Inhibition of α-adrenergic vasoconstriction in exercising human thigh muscles

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The mechanisms underlying metabolic inhibition of sympathetic responses within exercising skeletal muscle remain incompletely understood. The aim of the present study was to test whether α_2-adrenoreceptor-mediated vasoconstriction was more sensitive to metabolic inhibition than α_1-vasoconstriction during dynamic knee-extensor exercise. We studied healthy volunteers using two protocols: (1) wide dose ranges of the α-adrenoreceptor agonists phenylephrine (PE, α_1 selective) and BHT-933 (BHT, α_2 selective) were administered intra-arterially at rest and during 27 W knee-extensor exercise ($n = 13$); (2) flow-adjusted doses of PE (0.3 μg kg^{-1} l^{-1}) and BHT (15 μg kg^{-1} l^{-1}) were administered at rest and during ramped exercise (7 W to 37 W; $n = 10$). Ultrasound Doppler and thermodilution techniques provided direct measurements of femoral blood flow (FBF). PE (0.8 μg kg^{-1}) and BHT (40 μg kg^{-1}) produced comparable maximal reductions in FBF at rest (-58 ± 6 *versus* $-64 \pm 4\%$). Despite increasing the doses, PE (1.6 μg kg^{-1} min^{-1}) and BHT (80 μg kg^{-1} min^{-1}) caused significantly smaller changes in FBF during 27 W exercise (-13 ± 4 *versus* $-3 \pm 5\%$). During ramped exercise, significant vasoconstriction at lower intensities (7 and 17 W) was seen following PE (-16 ± 5 and $-16 \pm 4\%$), but not BHT (-2 ± 4 and $-4 \pm 5\%$). At the highest intensity (37 W), FBF was not significantly changed by either drug. Collectively, these data demonstrate metabolic inhibition of α-adrenergic vasoconstriction in large postural muscles of healthy humans. Both α_1- and α_2-adrenoreceptor agonists produce comparable vasoconstriction in the resting leg, and dynamic thigh exercise attenuates α_1- and α_2-mediated vasoconstriction similarly. However, α_2-mediated vasoconstriction appears more sensitive to metabolic inhibition, because α_2 is completely inhibited even at low workloads, whereas α_1 becomes progressively inhibited with increasing workloads.

Vascular tone is controlled by interactions of sympathetic nervous and local vascular control mechanisms (Laughlin *et al.* 1996). In resting muscle, the importance of sympathetic activity for vascular tone is exemplified by the 50–100% increase in blood flow following sympathetic denervation or α-adrenergic blockade (Delp & Armstrong, 1988; Laughlin *et al.* 1996; Dinenno *et al.* 2002*b*). In exercising muscle, metabolites accumulate and become involved in the control of local blood flow. During exercise the need for dramatic increases in perfusion and oxygen delivery to the contracting units is met by increased cardiac output and redistribution of blood flow. The latter is accomplished primarily by increasing sympathetic vasoconstriction in internal organs as well as resting muscle vascular beds. The increased sympathetic

vasoconstrictor stimulus is delivered in parallel to the exercising muscle (Hansen *et al.* 1994), but the effectiveness of this stimulus in the face of local metabolic vasodilatation has been a matter of some controversy. In some models of exercise, evidence of sympathetic vasoconstriction has been reported (Secher *et al.* 1977; O'Leary *et al.* 1991*b*; Joyner *et al.* 1992; Buckwalter & Clifford, 1999), whereas other studies find evidence for metabolic blunting of sympathetic vasoconstriction in the exercising muscle (Thomas *et al.* 1994; Richardson *et al.* 1995; Hansen *et al.* 1996; Thomas *et al.* 1998; Sander *et al.* 2000; Ruble *et al.* 2000, 2002; Chavoshan *et al.* 2002; Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003). These seemingly conflicting results are not mutually exclusive, since metabolic blunting of sympathetic vasoconstriction

does not exclude residual sympathetic effects, but rather implies less vasoconstriction than would be expected for a given sympathetic input.

In recent years there seems to be a growing consensus that metabolic inhibition of sympathetic vasoconstriction is a factor in several models of exercise in both animals and humans. Although the underlying mechanisms are incompletely understood, several lines of evidence point to inhibition of postjunctional adrenergic signalling via metabolites from active skeletal muscle. The α-adrenergic receptors can be divided into the α_1 and α_2 subtypes, each with vasoconstrictor properties *in vivo*. Microvascular preparations have identified α_1-adrenoreceptors primarily in proximal and α_2-adrenoreceptors primarily in the distal vasculature of skeletal muscle (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991). In both isolated microvascular preparations and intact animal models, α_2-vasoconstriction seems more sensitive to metabolic inhibition than α_1-vasoconstriction (Anderson & Faber, 1991; Thomas *et al.* 1994). However, the role of α-adrenoreceptor subtypes in humans remains unclear.

Previous studies considering the effect of exercise on α-adrenoreceptor-mediated vasoconstriction have been limited to the animal hindlimb muscles (Thomas *et al.* 1994; Thomas *et al.* 1998; Buckwalter *et al.* 2001), and the human forearm during rhythmic handgrip (Hansen *et al.* 1996; Sander *et al.* 2000; Chavoshan *et al.* 2002; Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003). However, to our knowledge no previous human studies have examined α-adrenergic function in a large postural muscle group such as the quadriceps during graded, dynamic exercise. This is an important consideration, since the leg represents a vascular bed with the potential to vasodilate to such a degree that it may challenge maintenance of systemic arterial blood pressure during exercise (Saltin *et al.* 1998; Volianitis *et al.* 2003), and so may be considered more haemodynamically significant than smaller muscle vascular beds.

The aims of this study were to test the following hypotheses: (1) metabolic inhibition of α-adrenergic vasoconstriction is present in exercising human thigh muscle, and (2) α_2-mediated vasoconstriction is more sensitive than α_1-mediated vasoconstriction to metabolic inhibition in exercising human thigh muscle. To accomplish this, femoral blood flow was measured directly at rest and during steady-state knee-extensor exercise with superimposed administration of the selective α-receptor subtype agonists phenylephrine (PE, an α_1 agonist) and BHT-933 (BHT, an α_2 agonist).

Methods

Subjects and general procedures

Seventeen healthy young men (25 ± 1 years) participated in the present study. Written informed consent was obtained from all participants, and experiments were approved by the local ethics committee of Copenhagen and Frederiksberg and conformed to the Declaration of Helsinki. All studies were performed in a thermoneutral environment, with subjects in a semirecumbent position (approximately 30 deg reclined). Heart rate (HR) was recorded from an ECG (ADInstruments), and respiratory excursions from a strain gauge belt (Respitrace, ADInstruments). Blood pressure was determined both non-invasively by automated sphygmomanometry (Dinamap), and invasively (see below). Knee-extensor exercise with one leg was performed at 60 r.p.m. on a modified cycle ergometer as previously described (Andersen *et al.* 1985). The knee extensor force and rhythm were recorded via a strain gauge attached to the ergometer lever arm (customized signal processor, FBJ Industries). All measurements at rest were performed during vascular occlusion of the lower leg (pneumatic cuff below the knee inflated to 280 mmHg). With this procedure, a very large fraction of blood flow measured at the level of the groin is supplying the thigh muscle vascular bed.

Catheterization

The femoral artery and vein of the exercising leg were cannulated under local anaesthesia (lidocaine, 5 ml, 20 mg ml^{-1}). The arterial (Arrow, 20 gauge) and venous (Cook, 18 gauge) catheters were inserted in the proximal direction $\sim$5 cm below the inguinal ligament. The arterial catheter was used for intra-arterial infusions of drugs and phasic invasive blood pressure measurements (calibrated to the level of the aortic valve and corrected for pump pressure artifacts during infusions). The venous catheter was used to perform thermodilution measurements of leg blood flow, as described below.

Blood flow measurements

Thermodilution. A sterile tip-thermistor (model 94-030-2.5-Fr, Baxter) was inserted through the femoral venous catheter and positioned 8 cm proximal to the catheter tip (at the level of the inguinal ligament). Another thermistor was placed at the venous catheter inlet and both thermistors were connected to a customized

dual temperature signal processor (custombuild, FBJ Industries). This allows continuous measurements of blood temperature during constant infusion of a sterile iced saline solution (around 2–3°C at the venous inlet) administered at 100 ml min^{-1} by a modified Harvard pump for periods of 20 s. Dilution of this indicator in the vein of the exercising leg causes blood temperature to decrease by 0.5–3°C, depending on blood flow. This thermodilution procedure has been described and validated in detail previously and provides very accurate and reproducible measurements of leg blood flow during knee extensor exercise (Andersen & Saltin, 1985). During rest, thermodilution was not used, because the method is unsuited for determination of very low levels of blood flow, as expected during vasoconstrictor infusions. Femoral blood flow (FBF$_{TD}$) was calculated by the formula:

$$ \mathrm{FBF_{TD}} = F_{\mathrm{sal}} \times \frac{C_{\mathrm{bl}} \times \rho_{\mathrm{bl}}}{C_{\mathrm{sal}} \times \rho_{\mathrm{sal}}} \times \frac{(t_{\mathrm{bd}} - t_{\mathrm{ic}})}{(t_{\mathrm{bb}} - t_{\mathrm{bd}})} $$

where F_{sal} is saline flow; C_{bl} and C_{sal} are specific heats for blood and saline, respectively; ρ_{bl} and ρ_{sal} are specific densities for blood and saline, respetively; t_{bb} and t_{bd} are temperatures of blood before and during the saline infusion, respectively; and t_{ic} is the saline infusate temperature, corrected to the level of the catheter tip.

Ultrasound imaging and Doppler. The ultrasound machine (model CFM 800, GE Medical) was equipped with a mechanical sector transducer operating at an imaging frequency of 7.5 MHz. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution is achieved (best theoretical resolution around 0.1 mm). The femoral artery was insonated distal to the inguinal ligament for dynamic recordings of diameter throughout a cardiac cycle. The maximum diameter (systole) was used for calculation of blood flow, because systole is the phase of blood propulsion in large arteries. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 4.0–6.0 MHz, operated in the high-pulsed repetition frequency mode (4–36 kHz) with a sample volume of 5 mm in depth. Care was taken to avoid aliasing especially during exercise. All blood velocity measurements were obtained with the probe at a constant 46 deg (or in some subjects 50 deg) angle to the femoral artery during simultaneous real time 2D vessel visualization to maintain insonation angle constant and sample volume centred. At all sample points we obtained both diameter of the femoral artery (FAD) and, approximately 20–30 s later, an angle-corrected, time- and space-averaged, and intensity-weighted mean

blood velocity (V_{mean}) (Echopac Software, GE Medical and PowerLab, ADInstruments). Using FAD and V_{mean}, femoral artery blood flow (FBF$_D$) was calculated from:

$$ \mathrm{FBF_D} = V_{\mathrm{mean}}\pi(\mathrm{FAD}/2)^2 $$

The Doppler method has been reported to yield reproducible femoral artery blood flow measurements at rest and during knee-extensor exercise, previously used at up to 70% of maximal work (Radegran, 1997). We obtained FBF$_D$ both during rest and exercise. However, *a priori* the thermodilution method was chosen as the method of choice during exercise, since variability for the thermodilution measurements was expected to be less compared to the Doppler measurements. Thus, for the present study, blood flow values during rest are from ultrasound Doppler measurements, while values during exercise are from the thermodilution technique. A direct comparison of the results obtained from the two methods during exercise was performed (see Results).

Drugs

Phenylephrine (PE; Danish county pharmaceutical corporation, SAD) was used as a specific α_1-adrenergic agonist. BHT-933 (BHT; Sigma-Aldrich, Denmark) was used as a specific α_2-adrenergic agonist. Propranolol (Prop; AstraZeneca, Sweden) was used as a non-specific β-adrenergic antagonist. In validation experiments, isoproterenol (Iso; Danish county pharmaceutical corporation, SAD) was used as a non-specific β-adrenergic agonist, while yohimbine (Sigma-Aldrich, Denmark) was used as an α_2-adrenergic antagonist. Drugs were dissolved and diluted as appropriate with normal saline, except yohimbine, which was diluted with sterile water. Intra-arterial infusion rates ranged from 0.2 to 6 ml min^{-1}.

Experimental protocols

To address the aims of the present study, four separate protocols were performed. Graphic presentations of the protocols are presented in Fig. 1. Protocols 1 and 2 were designed to validate the drugs administered for β-adrenergic blockade and α_2-adrenoreceptor activation, respectively. In protocol 3, wide dose ranges of the α_1-agonist PE and α_2-agonist BHT were administered at rest and during one level of moderate intensity (27 W) exercise. This design allows evaluation of the doses required to obtain a maximal drug response. In protocol 4, one flow-adjusted dose of PE and BHT was administered at rest and during graded (7 W to 37 W) exercise. This design allows evaluation of the exercise intensity required to

attenuate each drug at a concentration which was shown to yield submaximal effects at rest. Details regarding the specific objectives and measurements for each protocol are described below.

Protocol 1: Validation of β-adrenergic blockade. β-Blockade in the leg was used in one of the exercise protocols, because PE previously has been shown to have β-adrenergic agonist properties (Torp *et al.* 2001). Intra-arterial administration of propranolol has previously been shown to produce effective β-blockade in the human forearm after 10 min (Johnsson, 1967), and in validation experiments we extended this finding to the leg. The purpose of the present protocol was to validate the assumption that the maintenance dose of propranolol used

would sustain the β-blockade in the leg for several hours. We tested this in nine resting subjects by measuring blood pressure, HR and FBF during challenges with intra-arterial isoproterenol (Iso; 1, 2, 4, and 8 ng kg^{-1} min^{-1}, 2 min on each dose) before and after 150 min of intra-arterial administration of Prop (2.5 μg kg^{-1} min^{-1} for 5 min as bolus, followed by 0.125 μg kg^{-1} min^{-1} as maintenance). This dosing regimen for Prop was identical to that used in protocol 3 of the present study.

Protocol 2: Validation of α_2-adrenergic specificity of BHT-933. The α-adrenergic receptor subtype specificity of PE has been studied quite extensively. In contrast, the specificity of BHT has only been documented sparingly in humans (Jie *et al.* 1984). The purpose of this protocol was
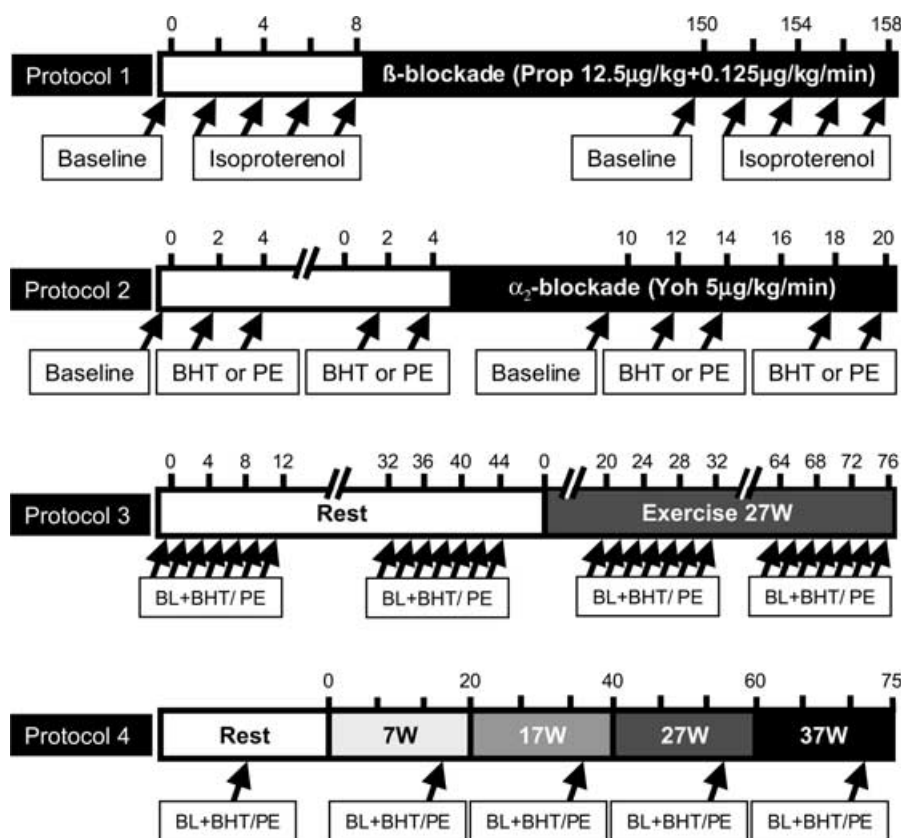

Figure 1. Experimental protocols 1–4 represented graphically
Time courses are given in minutes on the top axis of each panel. BL, baseline; BHT, BHT-933; PE, phenylephrine; Prop, propranolol; Yoh, yohimbine. Protocol 1: the β-receptor agonist isoproterenol (1, 2, 4 and 8 ng kg^{-1} min^{-1}, 2-min infusions) was administered before and after 150 min of β-blockade with propranalol (12.5 μg kg^{-1} bolus and 0.125 μg kg^{-1} min^{-1} maintenance). Protocol 2: the selective α_2-adrenoreceptor agonist BHT-933 (BHT, 20 and 40 μg kg^{-1} min^{-1}, 2-min infusions) and the selective α_1-agonist phenylephrine (PE, 0.4 and 0.8 μg kg^{-1} min^{-1}, 2-min infusions) were applied before and during the last 10 min of a 20 min infusion of the α_2-antagonist yohimbine (5 μg kg^{-1} min^{-1}). Protocol 3: dose–response for BHT (2.5–80 μg kg^{-1} min^{-1}, 2-min infusions) and PE (0.025–1.6 μg kg^{-1} min^{-1}, 2-min infusions) at rest and during 27 W knee extensor exercise. Protocol 4: flow-adjusted administration of BHT (15 μg kg^{-1} l^{-1}, 3-min infusions) and PE (0.3 μg kg^{-1} l^{-1}, 3-min infusions) at rest and during ramped exercise.

to validate the assumption that BHT in the dose range used is specific for α_2-adrenergic receptors in the human leg. We tested this in seven resting subjects by measuring blood pressure, HR and FBF during intra-arterial BHT (20 and 40 μg kg^{-1} min^{-1} each for 2 min) and PE (0.4 and 0.8 μg kg^{-1} min^{-1} each for 2 min) before and during the last 10 min of concomitant intra-arterial infusion of the α_2-adrenergic antagonist yohimbine (5 μg kg^{-1} min^{-1} for 20 min). If BHT specifically acts as an α_2-adrenergic receptor ligand, we would predict that yohimbine would inhibit the BHT response without affecting the PE response. All subjects participating in protocol 2 also participated in protocol 1 on the same day, and thus α-adrenergic activation and inhibition were performed during concomitant β-blockade.

Protocol 3: Subtype specific α-adrenergic agonists during rest and exercise. The purpose of this protocol was twofold: first, to determine to what degree metabolic inhibition of α-adrenergic vasoconstriction is evident in a large muscle mass during dynamic exercise in humans; second, to determine whether α_2-vasoconstriction is more sensitive to metabolic inhibition than α_1-vasoconstriction at a given work-load. Intra-arterial Prop was used to obtain local β-adrenergic blockade. In 13 subjects, we measured blood pressure, HR, FBF at rest and during 27 W one-legged knee-extensor exercise before and during intra-arterial administration of PE (0.025–1.6 μg kg^{-1} min^{-1}, 7 doses, 2 min each dose) and BHT (2.5–80 μg kg^{-1} min^{-1}, 6 doses, 2 min each dose). At rest, the highest dose of PE (1.6 μg kg^{-1} min^{-1}) and BHT (80 μg kg^{-1} min^{-1}) were only administered to a subset of the subjects ($n = 4$) due to systemic effects. The wide range of drug doses allowed evaluation of saturation kinetics at rest and during exercise, and *post hoc* comparison of doses normalized to flow, i.e. similar drug-concentration in femoral arterial blood despite large changes in predrug steady-state blood flow. The two agonists were separated by at least 15 min to allow washout and restoration of haemodynamic values, and the sequence of drug application was alternated. In this protocol, only the FBF measurements at rest could be performed during vascular occlusion of the lower leg, because in pilot experiments it was determined that prolonged lower leg ischaemia (around 15 min was needed for completion of the multiple doses of each drug) was too painful during knee-extensor exercise. During exercise, the first 10 min was without measurements to allow ample time for blood flow, HR and MAP to reach steady-state levels even at low to moderate exercise intensities (Radegran & Saltin, 1998). The exercise lasted a total of 80 min.

In pilot experiments, two prolonged infusions of BHT and PE were performed during supine rest with 60–90 min between infusions. There was no indication of tachyphylaxis to the vasoconstrictor effects of either drug. This is in accordance with previously published results which do not indicate development of tolerance towards intra-arterial administration of these α-agonists within the same study day (Jie *et al.* 1984; Lembo *et al.* 1994; Lembo *et al.* 1997).

Protocol 4: α-Adrenergic vasoconstriction during different levels of exercise. The purpose of this protocol was to determine whether metabolic inhibition of α-adrenergic vasoconstriction in the exercising thigh is dependent on exercise intensity. This protocol was performed without β-adrenergic blockade. In 10 subjects, we measured blood pressure, HR, FBF at rest and during 7, 17, 27 and 37 W one-leg knee-extensor exercise before and during intra-arterial administration of PE (flow-adjusted dosing to 0.3 μg kg^{-1} l^{-1}, 3 min infusions), and BHT (flow-adjusted dosing to 15 μg kg^{-1} l^{-1}, 3 min infusions). In a subset of the subjects ($n = 5$), the responses to increased doses of PE (0.6 μg kg^{-1} l^{-1}) and BHT (30 μg kg^{-1} l^{-1}) were determined during 7 W. This was done to determine if the responses seen during exercise were maximal. The brevity of drug infusions during this protocol allowed FBF measurements both at rest and during exercise to be performed during vascular occlusion of the lower leg. The subjects performed two separate bouts of exercise lasting 75–80 min, with 90 min rest between bouts. Subjects exercised for 12–15 min at each level (7 W to 37 W) using an incremental protocol. Blood flow was allowed to reach steady-state during the first 10 min of exercise at each level before measurements and drug administration began. The sequence of drug administration was alternated.

Data analysis and statistics

All data underwent analog-to-digital conversion and were sampled at 400 Hz, recorded on a PC, and analysed offline with signal processing software (PowerLab, ADInstruments). Mean arterial pressure was derived from the arterial pressure waveform and the sphygmomanometer readings (MAP = diastolic BP + 1/3 pulse pressure). Femoral vascular conductance (FVC) was calculated by the formula: FVC = FBF/MAP. During the last 30 s of each baseline or drug dose FBF$_{TD}$ was averaged over 5–10 s; FBF$_D$ over 8 s; and MAP, HR over at least 30 s. Within group differences were assessed by Student's paired *t* test and one- or two-way ANOVA for repeated measures (i.e. repeated measures for both drug and dose).

Post-hoc analysis was performed by Dunnett's procedure for one-way ANOVA, and by Tukey's HSD procedure for two-way ANOVA. The Doppler and thermodilution data obtained during exercise in protocols 3 and 4 were compared head to head by paired *t* tests. Data are expressed as the mean ± s.e.m., except when comparing the Doppler and thermodilution methods, where the mean ± 95% confidence intervals are used. Statistical significance was set at $P < 0.05$, adjusted by the Bonferroni method as appropriate.

Results

Lasting β-blockade in the human thigh (protocol 1)

The propranolol-bolus and maintenance dose provided virtually complete blockade of isoproterenol-induced vasodilatation, even 150 min after initiation (Fig. 2).

α_2-Adrenergic specificity of BHT-933 in the human thigh (protocol 2)

The local vasoconstrictor responses to high doses of BHT and PE both caused resting femoral blood flow to decrease from about 250 ml min^{-1} to 80 ml min^{-1}, a 60% decrease in flow (Fig. 3). During concomitant yohimbine administration to provide α_2-blockade, resting blood flow doubled. However, despite this increase in resting flow PE still caused flow to decrease to about 80 ml min^{-1}, while the effect of BHT was significantly depressed to accomplish only a 20% decrease in flow (Fig. 3).
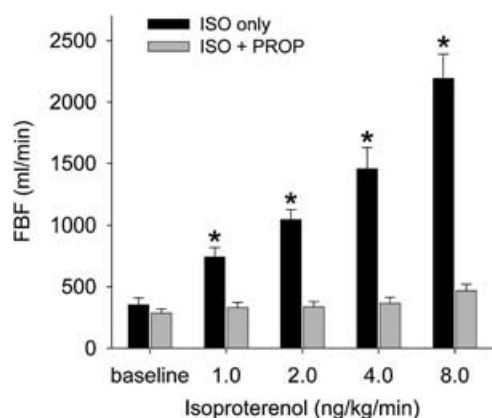
Dose–response relationship for phenylephrine and BHT at rest and during 27 W exercise (protocol 3)

The α-adrenoreceptor agonist dose–response relationships at rest and during 27 W dynamic knee extensor exercise are presented in Figs 4 and 5, respectively. There were no significant changes in blood pressure or heart rate (HR) during infusion of the agonist drugs at rest. FAD diameter decreased following PE administration (from 8.8 ± 0.2 to 5.6 ± 0.4 mm, rest *versus* PE 0.8 μg kg^{-1} min^{-1}, $P < 0.01$), while BHT caused a much smaller FAD change (from 8.9 ± 0.2 to 7.8 ± 0.3 mm, rest *versus* BHT 40 μg kg^{-1} min^{-1}, $P < 0.05$). The change in FAD was significantly greater during PE compared to BHT ($P < 0.01$). The highest doses of PE and BHT produced comparable reductions in FBF (-58.5 ± 6.3 *versus* $-64.4 \pm 4.3\%$ m l min^{-1}, PE 0.8 μg m l^{-1} min^{-1} *versus* BHT
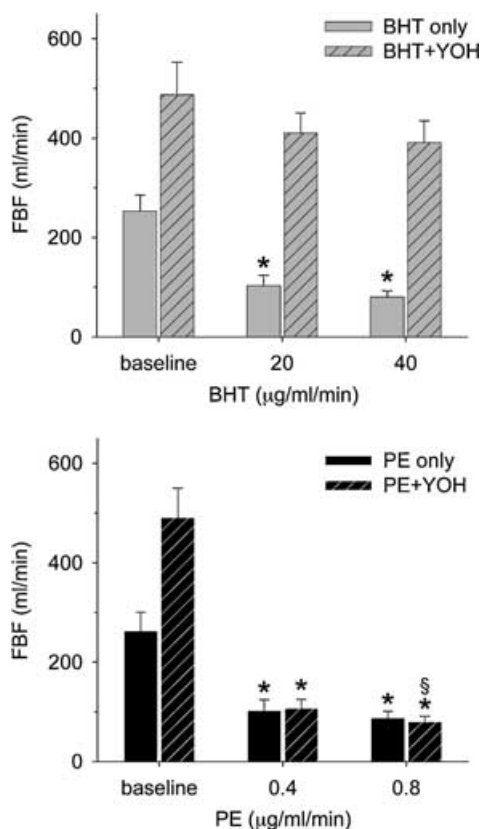
Figure 3. Specificity of BHT for α_2-adrenoreceptors was validated by measurements of femoral blood flow before and after selective α_2-blockade with yohimbine

Top panel, administration of the selective α_2-agonist BHT (20 and 40 μg kg^{-1} min^{-1}) produced significant vasoconstriction (grey bars), and this effect was inhibited following yohimbine (5 μg kg^{-1} min^{-1}) (hatched bars). Bottom panel, the selective α_1-agonist phenylephrine (PE, 0.4 and 0.8 μg kg^{-1} min^{-1}) produced significant vasoconstriction both before (black bars) and after administration of yohimbine ($n = 7$) (hatched bars). *$P < 0.05$ compared to baseline; §$P < 0.05$ compared to BHT responses after yohimbine.

Figure 2. Femoral blood flow (FBF) during infusion of the non-selective β-agonist isoproterenol (1–8 ng kg^{-1} min^{-1}) before (black bars), and 150 min after initiation of non-selective β-blockade with propranolol (grey bars)

Propranolol provided virtually complete blockade of isoproterenol-mediated vasodilatation ($n = 9$). *$P < 0.05$ compared to baseline and compared to responses after propranolol.

40 μg m l^{-1}min^{-1}) and FVC (-57.4 ± 6.6 *versus* $-62.4 \pm$ 4.1%, PE 0.8 *versus* BHT 40), with a clear plateau at the highest doses (Fig. 4*A* and *B*).

For higher doses of PE, the decrease in FAD dominated blood flow changes. For example, mean blood velocities during baseline and PE 0.8 μg kg^{-1} min^{-1} were not significantly different (7.7 ± 0.8 *versus* 7.6 ± 1.0 cm s^{-1}, n.s.), and despite similar decreases in flow, the mean blood velocity was significantly higher during PE 0.8 μg kg^{-1} min^{-1} compared to BHT 40 μg kg^{-1} min^{-1} (7.6 ± 1.0 *versus* 2.9 ± 0.4 cm s^{-1}, $P < 0.05$). We also observed a transient decrease (about 10 mmHg) in blood pressure and sleepiness following the highest dose of BHT, both lasting around 30 min with some individual variation.

Moderate intensity knee-extensor exercise (27 W) was accompanied by an increase in HR (58 ± 2 *versus* 78 ± 2 beats min^{-1}, rest *versus* exercise, $P < 0.01$) with no significant change in MAP (86 ± 2 *versus* 83 ± 2 mmHg). Exercising FBF reached about 3000 ml min^{-1} with no change in FAD (8.9 ± 0.2 *versus* 9.0 ± 0.2 mm, rest *versus* exercise). MAP, HR and FBF remained at steady-state levels throughout each exercise bout.

During 27 W exercise, PE infusion produced a small but statistically significant change in FAD (9.1 ± 0.2 *versus* 8.7 ± 0.3 mm, rest *versus* exercise, PE 0.8 μg kg^{-1} min^{-1}, $P < 0.05$), and significantly decreased FBF and FVC (Fig. 5*A*). However, the relative change in flow to the highest dose of PE (0.8 μg kg^{-1} min^{-1}) was significantly smaller than the
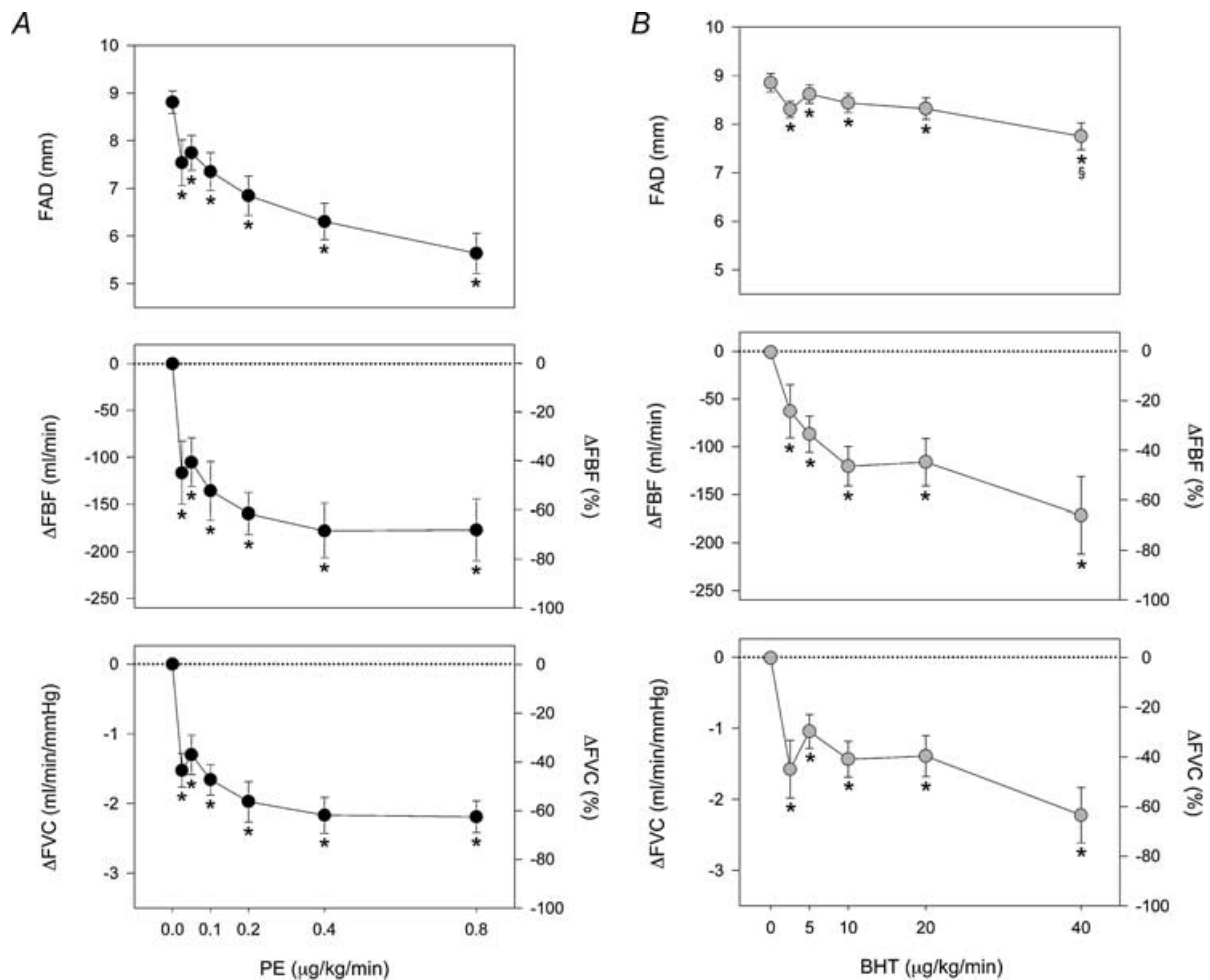

Figure 4. Dose–response relationships for intra-arterial PE and BHT at rest
A, during incremental doses of PE, femoral artery diameter (FAD, top) decreased 35%, with concomitant reductions in femoral blood flow (FBF, middle) and femoral vascular conductance (FVC, bottom). *B*, during incremental doses of BHT, FAD decreased only slightly and only at the highest doses. However, FBF and FVC both decreased significantly, and of similar magnitudes to the responses seen during PE. *$P < 0.05$ compared to resting baseline, §$P < 0.05$ between comparable doses of PE and BHT. On the lower four panels the mean $\pm$ s.e. symbols are accurate for the absolute changes, whereas the alternative y axis for relative changes has been included for easier comparison to Fig. 5.

response at rest (58 ± 6 *versus* $12 \pm 5\%$, rest *versus* exercise, $P < 0.01$), while the absolute change tended to be higher (188 ± 33 *versus* 380 ± 141 ml min^{-1}, rest *versus* exercise, n.s.). During the highest PE doses (PE 0.8 and 1.6 μg kg^{-1} min^{-1}) we observed significant increases in MAP (9 ± 2 and 15 ± 4 mmHg), with concomitant decreases in HR (-9 ± 1 and -14 ± 2 beats min^{-1}). In contrast, BHT infusion during exercise produced no significant changes in MAP, HR, FAD, FBF, or FVC (Fig. 5*B*). The difference in FBF response between PE and BHT did not reach significance at this level of exercise. However, the changes in FVC were

significantly larger for the last three doses of PE *versus* BHT (Fig. 5*A*).

Post-hoc 'flow-adjustment' was performed to calculate the drug doses yielding similar femoral arterial drug concentration at rest *versus* exercise. For PE, drug concentrations of 0.025, 0.05 and 0.1 (rest) *versus* 0.4, 0.8 and 1.6 μg kg^{-1} (exercise) were used, which resulted in drug deliveries of 0.12, 0.29 and 0.70 *versus* 0.13, 0.29 and 0.60 μg kg^{-1} l^{-1}, respectively, n.s. Similarly for BHT, 2.5 and 5 (rest) *versus* 40 and 80 μg kg^{-1} min^{-1} (exercise) gave deliveries of 12 and 32 *versus* 15 and
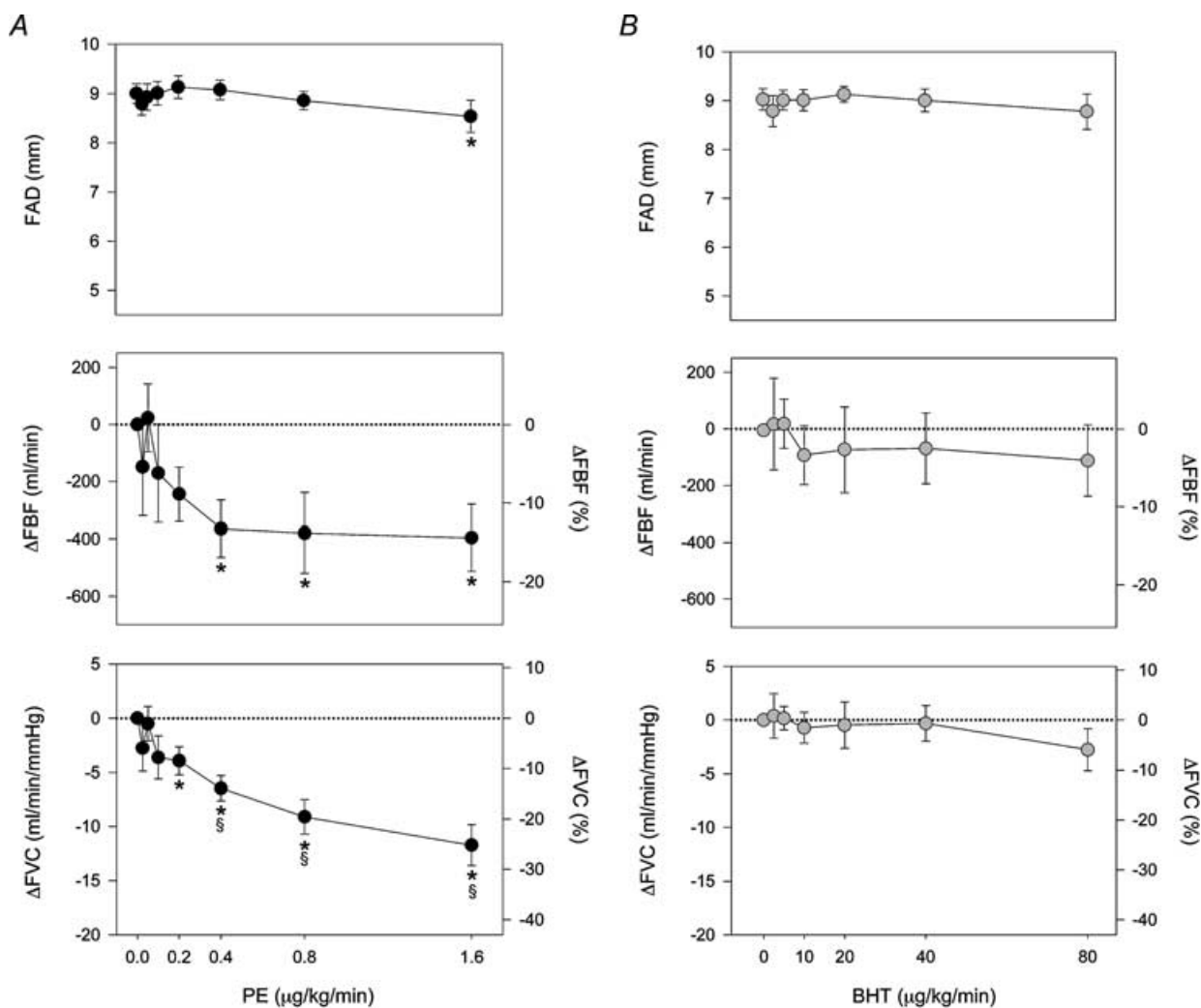

Figure 5. Dose–response relationships for intra-arterial PE and BHT during 27 W exercise
A, during exercise, PE reduced FAD only at the highest dose (top). The changes in FBF (middle) and FVC (bottom) during PE were blunted compared to rest. A significant change in FVC occurred at the highest doses of PE compared to baseline and compared to the highest dose of BHT, and FBF also fell slightly during high doses of PE. *B*, during exercise, the response to BHT was completely abolished, with no significant change in FAD, FBF, or FVC, even at the highest dose. *$P < 0.05$ compared to exercising baseline, §$P < 0.05$ between comparable doses of PE and BHT. On the lower four panels the mean ± s.e. symbols are accurate for the absolute changes, whereas the alternative y axis for relative changes has been included for easier comparison to Fig. 4.

$29 \ \mu g \ kg^{-1} \ l^{-1}$, respectively, n.s. When comparing the responses to these flow-adjusted doses, the PE-induced changes in FAD were significantly smaller during exercise compared to rest for all doses (Fig. 6A). However, only the FBF response to the highest flow-adjusted dose of PE was statistically reduced during exercise, and FVC responses at rest and during exercise were not statistically different for PE (Fig. 6A). In contrast, the FBF and FVC responses

to both flow-adjusted doses of BHT were significantly reduced during exercise (Fig. 6B).

Responses of phenylephrine and BHT-933 at rest and during increasing levels of exercise (protocol 4)

The responses of a single, flow-adjusted dose of the α-agonists PE ($0.3 \ \mu g \ kg^{-1} \ l^{-1}$) and BHT ($15 \ \mu g \ kg^{-1} \ l^{-1}$) during rest and ramped exercise from 7 W to
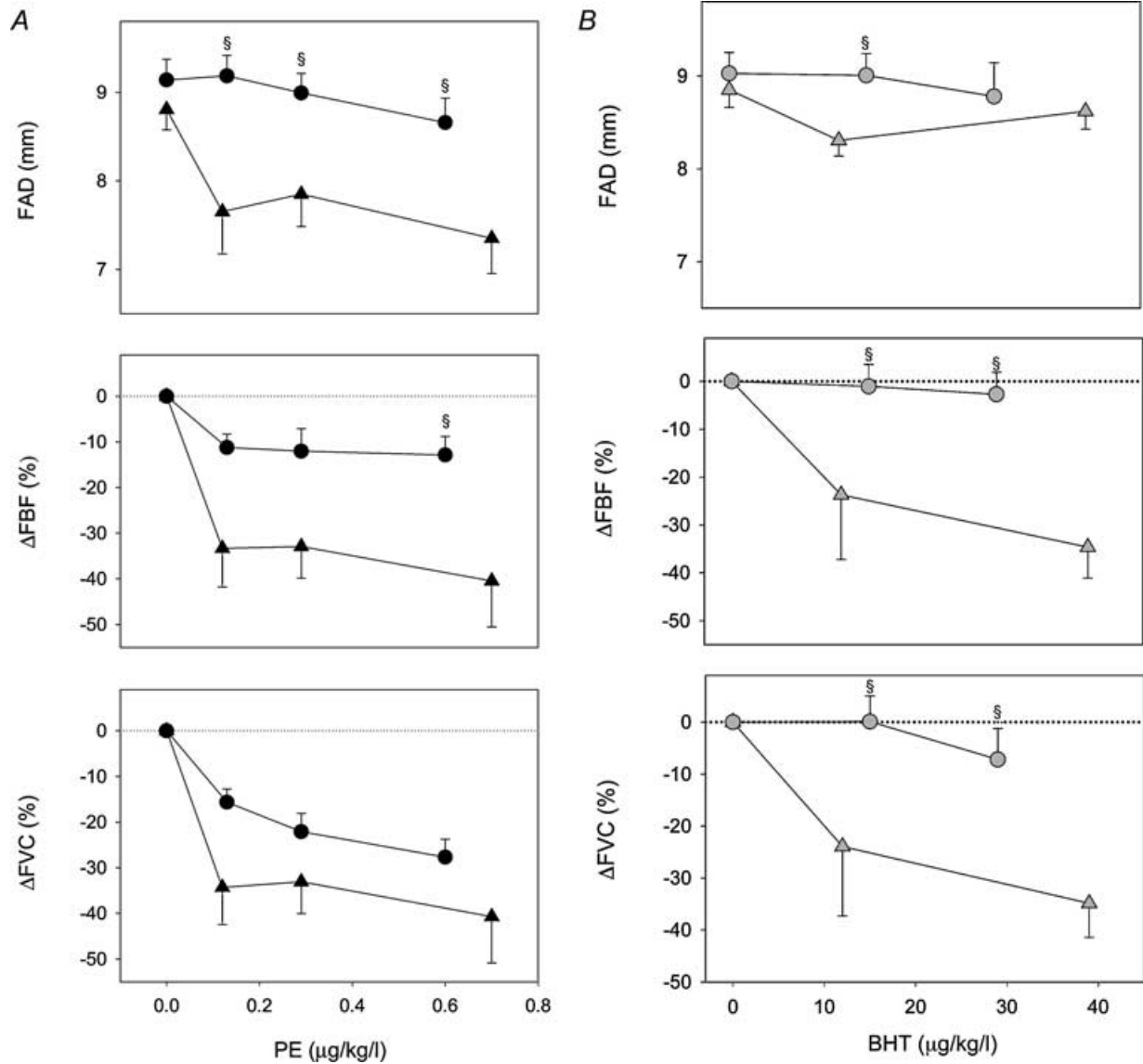

Figure 6. _Post-hoc_ flow-adjusted doses for PE and BHT at rest (triangles) and during 27 W exercise (circles)

_A_, PE-induced reduction in FAD (top) was significantly smaller during exercise compared to rest for all flow-adjusted doses. However, only the FBF response (middle) to the highest flow-adjusted dose of PE was statistically reduced during exercise, and FVC responses (bottom) at rest and during exercise were not statistically different during flow-adjusted doses of PE. _B_, BHT-induced reduction in FAD (top) was attenuated at the lower flow-adjusted dose, and the FBF (middle) and FVC (bottom) responses to both flow-adjusted doses of BHT were significantly reduced. §$P < 0.05$ compared to flow-adjusted dose–response at rest.

37 W are presented in Fig. 7*A* and *B*, respectively. At rest, FAD changed significantly during both PE (9.4 ± 0.3 *versus* 8.2 ± 0.5 mm, *P* < 0.01) and BHT (9.5 ± 0.3 *versus* 8.9 ± 0.3 mm, *P* < 0.01). The change in FAD was significantly greater with PE compared to BHT (*P* < 0.05). PE and BHT produced comparable reductions in FBF (44 ± 7 *versus* 50 ± 5%, PE *versus* BHT) and FVC (44 ± 7 *versus* 50 ± 5%, PE *versus* BHT).

Ramped exercise at 7–37 W significantly increased HR (57 ± 4 at rest to 65 ± 2, 72 ± 3, 77 ± 2 and 82 ± 2 beats min^{-1} during 7, 17, 27 and 37 W) with no significant change in MAP. FBF increased during exercise to approximately 1250, 2000, 2750 and 3400 ml min^{-1} (7, 17, 27 and 37 W, respectively) with no significant differences between the two bouts of exercise.

PE significantly reduced FAD at 7 W (from 9.4 ± 0.3 to 8.3 ± 0.5 mm, *P* < 0.01) and 17 W (from 9.5 ± 0.3 to 9.1 ± 0.4 mm, *P* < 0.05), decreased in

FBF at 7 and 17 W, and reduced FVC at 7, 17 and 27 W (Fig. 7*A*). At the highest workload (37 W), the effects of PE were abolished. The doses of PE used in this protocol caused no significant changes in MAP or HR. In contrast, BHT caused no significant differences in FAD, FBF or FVC at any intensity (Fig. 7*B*). BHT caused small (3–4 mmHg) but statistically significant decreases in MAP at 17 and 27 W without changing HR. While the relative changes in FBF and FVC were significantly attenuated at all levels of exercise compared to rest for both agonist drugs, the differences between PE and BHT reached statistical significance only at the 7 W intensity (Fig. 8).

In a subset of subjects in protocol 4 (*n* = 5), the effects of double dosing of PE and BHT were studied during 7 W exercise. For all parameters, this doubling of doses caused no significant changes in the responses to the drugs, indicating that the effects seen with the doses chosen were already near-maximal.
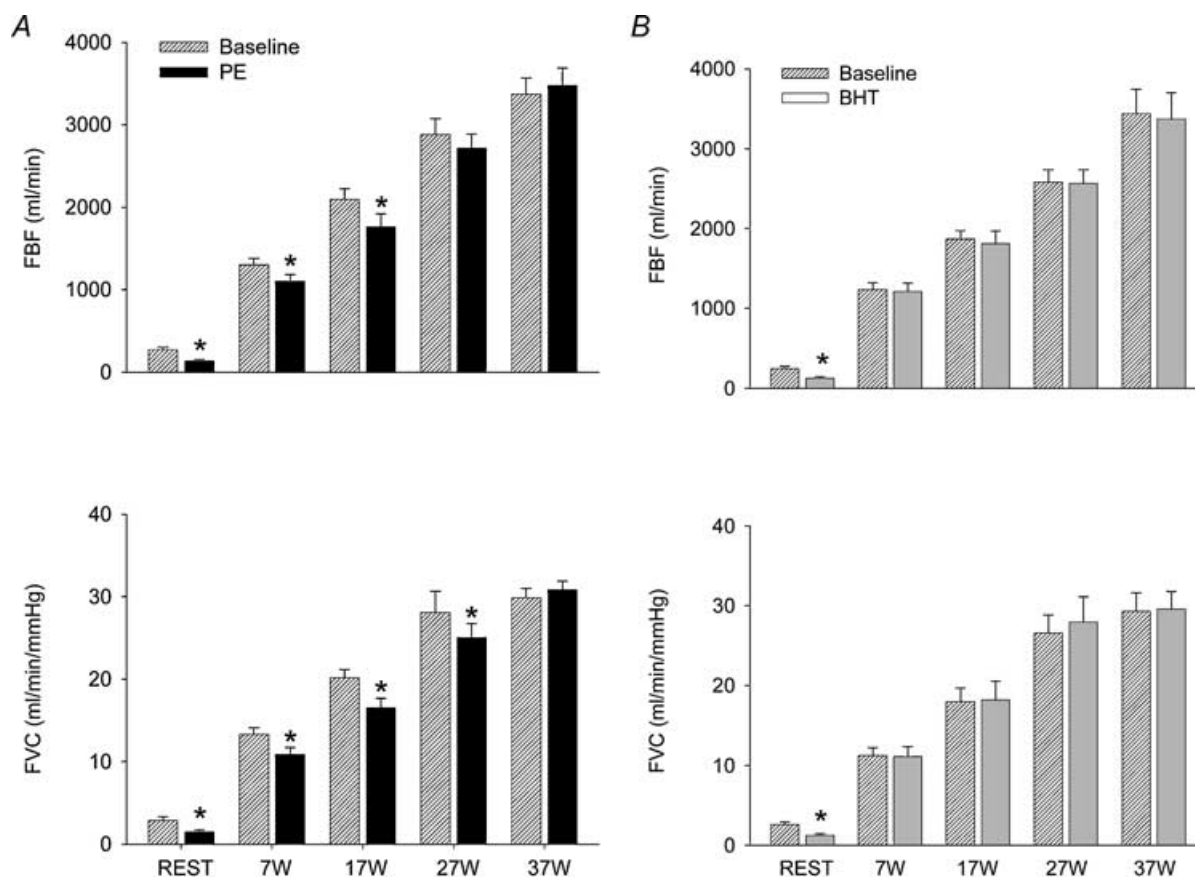

Figure 7. Absolute FBF and FVC during ramped exercise of 7–37 W with superimposed administration of PE (*A*) and BHT (*B*)
Both PE and BHT were flow-adjusted to the exercise-induced increase in FBF. *A*, at lower workloads (7 and 17 W), PE-induced decreases in FBF and FVC were seen (black bars), and this response was abolished at higher intensities. *B*, BHT administration caused no changes in FBF or FVC (grey bars) at any exercise intensity. *P* < 0.05 compared to predrug value for each level of exercise.

Comparison of Doppler and thermodilution for measuring femoral blood flow during exercise

Femoral blood flows measured during exercise with ultrasound Doppler and thermodilution were always performed within the same minute of steady-state exercise. The average FBF_{TD} and FBF_D during 27 W exercise before drug infusions were 2960 ± 240 ml min^{-1} and 2890 ± 400 ml min^{-1}, respectively (mean $\pm$ 95% confidence intervals) (n.s.). The PE and BHT-induced changes were small during exercise with both methods, and at no measurement point did the two methods yield significantly different results ($P \geq 0.29$ for all paired t tests). The difference in FVC between PE and BHT did not reach statistical significance when using FBF_D as the basis for calculating FVC, but the PE effect remained significant. The two methods also yielded very similar results during the ramped exercise protocol, and at no measurement point were the averages from the two methods statistically different ($P \geq 0.11$ for all paired t tests). The individual and summary data for simultaneous measurements of

FBF using Doppler and thermodilution during the first bout of ramped exercise at 7, 17, 27 and 37 W are compared in Fig. 9. As in protocol 3, FBF_{TD} showed less variability within the group than FBF_D with the exception of 37 W, where the variabilities were similar. The PE- and BHT-induced changes in blood flow were not different when comparing the two methods, and the PE-induced decreases in FBF_D and FVC during 7 and 17 W remained significant. The difference between PE and BHT responses for FBF_D during 7 W did not reach statistical significance.

Discussion

There are several major new findings from the present study. First, in dose–response studies α_1- and α_2-adrenoreceptor agonists produce comparable maximal vasoconstriction in the resting leg. Second, the small α_2-mediated compared to the large α_1-mediated decrease in femoral artery diameter provides functional evidence that α_2-receptors are located predominantly distal in the vascular tree. Third, we demonstrate for the first time metabolic inhibition of both α_1- and α_2-mediated vasoconstriction in the human leg. Finally, we show that α_2- is more sensitive than α_1-mediated vasoconstriction to metabolic inhibition in the exercising human thigh. This latter important finding is based on complete inhibition of α_2-mediated vasoconstriction even at low workloads, whereas
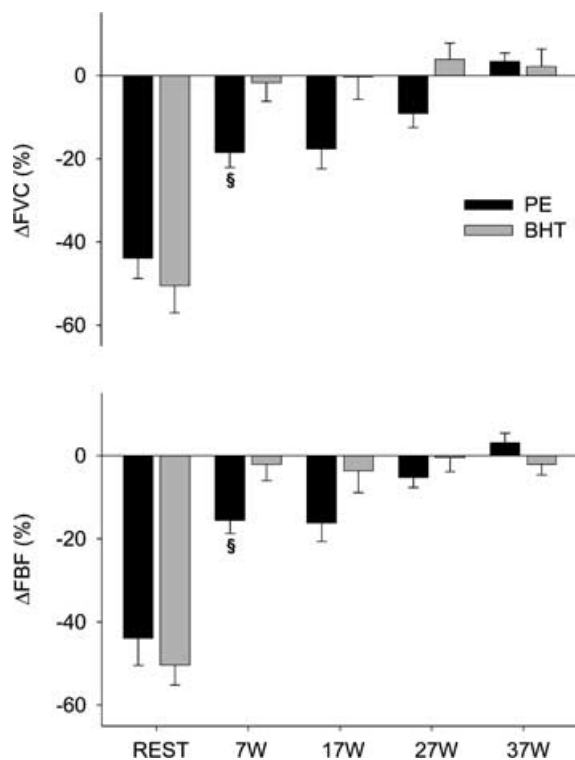

Figure 8. Changes in FBF (top) and FVC (bottom) during ramped exercise of 7–37 W with superimposed administration of PE (black bars) and BHT (grey bars)
All responses to BHT and PE during exercise were significantly smaller than at rest. At lower exercise intensities, PE produced significant changes in FBF and FVC, and these responses were significantly larger than the response to BHT at 7 W §$P < 0.05$ between PE and BHT at same level of exercise.
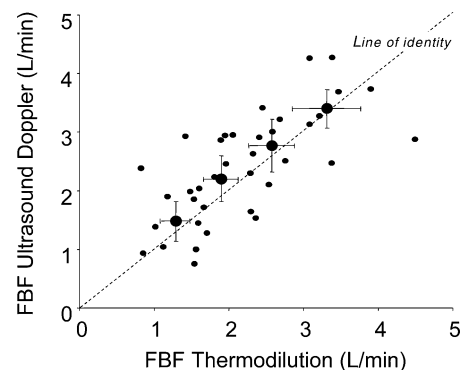

Figure 9. Comparison of ultrasound Doppler and thermodilution techniques for determining femoral blood flow during knee-extensor exercise
The individual data shown by small filled circles are obtained within the same minute for the two methods in 10 subjects during increasing levels of exercise intensity from 7 to 37 W before administration of α-agonists. The mean values obtained by the two methods, shown by the larger filled circles, did not differ significantly at any intensity of exercise. The variation of the data obtained by the Doppler method was generally larger than the variation of the thermodilution data. Summary data are shown as means $\pm$ 95% confidence intervals.

α_1-mediated vasoconstriction becomes progressively inhibited with increasing workloads. Collectively, these findings provide novel insight regarding the contribution of α-adrenoreceptor subtypes to the control of muscle blood flow at rest and during exercise.

Evidence for heterogeneous distribution of α-adrenoreceptors

Our finding that femoral artery diameter (FAD) was reduced to a much larger extent following α_1-compared to α_2-agonist administration provides functional evidence of a heterogeneous distribution of α-adrenoreceptors in the skeletal muscle vasculature of the human leg. This extends observations in the rat (Anderson & Faber, 1991) demonstrating a predominance of α_1-adrenoreceptors in the upstream, conduit arteries and α_2-receptors in the resistance arterioles of the microcirculation. Thus, the present study has advanced the concept first identified in animals (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991) that large upstream arteries contain both α_1- and α_2-receptors for the control of arterial blood pressure, while the nutrient arterioles contain predominantly α_2-receptors for the fine control of tissue perfusion.

α_1- *versus* α_2-mediated vasoconstriction at rest

The maximal reduction in resting FBF following α_2-agonist (BHT) application was similar to that seen following α_1-agonist (PE) administration. This extends similar observations in the dog hindlimb following intra-arterial agonist infusion of PE (selective for α_1) and clonidine (selective for α_2) at rest (Buckwalter *et al.* 2001). Conversely, local administration of selective α-antagonist agents in the resting human forearm indicated an apparent dominance of α_2-adrenoreceptor-mediated vascular tone (63% of basal tone) over α_1 (Dinenno *et al.* 2002b). However, calculated differences in control of resting flow do not exclude similar maximal responses to α_1- *versus* α_2-agonists. Furthermore, dissimilar α_1-responsiveness has recently been reported in the human arm and leg (Pawelczyk & Levine, 2002), suggesting that these two limbs exhibit differences in α_1-adrenoreceptor sensitivity and distribution.

Metabolic inhibition of local vasoconstriction in exercising muscle

To our knowledge, we have provided the first pharmacological evidence of metabolic attenuation of α-adrenergic vasoconstriction during dynamic exercise in the human thigh. These findings extend results from studies in the human forearm (Hansen *et al.* 1996, 2000; Sander *et al.* 2000; Chavoshan *et al.* 2002; Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003). We believe there are several major advantages of using knee-extensor exercise over handgrip in this context. First, direct and accurate measurements of blood flow during exercise are feasible by the thermodilution and ultrasound Doppler methods (Andersen & Saltin, 1985; Walloe & Wesche, 1988; Radegran & Saltin, 1998). Second, the fraction of blood flow in the femoral artery reaching the thigh muscles during knee-extensor exercise is more than 95% while using an occlusive cuff below the knee (Savard *et al.* 1988). In contrast, rhythmic handgrip cannot be sustained with an occlusive wrist cuff (Tschakovsky *et al.* 2002), and the fraction of brachial artery blood flow reaching contracting muscle at low intensity exercise is below 90% (Wahren, 1966). Third, knee-extensor exercise up to 30 W (around 40% of maximum) can be sustained for hours with steady-state blood flow and pressure, without fatiguing and with no significant activation of the sympathetic nervous system as measured by plasma noradrenaline (Turcotte *et al.* 1992; Steensberg *et al.* 2002). In contrast, to sustain rhythmic handgrip for more than 10 min requires very low intensities (10% of maximum) (Wahren, 1966; Rosenmeier *et al.* 2003). At higher intensities fatigue and evidence of increasing activation of muscle sympathetic nerve activity quickly become apparent (Tschakovsky *et al.* 2002). In the present study, these unique attributes of the human knee-extensor model of exercise allowed definition of the level of intensity required for metabolic inhibition of sympathetic vasoconstriction and definition of the pharmacological dose responses at a given intensity of thigh exercise.

Previous studies in humans and animals have pointed to a post-junctional site of interaction between skeletal muscle metabolic events and α-adrenergic vasoconstriction. In this regard, responses to reflex-mediated increases in muscle sympathetic nerve activity, direct sympatho-neural stimulation, indirect acting sympathomimetics (tyramine) and intra-arterial directly acting α-agonists are all attenuated during exercise (Thomas *et al.* 1994, 1998; Hansen *et al.* 1996, 2000; Sander *et al.* 2000; Buckwalter *et al.* 2001; Chavoshan *et al.* 2002; Tschakovsky *et al.* 2002; Ruble *et al.* 2002; Keller *et al.* 2003; Rosenmeier *et al.* 2003). A wide array of factors contributing to the observed alteration in vascular responsiveness during exercise have been proposed, including vasoactive substances such as adenosine (Laughlin *et al.* 1989; Saltin *et al.* 1998), prostaglandins and thromboxanes (Karamouzis *et al.*

2001), the indirect effect of increased muscle temperature (Cooke *et al.* 1984), hypoxia (Hansen *et al.* 2000), and acidosis (McGillivray-Anderson & Faber, 1990). These factors are not mutually exclusive.

It is noteworthy that several different protocols performed in both rodents and humans have identified skeletal muscle production of nitric oxide as an important metabolic event. Lack of skeletal muscle nitric oxide synthase or pharmacological blockade of nitric oxide production has been accompanied by loss of metabolic inhibition of sympathetic vasoconstriction (Thomas *et al.* 1998, 2003; Sander *et al.* 2000; Chavoshan *et al.* 2002; Fadel *et al.* 2003). One recent handgrip study was not able to demonstrate a significant effect of pharmacological blockade of nitric oxide synthase on the vasoconstriction induced by α-agonists in the human forearm (Dinenno & Joyner, 2003). This study clearly demonstrates exercise-induced inhibition of sympathetic vasoconstriction during handgrip and therefore suggests that nitric oxide may not be the only important signalling molecule mediating metabolic inhibition of sympathetic vasoconstriction.

Stimulation of the α-receptors in the exercising limb has often produced some degree of vasoconstriction. This was also the case in the present study for phenylephrine during lower exercise intensities. Conflicting evidence exists regarding the importance of functional sympatholysis *versus* sympathetic vasoconstriction in exercising skeletal muscle, and has been the subject of several comprehensive reviews (Laughlin *et al.* 1996; Rowell, 1997). The principal point of contention centres on the paradox of vasoconstriction of the exercising muscle vasculature, which seems counterproductive to the effort of increasing blood flow to meet the metabolic demands of the muscle. Several studies have addressed whether blood flow to exercising muscle or blood pressure is prioritized during heavy exercise of large muscle groups, with conflicting results (Secher *et al.* 1977; Savard *et al.* 1989; Richter *et al.* 1992; Strange, 1999). However, it is now well accepted that perfusion of the contracting skeletal muscle is ultimately a balance between metabolic vasodilatation and sympathetic vasoconstriction (VanTeeffelen & Segal, 2003; Joyner & Thomas, 2003). The underlying mechanisms guarding this balance are still incompletely understood.

α_1- *versus* α_2-mediated vasoconstriction during exercise

Experimental evidence in microvascular and animal preparations has suggested that α_2-mediated vasoconstriction is more sensitive to metabolic inhibition than α_1-mediated vasoconstriction (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Thomas *et al.* 1994; Buckwalter *et al.* 2001; Ruble *et al.* 2002). Thus, the vasoconstrictor responses to specific α_2-agonists such as UK 14304 and clonidine were more effectively inhibited during exercise than α_1-agonists such as phenylephrine and the non-specific agonist noradrenaline. Microvascular studies in rats have identified a heterogeneous distribution of α-receptors, with α_2-receptors located predominantly in the peripheral vascular bed (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991). Taken together, these findings have prompted the hypothesis that preserved α_1-responsiveness would help to maintain blood pressure by keeping vascular tone in arteries and large arterioles, while attenuation of α_2-responsiveness in smaller arterioles would help increase nutrient blood flow to the exercising muscle fibres.

In humans, mild handgrip exercise was recently shown to attenuate both phenylephrine- and clonidine-mediated vasoconstriction compared to rest (Rosenmeier *et al.* 2003). However, this study used only one level of exercise and one dose (flow-adjusted) of each drug, and therefore was not powered to detect differences between the attenuation of α_1- *versus* α_2-mediated vasoconstriction. The present study extends these findings to the human leg, because we also found evidence for attenuation of both α_1- and α_2-mediated vasoconstriction, and our dose–response curves provide evidence that even the maximal responses are attenuated. Using the ramped exercise protocol, we also demonstrated a residual vasoconstriction to phenylephrine (α_1-agonist) at low and moderate exercise intensity, whereas BHT-933 (α_2-agonist) did not produce vasoconstriction at any exercise intensity. Thus, a major contribution of the present study is the demonstration of a greater sensitivity of α_2- than α_1-adrenoreceptor subtypes to the metabolic byproducts produced during exercise in the human thigh.

The mechanisms underlying differential exercise-induced inhibition of α_1- than α_2-mediated vasoconstriction are not understood, but could be related to either ligand–receptor affinity or the signal transduction following receptor activation. Regarding the relative affinity, we used doses of BHT-933 that were about 50 times higher than the doses of phenylephrine. This is in accordance with the differences in dosing used in human forearm (Jie *et al.* 1984; Lembo *et al.* 1994) and dog carotid artery studies (Willems *et al.* 2001). It is unknown whether agonist binding to the receptors is decreased during exercise. Our dose–response curves for phenylephrine during exercise

argue against this mechanism for the attenuation of phenylephrine responses, because with a 'simple' affinity issue phenylephrine would be expected to have a similarly high maximum effect at rest and during exercise. The lack of significant responses to any dose of BHT-933 during exercise precludes any speculation on this putative mechanism for this agonist.

Degree of exercise-induced inhibition of α-adrenergic responses

Previous studies have demonstrated seemingly different degrees of exercise-induced attenuation of α-adrenergic vasoconstriction. For non-specific α-adrenergic or α_1-adrenergic agonists some previous animal studies have shown very little exercise-induced attenuation of the response (Thomas *et al.* 1994; Buckwalter *et al.* 1998). In contrast, more recent animal studies conclude that phenylephrine responses are attenuated during exercise (Buckwalter *et al.* 2001). Furthermore, recent human forearm studies (Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003), and the present study, agree that even mild exercise causes significant attenuation of the phenylephrine response such that maximal responses seen during exercise are around 10–20% of blood flow, compared to 50–60% at rest. The reason for this apparent development of results from earlier to more recent studies is not clear. For α_2-adrenergic agonists, animal studies report different degrees of exercise-induced attenuation of the response. In rat hindlimb, heavy evoked exercise caused a complete inhibition of the response to the α_2-agonist UK14304 (Thomas *et al.* 1994), whereas in the conscious dog, the hindlimb response to the α_2-agonist clonidine remained significant, around 15% at heavy running (Buckwalter *et al.* 2001). The differences in these studies were several, including species and choice of α_2-agonist. Regarding the latter though, *in vitro* studies have identified UK14304 as a full agonist but clonidine as a partial agonist (Wise *et al.* 1997), which would not explain why clonidine seems to cause larger *in vivo* effects than UK14304. In the recent human handgrip studies, the response to clonidine also remains at around 15% of brachial blood flow during mild exercise (Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003), whereas in the present study there was no detectable response in the thigh circulation to BHT-933 even during mild exercise. The obvious differences between these studies include the choice of limb and α_2-agonist. The choice of limb may explain part of the difference, because non-exercising muscle and tissue like the skin receive a larger proportion of brachial blood flow during handgrip than femoral blood

flow during knee-extensor exercise. The choice of α_2-agonist is also likely to be important, because clonidine has been reported to be less specific compared to BHT-933 (Jie *et al.* 1984). Thus, the higher flow-adjusted doses of clonidine used during exercise may cause α_1-adrenergic receptor stimulation.

Significance of functional sympatholysis in the leg

The leg represents a large muscle mass with an enormous capacity to vasodilate during exercise, reaching up to 2.5 l kg^{-1} min^{-1} (Andersen & Saltin, 1985) and often receiving the majority of cardiac output. Others have observed that the increase in skeletal muscle blood flow accompanied by the increase in fraction of cardiac output with exercise means that small changes in vascular conductance can greatly influence arterial pressure (O'Leary, 1991*a*; Tschakovsky *et al.* 2002). Thus, exercise produces a condition that demands a precise balance between neural and metabolic control of flow to maintain homeostasis. The vascular bed of the leg represents a 'sleeping giant' that may necessitate a haemodynamic response unique to this large muscle group (Andersen & Saltin, 1985). Accordingly, the observed attenuation of α-adrenergic vasoconstriction may be a protective mechanism, insuring adequate blood flow to the exercising tissue in the face of increased sympathetic outflow.

As noted above, muscle sympathetic nervous activity, measured by plasma noradrenaline, does not increase significantly at knee-extensor exercise intensities below 30 W (Turcotte *et al.* 1992; Steensberg *et al.* 2002). Since metabolic attenuation of α-adrenergic vasoconstriction is evident in our study even at intensities well below 30 W, it would seem sympathetic neural activation does not occur until well after the thigh muscle is 'protected' by functional sympatholysis. This extends similar findings in studies of handgrip exercise (Hansen *et al.* 1996; Sander *et al.* 2000).

Experimental limitations

The potential experimental limitations of the present study are related to the use of exogenous vasoconstrictors administered luminally. Physiological control of peripheral adrenergic receptors takes place at the abluminal side, where noradrenaline released from sympathetic boutons primarily acts on proximate receptors located on vascular smooth muscle cells. Sympathetic boutons are more abundant at the level of the resistance arterioles compared to large arteries. When agonists are administered intra-arterially both luminal endothelial receptors and abluminal smooth muscle

receptors are activated. However, in previous studies in the human forearm the responses to direct acting adrenergic agonists and the indirect sympathomimetic tyramine are attenuated to similar degrees during handgrip (Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003).

In these human studies, as well as in some animal studies, the sympathomimetic dosing has been 'flow-adjusted' to achieve similar intra-arterial drug concentration (Buckwalter *et al.* 1998; Ruble *et al.* 2002; Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003). Furthermore, in several studies α-adrenergic responses during exercise have been compared with the responses seen during local pharmacological vasodilatation administered to match exercise hyperaemia (Tschakowsky *et al.* 2002; Rosenmeier *et al.* 2003). When phenylephrine, clonidine and tyramine are superimposed on adenosine- or nitroprusside-induced vasodilatation, the result has invariably been large decreases in blood flow. This approach has served to validate that the diminished vasoconstrictor responses during exercise are not related to changes in drug delivery during increases in blood flow.

It should be noted that flow-adjusting the dose of sympathomimetic does not secure adjustment of drug concentration in the vascular wall interstitium or receptor occupancy. The higher absolute drug dose would tend to increase and the lower transit time to decrease receptor occupancy when using flow-adjusted dosing during exercise. This may be a concern, especially when the drugs are administered as bolus injections (Buckwalter *et al.* 1998; Ruble *et al.* 2002). Another caveat to flow-adjusted doses is that the resultant intra-arterial α-agonist concentration will be different during the latter part of drug administration in a condition where flow does not change much in response to the agonist drug (i.e. exercise) compared to a condition where flow decreases by about 50% in response to the agonist drug (i.e. resting pharmacological vasodilatation) (Rosenmeier *et al.* 2003). In the present study, the wide dose–response ranges in combination with flow-adjusted dosing (both by design and *post hoc*) have minimized these potential concerns of using intra-arterial infusion of agonists.

When using pharmacological tools, the specificity of the drugs should always be considered. Phenylephrine has the ability to activate β-adrenergic receptors (Torp *et al.* 2001). This effect is unlikely to be of major importance in the present study, since exercise attenuated phenylephrine responses to similar degrees both with and without complete β-blockade by the non-specific β-blocker propranolol (protocols 3 and 4). We chose phenylephrine, because it is well accepted to be a relatively

specific α_1-agonist. We chose BHT-933 as an α_2-agonist, rather than the classical drug clonidine, because in a previous study clonidine actions in the human forearm were inhibited by both the α_1- (prazosin) and α_2-antagonist (yohimbine), whereas BHT-933 actions were only inhibited by yohimbine (Jie *et al.* 1984). Our data (protocol 2) supported the specificity of PE for α_1-receptors since the α_2-antagonist yohimbine caused no decrease in the response to PE, and provided the first evidence in humans that BHT actions in the human thigh are largely inhibited by an α_2-antagonist.

When using subtype-selective agonists, the differential location of the receptors should also be considered. Specifically, α_1-receptors are almost exclusive located postsynaptically on vascular smooth muscle, while α_2-receptors have been identified both on smooth muscle and presynaptically on the sympathetic boutons. Activation of the presynaptic α_2-receptors is known to cause inhibition of noradrenaline release from the boutons, which may decrease effects on postsynaptic receptors (Guimaraes & Moura, 2001). In the present study, this presynaptic effect of BHT-933 is not dominant at rest, because the maximal vasoconstriction of BHT-933 is simlar to that of PE. During mild and moderate intensities of knee-extensor exercise, previous studies have demonstrated that noradrenaline levels remain similar to resting values (Turcotte *et al.* 1992; Steensberg *et al.* 2002). Furthermore, during increasing levels of sympathetic activation and noradrenaline release the presynaptic effects of α_2-agonists decrease, probably because noradrenaline has already activated the receptors (Guimaraes & Moura, 2001). Thus, the presynaptic effects of BHT-933 may contribute to but are unlikely to completely explain the lack of vasoconstriction during exercise in the present study.

One disadvantage of using the knee-extensor exercise model is that the maximal drug doses may have minor systemic effects. The highest intrafemoral doses of PE caused significant changes in blood pressure and heart rate during exercise, but not during rest. The explanation for this differential effect is likely to be related to the different transit time of PE during the two conditions. At rest the mean transit time during PE administration in the thigh is likely to be more than 15 s in the leg alone, while during exercise the transit time is decreased to 5 s (Bangsbo *et al.* 2000). Thus, the uptake and degradation of PE may be incomplete during exercise. It is worth emphasizing, that such a 'flow-through phenomenon' is unlikely to explain the exercise-induced inhibition of α-agonist effects, because simply increasing flow and lowering transit time by vasodilators in other studies have

not been sufficient to inhibit α-agonist vasoconstriction (Rosenmeier *et al.* 2003; Dinenno & Joyner, 2003).

In the present study, the dose–response and ramped exercise protocols both included the responses to phenylephrine at 0.3 μg kg^{-1} l^{-1} min^{-2} and to BHT-933 at 15 μg kg^{-1} l^{-1} min^{-2} at rest and during 27 W knee-extensor exercise. In both protocols, the overall findings were similar, because phenylephrine and BHT-933 caused large decreases in femoral blood flow and conductance at rest, and visibly smaller responses during exercise. It should be noted, however, that the blood flow decrease in response to phenylephrine during exercise reached significance only in the dose–response protocol, whereas the response to phenylephrine during exercise *versus* rest was only significant in the ramped exercise protocol. The reason for this difference in the responses to phenylephrine in the two protocols is unclear, but could be related to the difference in the duration and total dose of the phenylephrine infusion. The responses to BHT-933 during 27 W exercise were very similar in both protocols.

By design we have not attempted in the present study to address whether residual sympathetic vasoconstriction from endogenously released noradrenaline exists in the exercising human thigh. This could be addressed pharmacologically by using α-antagonists rather than agonists. In animals, previous studies using this approach have yielded conflicting results. The literature has been reviewed in the discussion of a recent study in exercising dogs which provided evidence for both residual α_1- and α_2-vasoconstriction in exercising legs (Buckwalter & Clifford, 1999). In humans, systemic α-blockade is not accompanied by increases in blood flow to exercising forearm (Hartling & Trap-Jensen, 1983). In contrast, 20–30% increases in plethysmographic blood flows were seen during heavy handgrip exercise after stellate block (Joyner *et al.* 1992). In pilot studies, we have found intra-arterial administration of the α_1-blocker prazosin into the femoral artery yields incomplete α_1-blockade in the human leg, even at doses that cause severe orthostatic hypotension. Thus, adjustments to the exercise model or to the pharmacological intervention are needed before α-antagonist studies can be performed in the human thigh.

Conclusions and potential clinical significance

We have identified a functionally differential distribution of α-adrenoreceptor subtypes in the vasculature of the human leg. Furthermore, we have demonstrated a higher sensitivity of α_2-mediated vasoconstriction compared to α_1-mediated vasoconstriction to the metabolic events taking place during mild thigh muscle exercise. Strikingly, we have observed that moderate intensity knee-extensor exercise completely inhibits the vasoconstrictor actions of intra-arterially administered exogenous sympathomimetics. Knowledge of these mechanisms may provide the clinician with a better understanding of the functional consequences of pathological vascular disorders associated with age, diabetes, heart failure and hypertension, and present possible diagnostic techniques and methods of treatment. For example, an age-related reduction in α-adrenergic responsiveness specific to α_1-receptors has recently been described (Dinenno *et al.* 2002*a*). This emphasizes the need for a better understanding of subtype contributions to peripheral vascular control. Furthermore, it has been demonstrated that patients suffering from congestive heart failure experience diminished skeletal muscle perfusion at rest and during submaximal and maximal exercise, with accompanying increased vascular resistance (Sullivan *et al.* 1989). Recent experimental and clinical trials indicate that this relative hypoperfusion is related to an inability of the skeletal muscle to adequately vasodilate in opposition to the increased sympathetic nerve activity that accompanies exercise (Johnson *et al.* 1999; Notarius *et al.* 1999; Thomas *et al.* 2001). Since these patients might benefit from exercise as prophylaxis and as part of a cardiac rehabilitation program (Monchamp & Frishman, 2002), knowledge of α-adrenoceptor contribution to the control of blood flow might provide a therapeutic target in this population.

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