ATP (Tyr + ATP) or adenosine (Tyr + Ado). Additionally, vasodilatory effects of ATP and adenosine during mild knee-extensor exercise without the presence of tyramine were ascertained (Ex + ATP and Ex + ATP + Ado). A, leg blood flow; B, arterial blood pressure; C, leg vascular conductance; D, leg O_2 delivery; E, leg a-v O_2 difference; F, leg $\dot{V}O_2$. Data are means \pm s.e.m. for 8 subjects. *Significantly different from exercise ($P < 0.05$). †Significantly different from exercise and tyramine ($P < 0.05$).

Vasodilatory and vasoconstrictor activities in the resting and maximally exercised leg

With a fixed-dose ATP infusion of $1 \mu\text{mol min}^{-1}$, total MSNA increased to $140 \pm 12\%$ ($n = 6$, $P < 0.05$). Incremental intrafemoral artery infusion of ATP in resting subjects resulted in dose-dependent increases in LBF, leg vascular conductance, circulating noradrenaline and total MSNA, all reaching a plateau at an infusion rate of $32 \mu\text{mol min}^{-1}$ (Figs 7 and 8). ATP infusion also resulted in progressive increases in heart rate with maintained mean arterial pressure. Femoral venous plasma ATP in the infused leg tended to decrease ($P = 0.067$) with

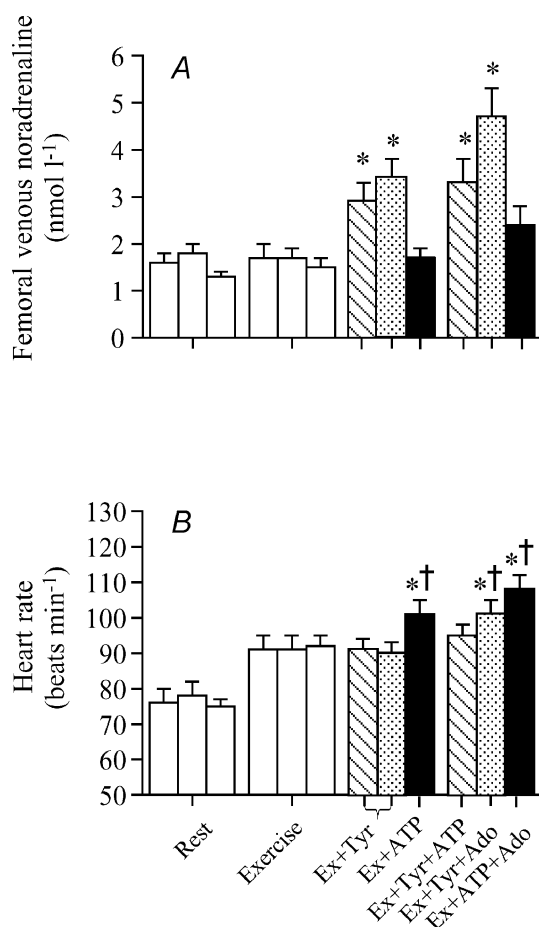


Figure 6. Circulating noradrenaline and heart rate during exercise with superimposed vasoconstrictor and vasodilator activities

Results were obtained at rest, during mild knee-extensor exercise (Ex), during the superimposition of the vasoconstrictor drug tyramine (Tyr) and during superimposition of ATP (Tyr + ATP) or adenosine (Tyr + Ado). Additionally, vasodilatory effects of ATP and adenosine during mild knee-extensor exercise without the presence of tyramine were ascertained (Ex + ATP and Ex + ATP + Ado). A, femoral venous noradrenaline concentration; B, heart rate. Data are means \pm S.E.M. for 8 subjects. *Significantly different from exercise ($P < 0.05$).

†Significantly different from exercise and tyramine ($P < 0.05$).

increasing ATP infusion rate, while arterial plasma ATP remained unchanged. During incremental cycling exercise, LBF and leg vascular conductance increased progressively and reached a peak value at 90% of peak power. In contrast, circulating noradrenaline increased exponentially after 60% of peak power was reached (Fig. 7). Maximal LBF during ATP infusion accounted for $78 \pm 2\%$ of the peak LBF during maximal exercise (7.2 ± 0.3 versus $9.3 \pm 0.7 \text{ l min}^{-1}$, respectively; $n = 4$). By comparison, femoral venous noradrenaline was 9-fold lower at peak vasodilatation during ATP infusion than during maximal bicycle exercise (6.1 ± 0.5 versus $59.9 \pm 8.7 \text{ nmol l}^{-1}$, respectively).

Discussion

A major finding of the present study is that ATP infusion in the resting leg fully blunts the effects of increased sympathetic vasoconstrictor activity in a manner similar to exercise, whereas adenosine infusion does not. Moreover, ATP infusion augments leg MSNA and circulating noradrenaline, but still evokes profound leg vasodilatation and elevates O_2 delivery to levels normally observed during intense dynamic leg exercise. Together, these novel observations indicate that circulating ATP by itself is sufficient to engender inhibition of sympathetic vasoconstriction in the skeletal muscle vasculature in the absence of metabolites from contracting muscle. This study also reveals that ATP infusion during exercise causes further vasodilatation and increases O_2 delivery to contracting skeletal muscle, regardless of the presence or absence of tyramine. Yet, increasing O_2 delivery to resting or exercising skeletal muscle when the O_2 supply is not limiting does not enhance muscle aerobic metabolism. Collectively, our results demonstrate that circulating ATP is capable of regulating blood flow and O_2 delivery in resting and exercising skeletal muscle by causing substantial vasodilatation, which offsets any concurrent increase in sympathetic vasoconstrictor drive.

Circulatory ATP attenuates sympathetic vasoconstriction in a manner similar to exercise while circulating adenosine does not

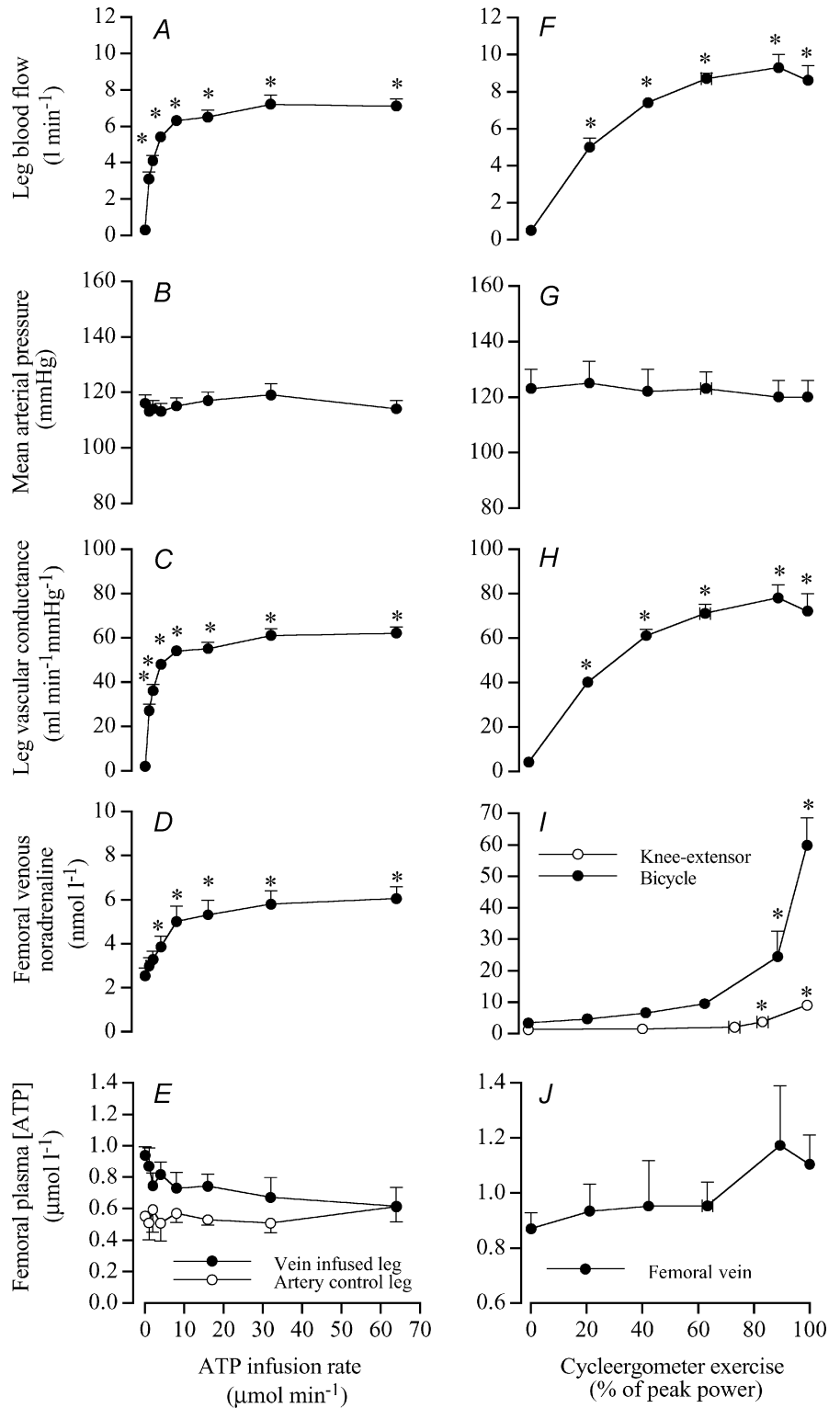
Despite augmented α -adrenoreceptor stimulation evoked by tyramine infusion, no reduction in blood flow or vascular conductance was observed during ATP infusion or exercise, yet a substantial vasoconstrictor response was seen during adenosine infusion. Leg a-v O_2 difference was also unchanged with a combined ATP and tyramine infusion but was elevated with a

combined adenosine and tyramine infusion such that leg \dot{V}_{O_2} was maintained at resting levels in both hyperaemic conditions. While modulation of α -adrenergic vasoconstriction has generally been associated with muscle contraction (McGillivray-Anderson & Faber, 1990;

Nishigaki *et al.* 1991; Ohyanagi *et al.* 1992; Hansen *et al.* 1996, 2000; Thomas & Victor, 1998; Thomas *et al.* 1998; Ruble *et al.* 2002; Tschakovsky *et al.* 2002; Chavoshan *et al.* 2002; Rosenmeier *et al.* 2003a,b; Dinneno & Joyner, 2003; Wray *et al.* 2004; Fadel *et al.* 2004), the present

Figure 7. Leg haemodynamics and circulating noradrenaline and ATP with ATP infusion and maximal exercise

Results were obtained during intrafemoral artery infusion of ATP in subjects seated upright ($n = 6$) and during incremental upright cycleergometer exercise to exhaustion ($n = 4$). *A*, leg blood flow with ATP infusion; *B*, arterial blood pressure with ATP infusion; *C*, leg vascular conductance with ATP infusion; *D*, femoral venous noradrenaline concentration with ATP infusion; *E*, femoral plasma ATP concentration with ATP infusion; *F*, leg blood flow during maximal exercise; *G*, arterial blood pressure during maximal exercise; *H*, leg vascular conductance during maximal exercise; *I*, femoral venous noradrenaline concentration during maximal exercise; *J*, femoral plasma ATP concentration during maximal exercise, *Significantly different from baseline control values ($P < 0.05$). Note that noradrenaline concentration during incremental one-legged knee-extensor exercise, which increases LBF from $0.4 \pm 0.1 \text{ l min}^{-1}$ at rest to $5.6 \pm 0.5 \text{ l min}^{-1}$ at peak power, is also depicted ($n = 8$).



investigation demonstrates a similar modulation in association with ATP infusion. This finding is compatible with a more universal role of ATP as a circulating metabolite, which might be involved in the maintenance or elevation of limb blood flow under conditions of elevated MSNA produced by exercise alone or exercise in combination with stressors such as hypoxia, CO-hypoxia, anaemia, heat stress and dehydration. For example, recent evidence demonstrates that blood flow to the resting leg is maintained despite a 2- to 4-fold elevation in MSNA with handgrip exercise and/or hypoxic hypoxia and CO-hypoxia (Hanada *et al.* 2003). Previous (González-Alonso *et al.* 2002) and present findings also indicate that blood flow in the control leg is maintained during ATP infusion despite a 2.5-fold

elevation in sympathetic activity. Moreover, blood flow to the exercising limbs is elevated with hypoxia despite the concurrent enhanced sympathoexcitation (González-Alonso *et al.* 2001, 2002; Dinneno & Joyner, 2003). As hypothesized in the introduction, a prerequisite for demonstrating a pivotal role of a metabolite in exercise hyperaemia and resting limb blood flow control is its dual ability to cause marked vasodilatation and inhibit α -adrenergic vasoconstriction. Clearly, ATP fulfils such requirements as the haemodynamic responses to ATP infusion mimicked exercise responses, while also allowing the maintenance of resting limb blood flow in the face of a sympathetic vasoconstrictor challenge. Therefore, the present results support a central role of circulating ATP in the attenuation of α -adrenergic vasoconstriction observed in exercising and resting skeletal muscle. Nevertheless, experiments reducing ATP levels in plasma and inhibiting the ATP receptor sites in the vascular endothelium are warranted to conclusively prove that endogenous ATP is the putative signal leading to inhibition of α -adrenergic vasoconstriction.

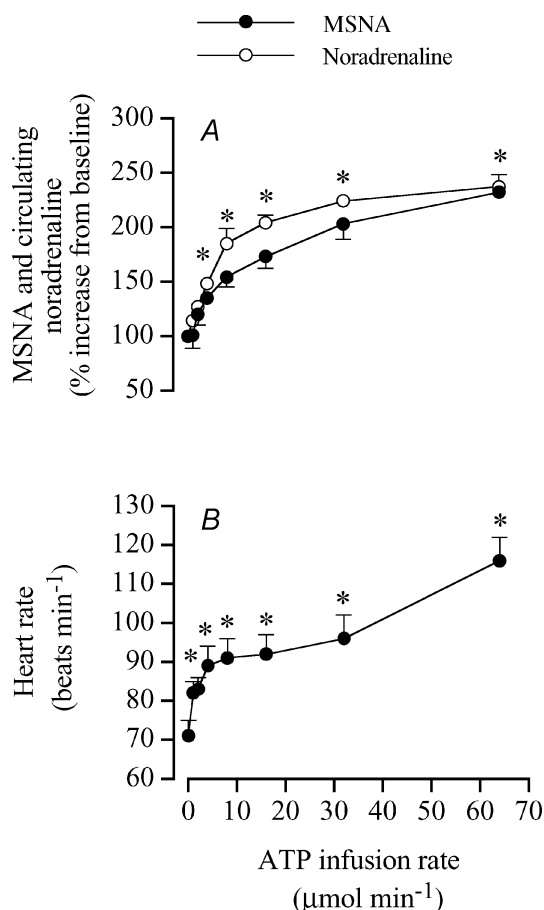


Figure 8. Muscle sympathetic nerve activity, circulating noradrenaline and heart rate with ATP infusion and maximal exercise

Results were obtained during intrafemoral artery infusion of ATP and during incremental cycle ergometer exercise to exhaustion. A, plasma noradrenaline concentration ($n = 5$); B, muscle sympathetic nerve activity (MSNA) ($n = 3$); C, heart rate ($n = 6$) during intrafemoral artery infusion of ATP. *Significantly different from baseline control values ($P < 0.05$).

ATP infusion mimics exercise-induced increases in skeletal muscle blood flow despite increasing MSNA

Another major finding of this study is that ATP infusion augments leg MSNA and circulating noradrenaline, while evoking profound leg vasodilatation and elevating O_2 delivery to levels normally observed during intense dynamic leg exercise in humans. The potency of ATP as a vasodilator *in vitro* and *in vivo* conditions is well documented (Folkow, 1949; Rongen *et al.* 1994; Ellsworth *et al.* 1995; González-Alonso *et al.* 2002) and is further supported by the present finding in healthy young subjects that peak LBF during ATP infusion is only 22% lower than the 9.31 min^{-1} peak LBF during maximal bicycling exercise, being similar to the maximal LBF values reported during one-legged cycling and one-legged knee-extensor exercise (Klausen *et al.* 1982; Andersen & Saltin, 1985). While its vasodilatory potency is well established, the capacity of circulating ATP to increase MSNA and circulating noradrenaline has not been reported to our knowledge. We have considered several possible underlying mechanisms for this increase. First, it is possible that ATP could directly activate the chemosensitive group III and IV afferent afferent nerve endings in the muscle interstitium also involved in the activation of the muscle metaboreflex (Mitchell *et al.* 1983; Li & Sinoway, 2002; Hanna *et al.* 2002; Hanna & Kaufman, 2003). However, femoral venous plasma ATP in the infused leg tended to decline during ATP infusion in association

with the increase in O_2 saturation from 70% at baseline to 97% at peak ATP infusion, while arterial ATP remained unchanged with maintained O_2 saturation ($\sim 98\%$). Thus, it is unlikely that ATP infusion exerted its sympathoexcitatory effect via direct increases in leg muscle interstitial ATP concentrations, because an elevation in extracellular and thus femoral venous [ATP] is required for this to happen (Mo & Ballard, 2001).

In the absence of an increase in femoral venous [ATP] it is also unlikely that the sympathoexcitatory actions of ATP involved direct effects on receptors located in the central nervous system or in the central circulation. Likewise, a dominant role of arterial baroreceptors seems improbable since ATP infusion did not reduce mean arterial pressure. More likely, indirect effects of ATP infusion causing unloading of cardiopulmonary baroreceptors might play a role, as the $\sim 7\text{ l min}^{-1}$ increase in LBF was quite probably mirrored by a parallel increase in cardiac output, as suggested by the simultaneous rise in heart rate, and possibly a reduction in central venous pressure. Results from experiments selectively depressing central venous pressure using low levels of lower body negative pressure strongly support the ability of cardiopulmonary baroreceptors to increase MSNA in the absence of changes in arterial blood pressure (Jacobsen *et al.* 1993). Although the precise mechanism underlying the ATP infusion-mediated sympathoexcitation requires further experimentation, the present findings clearly demonstrate the ability of circulating ATP to directly or indirectly augment MSNA (Fig. 9).

Interactions between tyramine, adenosine and ATP in contracting skeletal muscle

In the presence or absence of tyramine during exercise, ATP infusion evoked a remarkably similar elevation in blood flow and O_2 delivery to the exercising leg, even though circulating noradrenaline was different by a factor of 2. Similarly, adenosine infusion during exercise and tyramine infusion almost doubled blood flow and O_2 delivery, indicating that exercise completely nullified the effect of tyramine and concomitant elevated circulating noradrenaline seen with adenosine alone at rest. Intra-arterial infusion of adenosine evokes a rapid and potent dose-dependent vasodilatation in the leg to a magnitude comparable with that found here with ATP infusion (Rådegran & Calbet, 2001). Of note is that both adenine nucleotides cause a more potent vasodilatation when infused in the resting human leg than the infusion of the NO donor sodium nitroprusside or acetylcholine

($2\text{--}5\text{ l min}^{-1}$), and they do so without the profound hypotension produced by the latter vasodilators (Rådegran & Saltin, 1999). This makes adenosine an ideal enhancer of vasodilatation, together with ATP, as demonstrated in our study by the further increase in LBF with the combined infusion of ATP and adenosine as compared to infusion of ATP alone. However, it is important to note that the rate of adenosine infusion needed to achieve this effect was very high ($16\text{ 000 nmol min}^{-1}$) in comparison with the plasma adenosine concentrations during exercise ($10\text{--}20\text{ nmol l}^{-1}$) (Saito *et al.* 1999; Tune *et al.* 2000) and the $1000\text{ nmol min}^{-1}$ ATP infusion needed to increase LBF to the same level. This supports a contributory rather than essential role of adenosine in exercise hyperaemia, because ATP does not seem to be largely degraded to adenosine in the circulation as previously demonstrated using P_1 receptor blockade with theophylline during ATP infusion (Rongen *et al.* 1994). Adenosine blockade did not prevent ATP-induced vasodilatation, suggesting that ATP exerts its vasodilatory effect primarily via P_{2y} stimulation rather than P_1 stimulation via adenosine (Rongen *et al.* 1994). Together, these data show the capacity of circulating ATP to enhance vasodilatation and O_2 delivery in contracting skeletal muscle and the potential of circulating adenosine to augment this response.

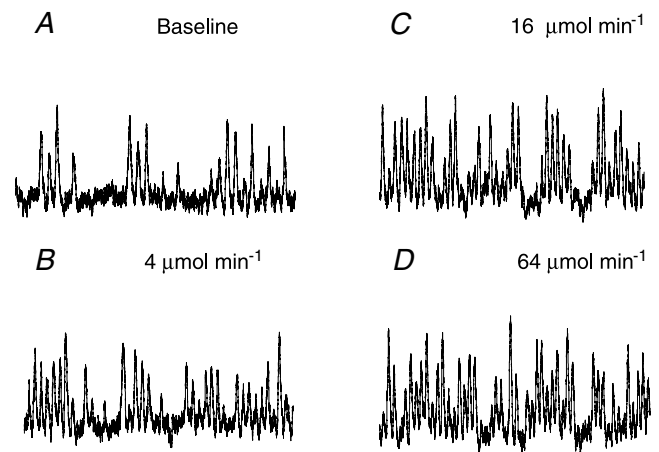


Figure 9. Representative recording of leg muscle sympathetic nerve activity

Segments of the original integrated neurogram (30 s) from one subject seated upright before (A) and during the infusion of 4 (B), 16 (C), and 64 (D) $\mu\text{mol min}^{-1}$ of ATP into the femoral artery. Note the marked increase in sympathetic nerve burst frequency from $39\text{ bursts min}^{-1}$ at baseline (mean \pm S.E.E. $43 \pm 2\text{ bursts min}^{-1}$) to $86\text{ bursts min}^{-1}$ with ATP infusion rate of $64\text{ }\mu\text{mol min}^{-1}$ (mean \pm S.E.M. $88 \pm 15\text{ bursts min}^{-1}$).

Modulation of sympathetic vasoconstriction by circulating ATP: potential mechanisms

Although the precise mechanism by which ATP overrides the increases in vasoconstrictor drive is not readily evident, the present difference in vasoconstrictor responses between ATP and adenosine at rest does not involve a differential reduction in presynaptic release of noradrenaline, since the venous noradrenaline increased to the same level during tyramine infusion in both conditions. Because neither ATP nor adenosine can readily cross the endothelium (Mo & Ballard, 2001), it is unlikely that ATP acts via direct modulation of α -adrenoreceptors located on vascular smooth muscle cells. Another argument against this direct pathway is that extraluminal ATP would cause vasoconstriction by binding to abundant vasoconstrictor P_{2X} purinergic receptors in vascular smooth muscle (Buckwalter *et al.* 2003), an unlikely possibility here given that blood flow and vascular conductance were unchanged with infusion of ATP and tyramine. Rather, the sympatho-inhibitory difference between these two related metabolites is probably due to activation of different signal transduction pathways and receptor types (Burnstock & Kennedy, 1986). On one hand, adenosine binds avidly to P_1 purinergic receptors inducing vasodilatation by releasing endothelial prostaglandin and/or NO (Ray *et al.* 2002). On the other hand, ATP binds strongly to P_2 purinergic receptors located on vascular endothelial cells inducing vasodilatation by triggering the release of endothelium-derived hyperpolarization factors (EDHF), NO and/or prostaglandins (Ellsworth *et al.* 1995; Wihlborg *et al.* 2003). Because prostaglandin has not been demonstrated to participate in functional sympatholysis in humans (Hansen *et al.* 2000; Frandsen *et al.* 2000), and since there still is conflicting evidence with regard to NO's role in this phenomenon (Rosenmeier *et al.* 2003b; Dinneno & Joyner, 2003; Buckwalter *et al.* 2004), the possibility exists that ATP primarily acts via EDHF and that this signalling pathway explains the distinctly different actions of ATP and adenosine. In support of this, the ATP-induced vasodilatation has been shown to be unaltered despite concomitant infusion of the NO synthase (NOS) inhibitor L-NMMA in the forearm (Rongen *et al.* 1994). Furthermore, EDHF has been shown to activate K_{ATP} channels located both on endothelial cells and the vascular smooth muscle cells (Brayden, 1990). These K_{ATP} channels have previously been implicated in exercise-induced attenuation of α -adrenergic vasoconstriction during exercise (Thomas *et al.* 1997). Of note, the K_{ATP} channel opener diazoxide is the only

other compound to our knowledge with the ability to abolish sympathetic vasoconstriction in quiescent skeletal muscle, as demonstrated in anaesthetized rats (Thomas *et al.* 1997). Future studies should elucidate if ATP exerts its dual vasodilatory and sympatholytic actions via EDHF or other unknown signal-transduction mechanism.

Integration of the vasoconstrictor and vasodilatory activities in the control of O_2 delivery to resting and contracting skeletal muscle: role of the erythrocyte

Compelling evidence in humans indicates that blood flow to contracting and resting skeletal muscle is exquisitely regulated to maintain O_2 delivery in a variety of conditions that drastically alter blood O_2 content and MSNA such as hypoxia, CO-hypoxia, hyperoxia, heat stress and dehydration (González-Alonso *et al.* 1998, 2001, 2002, 2004; González-Alonso & Calbet, 2003). Human studies independently manipulating the amount of O_2 circulating in arterial plasma (P_aO_2) and the amount of O_2 bound to haemoglobin (O_2Hb) with normoxia, hypoxia, hyperoxia, CO + normoxia and CO + hyperoxia also show that changes in blood flow, MSNA and circulating ATP are closely linked to alteration in O_2Hb but unrelated to large changes in P_aO_2 (i.e. 40–600 mmHg) (González-Alonso *et al.* 2001, 2002; Hanada *et al.* 2003). This suggests that the main vascular O_2 -sensing locus is located in the erythrocyte itself and that the red blood cell senses and signals its O_2 availability, thereby matching O_2 delivery to tissue O_2 demand (Ellsworth *et al.* 1995; Dietrich *et al.* 2000; Jagger *et al.* 2001; González-Alonso *et al.* 2002; Hanada *et al.* 2003; Ellsworth, 2004). The present observation that increasing O_2 delivery to resting or exercising skeletal muscle does not enhance muscle aerobic metabolism implies that excessive O_2 delivery has no beneficial effects in muscle, but rather could be counterproductive as it unnecessarily taxes the heart. In this context, the O_2 -sensing and -signalling erythrocyte provides an optimal mechanism to regulate local skeletal muscle blood flow and O_2 delivery by releasing ATP into the circulation in direct proportion to the number of unoccupied O_2 binding sites in the haemoglobin molecule (Jagger *et al.* 2001; González-Alonso *et al.* 2002). Although human experiments blocking ATP release from the erythrocyte and inhibiting the ATP receptor sites in the vascular endothelium need to be performed to conclusively prove a role for circulating ATP and the erythrocyte in skeletal muscle hyperaemia, available *in vitro* data strongly support this theory by demonstrating that: (1) ATP is

released from red blood cells with exposure to hypoxia in the presence of hypercapnia (Bergfeld & Forrester, 1992), hypoxia alone (Ellsworth *et al.* 1995) and mechanical deformation (Sprague *et al.* 1996), and (2) ATP infused locally in first- and second-order arterioles produces a potent vasodilatory response which is conducted upstream (Ellsworth *et al.* 1995; McCullough *et al.* 1997; Ellsworth, 2004). Furthermore, the finding presented here that in healthy humans circulating ATP is capable of modulating sympathetic vasoconstriction supports a novel role of ATP released from the erythrocyte as a modulator of sympathetic vasoconstriction in exercising or resting skeletal muscle. The present observations in normal humans may have broader implications for our understanding of disordered neurocirculatory control in disease states accompanied by excessive muscle hypoxia and intense sympathetic activation at rest or during exercise, such as severe pulmonary disease, congestive heart failure or in cardiogenic shock.

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