Graded sympatholytic effect of exogenous ATP on postjunctional α-adrenergic vasoconstriction in the human forearm: implications for vascular control in contracting muscle

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Recent evidence suggests that adenosine triphosphate (ATP) can inhibit vasoconstrictor responses to endogenous noradrenaline release via tyramine in the skeletal muscle circulation, similar to what is observed in contracting muscle. Whether this involves direct modulation of postjunctional α-adrenoceptor responsiveness, or is selective for α_1- or α_2-receptors remains unclear. Therefore, in Protocol 1, we tested the hypothesis that exogenous ATP can blunt direct postjunctional α-adrenergic vasoconstriction in humans. We measured forearm blood flow (FBF; Doppler ultrasound) and calculated the vascular conductance (FVC) responses to local intra-arterial infusions of phenylephrine (α_1-agonist) and dexmedetomidine (α_2-agonist) during moderate rhythmic handgrip exercise (15% maximum voluntary contraction), during a control non-exercise vasodilator condition (adenosine), and during ATP infusion in eight young adults. Forearm hyperaemia was matched across all conditions. Forearm vasoconstrictor responses to direct α_1-receptor stimulation were blunted during exercise *versus* adenosine (ΔFVC $= -11 \pm 3\%$ *versus* $-39 \pm 5\%$; $P < 0.05$), and were abolished during ATP infusion ($-3 \pm 2\%$). Similarly, vasoconstrictor responses to α_2-receptor stimulation were blunted during exercise *versus* adenosine ($-13 \pm 4\%$ *versus* $-40 \pm 8\%$; $P < 0.05$), and were abolished during ATP infusion ($-4 \pm 4\%$). In Protocol 2 ($n = 10$), we tested the hypothesis that graded increases in ATP would reduce α_1-mediated vasoconstriction in a dose-dependent manner compared with vasodilatation evoked via adenosine. Forearm vasoconstrictor responses during low dose adenosine ($-38 \pm 3\%$) and ATP ($-33 \pm 2\%$) were not significantly different from rest ($-40 \pm 3\%$; $P > 0.05$). In contrast, vasoconstrictor responses during moderate ($-22 \pm 6\%$) and high dose ATP ($-8 \pm 5\%$) were significantly blunted compared with rest, whereas the responses during adenosine became progressively greater (moderate $= -48 \pm 4\%$, $P = 0.10$; high $= -53 \pm 6\%$, $P < 0.05$). We conclude that exogenous ATP is capable of blunting direct postjunctional α-adrenergic vasoconstriction, that this involves both α_1- and α_2-receptor subtypes, and that this is graded with ATP concentrations. Collectively, these data are consistent with the conceptual framework regarding how muscle blood flow and vascular tone are regulated in contracting muscles of humans.

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Blood flow and oxygen delivery increase in proportion to the oxygen demand of contracting skeletal muscle, a complex response involving competing influences of local vasodilator signals and sympathetic neural vasoconstriction particularly as the intensity of exercise and amount of muscle mass engaged increase (Saltin *et al.* 1998). Although it is clear that sympathetic restraint of active muscle blood flow is imperative for appropriate blood pressure regulation (Marshall *et al.* 1961; Rowell, 1997), it has also been repeatedly demonstrated that the vasoconstrictor responses in contracting muscle are significantly blunted compared with the responses under resting (quiescent) conditions (Remensnyder *et al.* 1962; Anderson & Faber, 1991; Thomas & Victor, 1998;

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Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003). A variety of substances/mechanisms have been proposed in this local modulation of sympathetic vasoconstriction including activation of ATP-sensitive potassium channels (K_{ATP}) (Thomas *et al.* 1997; Keller *et al.* 2004), adenosine (Nishigaki *et al.* 1991), nitric oxide (NO) (Thomas & Victor, 1998; Chavoshan *et al.* 2002), and vasodilating prostaglandins (PGs) (Faber *et al.* 1982). However, recent data indicate that independent inhibition of NO and PGs does not influence the ability of contractions to blunt sympathetic vasoconstriction (Dinenno & Joyner, 2003, 2004), and that combined inhibition of these substances only slightly augments the constrictor response to α-adrenoceptor stimulation (Dinenno & Joyner, 2004). Further, exogenous infusion of adenosine to mimic exercise hyperaemia does not blunt sympathetic vasoconstriction (Tschakovsky *et al.* 2002). Thus, identifying *the* sympatholytic factor associated with muscle contractions has proven difficult.

It has recently been proposed that circulating adenosine triphosphate (ATP) could be involved in matching muscle perfusion to oxygen demand during exercise (Ellsworth, 2000; Gonzalez-Alonso *et al.* 2002; Ellsworth, 2004). Although ATP can be produced in many cells, one source of ATP release during mismatches in oxygen delivery and demand appears to be the red blood cell, where ATP release is coupled with the level of deoxygenated haemoglobin (Ellsworth, 2000, 2004; Jagger *et al.* 2001; Gonzalez-Alonso *et al.* 2002), and can also be augmented by hypercapnia, acidosis and mechanical deformation (Bergfeld & Forrester, 1992; Ellsworth *et al.* 1995; Sprague *et al.* 1998). In addition to directly evoking vasodilatation via binding to P_{2y}-receptors on the endothelium (Burnstock & Kennedy, 1986; Rongen *et al.* 1994), work by Rosenmeier *et al.* (2004) indicate that ATP could assist in matching oxygen delivery with tissue demand by modulating local sympathetic vasoconstrictor tone. Indeed, data derived from this study suggest that circulating ATP can override sympathetic vasoconstriction in the leg circulation evoked via intra-arterial administration of tyramine (evokes endogenous noradrenaline (NA) release), similar to that observed in contracting muscle (Rosenmeier *et al.* 2004). This occurred despite similar increases in femoral venous NA concentrations, suggesting that this modulatory effect of ATP is at the level of postjunctional α-adrenoceptors. However, it must be emphasized that changes in venous NA concentrations do not always accurately reflect NA release from sympathetic nerve endings especially when there are marked changes in regional blood flow (Esler *et al.* 1990). Therefore, whether circulating ATP modulates direct postjunctional α-adrenoceptor responsiveness and whether this is selective for α_1- or α_2-adrenoceptors is unclear.

An additional question related to the role of circulating ATP in modulating sympathetic vasoconstriction is whether this is graded with the levels of circulating ATP. In this context, several studies utilizing isolated limb models have clearly demonstrated that the magnitude of sympatholysis during exercise is graded with exercise intensity, such that mild levels of muscle contraction ($< 10\%$ maximal effort) do not interfere with sympathetic vasoconstriction whereas progressive increases in exercise intensity above this level lead eventually to robust blunting of the vasoconstrictor response (Thomas *et al.* 1994; Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002; Kirby *et al.* 2005). Thus, if circulating ATP does indeed play a role in muscle blood flow regulation during contractions, one would predict that low levels of ATP are not sympatholytic, whereas increasing circulating levels of ATP would lead to a progressive reduction in sympathetic vasoconstrictor responsiveness. This is an important hypothesis to test, as the only data regarding how ATP interacts directly with sympathetic vasoconstriction indicate that ATP completely overrides the vasoconstrictor response (Rosenmeier *et al.* 2004). As such, if ATP release from red blood cells occurred in all active muscle during high intensity large muscle mass exercise, and this completely abolishes sympathetic vasoconstriction as recently demonstrated (Rosenmeier *et al.* 2004), the vasodilatory capacity of exercising muscle would outstrip cardiac pumping capacity and arterial pressure would fall (Joyner & Thomas, 2003; Calbet *et al.* 2004). In healthy humans, this does not occur.

Therefore, in the present study we tested the hypothesis that exogenous ATP infusion blunts direct postjunctional α-adrenergic vasoconstriction in humans and determined whether this is selective for α_1- or α_2-adrenoceptors. Further, given that the ability of muscle contractions to blunt a known sympathetic vasoconstrictor stimulus is graded with exercise intensity, we also tested the hypothesis that graded increases in arterial ATP concentrations would cause graded inhibition of sympathetic α-adrenergic vasoconstriction.

Methods

Subjects

With Institutional Review Board approval and after written informed consent, a total of 18 young healthy adults (12 men, 6 women; age $= 22 \pm 1$ years; weight $= 72.6 \pm 3.0$ kg; height $= 176 \pm 2$ cm; body mass index $= 23.1 \pm 0.7$ kg m^{-2}; means $\pm$ S.E.M.) participated in the present study. All were non-smokers, non-obese, normotensive, and not taking any medications. Studies were performed after a 4 h fast with the subjects in the supine position. All studies were performed according to the *Declaration of Helsinki*.

Arterial catheterization

A 20 gauge, 7.6 cm catheter was placed in the brachial artery of the non-dominant arm under aseptic conditions after local anaesthesia (2% lidocaine) for local administration of study drugs. The catheter was connected to a 3-port connector as well as a pressure transducer for mean arterial pressure (MAP) measurement and continuously flushed at 3 ml h^{-1} with heparinized saline (Dinenno & Joyner, 2003, 2004; Dinenno *et al.* 2005). The two side ports were used for infusions of vasoactive drugs.

Forearm blood flow and vascular conductance

A 4 MHz pulsed Doppler probe (Model 500V, Multigon Industries, Mt Vernon, NY, USA) was used to measure brachial artery mean blood velocity (MBV) with the probe securely fixed to the skin over the brachial artery proximal to the catheter insertion site as previously described by our laboratory (Dinenno & Joyner, 2003, 2004; Dinenno *et al.* 2005). The probe insonation angle relative to the skin was 45 deg. A linear 12 MHz echo Doppler ultrasound probe (GE Vingmed Ultrasound Vivid7, Horten, Norway) was placed in a holder securely fixed to the skin immediately proximal to the velocity probe to measure brachial artery diameter. Forearm blood flow was calculated as:

$$\text{FBF} = \text{MBV} \times \pi(\text{brachial artery diameter}/2)^2 \times 60,$$

where the FBF is in ml min^{-1}, the MBV is in cm s^{-1}, the brachial diameter is in cm, and 60 is used to convert from ml s^{-1} to ml min^{-1}. Forearm vascular conductance (FVC) was calculated as (FBF/MAP) × 100, and expressed as ml min^{-1} (100 mmHg)$^{-1}$.

Rhythmic handgrip exercise

Maximum voluntary contraction (MVC) was determined for each subject as the average of at least three maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL, USA) that were within 3% of each other. For the exercise trials, weights corresponding to 15% MVC were attached to a pulley system and lifted 4–5 cm over the pulley at a duty cycle of 1 s contraction–2 s relaxation (20 contractions per minute) using audio and visual signals to ensure the correct timing (Dinenno & Joyner, 2003, 2004). We chose this moderate workload because it significantly blunts, but does not abolish, sympathetic vasoconstriction in contracting muscle (Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003; Dinenno *et al.* 2005).

Sympathetic α-adrenergic vasoconstrictor drugs

In male subjects, phenylephrine (a selective α_1-agonist; Baxter, Irvine, CA, USA) was infused at 0.0625 μg (dl forearm volume)$^{-1}$ min^{-1} and dexmedetomidine (a selective α_2-agonist; Hospira, Lake Forest, IL, USA) was infused at 6.25 ng (dl forearm volume)$^{-1}$ min^{-1}. The doses of phenylephrine and dexmedetomidine were chosen based on our experience at rest (Dinenno *et al.* 2002; Smith *et al.* 2007) and during handgrip exercise (Dinenno & Joyner, 2003, 2004; Rosenmeier *et al.* 2003*a*). Because young women typically have reduced vasoconstrictor responses to α-receptor stimulation (Kneale *et al.* 2000), the doses of phenylephrine and dexmedetomidine were doubled for the female participants. All vasoconstrictor drug infusions were adjusted for the hyperaemic conditions as previously described (see below) (Dinenno & Joyner, 2003, 2004).

Given that exercise increases forearm blood flow, adenosine was infused to elevate resting forearm blood flow to similar levels observed during exercise. We have previously demonstrated that exercise blunts the vasoconstrictor responses to direct α_1- and α_2-adrenoceptor stimulation, whereas these vasoconstrictor responses are maintained when blood flow is elevated with adenosine and hence it was used to create a 'high flow' control state (Dinenno & Joyner, 2003, 2004; Rosenmeier *et al.* 2003*a*). In an effort to normalize the concentration of each vasoconstricting drug in the blood perfusing the forearm, the infusions were adjusted on the basis of forearm blood flow and forearm volume (measured via regional analysis of whole-body DEXA scans). Various concentrations of each compound were available to keep the absolute infusion rates less than 3 ml min^{-1} in every trial.

Experimental protocols

General experimental protocol. Figure 1 is an example of a time-line for the specific trials. In the supine position, subjects either performed a bout of handgrip exercise, or received intra-arterial adenosine (Sicor, Irvine, CA, USA) or ATP (Sigma, USA); the total time for each trial was 8 min. After 2 min of baseline measurements, exercise or vasodilator infusion was initiated and steady-state FBF was reached within 3 min. Between 3 and 4 min of hyperaemia (minutes 5 and 6 of Fig. 1) the dose of the α_1- or α_2-agonist (vasoconstrictor) was calculated on the basis of forearm volume and blood flow. The vasoconstrictor infusion began at the 6-minute mark and lasted for 2 min.

Protocol 1. Effects of exogenous ATP on postjunctional α-adrenergic vasoconstrictor responsiveness. The purpose of this protocol was to determine whether exogenous ATP blunts direct postjunctional α-adrenergic responsiveness, and whether this is selective for α_1- or α_2-adrenoceptors. Therefore, in eight

subjects (6 men, 2 women), the vasoconstrictor responses to direct α_1- and α_2-adrenoceptor stimulation (via phenylephrine and dexmedetomidine, respectively) were assessed during control vasodilator infusion of adenosine, during moderate rhythmic handgrip exercise (15% MVC), and during infusion of ATP. In total, there were six experimental trials for each subject. In this protocol, the goal was to match steady-state FBF during infusion of adenosine or ATP with that observed during exercise. To do so, adenosine (45 nmol (dl forearm volume)$^{-1}$ min^{-1}) and ATP (5 nmol (dl forearm volume)$^{-1}$ min^{1}) were initially infused and doses were increased to elevate FBF accordingly. The final average doses of adenosine and ATP were 73 ± 8 and 11 ± 2 nmol (dl forearm volume)$^{-1}$ min^{-1}, respectively. The order of the adenosine, exercise and ATP trials were counterbalanced across subjects. Thus, for subjects that did not perform the exercise trial first, we had them perform 3–4 min of rhythmic handgrip exercise prior to any experimental trials with α-agonists to determine their individual steady-state FBF for this exercise intensity. Additionally, in one-half of the subjects, vasoconstrictor responses to α_1-adrenoceptor stimulation were determined under each hyperaemic condition, followed by the trials for α_2-receptor stimulation. This order was reversed in the other four subjects, and all subjects rested for 15 min between each trial.

Protocol 2. Effects of graded infusions of ATP on postjunctional α-adrenergic vasoconstrictor responsiveness. The ability of muscle contractions to blunt a known sympathetic vasoconstrictor stimulus is graded with exercise intensity, such that greater inhibition of α-mediated vasoconstriction (greater sympatholysis) is observed with increasing workloads (Thomas *et al.* 1994; Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002; Kirby *et al.* 2005). Therefore, the purpose of this protocol was to determine whether graded increases in exogenous ATP caused graded sympatholysis as has been observed during exercise. In 10 subjects (6 men, 4 women), we determined the vasoconstrictor responses to direct α_1-adrenoceptor stimulation via phenylephrine at rest (saline control), as well as during graded increases in ATP and adenosine. In total, there were seven experimental trials for each subject and each trial was performed in a similar fashion as outlined in Protocol 1. For this protocol, the doses of ATP were calculated (based on resting forearm blood flow) to increase arterial concentrations by 500, 1000 and 2000 nmol l^{-1} ('low', 'moderate', and 'high') provided no ATP degradation were to occur, and this was based on data obtained from the femoral vein during graded knee extensor exercise (Gonzalez-Alonso *et al.* 2002). Similar to Protocol 1, we infused adenosine as a control vasodilator at concentrations required to match the increase in FBF evoked via these doses of ATP. Thus, to do so, ATP trials were always performed prior to adenosine trials, but the order of ATP and adenosine doses (low, moderate, and high) were counterbalanced across subjects. All trials were separated by 15 min of rest.

Data acquisition and analysis

Data were collected and stored on computer at 250 Hz and analysed off-line with signal-processing software (WinDaq, DATAQ Instruments, Akron, OH, USA). Mean arterial pressure (MAP) was determined from the arterial pressure waveform. Baseline FBF, HR and MAP represent an average of the last minute of the resting time period, the steady-state hyperaemic values represent an average of minutes 3–4 (minutes 5–6 of Fig. 1; pre-vasoconstrictor) during exercise, adenosine, or ATP, and the effects of

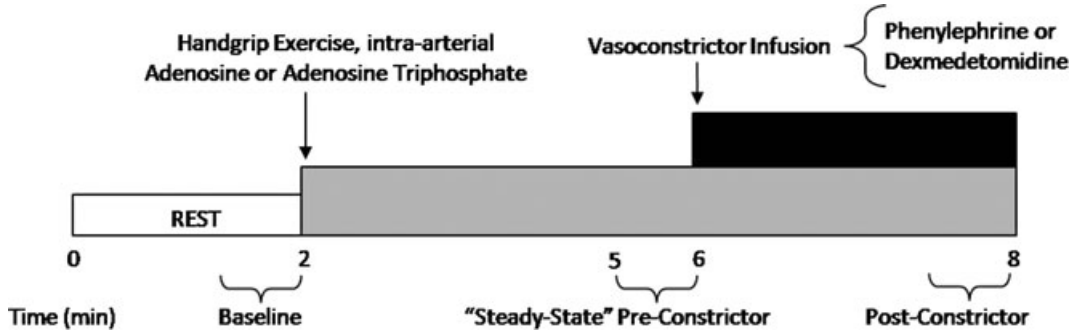

Figure 1. General experimental trial
Each trial consisted of a 2 min baseline period. After this time period, subjects either began rhythmic handgrip exercise or received intra-arterial adenosine or adenosine triphosphate (ATP) to elevate resting forearm blood flow to levels observed during exercise. During minutes 5–6 (pre-constrictor), the doses of the α_1- or α_2-adrenoceptor agonists (phenylephrine or dexmedetomidine, respectively) were calculated on the basis of steady-state hyperaemic forearm blood flow and forearm volume. Subsequently, the α-agonist was infused for 2 min until minute 8. An average of forearm blood flow and mean arterial blood pressure during the final 30 s of α-agonist infusion was used to calculate the vasoconstrictor effect during all hyperaemic conditions.

Table 1 Protocol 1. Forearm and systemic haemodynamics

Condition	Forearm blood flow (ml min^{-1})	Mean arterial pressure (mmHg)	Forearm vascular conductance (ml min^{-1} (100 mmHg)$^{-1}$)	Heart rate (beats min^{-1})
A. Phenylephrine trials				
Baseline				
Adenosine	30 ± 4	92 ± 3	32 ± 4	56 ± 3
Exercise	26 ± 4	94 ± 3	28 ± 4	55 ± 4
ATP	29 ± 5	92 ± 2	31 ± 5	54 ± 4
Pre-phenylephrine				
Adenosine	143 ± 16*	93 ± 3	156 ± 19*	57 ± 3
Exercise	154 ± 13*	96 ± 3	160 ± 12*	60 ± 4*
ATP	138 ± 19*	94 ± 2	149 ± 21*	56 ± 3
Phenylephrine				
Adenosine	89 ± 9*†	98 ± 3*	92 ± 10*†	56 ± 3
Exercise	136 ± 12*†‡	96 ± 3	141 ± 10*†‡	60 ± 3*
ATP	133 ± 19*‡	93 ± 1	143 ± 21*‡	54 ± 3
B. Dexmedetomidine trials				
Baseline				
Adenosine	27 ± 4	95 ± 2	28 ± 4	53 ± 3
Exercise	30 ± 6	92 ± 3	33 ± 7	54 ± 4
ATP	30 ± 6	97 ± 2	31 ± 6	54 ± 3
Pre-dexmedetomidine				
Adenosine	141 ± 25*	98 ± 3	144 ± 25*	54 ± 4
Exercise	150 ± 16*	94 ± 2	158 ± 15*	57 ± 4*
ATP	153 ± 20*	95 ± 2	162 ± 21*	54 ± 3
Dexmedetomidine				
Adenosine	85 ± 15*†	101 ± 3	84 ± 15*†	55 ± 3
Exercise	132 ± 12*†‡	95 ± 2	136 ± 12*†‡	57 ± 4*
ATP	152 ± 23*‡	96 ± 2	159 ± 24*‡	53 ± 3

*$P < 0.05$ *vs* baseline within condition; †$P < 0.05$ *vs* steady-state (Pre-vasoconstrictor; Phenylephrine/Dexmedetomidine) within condition; ‡$P < 0.05$ *vs* adenosine during α-agonist infusion. Forearm vascular conductance was calculated as (forearm blood flow/mean arterial pressure) × 100.

the α-agonists represent an average of the final 30 s of drug infusion (post-vasoconstrictor). The percentage reduction in FBF during vasoconstrictor administration was calculated as:

$$((\mathrm{FBF}_{\text{post-constrictor}} - \mathrm{FBF}_{\text{pre-constrictor}}) / (\mathrm{FBF}_{\text{pre-constrictor}})) \times 100.$$

We also calculated percentage reduction in FVC as our standard index to compare vasoconstrictor responses to the α-agonists across conditions, as this appears to be the most appropriate way to compare vasoconstrictor responsiveness under conditions where there might be differences in vascular tone (Lautt, 1989; O'Leary, 1991; Thomas *et al.* 1994; Tschakovsky *et al.* 2002). In an effort to be comprehensive, we have also presented absolute values of forearm haemodynamics for all conditions in tabular form.

Statistics

All values are reported as means ± S.E.M. Specific hypothesis testing within each of the exercise, adenosine, or ATP trials with the two different α-agonist infusions was performed using repeated measures ANOVA. Comparison of the haemodynamic values at specific time points between the exercise, adenosine and ATP conditions was made with Student's *t* test for unpaired data, and the values within each hyperaemic condition (exercise, adenosine, or ATP) with Student's *t* test for paired data. Significance was set at $P < 0.05$.

Results

Protocol 1. Effects of exogenous ATP on postjunctional α-adrenergic vasoconstrictor responsiveness

Forearm haemodynamics, HR, and MAP for Protocol 1 are presented in Table 1. Intra-arterial infusion

of both adenosine and ATP, as well as handgrip exercise, significantly increased FBF and FVC from baseline ($P < 0.05$). As desired by experimental design, steady-state (pre-vasoconstrictor) FBF responses to adenosine and ATP infusion were effectively matched to that observed during 15% MVC handgrip exercise within both phenylephrine (Fig. 2*A*) and dexmedetomidine conditions (Fig. 2*B*; $P = 0.5$–0.9). Infusion of phenylephrine (α_1-agonist) significantly reduced FBF from steady-state hyperaemia during adenosine and exercise ($P < 0.05$), whereas FBF was unchanged during ATP (NS; Fig. 2*A*). Similarly, infusion of dexmedetomidine (α_2-agonist) significantly reduced FBF from steady-state hyperaemia during adenosine and exercise ($P < 0.05$), whereas FBF was unchanged during ATP (NS; Fig. 2*B*).

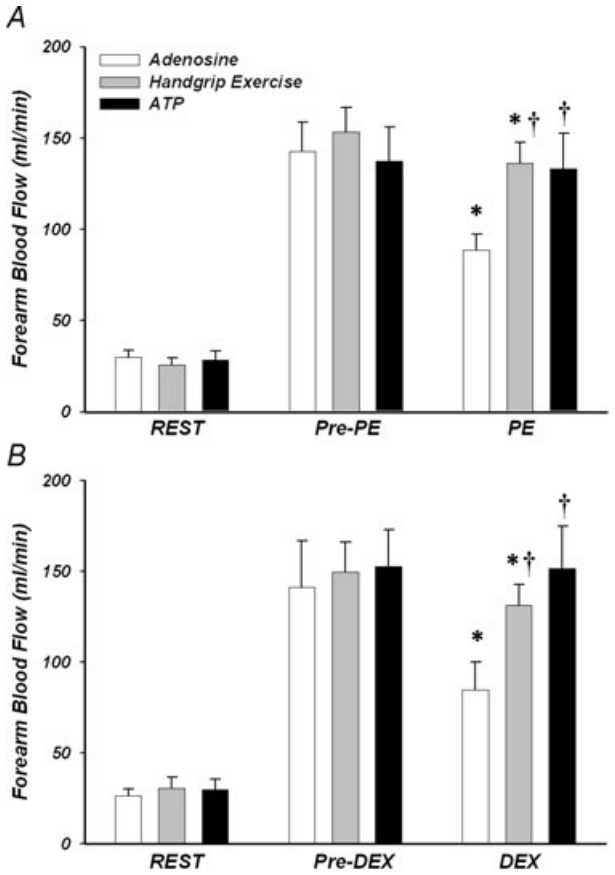

Figure 2. Forearm blood flow at rest, during each hyperaemic condition and during infusion of α-agonists
Steady-state hyperaemia was similar during rhythmic handgrip exercise, adenosine and ATP infusions for trials involving the α_1-agonist phenylephrine (*A*; Pre-PE) and the α_2-agonist dexmedetomidine (*B*; Pre-Dex). Forearm blood flow was reduced significantly with both α-agonists during adenosine and exercise, but the response was attenuated during exercise. In contrast, α-agonist infusion did not significantly reduce forearm blood flow during ATP. $^*P < 0.05$ *vs.* steady state (Pre-vasoconstrictor; PE/Dex) within condition; †$P < 0.05$ *vs.* adenosine during α-agonist infusion.

The forearm vasoconstrictor responses to direct α_1-adrenoceptor stimulation were blunted during steady-state exercise *versus* adenosine (ΔFVC = –11 ± 3% *versus* −39 ± 5%; $P < 0.05$), and were abolished during ATP infusion (−3 ± 2%; $P = 0.2$ *versus* zero; Fig. 3*A*). Similarly, vasoconstrictor responses to α_2-receptor stimulation were blunted during exercise *versus* adenosine (−13 ± 4% *versus* −40 ± 8%; $P < 0.05$), and were abolished during ATP infusion (−4 ± 4%; $P = 0.5$ *versus* zero; Fig. 3*B*). MAP changed minimally within and between conditions (Table 1A and B), thus FBF responses were similar to FVC. Heart rate increased in response to exercise ($P < 0.05$), but otherwise was not significantly between or within trials and conditions (Table 1).

Protocol 2. Effects of graded infusions of ATP on postjunctional α-adrenergic vasoconstrictor responsiveness

Forearm haemodynamics, HR, and MAP for Protocol 2 are presented in Table 2. Graded increases in exogenous

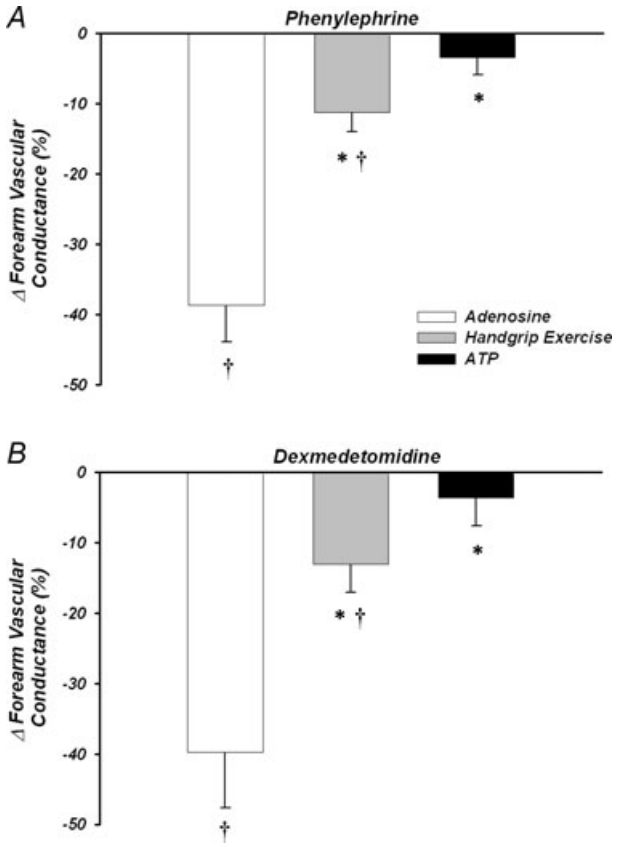

Figure 3. Forearm vasoconstrictor responses to α_1- and α_2-adrenoceptor stimulation
Percentage reductions in forearm vascular conductance to phenylephrine (α_1-agonist) (*A*) were significantly blunted during exercise and abolished during ATP compared with adenosine infusions. Similar data were obtained in response to α_2-adrenoceptor stimulation via dexmedetomidine (*B*). $^*P < 0.05$ *vs.* adenosine; †$P < 0.05$ *vs.* zero.

Table 2 Protocol 2. Forearm and systemic haemodynamics during graded infusions of ATP and adenosine

Condition/dose	Forearm blood flow (ml min^{-1})	Mean arterial pressure (mmHg)	Forearm vascular conductance (ml min^{-1} (100 mmHg)$^{-1}$)	Heart rate (beats min^{-1})
Baseline				
Adenosine 1	21 ± 4	86 ± 3	25 ± 5	58 ± 5
ATP 1	23 ± 3	86 ± 3	27 ± 4	59 ± 5
Adenosine 2	23 ± 3	86 ± 3	27 ± 4	58 ± 5
ATP 2	22 ± 3	86 ± 3	26 ± 4	59 ± 4
Adenosine 3	23 ± 3	86 ± 3	28 ± 4	60 ± 5
ATP 3	24 ± 3	86 ± 3	28 ± 4	59 ± 5
Pre-phenylephrine				
Adenosine 1	51 ± 8*	86 ± 3	61 ± 9*	60 ± 5
ATP 1	50 ± 9*	84 ± 3	61 ± 12*	58 ± 5
Adenosine 2	66 ± 9*	87 ± 2	77 ± 11*	59 ± 5
ATP 2	66 ± 9*	86 ± 3	78 ± 12*	59 ± 5
Adenosine 3	90 ± 12*	87 ± 3	105 ± 14*	60 ± 5
ATP 3	88 ± 13*	84 ± 3	106 ± 17*	59 ± 5
Phenylephrine				
Adenosine 1	33 ± 6*†	88 ± 4	38 ± 7*†	60 ± 5
ATP 1	35 ± 7*†	87 ± 3	41 ± 9*†	59 ± 5
Adenosine 2	36 ± 6*†	90 ± 3*	41 ± 8*†	59 ± 5
ATP 2	53 ± 10*†	88 ± 4	62 ± 12*†	58 ± 5
Adenosine 3	43 ± 8*†	90 ± 4*	50 ± 10*†	60 ± 5
ATP 3	79 ± 11*‡	86 ± 3	94 ± 14*‡	59 ± 5

*$P < 0.05$ vs baseline within condition; †$P < 0.05$ vs steady-state (Pre-vasoconstrictor; Phenylephrine) within condition; ‡$P < 0.05$ *vs* adenosine during phenylephrine (α_1-agonist) infusion. 1 = Low dose condition; 2 = Moderate dose condition; 3 = High dose condition (see text for details). Forearm vascular conductance was calculated as (forearm blood flow/mean arterial pressure) × 100.

ATP evoked a dose-dependent increase in FBF (Fig. 4). Specifically, increasing the arterial concentration by 500, 1000 and 2000 nmol l^{-1} increased FBF by ∼2, 3, and 4-fold, respectively (all $P < 0.05$ *versus* baseline). As desired by experimental design, steady-state (pre-vasoconstrictor) FBF responses to adenosine infusion were effectively matched to that observed within each dose condition of ATP (Fig. 4; $P = 0.9$–1.0). Infusion of phenylephrine (α_1-agonist) significantly reduced FBF from steady-state hyperaemia during low and moderate dose adenosine and ATP (both $P < 0.05$; Fig. 4*A* and *B*). However, although phenylephrine also reduced FBF during high dose adenosine ($P < 0.05$), it did not significantly affect FBF during high dose ATP ($P = 0.16$; Fig. 4*C*).

The forearm vasoconstrictor responses to direct α_1-adrenoceptor stimulation (ΔFVC) under control resting conditions were −40 ± 3%. The vasoconstrictor responses during low dose adenosine (−38 ± 3%) and ATP (−33 ± 2%) were not significantly different from one another, and were similar to the responses observed at rest ($P = 0.3$–0.9). The vasoconstrictor responses during the moderate dose of adenosine tended to be greater *versus* resting conditions (−48 ± 4%; $P = 0.07$), and the responses during high dose adenosine were significantly greater than rest conditions (−53 ± 6%; $P < 0.05$), most likely due to the absolute amount of phenylephrine infused as part of the flow adjustment process. In contrast, the vasoconstrictor responses during moderate dose ATP (−22 ± 6%) were significantly blunted compared with rest, and the responses during high dose ATP (−8 ± 5%) were significantly blunted compared with rest and those observed during low dose ATP (both $P < 0.05$; Fig. 5). Importantly, the vasoconstrictor responses during moderate and high dose ATP were significantly blunted compared with the responses during the conditions of matched forearm hyperaemia via adenosine (both $P < 0.05$). MAP changed minimally within and between conditions, and thus FBF responses were similar to FVC. Heart rate and MAP were not significantly different between trials or conditions (Table 2).

Discussion

The primary findings from the present investigation are as follows. First, exogenous infusions of ATP required to match steady-state hyperaemia observed during

moderate intensity dynamic handgrip exercise (15% MVC) abolishes postjunctional α-adrenoceptor mediated vasoconstriction in humans. Second, the ability of ATP to modulate α-mediated vasoconstriction under these conditions is not selective for α_1- or α_2-adrenoceptors, as both were similarly abolished by exogenous ATP. Third, increasing arterial ATP concentrations to mimic levels observed within the physiological range during dynamic exercise elevates resting forearm blood flow in a dose-dependent manner. Finally, low dose ATP infusion is not sympatholytic in the human forearm, but graded increases in ATP infusions progressively blunt postjunctional α-adrenergic vasoconstriction. Importantly, these data cannot be explained simply by the vasodilator properties *per se* of ATP, as forearm hyperaemia was matched via infusions of adenosine, which did not blunt sympathetic α-adrenergic vasoconstriction. The physiological implications of these findings will now be discussed.

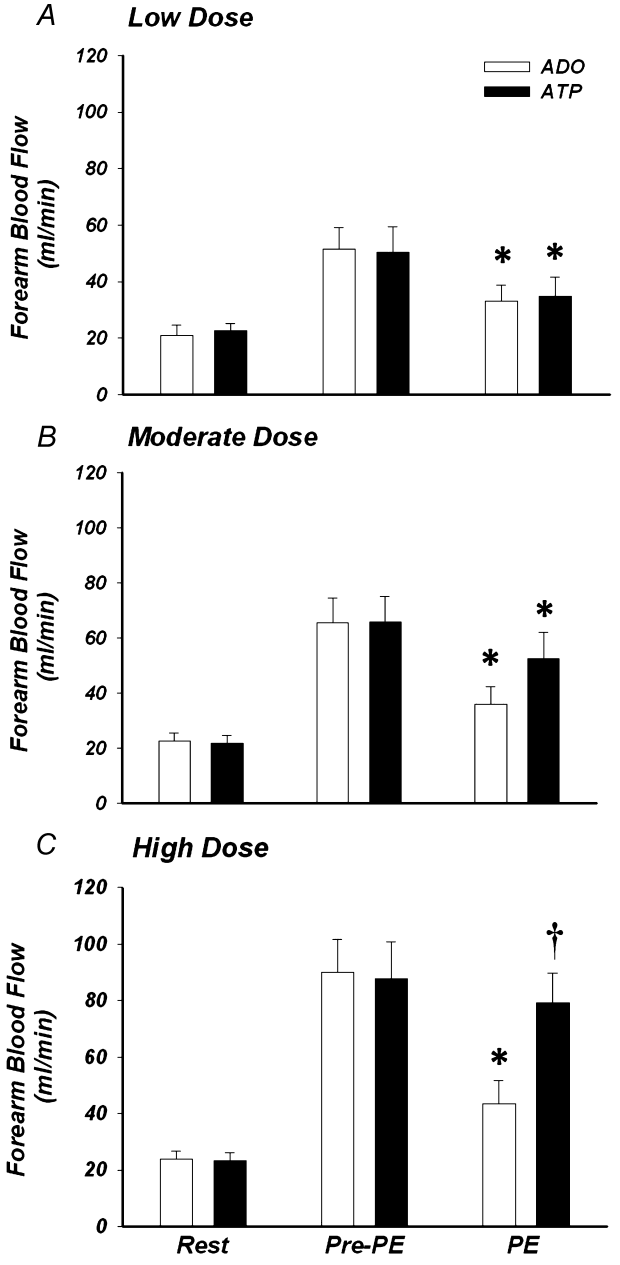

Figure 4. Forearm blood flow at rest, during the adenosine and ATP hyperaemic conditions, and during infusion of the α_1-agonist
Forearm blood flow was significantly elevated in a dose-dependent manner with exogenous ATP ($P < 0.05$), and forearm hyperaemia was effectively matched via infusion of adenosine. α_1-adrenoceptor stimulation with phenylephrine (PE) significantly reduced forearm blood flow during all doses (low, moderate, high) of adenosine. In contrast, phenylephrine significantly reduced forearm blood flow during low and moderate dose ATP, whereas this was not significant during high dose ATP. *$P < 0.05$ *vs.* steady state (Pre-PE) within condition; †$P < 0.05$ *vs.* adenosine during phenylephrine.

Exogenous ATP and postjunctional α-adrenergic vasoconstriction

Data derived from experimental studies using a variety of approaches in both animals and humans have clearly demonstrated a unique ability of contracting muscle to blunt sympathetic vasoconstriction, a phenomenon believed to optimize blood flow and oxygen delivery to active muscle when the sympathetic nervous system is engaged (Joyner & Thomas, 2003; VanTeeffelen & Segal, 2003). Several potential modulators of sympathetic vasoconstriction have been suggested to contribute to this phenomenon including adenosine, NO and PGs, although elucidating a clear mechanism in humans

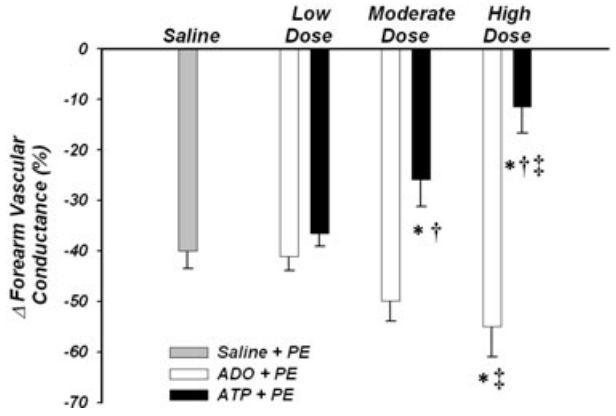

Figure 5. Forearm vasoconstrictor responses to α_1-adrenoceptor stimulation
Percentage reduction in forearm vascular conductance in response to α_1-adrenoceptor stimulation (via phenylephrine; PE) during low dose infusion of adenosine and ATP were not significantly different from during saline (control) conditions. α_1-mediated vasoconstriction was greater during high dose infusion of adenosine compared with control, whereas the vasoconstrictor responses were blunted during moderate and high dose ATP. *$P < 0.05$ *vs.* saline (control); †$P < 0.05$ *vs.* adenosine within dose condition; ‡$P < 0.05$ *vs.* low dose within drug condition.

has proved difficult (Dinenno & Joyner, 2003, 2004). Recently, however, Rosenmeier *et al.* (2004) demonstrated that exogenous ATP infusions abolished regional vasoconstriction in the skeletal muscle circulation evoked via intra-arterial tyramine, similar to what was observed during isolated knee extensor exercise at 25% of peak power output. These data are of significant interest in that infusions of other vasodilators such as adenosine and sodium nitroprusside (NO donor) to mimic exercise hyperaemia do not interfere with sympathetic vasoconstriction in humans (Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003; Rosenmeier *et al.* 2003*b*). Further, emerging evidence that ATP released from red blood cells in proportion to deoxygenated haemoglobin might act to couple blood flow and oxygen delivery to demand during exercise make it an attractive candidate capable of directly causing vasodilatation and also limiting sympathetic vasoconstriction in active muscle. In this context, ATP appears to be a good candidate for explaining sympatholyis because it has a short half-life, and is released when erythrocytes become deoxygenated, which would occur in close proximity of active muscle fibres, and this would allow sympathetic vasoconstriction to occur in resistance vessels of less active fibres to maintain an appropriate match between oxygen demand and oxygen delivery at the microcirculatory level (Calbet *et al.* 2006; Lundby *et al.* 2008).

In the present study, we directly tested the hypothesis that exogenous ATP infusion blunts postjunctional α-adrenoceptor responsiveness similar to that observed during exercise. In the study by Rosenmeier *et al.* (2004), intra-arterial administration of tyramine was used to evoke endogenous NA release and cause subsequent vasoconstriction. Although we (Dinenno & Joyner, 2003, 2004) and others (Ruble *et al.* 2002; Wilkins *et al.* 2006) have also used this approach, it must be emphasized that measured changes in venous NA concentrations do not always accurately reflect NA release from sympathetic nerve endings especially when there are marked changes in regional blood flow (Esler *et al.* 1990). Therefore, we determined the forearm vasoconstrictor responses to direct α_1- and α_2-adrenoceptor simulation (via phenylephrine and dexmedetomidine, respectively) during moderate handgrip exercise (15% MVC), during control vasodilator infusion of adenosine, and during infusion of ATP required to match forearm hyperaemia during exercise (Protocol 1). As expected, there was marked vasoconstriction to both α-agonists during infusion of adenosine, whereas the responses were significantly blunted (but not abolished) during handgrip exercise. These data are consistent with our previous work in this area of investigation (Dinenno & Joyner, 2003, 2004). Somewhat similar to our observations during exercise, the vasoconstrictor responses to direct α-adrenoceptor stimulation were abolished during infusion of exogenous ATP. To the best of our knowledge, these data provide the first experimental evidence that exogenous ATP modulates direct postjunctional α-adrenoceptor vasoconstriction in humans.

With respect to the α-adrenoceptor subtypes, we observed no difference in the ability of muscle contractions to blunt α_1- and α_2-mediated vasoconstriction in the human forearm. Similarly, exogenous ATP abolished both α_1- and α_2-mediated vasoconstriction with no apparent selectivity for the α-receptor subtypes. These data are consistent with previous studies indicating that both postjunctional α_1- and α_2-adrenoceptor responsiveness are significantly blunted during moderate forearm exercise in humans (Dinenno & Joyner, 2003; Rosenmeier *et al.* 2003*a*; Dinenno & Joyner, 2004). It should be noted that there are data in humans performing knee extensor exercise suggestive that α_2-adrenoceptors are more sensitive to metabolic inhibition than α_1-receptors (Wray *et al.* 2004), and this is conceptually similar with data derived from various experimental animal models (Anderson & Faber, 1991; Buckwalter *et al.* 2001). However, in this particular study in humans, there were marked pressor effects (and thus baroreflex activation) during infusion of the α-agonists that preclude clear interpretation of these data (Wray *et al.* 2004). Nevertheless, if α_2-adrenoceptors are indeed more sensitive to metabolic inhibition in the leg circulation, it would be of interest to determine whether ATP has a greater modulatory effect on α_2- *versus* α_1-adrenoceptor mediated vasoconstriction in the leg vasculature.

Exogenous ATP and graded modulation of postjunctional α-adrenergic vasoconstriction

In Protocol 1, we demonstrated that exogenous ATP required to match steady-state hyperaemia observed during moderate dynamic handgrip exercise (15% MVC) completely abolished postjunctional α_1- and α_2-mediated vasoconstriction. From a physiological standpoint, however, if circulating ATP were to completely override sympathetic vasoconstriction in active muscle during large muscle mass exercise, excess vasodilatation would occur and arterial pressure would be compromised (Marshall *et al.* 1961; Rowell, 1997; Joyner & Thomas, 2003). Thus, in Protocol 2, we determined whether graded ATP infusions evoked a dose-dependent modulation of α-adrenergic vasoconstriction, as has been demonstrated during graded levels of exercise (Thomas *et al.* 1994; Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002; Kirby *et al.* 2005). Interestingly, we found that low dose ATP infusion (500 nmol l^{-1}) sufficient to increase resting forearm blood flow 2-fold did not blunt α-adrenergic vasoconstriction. This is strikingly similar to what is observed during very mild muscle contractions (5% MVC) that elevate

forearm blood flow to a similar extent (Kirby *et al.* 2005). However, at moderate (1000 nmol l^{-1}) and higher (2000 nmol l^{-1}) doses of ATP selected to increase arterial concentrations similar to that observed in the femoral vein draining skeletal muscle during graded knee extensor exercise (Gonzalez-Alonso *et al.* 2002), α-adrenoceptor responsiveness was progressively blunted. In this context, the ability of graded ATP infusions to limit postjunctional α-adrenoceptor mediated vasoconstriction is quite similar to what is observed with graded exercise intensity, and provides further support for the hypothesis that circulating ATP could play a significant role in regulating muscle blood flow and vascular tone during exercise.

Potential mechanisms

The mechanism(s) by which circulating ATP can modulate sympathetic α-adrenergic vasoconstriction in the skeletal muscle circulation are at present unknown. What is clear, however, is that this does not simply reflect the vasodilator properties of ATP *per se*, as infusions of other vasodilators to mimic exercise hyperaemia do not blunt sympathetic vasoconstriction (Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003*b*). Indeed, in the present study (Protocol 2), adenosine was not capable of inhibiting α-adrenergic vasoconstriction. Thus, we speculate that cellular mechanisms by which ATP-induced P_{2y} receptor activation evokes smooth muscle cell relaxation are involved. In this context, recent studies indicate that ATP-mediated vasodilatation in humans is independent of NO and PGs (van Ginneken *et al.* 2004), which is consistent with recent findings that independent inhibition of NO (Dinenno & Joyner, 2003) and PGs (Hansen *et al.* 2000; Dinenno & Joyner, 2004) does not have a significant impact on functional sympatholysis in humans. Further, when we performed combined NO and PG inhibition, sympathetic vasoconstriction in contracting muscle was only slightly augmented compared with control conditions, suggesting other modulatory factors were involved (Dinenno & Joyner, 2004). Taken together, it seems plausible to speculate that ATP evokes smooth muscle cell hyperpolarization, and this in turn blunts sympathetic α-adrenergic vasoconstriction. Future studies will be needed to determine the exact mechanism underlying the sympatholytic effect of circulating ATP.

Experimental considerations

In Protocol 2 of the present investigation, we chose to only use phenylephrine (α_1-agonist) to test whether the ability of circulating ATP to blunt postjunctional α-adrenoceptor vasoconstriction was graded with ATP concentrations, as opposed to using both α_1- and α_2-adrenoceptor agonists. However, in Protocol 1, our data indicated that exogenous infusion of ATP at concentrations to match forearm hyperaemia observed during moderate intensity handgrip exercise abolished both α_1- and α_2-mediated vasoconstriction, with no apparent difference between the receptor subtypes. Additionally, we were concerned about giving dexmedetomidine (α_2-agonist) over seven experimental trials, as this would significantly increase the risk of α_2-adrenoceptor effects on the central nervous system (e.g. hypotension, sedation). This is important not only from a subject safety standpoint, but also if there were central α_2 effects, this would inhibit basal sympathetic outflow (Lang *et al.* 1997) and cloud interpretation of the data for the remaining experimental trials.

An additional consideration for Protocol 2 relates to the dose adjustment of phenylephrine we employed based on changes in forearm blood flow, which was performed to reduce any potential 'dilution effect' of the α_1-agonist during the various doses of adenosine and ATP. Our findings indicate that the vasoconstrictor responses during the moderate dose of adenosine tended to be greater, and that the vasoconstrictor responses during the high dose of adenosine were greater compared with control (saline) conditions. Although seemingly counterintuitive, this is consistent with what has been demonstrated when adjustments in the dose of tyramine were performed for similar reasons during hyperaemic conditions associated with adenosine infusions (Tschakovsky *et al.* 2002). Thus, although we were aware this might occur, we needed to be sure that any apparent sympatholytic effect of exogenous ATP was not due to a dilution effect, as this has never been determined before during ATP infusions. Regardless, our data clearly indicate a graded sympatholytic effect of exogenous ATP whether compared with the vasoconstriction observed during control conditions, or conditions of matched hyperaemia via adenosine.

Experimental limitations

One limitation of the present study relates to the use of moderate intensity exercise with a small muscle mass, and thus moderate hyperaemic conditions, to test our hypotheses. However, our experimental model allows for moderate intensity dynamic muscle contractions to be performed without increasing sympathetic outflow, and allows for well-controlled vasoactive drug infusions that do not alter arterial blood pressure and thus do not evoke baroreflex-mediated alterations in sympathetic outflow. This is important in that changes in sympathetic nervous system activity would cloud interpretation of our vasoconstrictor responses during the α-agonist infusions. Nevertheless, it is important to recognize that the potential interaction between ATP and sympathetic vasoconstriction during larger muscle mass, higher intensity exercise could be more complex than observed in our

studies as indicated by recent data demonstrating that some degree of local vasoconstriction is necessary for the precise matching of oxygen delivery to oxygen demand under such conditions (Calbet *et al.* 2006; Lundby *et al.* 2008).

Another limitation of the present study relates to the lack of measurement of circulating arterial or venous plasma ATP concentrations during the experimental trials. In Protocol 1, our goal was to match forearm blood flow to that observed during steady-state exercise hyperaemia, and thus we were not concerned with how much exogenous ATP was required to achieve this. However, in Protocol 2, our doses were chosen to mimic average increases in ATP concentrations observed in the venous circulation during graded knee extensor exercise in humans (Gonzalez-Alonso *et al.* 2002). We used data from the venous circulation (*versus* arterial) as ATP release from red blood cells would occur at the level of the microcirculation and thus venous concentrations would provide a better estimate of this. Using this approach, we were able to demonstrate a 2- to 4-fold increase in forearm blood flow across this range of exogenous ATP and further, that increasing ATP concentrations within this predicted range caused graded sympatholysis. However, it still remains unclear what exact concentrations of ATP at the level of the resistance vessel network are ultimately observed during exercise, and thus are sympatholytic under these conditions. A final limitation of the present investigation relates to the inability to inhibit P_{2y}-receptors in humans due to the lack of an available pharmacological agent. In this context, to definitively determine if ATP is mechanistically linked with the ability of muscle contractions to blunt sympathetic vasoconstriction, similar studies will need to be performed before and after P_{2y}-receptor blockade and demonstrate that muscle contractions are incapable of modulating sympathetic vasoconstriction under conditions of P_{2y}-receptor inhibition.

Conclusions

The results from the present investigation demonstrate that exogenous ATP infusions required to match the hyperaemic responses observed during moderate handgrip exercise abolish postjunctional α_1- and α_2-adrenoceptor responsiveness in the human forearm. Importantly, graded increases in arterial concentrations of ATP within the physiological range that evoke moderate limb hyperaemia causes graded inhibition of α-mediated vasoconstriction, such that low levels are not sympatholytic whereas progressive reductions in α-adrenoceptor mediated vasoconstriction are observed with increasing ATP concentrations. Collectively, these data are consistent with the conceptual framework regarding how muscle blood flow and vascular tone are regulated in contracting muscles of humans.

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