Evidence for nitric oxide-mediated sympathetic forearm vasodilatation in humans

Niki M. Dietz, Keith A. Engelke, Terri T. Samuel, Robert T. Fix and Michael J. Joyner*

Departments of Anesthesiology and Physiology & Biophysics, Mayo Clinic and Foundation, Rochester, MN 55905, USA

1. Our aim was to determine if sympathetic vasodilatation occurs in the human forearm, and if the vasodilating substance nitric oxide contributes to this dilatation. We also sought to determine if the nitric oxide might be released as a result of cholinergic stimulation of the vascular endothelium.

2. Blood flow was measured in the resting non-dominant forearm with venous occlusion plethysmography. To increase sympathetic traffic to the resting forearm, rhythmic handgrip exercise to fatigue followed by post-exercise ischaemia was performed by the dominant forearm. A brachial artery catheter in the non-dominant arm was used to selectively infuse drugs.

3. During control conditions, there was mild vasodilatation in the resting forearm during exercise followed by constriction during post-exercise ischaemia. When exercise was performed after brachial artery administration of bretylium (to block noradrenaline release) and phentolamine (an α-adrenergic antagonist), profound vasodilatation was seen in the resting forearm during both exercise and post-exercise ischaemia.

4. When the nitric oxide synthase blocker N^G-monomethyl-L-arginine (L-NMMA) was administered in the presence of bretylium and phentolamine prior to another bout of handgripping, little or no vasodilatation was seen either during exercise or post-exercise ischaemia. Atropine also blunted the vasodilator responses to exercise and post-exercise ischaemia after bretylium and phentolamine.

5. These results support the existence of active sympathetic vasodilatation in the human forearm and the involvement of nitric oxide in this phenomenon. They also suggest nitric oxide might be released as a result of cholinergic stimulation of the vascular endothelium.

Sympathetically mediated nitric oxide release may be the mechanism responsible for the active vasodilatation seen in forearm skeletal muscle during mental stress in humans (Dietz, Rivera, Eggener, Fix, Warner & Joyner, 1994*a*). While intact sympathetic innervation of the upper extremity is required to see forearm vasodilatation during mental stress, the vasodilator responses are variable, and the accompanying pattern of sympathetic traffic is poorly understood (Blair, Glover, Greenfield & Roddie, 1959; Roddie, 1977; Anderson, Wallin & Mark, 1987). In isolated tissues and anaesthetized animal preparations, nitric oxide released directly from autonomic nerves or by neurally mediated cholinergic stimulation of the vascular endothelium can cause vasodilatation (Toda & Okamura, 1991; Broten, Miyashiro, Moncada & Feigl, 1992; McMahon, Hood & Kadowitz, 1992). The purpose of this study was to investigate the existence of sympathetically mediated vasodilatation in human forearm skeletal muscle and to determine if nitric oxide contributes to this dilatation.

In contrast to mental stress, rhythmic forearm handgrip exercise to fatigue elicits a well-known pattern of muscle sympathetic nerve activity to both the upper and lower extremities (Mark, Victor, Nerhed & Wallin, 1985; Victor, Bertocci, Pryor & Nunnally, 1988; Wallin, Victor & Mark, 1989). At the onset of handgripping there is little or no rise in muscle sympathetic nerve activity but a mild atropine-sensitive vasodilator response in the contralateral resting forearm is seen in many subjects (Sanders, Mark & Ferguson, 1989). As the exercise continues there is a rise in muscle sympathetic nerve traffic which is maintained during post-exercise ischaemia of the previously active forearm. In some subjects progressive vasoconstriction is seen in the resting

* To whom correspondence should be addressed.

forearm, as muscle sympathetic nerve activity rises during exercise. In other subjects there can be a modest vasodilatation as a result of unintended contractions in the 'resting' forearm (Cotzias & Marshall, 1993). In all subjects there is marked forearm vasoconstriction during post-exercise ischaemia. By contrast, ischaemic handgrip exercise and post-exercise ischaemia do not evoke sustained increases in vasoconstrictor skin sympathetic activity (Vissing, Scherrer & Victor, 1991).

With this information as a background, we measured the blood flow responses in the resting forearm during contralateral handgrip exercise and post-exercise ischaemia in healthy human volunteers before and after selective administration of bretylium and phentolamine to the forearm. We reasoned that selective administration of these drugs to block the release (bretylium), and postsynaptic actions of noradrenaline (phentolamine) would allow us to observe the effects of any vasodilating substance released by the sympathetic nerves. Such a strategy permits a normal rise in sympathetic traffic to the forearm muscles during handgrip exercise and post-exercise ischaemia while eliminating noradrenaline-mediated vasoconstriction. Subsequent administration of other compounds would then allow us to gain insight into the mechanisms responsible for any vasodilatation observed. Our results are consistent with the existence of nitric oxide-mediated sympathetic vasodilatation in human forearm muscle.

METHODS

Subjects

Twelve healthy subjects (6 females and 6 males) between the ages of 19 and 29 were studied. The study was approved by the Institutional Review Board and each subject gave written informed consent. Female subjects had a negative pregnancy test within 48 h prior to the study. Prior to the beginning of the study, we obtained United States Food and Drug Administration permission to administer N^G-monomethyl-L-arginine (L-NMMA) to humans.

Subjects were studied in the supine position with the arms above heart level. Some studies were performed in the morning and others in the afternoon. The subjects were instructed to abstain from caffeine and avoid eating a heavy meal for a period of at least 3 h prior to the study. They were also instructed to abstain from non-steroid anti-inflammatory agents for 72 h prior to the study since these compounds interfere with prostaglandins which can influence the vasodilator responses to a variety of stimuli (Carlsson & Wennmalm, 1983). Throughout all studies, the laboratory temperature was maintained between 22 and 24 °C.

Arterial catheterization

A 20 gauge, 5 cm brachial artery catheter was placed in the non-dominant forearm, using an aseptic technique, after local anaesthesia (1% lidocaine (lignocaine)). The catheter was connected to a pressure transducer and flushed continuously at 3 ml h^{-1} with saline containing 2 units heparin ml^{-1}. A three-port connector was placed in series with the catheter–transducer system. One port was used to measure arterial pressure and the two other ports were used for drug infusions (Dietz, Rivera, Warner & Joyner, 1994*b*). The arterial wave form from the pressure transducer was used to measure heart rate and mean arterial pressure was obtained electronically.

Forearm blood flow

Forearm blood flow was measured in the non-dominant forearm using venous occlusion plethysmography with mercury in siliconized strain gauges (Greenfield, Whitney & Mowbray, 1963). The venous collecting pressure was set at 50 mmHg and care was taken to ensure that the collecting pressure did not alter arterial pressure. When forearm blood flow was measured, flow to the hand was excluded by inflating a wrist cuff to suprasystolic pressure (250 mmHg). Blood flow was measured 4 times each minute.

Rhythmic handgrip to fatigue

Subjects were instructed to squeeze a commercially available spring-loaded handgrip strengthening device rhythmically with the non-instrumented (dominant) forearm and hand to fatigue. Just prior to exercise, a cuff was inflated around the upper arm to suprasystolic pressure. Subjects were allowed to select their own rate of handgripping. To ensure maximal effort, subjects were encouraged by the investigators to continue squeezing until they reached fatigue. Handgripping to fatigue evokes a large increase in muscle sympathetic nerve activity and the sympathetic responses at the end of repeated bouts of fatiguing handgrip exercise are similar (Seals & Enoka, 1989). Post-exercise ischaemia was used to maintain the rise in sympathetic traffic to the forearm for two minutes following the end of exercise (Mark *et al.* 1985). We reasoned that exercise would cause a marked rise in the ischaemic metabolites which stimulate the muscle chemoreflex and evoke a rise in muscle sympathetic nerve activity that would be maintained during post-exercise ischaemia.

Drug preparation and administration

Acetylcholine (Miochol, IOLAB Corp., Claremont, CA, USA) was administered in doses of 64 $\mu g\ min^{-1}$ given intra-arterially to stimulate the release of nitric oxide from the vascular endothelium (Vallance, Collier & Moncada, 1989). A marked reduction in resting forearm blood flow and a blunting of the vasodilator response to acetylcholine is consistent with inhibition of nitric oxide synthase in the vascular endothelium (Vallance *et al.* 1989). Sodium nitroprusside (Elkins-Sinn, Cherry Hill, NJ, USA) was administered intra-arterially in doses of 10 $\mu g\ min^{-1}$ to test the continued ability of the forearm vasculature to dilate after L-NMMA administration (Dietz *et al.* 1994*a*,*b*). Both the acetylcholine and sodium nitroprusside infusions were continued until three consecutive peaks in forearm blood flow were observed after a minimum of 90 s of drug administration.

Bretylium (American Regent Laboratories Inc., Shirley, NY, USA) was administered intra-arterially in doses of 2·5 mg min^{-1} for 5 min to block the presynaptic release of noradrenaline in the forearm (Blair, Glover, Kidd & Roddie, 1960). This dose of bretylium is known to block forearm vasoconstrictor responses to a variety of physiological stimuli for many hours (Blair *et al.* 1960). The forearm was also treated with phentolamine (Regitine®, Ciba Pharmaceutical Co., Summit, NJ, USA) in doses of 100 $\mu g\ min^{-1}$ for 5 min intra-arterially to block postsynaptic α-adrenergic receptors (Eklund & Kaijser, 1976). Additionally, in pilot studies these drugs selectively blocked the vasoconstrictor response to venous pooling with moderate (< 40 mmHg) lower body suction. Finger temperature was also measured to evaluate the adequacy of forearm α-adrenergic blockade. When repeated bouts of exercise were performed, low dose intra-arterial infusions of both drugs (bretylium and phentolamine) were used to ensure that the pharmacological sympathectomy was maintained.

The nitric oxide synthase inhibitor L-NMMA (Calbiochem, La Jolla, CA, USA) was dissolved, using a strict aseptic technique, in preservative-free, sterile normal saline (0·9% NaCl) and filtered

through a 0·22 μm filter. A total of 50 mg of L-NMMA was given intraarterially in divided doses. This dose of L-NMMA causes a marked reduction in baseline forearm flow, a variable blunting of the vasodilator responses to intra-arterial acetylcholine, and reduces the rise in forearm blood flow during mental stress by approximately 70% (Dietz *et al.* 1994*a*, *b*).

Atropine sulphate (American Regent Laboratories Inc.) was administered intra-arterially in doses of 0·2 mg to selectively block cholinergic muscarinic receptors (Roddie, Shepherd & Whelan, 1957). This dose of atropine was administered over 1 min at the same pump flow rate as had been used for all other drugs. Cholinergic blockade with this dose of atropine lasts many minutes, and forearm blood flow was measured immediately (1–2 min) after the conclusion of the infusion. Propranolol (SoloPak Laboratories Inc., Elk Grove Village, IL, USA) was given in a dose of 1 mg intra-arterially to block β-adrenergic receptors in the forearm. These doses have been shown previously to block the forearm vasodilator response to acetylcholine and the β-adrenergic agonist isoproterenol (Eklund & Kaijser, 1976).

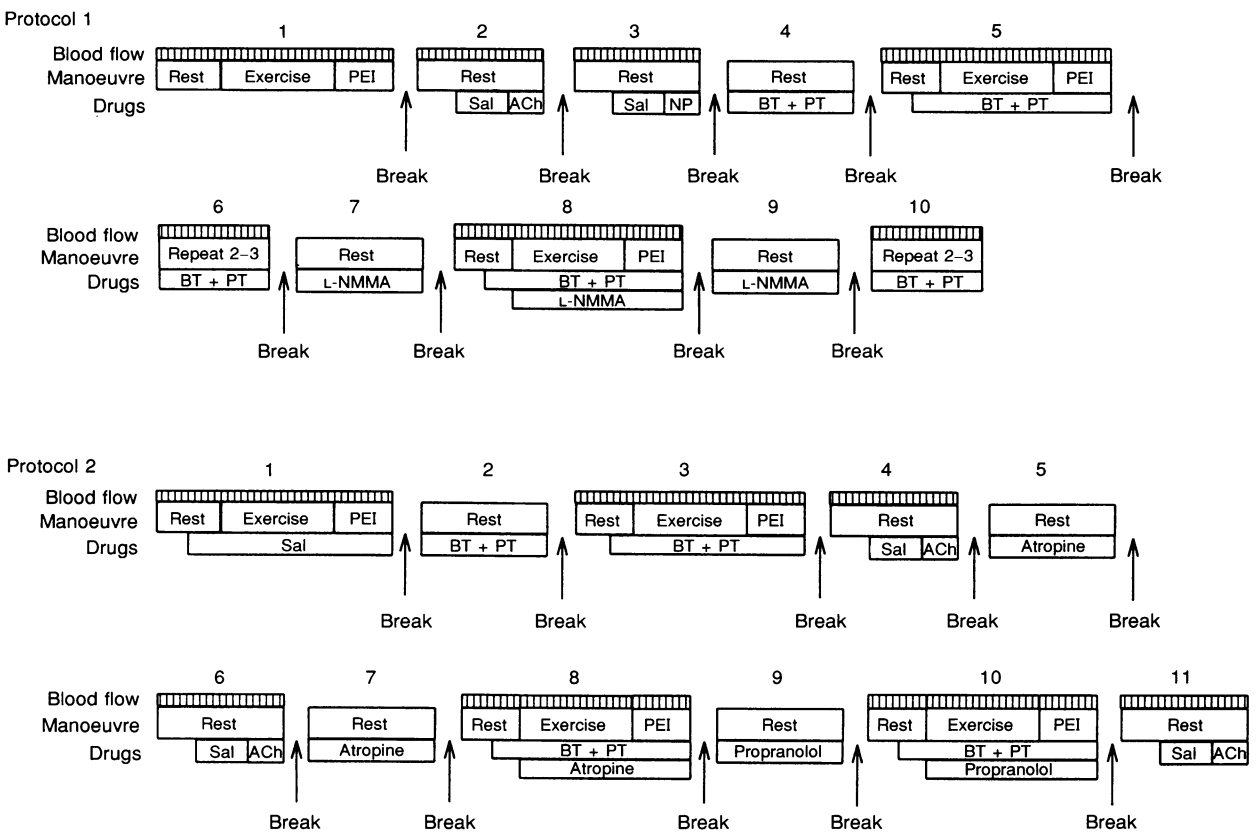

Figure 1. Schematic timeline of protocols 1 and 2

Schematic representation of the time course of blood flow measurements (hatched bars), physiological manoeuvres and drug administration during protocols 1 and 2. Protocol 1: step 1, control trial consisting of forearm blood flow measurements at rest, during ischaemic handgrip (Exercise) and during post-exercise ischaemia (PEI) with no pharmacological interventions. During steps 2 and 3, the vasodilator responses to acetylcholine (ACh) and nitroprusside (NP) were measured. Step 4, pharmacological sympathectomy of the forearm was then performed with the administration of bretylium (BT) and phentolamine (PT). Step 5, a second trial of forearm blood flow measurements at rest, during ischaemic handgrip, and during PEI with continued BT and PT administration. Step 6, ACh and NP infusions were then repeated during BT and PT administration. Step 7, the nitric oxide synthase inhibitor, L-NMMA, was infused. Step 8, a trial of rest, ischaemic handgrip and PEI was then repeated during continued L-NMMA infusion. Step 9, a final dose of L-NMMA was given. Step 10, ACh and NP infusions were then repeated. Protocol 2: step 1, control trial consisting of forearm blood flow measurements at rest, during ischaemic handgrip, and during PEI during saline (Sal) administration. Step 2, pharmacological sympathectomy of the forearm was then performed with the administration of BT and PT. Step 3, a second trial of forearm blood flow measurements was performed at rest, during ischaemic handgrip, and during PEI with continued BT and PT administration. During step 4, the vasodilator response to ACh was measured. Step 5, the muscarinic blocker, atropine, was infused. Step 6, the vasodilator response to ACh was once again measured. Step 7, a second dose of atropine was administered. Step 8, forearm blood flow was measured during a third trial of rest, ischaemic handgrip and PEI with BT, PT and atropine infusion. Step 9, the β-adrenergic blocker, propranolol, was then administered. Step 10, a final trial of forearm blood flow measurements was then performed at rest, during ischaemic handgrip and during PEI with BT, PT and propranolol administration. Step 11, a final ACh infusion was performed.

Table 1. Forearm blood flow responses during protocol 1

	Control	BT + PT	BT + PT + L-NMMA
Baseline FBF	1·9 ± 0·3	2·9 ± 0·3*	1·7 ± 0·1†
Peak FBF during:			
Exercise	4·0 ± 0·5‡	8·8 ± 1·7*‡	3·4 ± 0·4†‡
Post-exercise ischaemia	1·2 ± 0·2‡	8·9 ± 1·5*‡	2·3 ± 0·3†‡
Acetylcholine	19·9 ± 1·6‡	25·8 ± 3·5*‡	22·0 ± 2·2†‡
Sodium nitroprusside	16·2 ± 1·3‡	17·9 ± 2·0‡	17·5 ± 1·9‡

FBF, forearm blood flow (ml (100 ml)$^{-1}$ min^{-1}); BT, bretylium; PT, phentolamine. * $P < 0·05$ *vs.* control value. † $P < 0·05$ *vs.* BT and PT value. ‡ $P < 0·05$ *vs.* baseline value (same trial).

Specific protocols

Protocol 1. Effects of L-NMMA on forearm blood flow during handgrip exercise to fatigue after treatment with bretylium and phentolamine. The purpose of this protocol was to determine if there is sympathetic vasodilatation in the human forearm, and if nitric oxide is involved in this phenomenon. Six subjects (3 females and 3 males) participated in the protocol. Figure 1 contains a schematic timeline of protocol 1. First, a brachial artery catheter was placed in the non-dominant arm. The arm was then instrumented to measure forearm blood flow and resting values were obtained. After additional baseline measures were taken, the first rhythmic handgrip to fatigue was performed followed by 2 min of post-exercise ischaemia. Blood flow measurements in the non-dominant resting forearm were made throughout the manoeuvres. After a rest period of 5–10 min, flow measurements were taken to demonstrate the flow had returned to baseline. Next, acetylcholine was administered (64 μg min^{-1}) and changes in forearm blood flow measured. After a break (5–10 min) to allow forearm blood flow to return to baseline, sodium nitroprusside was administered (10 μg min^{-1}) and changes in blood flow measured.

When blood flow returned to baseline, bretylium was administered at 2·5 mg min^{-1} for 5 min followed by a 25 min rest period. This rest period was chosen so that forearm blood flow measurements would not be taken during the initial vasoconstriction seen with the administration of bretylium (Blair *et al.* 1960). During the bretylium infusion, the subject was instructed to perform gentle hand contractions with the arm receiving the drug to facilitate more uniform drug distribution throughout the forearm muscles. After the 25 min rest period, additional doses of bretylium at 2·5 mg min^{-1} and phentolamine at 100 μg min^{-1} were administered for 5 min. Low dose (bretylium, 0·625 mg min^{-1} and phentolamine, 25 μg min^{-1}) infusions of both drugs were then continued throughout the second bout of contralateral handgrip exercise.

Baseline measures were then taken and followed by the second bout of handgrip exercise to fatigue and post-exercise ischaemia while flow was measured as in the first bout of exercise. Acetylcholine and sodium nitroprusside were then administered as previously described.

When blood flow returned to baseline, L-NMMA was infused intra-arterially at 4 mg min^{-1} for 5 min with the wrist cuff inflated. After a 1 min rest, a second dose of L-NMMA was infused intra-arterially at 4 mg min^{-1} for 5 min with the wrist cuff inflated to saturate the forearm. The subject was instructed to perform gentle contractions during the infusion of L-NMMA to ensure distribution throughout the forearm. After a short rest, baseline measurements were taken and the third handgrip to fatigue and post-exercise ischaemia followed. Again, low dose infusions of bretylium (0·625 mg min^{-1}) and phentolamine (25 μg min^{-1}) were administered throughout the third bout of handgrip exercise. After a 5–10 min rest, a supplemental dose of L-NMMA was infused intra-arterially at 2 mg min^{-1} for 5 min with the wrist cuff down to replace any drug which may have washed out or been metabolized during the exercise. This supplemental dose of L-NMMA was chosen after taking into account the length of time elapsed from the initial dose, the physiological half-life of L-NMMA which is 30–60 min, and previous experience with L-NMMA. Following the infusion, an acetylcholine trial (64 μg min^{-1}) was conducted to test the nitric oxide synthase blockade. Following a 5 min rest period, a sodium nitroprusside trial (10 μg min^{-1}) was conducted to demonstrate that the forearm could still dilate in the presence of L-NMMA.

Protocol 2. Effects of atropine and propranolol on forearm blood flow during handgrip exercise to fatigue after sympathectomy with bretylium and phentolamine. The purpose of this protocol was to determine if cholinergic or β-adrenergic mechanisms play a role in any vasodilatation observed in a resting forearm during contralateral handgrip to fatigue and post-exercise ischaemia after bretylium and phentolamine. We reasoned that if nitric oxide contributes to sympathetic vasodilator responses, it might be released as a result of cholinergic stimulation of the vascular endothelium (Broten *et al.* 1992; McMahon *et al.* 1992). Six subjects (3 females and 3 males) participated in the protocol. Figure 1 contains a schematic timeline of protocol 2. Subjects were instrumented as in protocol 1 and performed a bout of rhythmic handgrip to fatigue followed by 2 min of post-exercise ischaemia while blood flow was measured.

When blood flow returned to baseline, bretylium and phentolamine were administered as in the first protocol. This was followed by a bout of rhythmic forearm handgrip exercise to fatigue and post-exercise ischaemia. After a 5 min rest period, acetylcholine was administered and changes in blood flow measured.

When forearm blood flow returned to normal after acetylcholine, atropine (0·2 mg) was administered and blood flow changes to acetylcholine again determined. Another dose of atropine (0·2 mg) was followed by a third trial of rhythmic forearm handgrip exercise to fatigue and post-exercise ischaemia. This bout of exercise served to test the role of cholinergic stimulation of the vascular endothelium on vasodilatation. After this trial, propranolol was infused along with another dose of atropine. The fourth bout of exercise followed by post-exercise ischaemia was then conducted and forearm blood flow in the resting arm measured during the procedure. This bout of exercise served to test the role of β-adrenergic stimulation on vasodilatation. Supplemental doses of bretylium and phentolamine

were given before and during the third and fourth bouts of exercise to ensure that the pharmacological sympathectomy in the experimental arm remained constant during these bouts of exercise.

Data analysis

'Baseline' blood flow, arterial pressure and heart rate values are the mean values obtained during the 1 min of instructions prior to the start of the rhythmic handgrip exercise. 'Peak' changes in forearm blood flow were determined for each individual trial on the basis of the largest changes seen in the infusion forearm during the final 1·5 min of the forearm handgrip exercise and during post-exercise ischaemia. Mean arterial pressure and heart rate were measured during the final 15 s of exercise and also during each minute of post-exercise ischaemia. Data from only the final 1·5 min of exercise are presented because the time at which fatigue occurred was highly variable between the subjects (between 1·5 and 4 min to fatigue). While this approach to data analysis may seem flexible, it was utilized to present the data from each subject at a common, reproducible end-point (Taylor, Hand, Johnson & Seals, 1991). Forearm vascular conductance was calculated as forearm blood flow/mean arterial pressure.

Statistics

In the study, each subject served as their own control. Data are expressed as means ± S.E.M. When appropriate, the comparisons were made using simple Student's paired t tests or repeated measures analysis of variance (treatment and time). Significance was set at $P < 0·05$ for the study.

RESULTS

Protocol 1

The effects of bretylium and phentolamine with and without nitric oxide synthase blockade on forearm blood flow responses during protocol 1 are shown in Table 1. Baseline forearm blood flow rose after bretylium and phentolamine in a manner consistent with forearm sympathectomy. Finger temperature also rose from 33·5 ± 0·5 to 35·7 ± 0·3 °C after bretylium and phentolamine ($P < 0·05$). Baseline forearm blood flow after L-NMMA, bretylium and phentolamine was lower than the value seen after bretylium and phentolamine alone. The rise in forearm blood flow during acetylcholine increased after bretylium and phentolamine. L-NMMA caused a small reduction in the subsequent dilator response to acetylcholine (Table 1). The forearm blood flow responses to sodium nitroprusside were unaffected by any of the drugs. These responses indicate that L-NMMA reduced both the basal and stimulated release of nitric oxide but did not alter the ability of the forearm vessels to dilate.

During control conditions there was a mild increase in forearm blood flow at the onset of exercise followed by vasoconstriction during post-exercise ischaemia. After bretylium and phentolamine, there were large increases in forearm blood flow which occurred during the final minutes of

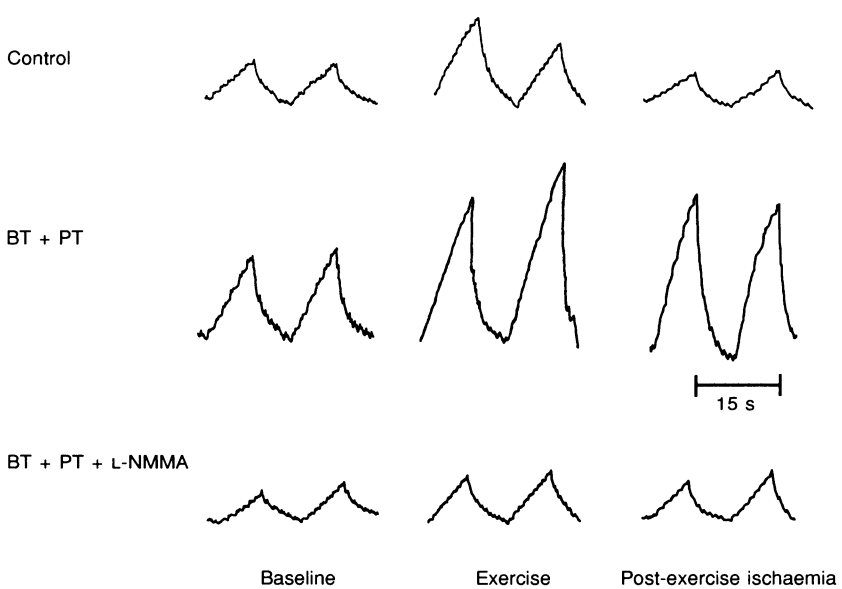

Figure 2. Experimental record of blood flow responses to ischaemic handgrip exercise and post-exercise ischaemia

Original plethysmographic record of the forearm blood flow responses to ischaemic handgrip exercise and post-exercise ischaemia. Forearm blood flow is proportional to the slope of the plethysmographic tracing. An increase in slope means that forearm blood flow rose. The top tracing shows the responses during control conditions. There was a mild increase in forearm blood flow at the onset of exercise followed by vasoconstriction during post-exercise ischaemia. The middle tracing shows blood flow responses after administration of bretylium (BT) and phentolamine (PT) to the forearm. Baseline forearm blood flow was increased above control conditions, and there was a large increase at the end of handgrip exercise which was sustained during post-exercise ischaemia. Following the infusion of L-NMMA (bottom tracing) with BT + PT, baseline forearm blood flow was lower than the value seen after BT and PT alone, and almost no increase in forearm blood flow with exercise or with post-exercise ischaemia was seen.

Table 2. Mean arterial pressure and heart rate responses during protocol 1

	Control	BT + PT	BT + PT + L-NMMA
Mean arterial pressure (mmHg)			
Baseline	91 ± 5	93 ± 6	97 ± 5
Exercise	110 ± 7‡	116 ± 7‡	118 ± 7‡
Post-exercise ischaemia	118 ± 8‡	118 ± 6‡	123 ± 6‡
Heart rate (beats min^{-1})			
Baseline	57 ± 4	56 ± 4	56 ± 4
Exercise	74 ± 7‡	77 ± 7‡	78 ± 6‡
Post-exercise ischaemia	60 ± 4	60 ± 6	60 ± 6

Blood pressure and heart rate values were obtained at the time of peak flow responses during each specified experimental condition. BT, bretylium; PT, phentolamine. ‡ $P < 0.05$ *vs.* baseline value (same trial).

exercise and were sustained during post-exercise ischaemia. Following infusion of L-NMMA to block nitric oxide synthase, almost no increase was seen in forearm blood flow during exercise or post-exercise ischaemia. Figure 2 is an individual record of the plethysmographic trace with representative blood flows displayed at various times during protocol 1.

Table 2 shows heart rate and blood pressure responses at the end of exercise and during the first minute of post-exercise ischaemia for each of the three conditions. There was a significant increase in blood pressure during exercise which was maintained during post-exercise ischaemia in all three conditions. Heart rate also increased during exercise but returned toward baseline values during post-exercise ischaemia.

Figure 3 shows forearm vascular conductance for 45 s at baseline, during the last 90 s of handgrip exercise prior to fatigue, and during 2 min of post-exercise ischaemia. During control conditions, forearm vascular conductance increased modestly during exercise and fell below baseline during

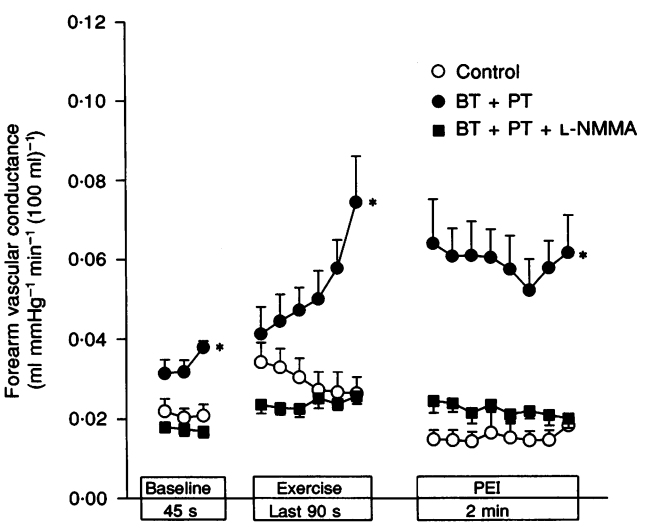

Figure 3. Time course of forearm vascular conductance responses to ischaemic handgrip exercise to fatigue and post-exercise ischaemia in protocol 1

Mean ± S.E.M. forearm vascular conductance responses at baseline, during the last 90 s of contralateral handgrip exercise prior to fatigue, and during 2 min of post-exercise ischaemia (PEI) in the 6 subjects in protocol 1. During control conditions, forearm vascular conductance increased modestly at the end of exercise and fell below baseline during post-exercise ischaemia. After infusion of bretylium and phentolamine (BT + PT), baseline vascular conductance was elevated above control at baseline and there was a marked rise ($P < 0.05$) at the end of handgrip exercise which remained elevated during post-exercise ischaemia. Following the infusion of L-NMMA along with additional BT and PT (BT + PT + L-NMMA), baseline forearm vascular conductance returned to that seen during control conditions and the rise in conductance seen with exercise and post-exercise ischaemia after BT and PT was nearly eliminated ($P < 0.05$). * $P < 0.05$ *vs.* control or BT + PT + L-NMMA.

Table 3. Forearm blood flow responses during protocol 2

	Control	BT + PT	BT + PT + AT	BT + PT + AT + PR
Baseline FBF	1·5 ± 0·2	2·9 ± 0·5*	3·4 ± 0·4*	3·4 ± 0·8*
Peak FBF during:				
Exercise	5·2 ± 1·4‡	12·4 ± 3·2*‡	7·7 ± 2·4†‡	5·9 ± 1·0†‡
Post-exercise ischaemia	2·0 ± 0·3	10·1 ± 2·6*‡	6·1 ± 1·3*†‡	5·2 ± 0·9*†‡
Acetylcholine	—	27·5 ± 5·0‡	12·35 ± 3·1†‡	—

FBF, forearm blood flow (ml (100 ml)$^{-1}$ min^{-1}). BT, bretylium; PT, phentolamine; AT, atropine; PR, propranolol. * $P < 0·05$ *vs.* control value. † $P < 0·05$ *vs.* BT and PT value. ‡ $P < 0·05$ *vs.* baseline value (same trial).

post-exercise ischaemia. After infusion of bretylium and phentolamine, baseline vascular conductance was elevated above control at baseline and there was a marked rise at the end of handgrip exercise which remained elevated during post-exercise ischaemia. Following the infusion of L-NMMA along with additional bretylium and phentolamine, baseline forearm vascular conductance returned to that seen during control conditions and the rise in conductance seen with exercise and post-exercise ischaemia after α-adrenergic blockade was nearly eliminated.

Protocol 2

The effects of bretylium and phentolamine, with subsequent administration of atropine and propranolol, on forearm blood flow responses during protocol 2 are shown in Table 3. As in protocol 1, baseline forearm blood flow rose after bretylium and phentolamine in a manner consistent with forearm sympathectomy. Baseline forearm blood flow was not changed after administration of atropine and then propranolol in addition to bretylium and phentolamine. The rise in forearm blood flow during acetylcholine increased

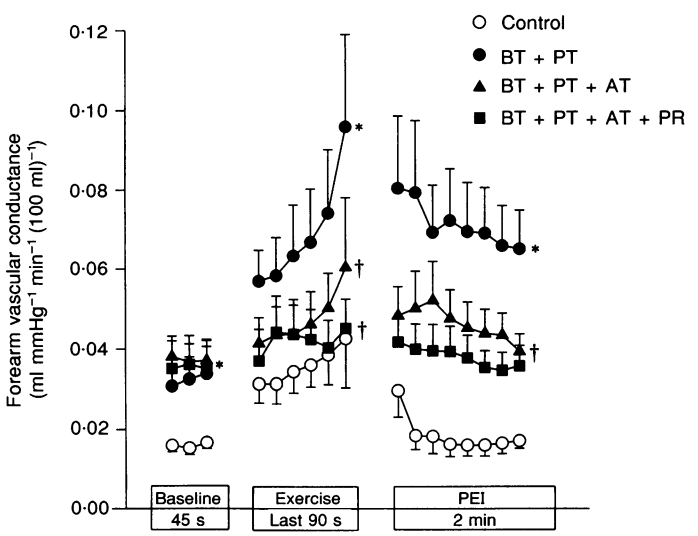

Figure 4. Time course of forearm blood flow and vascular conductance responses to ischaemic handgrip exercise to fatigue and post-exercise ischaemia in protocol 2

Mean ± S.E.M. forearm vascular conductance responses at baseline, during the last 90 s of contralateral handgrip exercise prior to fatigue, and during 2 min of post-exercise ischaemia (PEI) in the 6 subjects in protocol 2. During control conditions, there was a mild increase in forearm vascular conductance at the onset of exercise followed by vasoconstriction during post-exercise ischaemia. After administration of bretylium and phentolamine (BT + PT) to the forearm, baseline forearm vascular conductance was increased above control conditions ($P < 0·05$), and there was a large increase ($P < 0·05$) during handgrip exercise which was sustained during post-exercise ischaemia. Infusion of atropine (AT) with BT and PT (BT + PT + AT) did not change baseline forearm vascular conductance. However, forearm vascular conductance increased much less during exercise and post-exercise ischaemia than after BT and PT alone ($P < 0·05$). Following subsequent infusion of propranolol (PR) (BT + PT + AT + PR), baseline forearm vascular conductance did not change and the modest increases in conductance seen during handgrip exercise and post-exercise ischaemia after AT were nearly eliminated. * $P < 0·05$ *vs.* control. † $P < 0·05$, other treatments *vs.* BT + PT.

after bretylium and phentolamine. Atropine caused a 60% reduction in the subsequent dilator response to acetylcholine.

As in protocol 1, during control conditions there was a mild increase in forearm blood flow at the onset of exercise followed by vasoconstriction during post-exercise ischaemia. The pattern of changes in forearm blood flow seen during the trial conducted after bretylium and phentolamine were similar to those observed in protocol 1. Following infusion of atropine the increases in forearm blood flow with exercise and post-exercise ischaemia were reduced compared with those seen during bretylium and phentolamine alone ($P < 0.05$). The subsequent administration of propranolol did not alter the blood flow responses observed during the fourth bout of exercise.

Figure 4 shows forearm vascular conductance responses for 45 s at baseline, during the last 90 s of handgrip exercise prior to fatigue, and during the ensuing 2 min of post-exercise ischaemia. The changes in conductance during the control trial and following bretylium and phentolamine were similar to those seen in protocol 1. After infusion of atropine and additional bretylium and phentolamine, the vasodilator responses to acetylcholine but not nitroprusside were markedly blunted. Atropine did not change baseline forearm vascular conductance, and conductance increased much less during exercise than after bretylium and phentolamine alone. Following subsequent infusion of propranolol, baseline forearm vascular conductance did not change and no further changes in the vascular conductance response to handgrip exercise and post-exercise ischaemia were seen. The blood pressure, heart rate and finger temperature responses seen in protocol 2 were similar to those observed in protocol 1.

DISCUSSION

The major finding of this study is that the vasoconstrictor response normally seen in the contralateral 'resting' forearm during post-exercise ischaemia following handgrip exercise to fatigue becomes a vasodilator response after administration of bretylium and phentolamine. Additionally, marked dilatation in the resting forearm is also seen at the end of exercise. The time course of this vasodilatation is similar to the muscle sympathetic nerve activity responses during these manoeuvres (Mark *et al.* 1985; Victor *et al.* 1988; Wallin *et al.* 1989). Additionally, the vasodilatation seen after bretylium and phentolamine is eliminated by nitric oxide synthase inhibition with L-NMMA. These findings support the existence of active sympathetic vasodilatation in forearm skeletal muscle and suggest that nitric oxide contributes to this dilatation.

Results from protocol 2 also indicate that the vasodilatation seen during ischaemic handgrip exercise and post-exercise ischaemia after bretylium and phentolamine is attenuated by atropine administration. This suggests that sympathetic cholinergic nerves to the forearm might release acetylcholine, which can evoke nitric oxide release from the vascular endothelium. The lack of major changes in the vasodilator responses to ischaemic handgrip exercise and post-exercise ischaemia after administration of propranolol suggests that vasodilating β-adrenergic receptor stimulation either by locally released or circulating catecholamines is not essential to this response.

Nitric oxide synthase inhibition in humans

There are some inherent limitations to this study associated with our use of L-NMMA and various study drugs which must be addressed. First, it is often difficult to ensure that the changes seen in vascular tone are not due to some non-specific effect of L-NMMA or other compounds on the vascular smooth muscle. To address this issue, we studied the endothelium-independent vasodilator responses to sodium nitroprusside before and after administration of L-NMMA and bretylium and phentolamine. The sodium nitroprusside responses were not changed by administration of any of the study drugs, indicating that the effects of the various drugs on the vascular responses we investigated were not due to some non-specific drug effects.

Second, assessing the level of nitric oxide synthase inhibition after L-NMMA can be difficult. Administration of L-NMMA significantly decreased forearm blood flow below the baseline value observed after bretylium and phentolamine to an average value slightly below that seen during the control trial. This suggests that the basal release of nitric oxide was reduced. A blunting of the vasodilator response to acetylcholine is also consistent with blockade of nitric oxide synthase in the vascular endothelium (Vallance *et al.* 1989). However, the vasodilator responses to acetylcholine were blunted in a variable manner by L-NMMA administration, suggesting a less consistent inhibition of the stimulated release of nitric oxide. Additionally, several lines of evidence favour the concept that pharmacological doses of acetylcholine might also evoke release of a second vasodilator substance not subject to inhibition by L-NMMA (Rubanyi & Vanhoutte, 1987; Mügge, Lopez, Piegors, Breese & Heistad, 1991). Finally, in prior studies we have also seen variable forearm blood flow responses to acetylcholine after nitric oxide synthase blockade, even when consistent changes in the blood flow or vasodilator responses to other physiological stimuli were evident (Dietz *et al.* 1994*a*, *b*). When these limitations are considered in the context of the maintained vasodilator responses to sodium nitroprusside after L-NMMA, the reductions in basal forearm blood flow after L-NMMA, and the blocking by L-NMMA of the vasodilator responses to handgripping and mental stress, our contention that the doses of L-NMMA we used were sufficient to inhibit nitric oxide synthase seems reasonable.

Sympathetic nerve activity during ischaemic handgrip exercise

In this study we have made the assumption that the pattern of muscle sympathetic nerve activity during ischaemic handgrip exercise followed by post-exercise ischaemia was

similar to that previously reported, with little or no change in muscle sympathetic nerve activity seen at the onset of exercise followed by an increase as the exercise becomes fatiguing. This rise in muscle sympathetic nerve activity is then maintained by post-exercise ischaemia (Mark *et al.* 1985; Victor *et al.* 1988; Wallin *et al.* 1989). This pattern of sympathetic traffic also occurs in a consistent manner during repeated bouts of fatiguing exercise (Seals & Enoka, 1989). We have also had some subjects ($n = 4$) perform several bouts of handgripping to fatigue followed by post-exercise ischaemia after administration of bretylium and phentolamine and no additional drugs in the same time schedule as protocol 2. In these subjects the vasodilator responses observed during the first bout performed after bretylium and phentolamine were the same or actually increased with the subsequent bouts, indicating that the responses after L-NMMA were caused by the drug and were not the result of a diminished neural vasodilator stimulus during repeated bouts of exercise (N. M. Dietz, T. T. Samuel & M. J. Joyner, unpublished observations).

After our first observations of forearm vasodilatation during handgrip exercise and post-exercise ischaemia following bretylium and phentolamine we questioned the possibility of cutaneous vasodilatation. Several factors argue against this possibility. First, there can be an increase in vasoconstrictor skin sympathetic nerve activity at the onset of exercise, but this is not maintained during post-exercise ischaemia (Vissing *et al.* 1991). Second, laser Doppler data obtained from several subjects showed no increase in skin blood flow accompanying forearm vasodilatation during exercise and post-exercise ischaemia after bretylium and phentolamine. Taken together these observations support our interpretation that the forearm vasodilator responses we observed were due to active vasodilatation in forearm skeletal muscle.

Sympathetic vasodilatation and nitric oxide in animals

Previous studies have shown that electrical stimulation of sympathetic nerves to hindlimb skeletal muscles in anaesthetized animals normally causes vasoconstriction (Folkow, Haeger & Uvnäs, 1948; Uvnäs, 1954, 1966). However, following either pharmacological interventions that reduce catecholamine release from the nerves, or blockade of post-synaptic adrenergic receptors, stimulation of sympathetic nerves evokes a consistent pattern of hindlimb vasodilatation. Additionally, electrical stimulation of certain autonomic centres in the brainstem can evoke a so-called 'defence reaction' that consists of systemic hypertension, tachycardia and skeletal muscle vasodilatation (Abrahams, Hilton & Zbrozyna, 1964). In these preparations, the vasodilatation observed during sympathetic stimulation can be reduced or eliminated by selective intra-arterial infusion of cholinergic blocking drugs (e.g. atropine) to the vascular bed under study (Folkow *et al.* 1948; Uvnäs, 1954, 1966). As a result of these observations and histological evidence of cholinergic nerves in skeletal muscle blood vessels, the concept of active sympathetic vasodilatation in skeletal muscles was established in several species many years ago (Uvnäs, 1954, 1966).

Recently it has been shown that active sympathetic vasodilatation of skeletal muscles can also be blocked by administration of arginine analogues that inhibit nitric oxide synthase (Matsukawa, Shindo, Shirai & Ninomiya, 1993). There is also data suggesting that acetylcholine from autonomic nerves can stimulate nitric oxide release from the vascular endothelium (Broten *et al.* 1992; McMahon *et al.* 1992). These findings are consistent with our interpretation that sympathetic cholinergic release of nitric oxide contributed to the forearm vasodilator responses we observed. Since L-NMMA appeared to blunt these dilator responses more completely than atropine alone, it is also possible that nitric oxide released directly from nerves contributed to the vasodilatation (Toda & Okamura, 1991).

Sympathetic vasodilatation in humans

Observations beginning in the 1940s suggest that there can be neurally mediated skeletal muscle vasodilatation in humans (Barcroft & Edholm, 1945). First, during mental or emotional stress, there can be profound forearm vasodilatation that is confined to muscle and not skin (Blair *et al.* 1959; Roddie, 1977). Although the pattern of muscle sympathetic nerve activity associated with this manoeuvre appears to be minimal, the vasodilatation during mental stress is absent after surgical sympathectomy (Blair *et al.* 1959; Anderson *et al.* 1987). Second, in some subjects, the dilatation can exceed by a wide margin that seen during or immediately after surgical sympathectomy, nerve block or α-adrenergic blockade of the forearm (Blair *et al.* 1959; Roddie, 1977). Third, vasodilator responses seen during mental stress are also usually attenuated by about 50% after intra-arterial infusion of atropine (Blair *et al.* 1959; Dietz *et al.* 1994*a*). Fourth, a cold pressor test normally causes forearm vasoconstriction due to an increase in sympathetic outflow (Abboud & Eckstein, 1966). However, after α-adrenergic blockade of the forearm, there can be marked forearm vasodilatation during the cold pressor test that can be blunted by intra-arterial atropine (Abboud & Eckstein, 1966). More recently we have shown that the forearm vasodilator responses to mental stress are reduced by about 70% after brachial artery infusion of L-NMMA (Dietz *et al.* 1994*a*).

When these observations are viewed in the context of our current study, we believe we have clear evidence consistent with the existence of active vasodilatation in human skeletal muscle during a manoeuvre known to cause marked and consistent increases in muscle sympathetic nerve activity. It also appears that nitric oxide contributes to this dilatation and may be released (in part) as a result of sympathetic cholinergic stimulation of the vascular endothelium. The role of this vasodilating mechanism in the physiological regulation of muscle blood flow and arterial blood pressure in conscious humans remains to be determined.

Abboud, F. M. & Eckstein, J. W. (1966). Active reflex vasodilatation in man. *Federation Proceedings* **25**, 1611–1617.

Abrahams, V. C., Hilton, S. M. & Zbrozyna, A. W. (1964). The role of active muscle vasodilatation in the alerting stage of the defence reaction. *Journal of Physiology* **171**, 181–202.

Anderson, E. A., Wallin, B. G. & Mark, A. L. (1987). Dissociation of sympathetic nerve activity in arm and leg muscle during mental stress. *Hypertension* **9**, 114–119.

Barcroft, H. & Edholm, O. G. (1945). On the vasodilatation in human skeletal muscle during post-haemorrhagic fainting. *Journal of Physiology* **104**, 161–175.

Blair, D. A., Glover, W. E., Greenfield, A. D. M. & Roddie, I. C. (1959). Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *Journal of Physiology* **148**, 633–647.

Blair, D. A., Glover, W. E., Kidd, B. S. L. & Roddie, I. C. (1960). Peripheral vascular effects of bretylium tosylate in man. *British Journal of Pharmacology* **15**, 466–475.

Broten, T. P., Miyashiro, J. K., Moncada, S. & Feigl, E. O. (1992). Role of endothelium-derived relaxing factor in parasympathetic coronary vasodilation. *American Journal of Physiology* **262**, H1579–1584.

Carlsson, I. & Wennmalm, Å. (1983). Effect of different prostaglandin synthesis inhibitors on post-occlusive blood flow in human forearm. *Prostaglandins* **26**, 241–251.

Cotzias, C. & Marshall, J. M. (1993). Vascular and electromyographic responses evoked in forearm muscle by isometric contraction of the contralateral forearm. *Clinical Autonomic Research* **3**, 21–30.

Dietz, N. M., Rivera, J. M., Eggener, S. E., Fix, R. T., Warner, D. O. & Joyner, M. J. (1994*a*). Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. *Journal of Physiology* **480**, 361–368.

Dietz, N. M., Rivera, J. M., Warner, D. O. & Joyner, M. J. (1994*b*). Is nitric oxide involved in cutaneous vasodilation during body heating in humans? *Journal of Applied Physiology* **76**, 2047–2053.

Eklund, B. & Kaijser, L. (1976). Effect of regional α- and β-adrenergic blockade on blood flow in the resting forearm during contralateral isometric handgrip. *Journal of Physiology* **262**, 39–50.

Folkow, B., Haeger, K. & Uvnäs, B. (1948). Cholinergic vasodilator nerves in the sympathetic outflow to the muscles of the hind limbs of the cat. *Acta Physiologica Scandinavica* **15**, 401–411.

Greenfield, A. D. M., Whitney, R. J. & Mowbray, J. F. (1963). Methods for the investigation of peripheral blood flow. *British Medical Bulletin* **19**, 101–109.

McMahon, T. J., Hood, J. S. & Kadowitz, P. J. (1992). Pulmonary vasodilator response to vagal stimulation is blocked by N^{ω}-nitro-L-arginine methyl ester in the cat. *Circulation Research* **70**, 364–369.

Mark, A. L., Victor, R. G., Nerhed, C. & Wallin, B. G. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circulation Research* **57**, 461–469.

Matsukawa, K., Shindo, T., Shirai, M. & Ninomiya, I. (1993). Nitric oxide mediates cat hindlimb cholinergic vasodilation induced by stimulation of posterior hypothalamus. *Japanese Journal of Physiology* **43**, 473–483.

Mügge, A., Lopez, J. A., Piegors, D. J., Breese, K. R. & Heistad, D. D. (1991). Acetylcholine-induced vasodilatation in rabbit hindlimb *in vivo* is not inhibited by analogues of L-arginine. *American Journal of Physiology* **260**, H242–247.

Roddie, I. C. (1977). Human responses to emotional stress. *Irish Journal of Medical Science* **146**, 395–417.

Roddie, I. C., Shepherd, J. T. & Whelan, R. F. (1957). The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *Journal of Physiology* **136**, 489–497.

Rubanyi, G. M. & Vanhoutte, P. M. (1987). Nature of endothelium-derived relaxing factor: Are there two relaxing mediators? *Circulation Research* **61**, II-61–67.

Sanders, J. S., Mark, A. L. & Ferguson, D. W. (1989). Evidence for cholinergically mediated vasodilation at the beginning of isometric exercise in humans. *Circulation* **79**, 815–824.

Seals, D. R. & Enoka, R. M. (1989). Sympathetic activation is associated with increases in EMG during fatiguing exercise. *Journal of Applied Physiology* **66**, 88–95.

Taylor, J. A., Hand, G. A., Johnson, D. G. & Seals, D. R. (1991). Sympathoadrenal-circulatory regulation during sustained isometric exercise in young and older men. *American Journal of Physiology* **261**, R1061–1069.

Toda, N. & Okamura, T. (1991). Role of nitric oxide in neurally induced cerebroarterial relaxation. *Journal of Pharmacology and Experimental Therapeutics* **258**, 1027–1032.

Uvnäs, B. (1954). Sympathetic vasodilator outflow. *Physiological Reviews* **34**, 608–618.

Uvnäs, B. (1966). Cholinergic vasodilator nerves. *Federation Proceedings* **25**, 1618–1622.

Vallance, P., Collier, J. & Moncada, S. (1989). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **ii**, 997–1000.

Victor, R. G., Bertocci, L. A., Pryor, S. L. & Nunnally, R. L. (1988). Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *Journal of Clinical Investigation* **82**, 1301–1305.

Vissing, S. F., Scherrer, U. & Victor, R. G. (1991). Stimulation of skin sympathetic nerve discharge by central command. Differential control of sympathetic outflow to skin and skeletal muscle during static exercise. *Circulation Research* **69**, 228–238.

Wallin, B. G., Victor, R. G. & Mark, A. L. (1989). Sympathetic outflow to resting muscles during static handgrip and post-contraction muscle ischemia. *American Journal of Physiology* **256**, H105–110.

Acknowledgements

The authors express their gratitude to the subjects. Technical assistance was provided by Tamara Eickhoff. Expert secretarial help was provided by Ms K. Sankey. This work was supported by NIH Grants HL 46493, NS 32352, RR00585-24 and RR00585-24S3, the Jimmy Hueja Foundation and the Mayo Foundation.

Author's email address

M. J. Joyner: joyner.michael@mayo.edu

Received 18 March 1996; accepted 23 September 1996.