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Blunted sympathetic vasoconstriction in contracting skeletal muscle of healthy humans: is nitric oxide obligatory?

Frank A. Dinenno and Michael J. Joyner

Department of Anesthesiology and General Clinical Research Center, Mayo Clinic and Foundation, Rochester, MN 55905, USA

We tested the hypothesis that nitric oxide (NO) is responsible for blunting sympathetic α-adrenergic vasoconstriction in the active muscles of humans (functional sympatholysis). We measured forearm blood flow (Doppler ultrasound) and calculated the reductions in forearm vascular conductance (FVC) in response to α-adrenergic receptor stimulation during rhythmic handgrip exercise and during a control non-exercise vasodilator condition (intra-arterial adenosine), before and after local NO synthase (NOS) inhibition in healthy men. The forearm vasoconstrictor responses to endogenous noradrenaline release (intra-arterial tyramine) were significantly blunted during moderate exercise compared with adenosine, and these vasoconstrictor responses were not restored by NOS inhibition with N^G-monomethyl-L-arginine (L-NMMA; $n = 6$) or N^G-nitro-L-arginine methyl ester (L-NAME; $n = 8$). Similarly, L-NAME did not restore the vasoconstrictor responses to tyramine in contracting muscle during heavy rhythmic handgrip exercise ($n = 4$). In four additional subjects, we also found that the vasoconstrictor responses evoked by tyramine during exercise or adenosine were repeatable in the absence of NOS inhibition (i.e. time control). Finally, in five subjects the forearm vasoconstrictor responses to direct α_1-adrenergic (phenylephrine) and α_2-adrenergic (clonidine) receptor stimulation were blunted during moderate exercise compared with adenosine; these responses were also unaffected by L-NAME. Taken together, our results demonstrate that NO is not obligatory for functional sympatholysis in contracting skeletal muscles of healthy men.

During dynamic exercise, there is competition between local metabolic vasodilatation and sympathetic vasoconstriction as determinants of skeletal muscle vascular tone and blood flow. Although sympathetic vasoconstriction persists in active skeletal muscle and is important for appropriate blood pressure regulation during exercise (Marshall *et al.* 1961), the vascular responses to a variety of sympathetic α-adrenergic vasoconstrictor stimuli are blunted in active compared with resting muscle, a phenomenon known as 'functional sympatholysis' (Remensnyder *et al.* 1962; Anderson & Faber, 1991; Thomas *et al.* 1994; Buckwalter *et al.* 2001; Ruble *et al.* 2002; Tschakovsky *et al.* 2002). This observed reduction in vascular responsiveness to sympathetic stimulation might optimize blood flow distribution and oxygen delivery under conditions in which there are potential mismatches in oxygen supply and demand (Strandell & Shepherd, 1967; VanTeeffelen & Segal, 2003).

Skeletal muscle contractions evoke the release of a number of substances from both the active muscle and vascular endothelium that can potentially modulate sympathetic α-adrenergic vasoconstriction. Adenosine, prostaglandins,

and more recently, nitric oxide (NO) have been postulated as putative 'sympatholytic' factors that are involved in this integrative regulation of muscle blood flow during exercise (Hansen *et al.* 2000). Importantly, under a variety of experimental conditions, all of these substances can reduce the vasoconstrictor responses to sympathetic α-adrenergic stimulation (Lippton *et al.* 1981; Faber *et al.* 1982; Nishigaki *et al.* 1991; Ohyanagi *et al.* 1992). In this context, data derived from a series of studies in experimental animals suggest that NO is involved, at least in part, in the blunted α-adrenergic vasoconstrictor responses in active muscle (Thomas & Victor, 1998). The source of NO during muscle contractions appears to be derived from neuronal NO synthase (nNOS), and the postulate is that calcium released from contracting skeletal muscle stimulates nNOS activity (a calcium–calmodulin-dependent enzyme) which subsequently increases NO synthesis and release (Hansen *et al.* 2000; Grange *et al.* 2001). Under these conditions, NO appears to blunt α-adrenergic vasoconstriction by limiting smooth muscle regulatory light chain phosphorylation (Grange *et al.* 2001). Collectively, the available evidence from these studies in animals indicates that this elevation in NO

interferes with α-adrenergic vasoconstriction via activation of ATP-sensitive potassium (K_{ATP}) channels, but does not completely 'restore' this vasoconstriction (Thomas *et al.* 1997; Thomas & Victor, 1998).

To date, only two studies in humans have addressed whether NO is responsible for blunting sympathetic vaso-constriction in active skeletal muscle and have provided discrepant findings. Wilson & Kapoor (1993) used venous occlusion plethysmography to estimate forearm blood flow during wrist flexion exercise with and without concomitant intra-brachial noradrenaline infusion, and found that local NOS inhibition via N^G-monomethyl-L-arginine (L-NMMA) did not affect the blood flow responses. However, these data are difficult to interpret because blood flow measures obtained with this technique reflect post-exercise rather than active muscle blood flow, an issue that has received recent attention (Shoemaker *et al.* 1997; Frandsen *et al.* 2001). In contrast, Chavoshan *et al.* (2002) demonstrated that the blunted vasoconstrictor responses in contracting forearm muscle during reflex increases in sympathetic activity (estimated via reductions in muscle oxygenation with near infrared spectroscopy) were completely reversed after systemic NOS inhibition with N^G-nitro-L-arginine methyl ester (L-NAME). From this study it is unclear what effects NOS inhibition had on the forearm hemodynamic responses during exercise, which would seem of importance with respect to both muscle blood flow and systemic blood pressure regulation during exercise.

In addition to these potential limitations of each respective study, the discrepant findings might be related to the use of L-NMMA compared with L-NAME to inhibit NOS. Specifically, L-NAME is believed to be a more potent NOS inhibitor and might inhibit nNOS to a greater degree than L-NMMA and therefore, might be the more appropriate NOS inhibitor to use for studies involving muscle contractions (Frandsen *et al.* 2001).

With this information as a background, the purpose of the present investigation was to test the hypothesis that NO is responsible for the blunted α-adrenergic vasoconstrictor responses in the vascular beds of contracting skeletal muscle. To do so, we measured forearm hemodynamics (Doppler ultrasound) during rhythmic handgrip exercise and intra-arterial adenosine ('control' vasodilator), and determined the vasoconstrictor responses to α-adrenergic stimulation before and after local NOS inhibition. Utilizing several experimental approaches, our findings indicate that acute NOS inhibition does not restore α-adrenergic vasoconstrictor responses in contracting muscle, thus suggesting that NO is not obligatory to observe functional sympatholysis in healthy humans.

METHODS

Subjects

With Institutional Review Board approval and after giving written informed consent, a total of 28 young healthy men (age 25 ± 1 years; weight 77.6 ± 2.3 kg; height 180 ± 1 cm; body mass index 23.6 ± 0.5 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, 5 cm catheter was placed in the brachial artery of the non-dominant arm under aseptic conditions after local anaesthesia (1 % lidocaine (lignocaine)) for local administration of study drugs. The catheter was connected to a pressure transducer for mean arterial pressure (MAP) measurement and continuously flushed at 3 ml h^{-1} with heparinized saline (Dietz *et al.* 1994)

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 (Tschakovsky *et al.* 2002). The probe insonation angle was 60 deg. A linear 7.0 MHz echo Doppler ultrasound probe (Acuson 128XP, Mountain View, CA, USA) 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:

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

where the FBF is in millilitres per minute, the MBV is in centimetres per second, the brachial diameter is in centimetres, and 60 is used to convert from millilitres per second to millilitres per minute. Forearm vascular conductance (FVC) was calculated as (FBF/MAP) $\times$ 100, and expressed as millilitres per minute per 100 mmHg.

Rhythmic handgrip exercise

Rhythmic forearm handgrip exercise was performed using either a 6.4 kg weight that was ~10–15 % of maximal voluntary contraction (MVC), or a 12.1 kg weight that was approximately ~20–25 % MVC. The weight was lifted 4–5 cm over a pulley at a duty cycle of 1 s contraction–2 s relaxation (20 contractions per minute) using a signal light to insure the correct timing.

Sympathetic α-adrenergic vasoconstrictor drugs

The following drugs were infused via the brachial artery catheter: Tyramine was infused at 4 and 8 μg (per decilitre forearm volume per minute). These doses were based on the effects of forearm exercise on the tyramine vasoconstrictor dose-response curves reported by Tschakovsky *et al.* (2002). Tyramine evokes endogenous noradrenaline release from sympathetic nerve endings (Frewin & Whelan, 1968) and subsequent post-junctional α_1- and α_2-adrenergic vasoconstriction (Jie *et al.* 1987). Importantly, tyramine does not have any direct vasoconstrictor effects (Frewin & Whelan, 1968), and the vascular responses to tyramine are abolished by non-selective α-adrenergic blockade (Dinenno *et al.* 2002*a,b*). Phenylephrine (a selective α_1 agonist) was infused at 0.0312 μg (dl forearm volume)$^{-1}$ min^{-1} and

clonidine (an α_2 agonist) was infused at 0.15 μg (dl forearm volume)$^{-1}$ min^{-1}. The doses of phenylephrine and clonidine were based on our experience at rest (Dinenno *et al.* 2002*a*) and during handgrip exercise (Rosenmeier *et al.* 2003). All vasoconstrictor drug infusions were adjusted for the hyperaemic conditions (see below).

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 tyramine, phenylephrine and clonidine, 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 (Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003). 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 water displacement). Care was taken so that various concentrations of each compound were available and the absolute infusion rate was 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. The subjects performed either a bout of forearm exercise or they received intra-arterial adenosine; the total time for each trial was 9 min. After 2 min of baseline measurements, exercise or adenosine 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 vasoconstricting agent was calculated on the basis of forearm volume and blood flow. The vasoconstrictor infusion began at the 6-minute mark and lasted for 3 min.

Protocol 1. Effects of NOS inhibition with L-NMMA on sympathetic vasoconstrictor responses during handgrip exercise. In the first six subjects, the vasoconstrictor responses to tyramine (4 and 8 μg (dl forearm volume)$^{-1}$ min^{-1}) were assessed during control vasodilator infusion of adenosine (6.25 μg (dl forearm volume)$^{-1}$ min^{-1}) and during moderate rhythmic handgrip exercise (6.4 kg). The order of tyramine dose and exercise or adenosine was randomized, and the subjects rested for 15 min between each trial. After these four initial trials (both doses of tyramine during adenosine and exercise), L-NMMA was administered at 5 mg for 10 min (total dose = 50 mg) to inhibit NOS (Dietz *et al.* 1994). We have previously documented that this dose reduces basal FBF as well as the vasodilator responses to acetylcholine, consistent with effective NOS blockade (Dietz *et al.* 1994; Eisenach *et al.* 2002). A maintenance dose of L-NMMA (1 mg min^{-1}) was continued throughout the rest of the experimental protocol (Eisenach *et al.* 2002). Subsequently, the vasoconstrictor responses to tyramine were assessed again during adenosine and handgrip exercise in randomized order. In this protocol and those that follow, NOS inhibition was performed after the first set of adenosine and exercise trials due to the long half-life of NOS inhibition.

Protocol 2. Effects of NOS inhibition with L-NAME on sympathetic vasoconstrictor responses during handgrip exercise. L-NAME has been suggested to inhibit nNOS more effectively than L-NMMA, and thus might be the more appropriate NOS inhibitor for studies involving muscle contractions (Frandsen *et al.* 2001). We therefore repeated protocol 1 in eight additional subjects using L-NAME as the NOS inhibitor. In these subjects, the vasoconstrictor responses to tyramine (4 and 8 μg (dl forearm volume)$^{-1}$ min^{-1}) were assessed during control vasodilator infusion of adenosine (6.25 μg (dl forearm volume)$^{-1}$ min^{-1}) and during moderate rhythmic handgrip exercise (6.4 kg). The order of tyramine dose and exercise or adenosine was randomized, and the subjects rested for 15 min between each trial. After these initial trials, L-NAME was administered at 5 mg for 10 min (total dose = 50 mg) to inhibit NOS. A maintenance dose of L-NAME (1 mg min^{-1}) was continued throughout the rest of the experimental protocol. The efficacy of this dose of L-NAME to inhibit NOS was tested in the subjects who participated in protocol 4 (see below). Subsequently, the vasoconstrictor responses to tyramine were assessed during adenosine and handgrip exercise in randomized order.

Protocol 3. Repeatability of vasoconstrictor responses to tyramine. The purpose of this protocol was to determine whether the forearm vasoconstrictor responses to tyramine were consistent over time throughout the length of the experimental protocol in the absence of NOS inhibition (time control). In four additional subjects, the vasoconstrictor responses to tyramine (4 and 8 μg (dl forearm volume)$^{-1}$ min^{-1}) were assessed during control vasodilator infusion of adenosine (6.25 μg (dl forearm volume)$^{-1}$ min^{-1}) and during moderate rhythmic handgrip exercise (6.4 kg). The order of tyramine dose and exercise or adenosine was randomized, and the subjects rested for 15 min between each trial. In these subjects we did not administer a NOS inhibitor. The vasoconstrictor responses to tyramine were then repeated during adenosine and handgrip exercise in randomized order.

Protocol 4. Effects of L-NAME on sympathetic vasoconstrictor responses during heavy handgrip exercise. It is well known that the blunted sympathetic vasoconstrictor responses during exercise are graded with the level of exercise intensity such that a greater reduction in vasoconstriction (greater sympatholysis) is observed during higher workloads (Anderson & Faber, 1991;
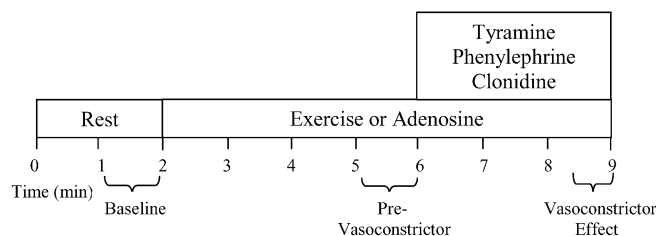
Figure 1. Experimental trial

Each trial consisted of a 2-min rest (baseline) period. After this time period, subjects either began rhythmic forearm exercise or received intra-arterial adenosine to elevate resting forearm blood flow to similar levels observed during exercise (control non-exercise vasodilator). During minutes 5 and 6 (pre-vasoconstrictor), the dose of the α-adrenergic agonist was calculated on the basis of steady state hyperaemic forearm blood flow and forearm volume. Subsequently, the α-agonist (tyramine, phenylephrine or clonidine) was infused at minute 6 and lasted for 3 min. An average of the forearm blood flow and mean arterial blood pressure during the last 30 s of α-agonist infusion was used to calculate the vasoconstrictor effect during both hyperaemic conditions.

Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002). Additionally, in rats it appears that nNOS might be preferentially located in fast-twitch muscle fibres (Kobzik *et al.* 1994), although this might not be the case in humans (Frandsen *et al.* 1996). Thus, it is possible that nNOS might be activated to a greater extent during heavier workloads when a greater proportion of fast-twitch muscle fibres are recruited during contractions. Therefore, the purpose of this protocol was to determine whether NO blunts sympathetic vasoconstriction during heavy exercise. In four additional subjects, the vasoconstrictor responses to tyramine (8 μg (dl forearm volume)$^{-1}$ min^{-1}) were assessed during control vaso-dilator infusion of adenosine (12.50 μg (dl forearm volume)$^{-1}$ min^{-1}) and during heavy rhythmic handgrip exercise (12.1 kg). Only one dose of tyramine was administered in these subjects to reduce the number of exercise trials in an effort to minimise any potential effects of muscle fatigue associated with this workload. L-NAME was then administered at 5 mg min^{-1} for 10 min (total dose = 50 mg) and a maintenance dose of L-NAME (1 mg min^{-1}) was continued throughout the rest of the experimental protocol. In previous studies in humans utilizing L-NAME to inhibit NOS during exercise, a systemic dose was administered intravenously over 1 h and NOS activity was reduced by ~70 % (Frandsen *et al.* 2001). Therefore, in this protocol, the vasoconstrictor responses to tyramine were assessed during adenosine and heavy handgrip exercise after 1 h of the termination of the 10 min loading dose of L-NAME to ensure maximal reduction in NOS activity.

Because we are unaware of any studies in humans that have administered intra-arterial L-NAME, we tested the efficacy of NOS inhibition in these subjects by determining the vasodilator responses to brachial artery infusions of acetylcholine (which is partly NO mediated) before and after L-NAME. Therefore, 20 min after the initial adenosine and exercise trials, acetylcholine (1, 2 and 4 μg (dl forearm volume)$^{-1}$ min^{-1}) was infused for 2 min at each dose, and these infusions were subsequently repeated 10 min after L-NAME administration.

Protocol 5. Effects of L-NAME on post-junctional α-adrenergic vasoconstrictor responses during handgrip exercise. The purpose of this protocol was to determine whether NOS inhibition restores post-junctional α-adrenergic vasoconstriction during exercise. Because the tyramine-induced release of noradrenaline during adenosine infusions and exercise (both before and after NOS inhibition) is difficult to assess, in five additional subjects we determined the vasoconstrictor responses to direct α-adrenergic stimulation via phenylephrine (selective α_1 agonist) and clonidine (α_2 agonist). Four subjects received both phenylephrine and clonidine, and one subject received clonidine only. The vasoconstrictor responses to phenylephrine and clonidine were assessed during control vasodilator infusion of adenosine (6.25 μg (dl forearm volume)$^{-1}$ min^{-1}) and during moderate rhythmic handgrip exercise (6.4 kg). The order of α-agonist administration and exercise or adenosine was randomized, and the subjects rested for 15 min between each trial. After these initial trials, our standard L-NAME loading dose was administered and a maintenance dose was continued throughout the rest of the experimental protocol. Subsequently, the vaso-constrictor responses to phenylephrine and clonidine were assessed during adenosine and handgrip exercise, but the exercise trials were performed last to make sure that these were performed 1 h post L-NAME administration.

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 and MAP represent an average of the last minute of the resting time period, the hyperaemic values represent an average of minutes 3–4 (minutes 5–6 of Fig. 1; pre-vaso-constrictor) during adenosine or exercise, and the effects of the α-agonists represent an average of the final 30 s of drug infusion (post-vasoconstrictor).

The percentage reduction in FVC during vasoconstrictor administration was calculated as:

$$\frac{\text{FVC post constrictor} - \text{FVC pre constrictor}}{\text{FVC pre constrictor}} \times 100.$$

We used percentage reduction in FVC as our standard index to compare vasoconstriction across conditions when forearm blood flow and vascular conductance might differ. After much discussion this index has emerged as the most appropriate way to compare interventions that cause vasodilatation or vaso-constriction under conditions where there might be marked difference in baseline blood flow (Lautt, 1989; O'Leary, 1991; Thomas *et al.* 1994; Tschakovsky *et al.* 2002).

Statistics

All values are reported as means ± S.E.M. Specific hypothesis testing within each of the exercise or adenosine trials with the three different drug infusions was performed using repeated measures ANOVA. Comparison of the haemodynamic values at specific time points between the exercise and adenosine conditions were made with unpaired *t* tests, and the values within each hyperaemic condition (exercise or adenosine) before and after NOS inhibition were made with paired *t* tests. Significance was set at $P < 0.05$.

RESULTS

Protocol 1. Effects of NOS inhibition with L-NMMA on sympathetic vasoconstrictor responses during handgrip exercise

Forearm haemodynamics and MAP are presented in Table 1. Adenosine increased FBF and FVC significantly, but the steady-state forearm haemodynamics were slightly less than achieved during exercise. The vasoconstrictor responses to the low and high doses of tyramine were significantly blunted during exercise (ΔFVC = -21 ± 4 and -20 ± 4 %, respectively) compared with the responses during adenosine (-44 ± 6 and -64 ± 5 %; $P < 0.001$ *vs.* exercise; Fig. 2). NOS inhibition via L-NMMA reduced baseline FVC ~40 %, and the steady-state FVC during adenosine (~15–30 %) and handgrip exercise (~10 %). However, the low and high doses of tyramine evoked similar vasoconstrictor responses during adenosine (ΔFVC = -45 ± 6 and -62 ± 6 %, respectively), and this reduction was still significantly blunted during exercise (-20 ± 2 and -22 ± 2 %; Fig. 2). Importantly, the tyramine-induced vasoconstriction was similar during exercise before and after L-NMMA ($P > 0.05$).

Table 1. Forearm and systemic haemodynamics for moderate exercise before and after
N^G**-monomethyl-L-arginine (L-NMMA) (protocol 1:** $n = 6$**)**

| Variable	Tyramine dose	Time	Adenosine		Exercise	
			Pre L-NMMA	Post L-NMMA	Pre L-NMMA	Post L-NMMA
FBF (ml min^{-1})	Low	Baseline	75 ± 11	50 ± 6*	61 ± 9	44 ± 6*
		Pre-tyramine	232 ± 47	187 ± 33	297 ± 27	282 ± 32
		Post-tyramine	123 ± 20	96 ± 9	240 ± 27†	231 ± 25†
	High	Baseline	73 ± 11	43 ± 6*	65 ± 13	45 ± 8
		Pre-tyramine	224 ± 46	217 ± 45	296 ± 21	284 ± 31
		Post-tyramine	76 ± 11	75 ± 11	245 ± 25†	232 ± 29†
FVC (ml min^{-1} (100 mmHg)$^{-1}$)	Low	Baseline	85 ± 13	51 ± 6*	68 ± 11	45 ± 6*
		Pre-tyramine	263 ± 54	193 ± 35	315 ± 27	283 ± 30*
		Post-tyramine	133 ± 21	98 ± 10	248 ± 25†	225 ± 23†
	High	Baseline	79 ± 13	43 ± 6*	71 ± 15	45 ± 8*
		Pre-tyramine	253 ± 57	213 ± 43	307 ± 19	280 ± 34
		Post-tyramine	81 ± 11‡	72 ± 9	247 ± 24†	221 ± 29†
MAP (mmHg)	Low	Baseline	88 ± 1	98 ± 4*	89 ± 2	98 ± 3
		Pre-tyramine	89 ± 1	97 ± 3*	94 ± 3	99 ± 4
		Post-tyramine	92 ± 2	98 ± 4	97 ± 3	103 ± 3
	High	Baseline	93 ± 4	100 ± 4*	92 ± 3	99 ± 3
		Pre-tyramine	91 ± 4	101 ± 3*	96 ± 3	103 ± 3
		Post-tyramine	94 ± 3	103 ± 5*	99 ± 3	106 ± 2

FBF, forearm blood flow; FVC, forearm vascular conductance; MAP, mean arterial pressure. * $P < 0.05$ post *vs.* pre L-NMMA within same hyperaemic condition; † $P < 0.05$ exercise *vs.* adenosine within same L-NMMA condition; ‡ $P < 0.05$ *vs.* tyramine low during adenosine and within same L-NMMA condition.

Protocol 2. Effects of NOS inhibition with L-NAME on sympathetic vasoconstrictor responses during handgrip exercise

Forearm haemodynamics and MAP are presented in Table 2. Steady-state FBF and FVC during adenosine and exercise were similar. The vasoconstrictor responses to the low and high doses of tyramine were significantly blunted during exercise (ΔFVC = −16 ± 3 and −24 ± 2 %, respectively) compared with the responses during adenosine (−54 ± 6 and −74 ± 5 %; $P < 0.001$ *vs.* exercise; Fig. 3). NOS inhibition via L-NAME reduced baseline FVC ~50 %, and the steady-state FVC during adenosine (~55 %) and handgrip exercise (~20 %). However, the low and high doses of tyramine evoked similar vasoconstrictor responses during adenosine (ΔFVC = −47 ± 4 and −67 ± 5 %, respectively), and this reduction was still significantly blunted during exercise (−16 ± 3 and −26 ± 2 %; Fig. 3). Importantly, the tyramine-induced vasoconstriction was similar during exercise before and after L-NAME ($P > 0.05$).

Protocol 3. Repeatability of vasoconstrictor responses to tyramine

Forearm haemodynamics and MAP are presented in Table 3. In trial 1, adenosine increased FBF and FVC significantly, but the steady-state forearm haemodynamics were slightly less than achieved during exercise. The vasoconstrictor responses to the low and high doses of
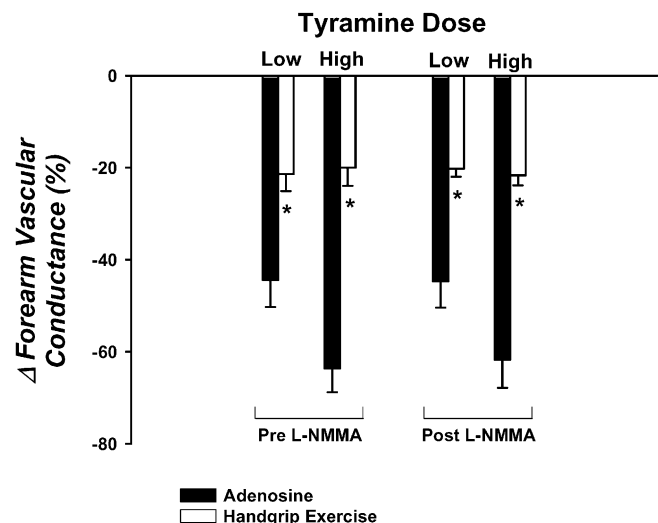
Figure 2. Effects of L-NMMA on forearm vasoconstrictor responses to tyramine

The vasoconstrictor responses to tyramine are significantly blunted during rhythmic handgrip exercise (open bars) compared with a control vasodilator condition (adenosine; filled bars). Intra-arterial administration of L-NMMA to inhibit nitric oxide synthase (NOS) does not augment the vasoconstrictor responses during adenosine or (more importantly) during handgrip exercise. * $P < 0.05$ *vs.* adenosine for given tyramine dose within same L-NMMA condition.

Table 2. Forearm and systemic haemodynamics for moderate exercise before and after L-NAME (protocol 2: $n = 8$)

Variable	Tyramine dose	Time	Adenosine		Exercise	
			Pre L-NAME	Post L-NAME	Pre L-NAME	Post L-NAME
FBF (ml min^{-1})	Low	Baseline	69 ± 9	$43 \pm 4^*$	72 ± 13	$36 \pm 4^*$
		Pre-tyramine	267 ± 26	$134 \pm 16^*$	277 ± 21	252 ± 25†
		Post-tyramine	119 ± 13	$69 \pm 5^*$	238 ± 16†	$215 \pm 16^*$†
	High	Baseline	69 ± 10	$46 \pm 5^*$	64 ± 10	$39 \pm 3^*$
		Pre-tyramine	272 ± 38	$149 \pm 18^*$	282 ± 31	266 ± 22†
		Post-tyramine	68 ± 9‡	$48 \pm 5^*$‡	219 ± 24†	204 ± 19†
FVC (ml min^{-1} (100 mmHg)$^{-1}$)	Low	Baseline	75 ± 9	$40 \pm 6^*$	74 ± 12	$33 \pm 3^*$
		Pre-tyramine	287 ± 22	$125 \pm 14^*$	283 ± 16	$227 \pm 18^*$†
		Post-tyramine	126 ± 14	$63 \pm 4^*$	236 ± 12†	$187 \pm 10^*$†
	High	Baseline	75 ± 10	$42 \pm 4^*$	69 ± 10	$36 \pm 3^*$
		Pre-tyramine	298 ± 41	$137 \pm 14^*$	286 ± 25	$241 \pm 15^*$†
		Post-tyramine	72 ± 11‡	$44 \pm 4^*$‡	217 ± 18†	$179 \pm 14^*$†
MAP (mmHg)	Low	Baseline	92 ± 2	$107 \pm 4^*$	95 ± 3	$107 \pm 2^*$
		Pre-tyramine	92 ± 3	$107 \pm 3^*$	98 ± 4	$110 \pm 3^*$
		Post-tyramine	95 ± 3	$109 \pm 2^*$	101 ± 4	$114 \pm 4^*$
	High	Baseline	91 ± 2	$108 \pm 3^*$	93 ± 3	$107 \pm 3^*$
		Pre-tyramine	91 ± 2	$107 \pm 3^*$	98 ± 4	$110 \pm 3^*$
		Post-tyramine	96 ± 3	$109 \pm 3^*$	100 ± 4	$113 \pm 4^*$

$^*P < 0.05$ post *vs.* pre L-NAME within same hyperaemic condition; †$P < 0.05$ exercise *vs.* adenosine within same L-NAME condition; ‡$P < 0.05$ *vs.* tyramine low during adenosine and within same L-NAME condition.
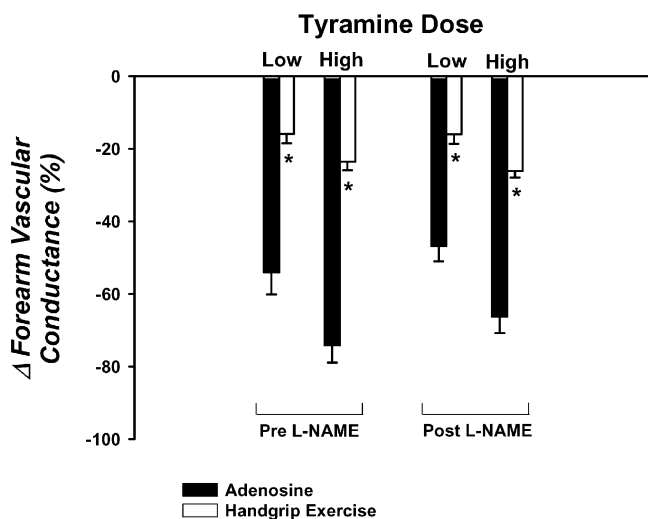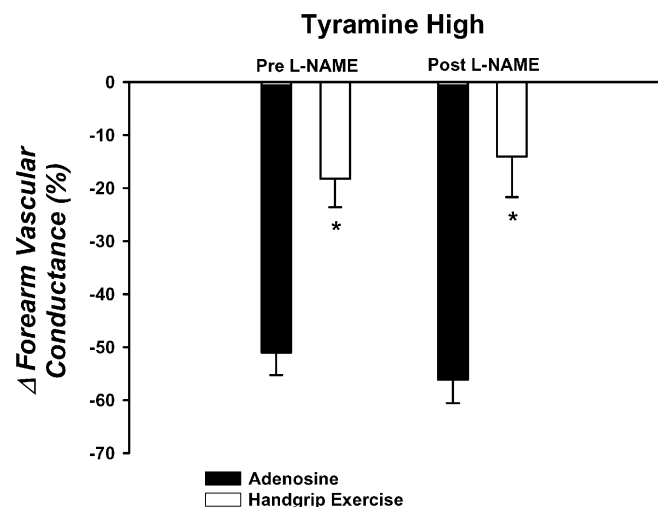

Figure 3. Effects of L-NAME on forearm vasoconstrictor responses to tyramine

The vasoconstrictor responses to tyramine are significantly blunted during rhythmic handgrip exercise (open bars) compared with a control vasodilator condition (adenosine; filled bars). Intra-arterial administration of L-NAME to inhibit NOS does not augment the vasoconstrictor responses during adenosine or (more importantly) during handgrip exercise. $^* P < 0.05$ *vs.* adenosine for given tyramine dose within same L-NAME condition.

Figure 4. Effects of L-NAME on forearm vasoconstrictor responses to tyramine during heavy rhythmic handgrip exercise

The vasoconstrictor responses to tyramine are significantly blunted during heavy handgrip exercise (open bars) compared with a control vasodilator condition (adenosine; filled bars). NOS inhibition with L-NAME does not augment the vasoconstrictor responses during either adenosine or exercise. $^* P < 0.05$ *vs.* adenosine within same L-NAME condition.

Table 3. Forearm and systemic haemodynamics for time control trials (protocol 3: $n = 4$)

Variable	Tyramine dose	Time	Adenosine		Exercise	
			Trial 1	Trial 2	Trial 1	Trial 2
FBF (ml min^{-1})	Low	Baseline	37 ± 5	41 ± 6	31 ± 3	40 ± 6
		Pre-tyramine	118 ± 18	$166 \pm 26*$	180 ± 23	192 ± 19
		Post-tyramine	62 ± 7	92 ± 17	163 ± 22	175 ± 16
	High	Baseline	41 ± 6	38 ± 3	33 ± 5	36 ± 5
		Pre-tyramine	142 ± 19	144 ± 33	183 ± 24	193 ± 23
		Post-tyramine	54 ± 6	54 ± 8	157 ± 22	171 ± 29
FVC (ml min^{-1} (100 mmHg)$^{-1}$)	Low	Baseline	41 ± 5	45 ± 6	35 ± 4	42 ± 6
		Pre-tyramine	133 ± 19	179 ± 24	190 ± 17	198 ± 11
		Post-tyramine	70 ± 7	96 ± 14	164 ± 5	171 ± 14
	High	Baseline	46 ± 8	41 ± 4	38 ± 7	39 ± 5
		Pre-tyramine	157 ± 19	156 ± 36	195 ± 19	198 ± 13
		Post-tyramine	58 ± 6	58 ± 8	160 ± 19	162 ± 15
MAP (mmHg)	Low	Baseline	88 ± 2	91 ± 3	89 ± 2	94 ± 2
		Pre-tyramine	88 ± 3	91 ± 3	94 ± 5	97 ± 4
		Post-tyramine	89 ± 4	94 ± 4	99 ± 6	101 ± 7
	High	Baseline	90 ± 2	93 ± 1	90 ± 3	94 ± 2
		Pre-tyramine	90 ± 3	92 ± 1	93 ± 4	97 ± 5
		Post-tyramine	93 ± 5	94 ± 1	98 ± 8	104 ± 9

$*P < 0.05$ trial 2 *vs.* trial 1 within same hyperaemic condition.

tyramine were significantly blunted during exercise (ΔFVC $= -14 \pm 2$ and $-18 \pm 5\%$, respectively) compared with the responses during adenosine (-47 ± 5 and $-61 \pm 4\%$; $P < 0.001$ *vs.* exercise). In trial 2, the steady-state forearm haemodynamics were similar to trial 1. Additionally, the reductions in FVC to the low and high dose of tyramine during adenosine (-46 ± 6 and $-61 \pm 4\%$, respectively) as well as during exercise (-14 ± 3 and $-19 \pm 4\%$, respectively) were similar during both trials. These data indicate that the vasoconstrictor responses to tyramine are consistent throughout the experimental protocol, and any lack of effect of NOS inhibition in protocols 1 and 2 cannot be explained by tachyphylaxis to tyramine.

Protocol 4. Effects of L-NAME on sympathetic vasoconstrictor responses during heavy handgrip exercise

Forearm haemodynamics and MAP are presented in Table 4. Adenosine increased FBF and FVC significantly, but the steady-state forearm haemodynamics were less than achieved during heavy exercise. The vasoconstrictor responses to the high dose of tyramine were significantly blunted during exercise (ΔFVC $= -18 \pm 5\%$) compared with the responses during adenosine ($-51 \pm 4\%$; $P < 0.001$ *vs.* exercise; Fig. 4). NOS inhibition via L-NAME reduced baseline FVC $\sim35\%$, and the steady-state FVC during adenosine ($\sim36\%$) and handgrip exercise ($\sim10\%$). However, the high dose of tyramine evoked similar reductions in FVC during adenosine as before L-NAME ($-56 \pm 4\%$), and this reduction was still significantly

blunted during exercise ($-14 \pm 7\%$; Fig. 4). Importantly, the tyramine-induced vasoconstriction was blunted to a similar extent during heavy exercise before and after L-NAME ($P > 0.05$).

In these subjects, L-NAME reduced baseline FVC by $\sim37\%$ before acetylcholine infusion. Additionally, the vaso-dilator responses to all three doses of acetylcholine were significantly reduced after L-NAME (ΔFVC before L-NAME $= 194 \pm 39$, 222 ± 40 and 291 ± 41 ml min^{-1} (100 mmHg)$^{-1}$; ΔFVC after L-NAME $= 62 \pm 16$, 71 ± 18
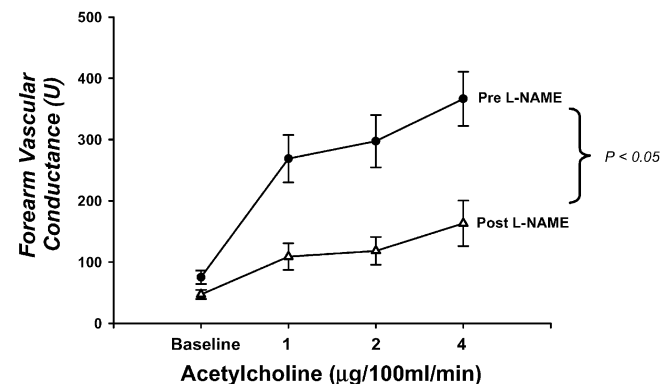
Figure 5. Effects of intra-arterial L-NAME on forearm vasodilator responses to acetylcholine

Baseline forearm vascular conductance (FVC) is reduced $\sim40\%$ and the increases in FVC to acetylcholine are significantly blunted after NOS inhibition with L-NAME. Taken together, these data are consistent with NOS inhibition in the human forearm.

Table 4. Forearm and systemic haemodynamics for heavy exercise before and after L-NAME (protocol 4: *n* = 4)

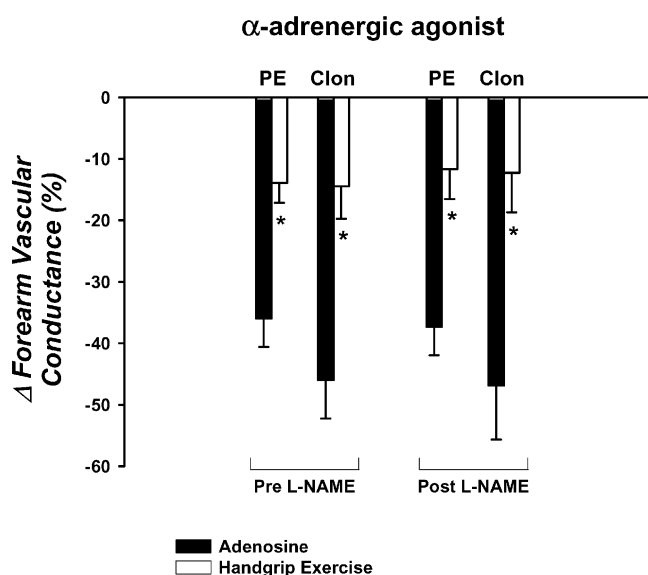| Variable	Tyramine dose	Time	Adenosine		Exercise	
			Pre L-NAME	Post L-NAME	Pre L-NAME	Post L-NAME
FBF (ml min^{-1})	High	Baseline	53 ± 11	41 ± 7	54 ± 10	$38 \pm 7^*$
		Pre-tyramine	238 ± 59	171 ± 40	353 ± 62	370 ± 66†
		Post-tyramine	115 ± 27	72 ± 15	317 ± 74†	338 ± 80†
FVC (ml min^{-1} (100 mmHg)$^{-1}$)	High	Baseline	59 ± 11	$40 \pm 6^*$	59 ± 10	$36 \pm 7^*$
		Pre-tyramine	267 ± 62	170 ± 38	348 ± 61	326 ± 54†
		Post-tyramine	125 ± 25	$71 \pm 15^*$	289 ± 62	289 ± 68†
MAP (mmHg)	High	Baseline	88 ± 5	$101 \pm 4^*$	90 ± 3	$104 \pm 1^*$
		Pre-tyramine	87 ± 4	$100 \pm 2^*$	$102 \pm 4^*$	$113 \pm 3^*$†
		Post-tyramine	89 ± 5	101 ± 2	$109 \pm 6^*$	$116 \pm 5^*$†

$^*P < 0.05$ post *vs.* pre L-NAME within same hyperaemic condition; †$P < 0.05$ exercise *vs.* adenosine within same L-NAME condition.

and 116 ± 22 ml min^{-1} (100 mmHg)$^{-1}$; $P < 0.05$; Fig. 5). Collectively, these data indicate that the dose of L-NAME used in our studies effectively inhibits NOS.

Protocol 5. Effects of L-NAME on post-junctional α-adrenergic vasoconstrictor responses during handgrip exercise

Forearm haemodynamics and MAP are presented in Table 5. Adenosine increased FBF and FVC significantly, but the steady-state forearm haemodynamics were slightly less than achieved during exercise. Phenylephrine

α-adrenergic agonist

Figure 6. Effects of L-NAME on forearm vasoconstrictor responses to phenlyephrine and clonidine
The vasoconstrictor responses to the direct α_1-agonist (phenylephrine; PE) and α_2-agonist (clonidine; Clon) are significantly blunted during exercise compared with adenosine. Local NOS inhibition with L-NAME does not augment the vasoconstrictor responses to either α-agonist during adenosine or rhythmic handgrip exercise. $^* P < 0.05$ *vs.* adenosine for specific α-agonist within same L-NAME condition.

$(-36 \pm 4\%)$ and clonidine $(-46 \pm 6\%)$ evoked significant reductions in FVC during adenosine, and this reduction was significantly blunted during exercise $(-14 \pm 3$ and $-15 \pm 5\%$, respectively; $P < 0.05$; Fig. 6). NOS inhibition via L-NAME reduced baseline FVC ~40 %, and the steady-state FVC during adenosine (~40%) and handgrip exercise (~15%). However, phenylephrine and clonidine evoked similar reductions in FVC during adenosine as before L-NAME $(-37 \pm 4$ and $-47 \pm 9\%$, respectively), and this reduction was still significantly blunted during exercise $(-12 \pm 5$ and $-12 \pm 6\%$; Fig. 6). Importantly, the vasoconstrictor responses to phenylephrine and clonidine were similar during exercise before and after L-NAME $(P > 0.05)$.

DISCUSSION

Recent studies in both experimental animals and conscious humans have clearly demonstrated that the vasoconstrictor responses to sympathetic α-adrenergic stimulation are blunted in contracting compared with resting (quiescent) skeletal muscle (Thomas *et al.* 1994; Buckwalter *et al.* 2001; Tschakovsky *et al.* 2002). What is less clear is the mechanism(s) by which skeletal muscle contractions can interfere with or blunt sympathetic vasoconstriction. Despite evidence that NO is involved in functional sympatholysis in experimental animals (Thomas & Victor, 1998), the role of NO in blunting sympathetic vasoconstriction in contracting muscle of healthy humans has been more difficult to determine. The primary new finding from the present investigation is that local inhibition of NOS via both L-NMMA or L-NAME does not restore the vasoconstrictor responses to local endogenous noradrenaline release (via tyramine) during moderate or heavy rhythmic handgrip exercise. Importantly, these observations cannot be explained by any potential differences in neurotransmitter release because NOS inhibition did not restore the vasoconstrictor responses

Table 5. Forearm and systemic haemodynamics for moderate exercise before and after L-NAME (protocol 5)

Variable	Vasoconstrictor	Time	Adenosine		Exercise	
			Pre L-NAME	Post L-NAME	Pre L-NAME	Post L-NAME
FBF (ml min^{-1})	Phenylephrine	Baseline	28 ± 2	$20 \pm 4^{\star}$	31 ± 5	18 ± 7
	($n = 4$)	Pre-phenylephrine	143 ± 46	88 ± 24	185 ± 16	$164 \pm 15\dagger$
		Post-phenylephrine	96 ± 35	57 ± 19	163 ± 8	$151 \pm 22\dagger$
	Clonidine	Baseline	36 ± 7	26 ± 4	38 ± 7	$23 \pm 3^{\star}$
	($n = 5$)	Pre-clonidine	148 ± 31	$92 \pm 29^{\star}$	205 ± 21	$188 \pm 19\dagger$
		Post-clonidine	78 ± 15	$45 \pm 12^{\star}$	$185 \pm 25\dagger$	$171 \pm 25\dagger$
FVC (ml min^{-1} (100 mmHg)$^{-1}$)	Phenylephrine	Baseline	31 ± 2	$21 \pm 4^{\star}$	35 ± 5	$18 \pm 7^{\star}$
		Pre-phenylephrine	166 ± 52	94 ± 28	194 ± 14	$163 \pm 17^{\star}$
		Post-phenylephrine	107 ± 37	59 ± 20	167 ± 15	$146 \pm 22\dagger$
	Clonidine	Baseline	42 ± 8	$27 \pm 5^{\star}$	44 ± 9	$24 \pm 4^{\star}$
		Pre-clonidine	172 ± 35	$101 \pm 33^{\star}$	226 ± 27	$188 \pm 21^{\star}$
		Post-clonidine	89 ± 17	$47 \pm 12^{\star}$	$198 \pm 32\dagger$	$168 \pm 27^{\star}\dagger$
MAP (mmHg)	Phenylephrine	Baseline	90 ± 2	95 ± 2	90 ± 1	$99 \pm 3^{\star}$
		Pre-phenylephrine	86 ± 1	95 ± 2	95 ± 3	101 ± 2
		Post-phenylephrine	89 ± 2	$96 \pm 2^{\star}$	97 ± 4	$104 \pm 1\dagger$
	Clonidine	Baseline	89 ± 2	94 ± 1	88 ± 1	$99 \pm 2^{\star}$
		Pre-clonidine	86 ± 2	92 ± 1	91 ± 3	$100 \pm 3^{\star}\dagger$
		Post-clonidine	89 ± 3	$96 \pm 1^{\star}$	96 ± 4	$103 \pm 2\dagger$

$^{\star}P < 0.05$ post *vs.* pre L-NAME within same hyperaemic condition; $\dagger P < 0.05$ exercise *vs.* adenosine within same L-NAME condition.

to direct α_1- or α_2-adrenergic receptor stimulation during exercise. Taken together, our findings demonstrate that NO is not obligatory to observe blunted post-junctional α-adrenergic vasoconstriction (i.e. functional sympatholysis) during muscle contractions in healthy humans.

Early studies in the rat hindlimb demonstrated that sympathetic vasoconstrictor responses were blunted during muscle contractions, and that these responses could be (in part) 'normalized' by administration of the K_{ATP} channel antagonist glibenclamide (Thomas *et al.* 1997). These data indicated that muscle contractions activated these metabolically sensitive channels and inhibited the vasoconstrictor responses to sympathetic nerve stimulation, and that this might be specific for post-junctional α_2-adrenergic receptors. Additional studies by Thomas and colleagues (Thomas & Victor, 1998) also implicated a role for NO, but not adenosine or prostaglandins, in the blunted vasoconstrictor responses during exercise. Interestingly, in this latter study, blockade of K_{ATP} channels after NOS inhibition did not further augment the hindlimb sympathetic vasoconstrictor responses during contractions, strongly suggesting that the actions of NO interact with K_{ATP} channels to evoke functional sympatholysis.

Other studies performed in a mouse model of Duchenne muscular dystrophy (in which nNOS levels are substantially low), as well as in an nNOS knockout mouse model, have provided evidence that NO is involved in this phenomenon in animals (Thomas *et al.* 1998). A lack of functional sympatholysis has also been suggested (via near-infrared spectroscopy; see below) in children with Duchenne muscular dystrophy (in whom nNOS levels are greatly decreased), providing indirect evidence that NO derived from nNOS is an important modulator of sympathetic vasoconstriction in contracting muscle (Sander *et al.* 2000).

In healthy humans, a role for NO in functional sympatholysis has been more difficult to demonstrate. Wilson and Kapoor (1993) documented that intra-arterial infusions of noradrenaline during exercise evoked similar forearm vasoconstrictor responses before and after local NOS inhibition via L-NMMA. In this study, the investigators used venous occlusion plethysmography to estimate muscle blood flow, a technique that necessitates the interruption of muscle contractions during the time required for blood flow measurements. The major problem with this approach is that the regulatory mechanisms of muscle blood flow control studied using this technique reflect the control of post-exercise hyperaemia, and not active muscle blood flow regulation (Dyke *et al.* 1995; Shoemaker *et al.* 1997; Radegran & Saltin, 1999; Frandsen *et al.* 2001).

In contrast, Chavoshan *et al.* (2002) demonstrated that the blunted vasoconstrictor responses (estimated via near-infrared spectroscopy) normally observed during reflex

increases in muscle sympathetic nerve activity (via lower body negative pressure) in contracting skeletal muscle were completely reversed after systemic NOS inhibition via L-NAME. These data not only support a role for NO in functional sympatholysis in humans but also indicate that NO is *the* putative sympatholytic factor and contrasts the findings in experimental animals indicating that NO is only in part responsible for blunting sympathetic vasoconstriction. Unfortunately, the use of near-infrared spectroscopy does not provide measures of active muscle blood flow regulation or the whole-limb haemodynamic responses (flow and pressure) during exercise and subsequently during sympathetic vasoconstriction. This latter point is significant because much of the discussion regarding functional sympatholysis concerns how the sympathetic nerves and metabolic vasodilators interact to regulate blood pressure during exercise (Rowell, 1997).

In the present study, we sought to overcome some of the methodological concerns that have potentially contributed to these equivocal findings. First, we used Doppler ultrasound to measure active forearm blood flow during moderate and heavy dynamic handgrip exercise and determined the vasoconstrictor responses to intra-arterial infusions of tyramine to evoke local endogenous nor-adrenaline release and subsequent post-junctional α-adrenergic receptor stimulation. Second, we administered both L-NMMA and L-NAME to inhibit NOS in order to determine whether the discrepant findings from prior investigations in humans are related to the specific analogue for L-arginine used to inhibit NOS in these previous studies. Finally, we extended our findings utilizing tyramine to evoke local noradrenaline release by selectively stimulating post-junctional α_1- and α_2-adrenergic receptors (via phenylephrine and clonidine, respectively). Collectively, our data derived from the use of several approaches indicate that acute inhibition of NOS does not restore sympathetic α-adrenergic vaso-constriction in the vascular beds of contracting skeletal muscle of healthy humans. This finding is also consistent with recent data from our laboratory indicating that exogenous administration of sodium nitroprusside (NO donor) sufficient to elevate forearm blood flow to levels observed during exercise does not blunt α-adrenergic vasoconstriction (Tschakovsky *et al.* 2002).

Experimental considerations

The source of NO postulated to be involved in functional sympatholysis is derived primarily from skeletal muscle nNOS (Hansen *et al.* 2000). We have demonstrated either previously or in the present study that NOS inhibition (via L-NMMA and L-NAME) reduces resting forearm vascular conductance and the vasodilator responses to acetyl-choline, consistent with effective NOS inhibition. Despite this, it could be argued that the infusions of these L-arginine analogues only effectively inhibited eNOS (and

not nNOS) and therefore could possibly explain why we did not observe 'restored' vasoconstrictor responses in contracting muscle after NOS inhibition. However, the dose of L-NMMA or L-NAME administered directly into the forearm vasculature in the present study (total dose $\sim$110 mg kg^{-1}) is substantially greater than the dose of L-NAME infused systemically (4 mg kg^{-1} body mass) in humans which reduced skeletal muscle NOS activity by $\sim$70 % (Fransden *et al.* 2001). Given the nature of drug administration in the present study (intra-arterial), the concentration of either L-NMMA or L-NAME at the level of the tissue should have been much greater than in this previous study (Fransden *et al.* 2001). Therefore, we believe that our doses of L-NMMA and L-NAME effectively inhibited both eNOS and nNOS and that this should not limit the interpretations of our findings.

Another potential experimental consideration is our choice of using adenosine as a 'control' vasodilator in the present study. Previous studies in experimental animals have demonstrated that K$_{ATP}$ channels are involved in functional sympatholysis. Thus, given that adenosine can activate these channels, one could question the use of adenosine as a control vasodilator to study this phenomenon in humans. However, our rationale for using adenosine is based on previous findings from our laboratory which indicate that elevating resting forearm blood flow (via adenosine) similar to levels observed during exercise does not blunt sympathetic vaso-constriction (Tschakovsky *et al.* 2002; Rosenmeier *et al.* 2003). Further, it has recently been demonstrated that passive vasodilatation via the NO donor sodium nitro-prusside also does not blunt sympathetic vasoconstriction in both animals and humans (Tschakovsky *et al.* 2002; VanTeeffelen & Segal, 2003). Irrespective of which vaso-dilator is used to elevate resting forearm blood flow, the key finding from the present investigation is that local NOS inhibition does not restore the sympathetic α-adrenergic vasoconstrictor responses in contracting skeletal muscle.

Perspectives

Skeletal muscle blood flow control during muscle contractions is a complicated and highly integrated process involving the interactions of a number of locally produced vasodilating metabolites, mechanical factors (e.g. muscle pump), as well as sympathetic nerves (Saltin *et al.* 1998). With respect to the possible substance(s) that could interfere with sympathetic vasoconstriction in contracting muscle, several vasodilating factors (adenosine, prostaglandins, NO) have been demonstrated to blunt sympathetic vasoconstriction in a variety of experimental conditions (Lippton *et al.* 1981; Faber *et al.* 1982; Nishigaki *et al.* 1991; Ohyanagi *et al.* 1992). Therefore, the findings from the present study suggest that there are redundant pathways that can mediate functional sympatholysis

during exercise, as is probably the case for the metabolic vasodilating substances involved in exercise hyperaemia (Joyner & Proctor, 1999). For example, prostaglandins have been demonstrated to inhibit noradrenaline release (Malik & Sehic, 1990), as well as reduce post-junctional α-adrenergic vasoconstriction (Lippton *et al.* 1981; Malik & Sehic, 1990). Given that acute NOS inhibition can result in greater prostacyclin synthesis and release (Osanai *et al.* 2000), it appears reasonable to speculate that elevated levels of vasodilating prostaglandins might compensate for the impaired NO synthesis under these conditions and continue to blunt the sympathetic vasoconstrictor responses during exercise.

In this context, a recent study (Boushel *et al.* 2002) demonstrated that combined inhibition of NOS and prostaglandins reduces active muscle blood flow during dynamic knee extensor exercise, a finding that contrasts what has been demonstrated by only the selective inhibition of either substance alone. It is also of interest to note that the reduction in active muscle blood flow during combined prostaglandin and NO inhibition was greater during heavy compared with light exercise (Boushel *et al.* 2002), a condition associated with elevations in sympathetic vasoconstrictor nerve activity (Seals *et al.* 1988; Hansen *et al.* 1994). Therefore, whether or not the reduction in muscle blood flow under these conditions reflects less available vasodilator substances or an impaired ability to blunt sympathetic vasoconstriction in active muscle is unclear, but certainly deserves further study.

Conclusions

The findings from the present investigation challenge the hypothesis that NO plays an obligatory role in the blunted α-adrenergic vasoconstrictor responses observed in the vascular beds of contracting skeletal muscles of humans. Similar to exercise hyperaemia, it is likely that there are redundant mechanisms involved in functional sympatholysis. Future studies are needed to identify and determine the potential interactions between these 'sympatholytic' substances in healthy humans, and how these interactions might be altered in those populations that demonstrate impaired active muscle blood flow regulation during exercise (e.g. ageing, heart failure patients).

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