Evidence for Cholinergically Mediated Vasodilation at the Beginning of Isometric Exercise in Humans

Jeffrey S. Sanders, MD, Allyn L. Mark, MD, and David W. Ferguson, MD

With the technical assistance of Joan S. Kempf, LPN

Vasodilation occurs in the nonexercising forearm at the beginning of isometric handgrip despite activation of sympathetic vasoconstrictor reflexes. The mechanism of this response remains unclear. In 33 normal humans, age 24±1 years (mean±SEM), we measured mean arterial pressure, heart rate, and forearm blood flow (plethysmography) in the nonexercising arm during sustained contralateral isometric handgrip at 30% maximal voluntary contraction. Sympathetic nerve activity to calf muscles (microneurography) was also measured in 15 subjects. Handgrip resulted in increases in arterial pressure from 86±2 to 97±3 mm Hg (p<0.05). Despite increases in nerve activity to calf muscles from 229±43 to 337±66 units (p<0.005), which would be expected to produce forearm vasoconstriction, forearm vascular resistance in the contralateral resting arm decreased from 20±3 to 18±2 units (p<0.05). To determine the mechanism of this vasodilatory influence, additional studies were performed with regional autonomic blockade with intra-arterial administration of atropine (0.8 mg, 10 subjects) or propranolol (2.0 mg, eight subjects) into the nonexercising forearm before contraction. Propranolol and vehicle had no effect on forearm vascular responses in the resting arm during SHG in the other arm. In contrast, atropine blocked the vasodilatory response in the resting arm during contraction (Δ forearm vascular resistance during contraction, control=−2.1±0.6 units; postatropine=+0.2±0.9 units, p<0.05). Atropine did not attenuate the vasodilator response to isoproterenol or the vasoconstrictor response to norepinephrine. We conclude 1) a dissociation exists between sympathetic neural and forearm vascular responses to isometric exercise; 2) the vasodilatory response in the nonexercising forearm is not due to sympathetic withdrawal or β-adrenergic-mediated vasodilation; and 3) this response is mediated primarily by cholinergic mechanisms. These studies provide the first direct evidence for active, cholinergically mediated vasodilation during exercise in humans. (Circulation 1989;79:815–824)

Stimulation of sensory fibers located in skeletal muscle during isometric exercise initiates a pressor reflex that is associated with widespread vasoconstriction.1,2 However, it has also been demonstrated that during the early stages of unilateral isometric forearm exercise, blood flow to the contralateral forearm is augmented, due to vasodilation of resistance vessels.3–5 While some investigators have attributed this vascular response to metabolic vasodilation resulting from inadvertent muscle contractions in the “resting” arm,2,6 others have suggested that humoral3–5 or neurogenic3–5 vasodilator influences are involved. The present studies were performed to determine which of these potential mechanisms is principally responsible for the early vasodilatory response to isometric exercise that is seen in humans.

Methods

Subjects and Protocol Groups

Thirty-three subjects (30 men and three women), 24±1 years old (mean±SEM), were studied in three separate protocols. All subjects were healthy and were not receiving any medication. Written informed consent was obtained from all subjects, and the pro-

From the Clinical Cardiovascular Physiology Laboratory, Cardiovascular and Clinical Research Centers, Cardiovascular Division, Department of Internal Medicine, University of Iowa Hospitals and College of Medicine, Iowa City, Iowa.

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Address for correspondence: David W. Ferguson, MD, Cardiovascular Division, Department of Internal Medicine, University of Iowa Hospitals, Iowa City, IA 52242.

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tocol was approved by the Human Subjects Review Committee of the University of Iowa. All studies were performed in the supine, postabsorptive state.

In the first series of studies (protocol 1), 15 subjects performed sustained handgrip (SHG) and the cold pressor test, during which measurements were made of systemic hemodynamics, forearm blood flow (FBF) in the contralateral resting arm, and efferent sympathetic nerve activity to muscle (MSNA). In the second series of studies (protocol 2), an additional 10 subjects performed these same interventions before and after regional cholinergic blockade with intra-arterial administration of atropine into the resting forearm. In the third series of experiments (protocol 3), an additional eight subjects performed SHG and the cold pressor test before and after regional $\beta$-adrenergic blockade produced by intra-arterial administration of propranolol into the resting forearm.

**Measurements**

A direct writing, multichannel physiologic recorder was used to simultaneously record phasic and mean (MAP) arterial pressures, heart rate (HR), respiratory activity, and FBF (plethysmography) in the nonexercising arm. In subjects studied during protocol 1, additional measurements included central venous pressure (CVP) and MSNA. Arterial pressure was measured directly through an indwelling 4F polyethylene catheter inserted percutaneously into the brachial artery of the nondominant resting arm. An 18.5-gauge polyethylene catheter was inserted percutaneously through a median ante-cubital vein in the exercising arm and advanced to an intrathoracic position for measurement of CVP. HR and rhythm were recorded continuously by electrocardiography, and respiratory activity was measured by a strain gauge pneumograph. Zero reference point was defined at the midaxillary position. FBF (ml/min/100 ml) was measured in the nonexercising forearm in all subjects by venous occlusion plethysmography with a mercury-in-silastic Whitney strain-gauge apparatus as previously described.\(^7\) Measurements of FBF were obtained every 15 seconds, and the average value per minute was determined. Forearm vascular resistance (FVR) was derived by dividing MAP by FBF and is expressed in units.

**Microneurography**

Multiunit recordings of efferent postganglionic MSNA (skeletal muscle) were recorded from a muscle nerve fascicle in the peroneal nerve posterior to the fibular head. These techniques have been validated and extensively described in studies from our laboratory and elsewhere.\(^9\) In brief, recordings were obtained via percutaneous insertion of tungsten microelectrodes into the peroneal nerve. The electrodes were connected to a preamplifier, and the nerve signal was fed through a bandpass filter and routed through an amplitude discriminator to a storage oscilloscope and loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance-capacitance integrating network to obtain a mean voltage display of the neural activity. Standard criteria for acceptance of a recording of MSNA were achieved in all subjects.\(^9\) Resting MSNA was measured for up to 10 minutes before experiments were begun to ensure that a stable baseline level had been obtained. Sympathetic bursts were identified by inspection of the mean voltage neurogram. Individual burst amplitude was measured and total integrated MSNA was calculated as the total sum of burst amplitudes per minute and expressed as units per minute. Previous studies in our laboratory have determined an intraobserver variability of 5% with an interobserver variability of less than 10% in this expression of MSNA.\(^11\)

**Interventions**

All subjects performed SHG with the dominant arm at 30% of maximal voluntary contraction for 2 minutes with an exercise dynamometer. Maximal voluntary contraction was determined for each subject just before the beginning of the experimental session. Subjects were instructed to avoid a Valsalva maneuver during SHG. The response to post-handgrip muscle ischemia (PHMI) was studied by immediately inflating a pneumatic cuff on the upper portion of the exercising arm to suprasystolic levels 5 seconds before the end of handgrip. After the cuff was inflated, the subjects were instructed to release the SHG and measurements were obtained for an additional 2 minutes. The PHMI was performed to evaluate the influence of chemically sensitive muscle afferents without the concomitant influences of central command or mechanosensitive muscle afferents or both, which are also engaged during SHG.\(^11\)

Responses to the cold pressor test were assessed by immersion of the subject’s hand up to the wrist in ice water for 2 minutes.\(^13\) Subjects were instructed to avoid muscle contraction, performance of a Valsalva maneuver, or held expiration during this intervention. The cold pressor test was performed as an internal control for assessment of the sympathetic effector limb of the subject’s reflex responses, as well as a non-specific indicator of overall reflex responsiveness.

**Experimental Protocols**

The subjects were familiarized with the techniques and procedures to be used before the beginning of the study. A 20-minute rest period followed the insertion of all intravascular catheters, determination of maximal voluntary contraction, and location of a satisfactory recording site for MSNA. In all subjects, measurements were obtained for 2 minutes preceding (control) and 2 minutes after (recovery) each 2-minute intervention period.

In protocol 1 subjects, recordings of hemodynamics and MSNA were obtained during SHG followed by PHMI and during the cold pressor test. Responses
TABLE 1. Hemodynamic and MSNA Effects of Isometric Exercise and Cold Pressor Test in Protocol 1 Subjects

<table>
<thead>
<tr>
<th>Intervention</th>
<th>SAP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>CVP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>FBF (ml/min/100 ml)</th>
<th>FVR (units)</th>
<th>MSNA (bursts/min)</th>
<th>MSNA (units/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125.2±3.1</td>
<td>85.7±2.1</td>
<td>2.2±0.4</td>
<td>61.2±2.5</td>
<td>5.2±0.6</td>
<td>20.1±2.7</td>
<td>20.7±2.6</td>
<td>228.9±43.1</td>
</tr>
<tr>
<td>SHG</td>
<td>133.7±4.0*</td>
<td>97.2±3.1*</td>
<td>2.2±0.5</td>
<td>69.2±2.6*</td>
<td>6.4±0.6*</td>
<td>18.1±2.5*</td>
<td>25.1±2.5*</td>
<td>337.5±66.2*</td>
</tr>
<tr>
<td>PHMI</td>
<td>133.6±3.7*</td>
<td>93.7±2.4*†</td>
<td>2.2±0.5</td>
<td>62.7±2.7†</td>
<td>5.4±0.5†</td>
<td>20.5±2.8†</td>
<td>26.3±2.8*</td>
<td>344.8±59.1*</td>
</tr>
<tr>
<td>Recovery</td>
<td>127.3±2.8*</td>
<td>87.3±1.9</td>
<td>2.3±0.5</td>
<td>62.7±2.4</td>
<td>4.9±0.6</td>
<td>21.9±3.2</td>
<td>23.9±3.1</td>
<td>271.3±51.7</td>
</tr>
<tr>
<td>Control</td>
<td>129.2±3.0</td>
<td>88.1±1.8</td>
<td>2.1±0.4</td>
<td>61.3±2.8</td>
<td>4.8±0.6</td>
<td>21.8±2.5</td>
<td>19.5±3.2</td>
<td>207.2±47.6</td>
</tr>
<tr>
<td>CPT</td>
<td>142.0±3.2*</td>
<td>102.7±2.9*</td>
<td>2.3±0.5</td>
<td>66.8±3.1*</td>
<td>4.5±0.6</td>
<td>27.0±2.8*</td>
<td>32.2±3.8*</td>
<td>472.9±101.3*</td>
</tr>
<tr>
<td>Recovery</td>
<td>135.6±3.7*</td>
<td>94.2±3.4*</td>
<td>2.6±0.5*</td>
<td>61.6±2.8</td>
<td>4.8±0.5</td>
<td>21.9±1.9</td>
<td>27.8±3.4*</td>
<td>344.3±70.1*</td>
</tr>
</tbody>
</table>

Values are mean±SEM for the average response over 2-minute periods for n=15 subjects (protocol 1).
CVP, central venous pressure; CPT, cold pressor test; FBF, forearm blood flow; FVR, forearm vascular resistance; HR, heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; PHMI, posthandgrip muscle ischemia response; SAP, systolic arterial pressure; SHG, sustained hand grip.

*p<0.05 vs. control.
*p<0.05 SHG vs. PHMIR.

were measured for each minute and average values were determined for control, intervention, and recovery periods.

In protocol 2 subjects, SHG with PHMI and the cold pressor test were performed before and again after regional intra-arterial administration first of vehicle (dextrose 5% in water) and then of atropine sulfate (0.8 mg) into the brachial artery of the nonexercising arm. Both vehicle and atropine were infused by means of a Harvard continuous nonpulsatile pump at a rate of 0.6 ml/min. Previous studies in our laboratory have demonstrated that this rate of infusion of vehicle alone does not alter FBF.14,15 Intra-arterial administration of atropine in the doses used in our experiments has previously been shown to effectively block cholinergically mediated vasodilatory responses in human subjects.16

In protocol 3 subjects, responses to SHG with PHMI, cold pressor test, and intra-arterial infusion of isoproterenol (50 μg/min×4 min, infusion rate 0.6 ml/min) were evaluated before and after intra-arterial administration of propranolol (2.0 mg over 5 minutes). Propranolol was used to block peripheral β-adrenergic receptors and thereby evaluate the role of β-adrenergic vasodilation3 in the forearm vascular responses to contralateral isometric exercise.

In each group of subjects, SHG with PHMI and the cold pressor test were performed in a random order. The subjects were blinded as to which intra-arterial drug they were receiving. There was a 5–10-minute rest period between each intervention during which hemodynamic and MSNA parameters returned to control levels.

Statistical Analysis

Minute-by-minute and averaged hemodynamic and MSNA responses during control, intervention, and recovery periods were compared by analysis of variance with repeated measures. Comparison of control values for baseline versus postdrug studies were performed by paired t test. Statistical significance was taken as p<0.05. Values are presented in the text, tables, and figures as mean±SEM.

Results

Responses to Isometric Exercise Before Regional Autonomic Blockade

Hemodynamic and MSNA responses of the 15 subjects in protocol 1 are presented in Table 1 and Figures 1 and 2. Sustained handgrip resulted in significant increases in MAP (Δ MAP from control, Figure 1. Hemodynamic and sympathetic nerve activity to calf muscles (MSNA) responses of protocol 1 subjects during sustained handgrip (SHG) and posthandgrip muscle ischemia (PHMI). Isometric handgrip resulted in significant increases in mean arterial pressure (MAP) and heart rate (HR) with early forearm vasodilation despite significant increases in efferent muscle sympathetic nerve activity (MSNA). During PHMI, HR returned to control while MAP and MSNA remained elevated. Despite continued activation of somatic muscle afferents during PHMI, forearm blood flow (FBF) and forearm vascular resistance (FVR) returned to control levels. Results are mean±SEM for 15 subjects. C, minute 1 of control; SHG, minute 2 of SHG; R, recovery. *p<0.05 vs. control.
Regional blockade into the brachial artery of the resting forearm did not alter responses to SHG or PHMI (Table 2, Figure 3). After intra-arterial vehicle, FVR decreased significantly (−2.4±0.7 units) during SHG and increased significantly (+2.6±0.7 units) during PHMI.

Intra-arterial administration of atropine sulfate (0.8 mg) into the contralateral resting forearm resulted in a small increase in HR (60.2±3.1 to 66.3±3.5 beats/min, \( p < 0.01 \)) but there were no significant differences in resting arterial pressure, FBF, or FVR during the control periods for the baseline, vehicle, or atropine trials (Table 2). Administration of atropine had no effect on arterial pressure or HR responses to SHG or PHMI. However, atropine abolished the forearm vasodilatory response of the resting forearm to SHG in the contralateral arm. After atropine, SHG produced no significant change in FVR (Δ FVR, +0.2±0.9 units) (Figure 3). The vasoconstrictor response to PHMI was somewhat less after atropine, although the response was not significantly different from the pretreatment trials (Δ FVR during PHMI, +3.3±1.4 units before compared with +1.2±0.8 units after atropine, \( p = \text{NS} \)).

To determine if atropine might have produced a nonspecific decrease in vasodilator or vasoconstrictor...
TABLE 2. Effects of Vehicle and Atropine on Hemodynamic Responses to Isometric Exercise in Protocol 2 Subjects

<table>
<thead>
<tr>
<th>Intervention</th>
<th>SAP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>FBF (ml/min/100 ml)</th>
<th>FVR (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125.0±1.8</td>
<td>85.1±1.8</td>
<td>60.2±3.1</td>
<td>5.0±0.5</td>
<td>18.9±2.0</td>
</tr>
<tr>
<td>SHG</td>
<td>135.3±1.4*</td>
<td>94.8±2.4*</td>
<td>69.2±4.1*</td>
<td>6.3±0.6*</td>
<td>16.9±1.8*</td>
</tr>
<tr>
<td>PHMI</td>
<td>136.2±1.3*</td>
<td>95.3±1.9*</td>
<td>59.8±3.0†</td>
<td>5.1±0.7†</td>
<td>22.3±2.8†</td>
</tr>
<tr>
<td>Recovery</td>
<td>125.8±1.3</td>
<td>84.2±1.4</td>
<td>60.5±3.0</td>
<td>5.4±0.8</td>
<td>18.6±2.3</td>
</tr>
<tr>
<td><strong>Postvehicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>124.5±1.6</td>
<td>84.7±1.5</td>
<td>60.2±3.0</td>
<td>5.3±0.6</td>
<td>18.7±2.4</td>
</tr>
<tr>
<td>SHG</td>
<td>135.0±1.5*</td>
<td>94.6±1.9*</td>
<td>68.3±3.6*</td>
<td>6.6±0.7*</td>
<td>16.3±2.0*</td>
</tr>
<tr>
<td>PHMI</td>
<td>134.5±1.1*</td>
<td>93.3±1.4*</td>
<td>60.7±3.3†</td>
<td>5.0±0.6†</td>
<td>21.3±2.4†</td>
</tr>
<tr>
<td>Recovery</td>
<td>126.2±1.5</td>
<td>85.3±1.4</td>
<td>60.0±2.8</td>
<td>5.2±0.7</td>
<td>19.1±2.2</td>
</tr>
<tr>
<td><strong>Postatropine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>128.6±2.1</td>
<td>90.3±1.8</td>
<td>66.3±3.5‡</td>
<td>5.3±0.7</td>
<td>20.1±2.7</td>
</tr>
<tr>
<td>SHG</td>
<td>139.8±3.0*</td>
<td>101.7±3.0*</td>
<td>77.2±5.5*</td>
<td>6.0±0.8*</td>
<td>20.3±2.7</td>
</tr>
<tr>
<td>PHMI</td>
<td>139.5±3.1*</td>
<td>106.2±3.0*</td>
<td>71.8±4.7†</td>
<td>5.3±0.6†</td>
<td>21.2±2.5</td>
</tr>
<tr>
<td>Recovery</td>
<td>132.2±2.3</td>
<td>93.1±1.7*</td>
<td>68.8±3.9</td>
<td>5.3±0.7</td>
<td>21.0±2.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM for the average response over 2-minute periods for n=10 subjects (protocol 2).

FBF, forearm blood flow; FVR, forearm vascular resistance; HR, heart rate; MAP, mean arterial pressure; PHMI, posthandgrip muscle ischemia response; SAP, systolic arterial pressure; SHG, sustained handgrip.

*p<0.05 vs. control.
†p<0.05 SHG vs. PHMIR.
‡p<0.05 vs. baseline control.

ator responsiveness, a separate series of experiments was conducted in eight protocol 2 subjects. In these studies, forearm vascular responses were measured during intra-arterial infusions of isoproterenol (50 μg/min×4 min at 0.6 ml/min) and norepinephrine (50 μg/min, 0.6 ml/min) before and after intraarterial atropine (0.8 mg). Atropine caused no impairment in the ability of forearm vessels to vasodilate during isoproterenol (Δ FVR, −7.2±0.9 units preatropine compared with −8.5±1.1 units postatropine, p=NS) or vasoconstrict during norepinephrine infusion (Δ FVR, +6.1±0.6 units preatropine compared with +8.7±1.1 units postatropine, p=NS).

Responses to Isometric Exercise Before and After Regional β-Adrenergic Blockade

Responses of protocol 3 subjects to SHG and PHMI before and after regional β-adrenergic receptor blockade with intra-arterial administration of propranolol into the contralateral forearm are shown in Table 3. Before administration of propranolol, SHG produced vasodilation in the resting forearm that was of a similar magnitude (Δ FVR, −2.0±0.5 units) to that observed in protocol 1 and 2 subjects. Similarly, PHMI resulted in increases in FVR (+1.6±1.2 units), as was noted in protocol 1 and 2 subjects.

Infusion of propranolol into the brachial artery of the resting forearm resulted in an increase in control FVR (16.8±1.4 units before compared with 22.4±2.2 units postpropranolol, p<0.02) and MAP (86.3±3.1 to 88.4±2.6 mm Hg, p<0.02) and a decrease in resting HR (65.1±3.9 to 59.2±3.5 beats/min, p<0.02) but there was no significant change in FBF (Table 3).

Regional β-adrenergic receptor blockade had no effect on forearm vascular responses to isometric

Figure 3. Forearm vascular responses in the resting arm of protocol 2 subjects during contralateral sustained handgrip (SHG) and posthandgrip muscle ischemia (PHMI) during baseline studies, after intra-arterial administration of vehicle, and after intra-arterial administration of atropine. Vehicle had no effect on the early vasodilatory response to SHG. However, regional cholinergic blockade with atropine abolished the early vasodilatory response to SHG. Results are mean±SEM for 10 protocol 2 subjects. FVR, forearm vascular resistance in resting forearm; C1, minute 1 of control; SHG, minute 2 of SHG; R, recovery. *p<0.05 vs. control.
exercise. During SHG after intra-arterial propranolol, the forearm vasodilatory response (Δ FVR, −2.6±0.8 units) was not different from that before propranolol. Similarly, the forearm vasodilatory response to PHMI after propranolol (Δ FVR, +2.9±1.2 units) was not different from that before propranolol.

The adequacy of regional β-adrenergic blockade was determined by assessing forearm vasodilatory responses to intra-arterial infusion of isoproterenol (50 μg/min×4 min) before and again after administration of intra-arterial propranolol. During baseline trials, isoproterenol resulted in an increase in FBF of +5.6±1.5 ml/min/100 ml and a decrease in FVR of −9.6±1.6 units. After intra-arterial administration of propranolol, intra-arterial infusion of isoproterenol resulted in an increase in FBF of +5.0±0.2 units (p<0.01 compared with before propranolol) and a decrease in FVR of −1.9±1.4 units (p<0.01 compared with before propranolol), indicating that significant regional β-adrenergic blockade was achieved.

Responses to the Cold Pressor Test Before and After Regional Autonomic Blockade

Responses of protocol 1 subjects to the cold pressor test are summarized in Table 1. The cold pressor test resulted in significant increases in MAP (Δ MAP, +14.6±1.6 mm Hg), HR (Δ HR, +5.5±1.6 beats/min), MSNA (Δ MSNA, +265.7±76.3 units/min), and FVR (+5.2±1.4 units), while FBF and CVP did not change significantly.

Baseline (predrug) responses of protocol 2 and 3 subjects to the cold pressor test were similar to those of protocol 1 subjects noted above (data not shown). Regional autonomic blockade with intra-arterial administration of atropine (0.8 mg, protocol 2 subjects) or propranolol (2.0 mg, protocol 3 subjects) had no significant effect on responses to the cold pressor test with the exception of a relatively smaller increase in FVR during the cold pressor test performed after atropine (Δ FVR during cold pressor test, +4.0±1.0 units baseline, compared with Δ FVR, +1.2±0.8 units after atropine; p<0.05).

Discussion

The objective of the present study was to examine the forearm vascular responses of the nonexercising arm during contralateral isometric exercise and to determine the mechanisms responsible for these responses. Our studies confirm earlier observations that vasodilation characterizes the early vascular response to contralateral isometric handgrip5–5 and permit us to make several conclusions regarding these responses. First, a dissociation exists between the sympathetic neural and forearm vascular responses to isometric exercise, in that vasodilation occurs despite an increase in sympathetic vasoconstrictor activity. Second, this early vasodilation during contralateral handgrip does not result primarily from stimulation of peripheral β2-adrenergic receptors, nor does it result primarily from "metabolic vasodilation." Third, as previously proposed,6 central command is an important initiator of this vasodilatory response. Finally, cholinergic activation during isometric exercise is a principal mechanism responsible for the vasodilation observed in the contralateral resting forearm. The present studies provide the first direct evidence that cholinergic vasodilation contributes importantly to vascular responses during isometric exercise in humans.

Dissociation Between Neural and Vascular Responses to Isometric Exercise

The MSNA responses to SHG observed in our studies agree with those described previously.11,17,18 Mark et al11 demonstrated that after the first 30–60 seconds of SHG, there is a steady rise in MSNA that is maintained during posthandgrip ischemia. This increased efferent MSNA is initiated by chemosensitive receptors in exercising muscle that
are activated by ischemic metabolites and transmit excitatory afferent signals to brainstem cardiovascular centers.\textsuperscript{19–21}

In contrast to the neural responses to SHG, the vascular responses in the nonexercising forearm during isometric exercise follow a biphasic pattern of early vasodilation followed by a return of FVR to or above baseline levels.\textsuperscript{3,5,22} This vasodilatory response peaks within the 1st minute of exercise, although FVR remains below control throughout the 2nd minute of sustained contraction\textsuperscript{22} (Figures 1 and 3). When \(\alpha\)-adrenergically mediated vasoconstriction is pharmacologically blocked before initiation of SHG, FVR progressively decreases throughout the duration of contraction.\textsuperscript{4}

Our findings of forearm vasodilator responses in the resting arm during sustained contralateral handgrip essentially mimic those previously described by Eklund, Kajser, and colleagues\textsuperscript{3,4,22} and by Rusch et al.\textsuperscript{5} Furthermore, the forearm vasodilator response in the present study becomes even more interesting when compared with the neural responses in the same subjects. FVR decreased significantly despite the fact that efferent sympathetic vasoconstrictor activity (measured directly) was increasing (Figure 1). Although MSNA was, by necessity, measured in a resting leg in this study, while FVR was measured in the resting arm, previous human studies using identical techniques of microneurographic recording during SHG have demonstrated parallel increases of MSNA in both the arm and leg.\textsuperscript{18} Thus, forearm vascular and sympathetic neural responses during the early stages of isometric exercise are dissociated and the vasodilatory response is, therefore, not due to withdrawal of sympathetic vasoconstrictor activity. This dissociation appears not to be generalized to other reflex responses, since both MSNA and FVR increased simultaneously during the cold pressor test in the same group of subjects (Table 1).

**Role of Metabolic and \(\beta\)-Adrenergic–Mediated Vasodilation**

Lind and colleagues\textsuperscript{6} have attributed the vasodilatory response in the contralateral forearm to metabolic origins, resulting from the involuntary muscle contraction in the “resting” arm during exercise in the other limb. These investigators noted that FBF (FVR not reported) increased during contralateral handgrip only when electromyographic activity could be detected in the “resting” arm. However, these authors point out that little or no electromyographic activity occurred in the contralateral arm during the 1st minute of contraction. As noted in our studies and others, the vasodilatory response in question is at its greatest magnitude during the 1st minute of exercise. Rusch et al\textsuperscript{15} also noted that while exercise-induced vasodilation occurred early, electromyographic activity in the resting arm increased gradually and at a time when resistance began returning toward baseline. We also observed that the peak vasodilatory response occurs in the 1st minute of SHG (Figures 1 and 3), when subject fatigue and any potential inadvertent muscle contraction in the experimental arm would be expected to be at a minimum. The subjects reported here were instructed to completely relax their nonexercising arm throughout the interventions and the baseline of the plethysmographic recordings of FBF (a sensitive indicator of arm motion artifact) remained stable. Further evidence against metabolic vasodilation is that the peak vasodilatory response occurred in the 1st minute of SHG, whereas the peak response in MSNA (which is determined by the accumulation of ischemic metabolites) did not occur until the 2nd minute (Figure 1). Most importantly, metabolic vasodilation would not be expected to be abolished by atropine, as was the case in our experiments. Therefore, we believe that it is unlikely that metabolic vasodilation is the sole mechanism for the vascular responses observed during contralateral SHG. In support of this conclusion is a recent report by Anderson and Saltin,\textsuperscript{23} which examined blood flow in the lower extremities during rhythmic leg exercise. These investigators demonstrated an increase in blood flow before detectable changes occurred in metabolically derived substances in the venous effluent from the limb.

\(\beta\)-Adrenergically mediated vasodilation has also been proposed as a potential mechanism for the early decrease in FVR during contralateral isometric exercise. Eklund and Kajser\textsuperscript{4} demonstrated that the vasodilatory response was attenuated (although not abolished) after local \(\beta\)-blockade with propranolol. Therefore, they suggested that the blood flow increase in the resting forearm is mediated to a substantial degree, although not entirely, by \(\beta\)-adrenergic mechanisms. These investigators speculated that stimulation of peripheral \(\beta\)-adrenergic receptors, either by circulating epinephrine released from the adrenal medulla, or by adrenergic vasomotor nerve activity, was responsible for the vasodilation. However, they acknowledged that this explanation was not entirely satisfactory for two reasons. First, while circulating epinephrine is known to increase during SHG, the increase is small and appears only after the 1st minute.\textsuperscript{24} Second, increases in MSNA, as well as circulating norepinephrine, primarily produce vasoconstriction by preferential stimulation of \(\alpha\)-adrenergic receptors. In the absence of \(\alpha\)-adrenergic blockade, an increase in MSNA should produce an \(\alpha\)-receptor–mediated increase in FVR and not a \(\beta\)-receptor–mediated decrease in FVR.

The results of the present experiments do not support the concept of \(\beta\)-adrenergic vasodilation as an important component of the early vascular response to isometric exercise. In protocol 3 subjects, regional blockade of \(\beta\)-adrenergic (vasodilatory) receptors with intra-arterial administration of propranolol did not attenuate the vascular response to handgrip in the resting arm. The fact that the degree of vasodilation during SHG was the same
before and after propranolol was not due to inadequate regional β-blockade because isoproterenol-induced vasodilation was abolished after intra-
arterial administration of propranolol.

It is possible that with prolonged exercise and more substantial elevations of circulating epinephrine, β-adrenergic vasodilation may modulate α-adrenergic vasoconstriction. However, it does not appear that β-adrenergic vasodilation is responsible for the vasodilatory response that we and others have observed during the early stages of isometric exercise.

**Role of Central Command**

Central command denotes the activation of brainstem cardiovascular centers via descending central neural pathways that are involved in the initiation of somatomotor activity. The fact that FVR decreases almost immediately with the initiation of exercise and abruptly increases with the release of contraction (Figures 1 and 3) raises the probability that a central mechanism, related to the effort involved in sustaining the muscle contraction, is important in determining the vascular responses. This concept is supported by the fact that vasodilation is not present during posthandgrip muscle ischemia, when the influence of central command is eliminated.

Eklund and Kaijser examined vascular responses to prolonged handgrip (6 minutes) at both constant force (33% maximal voluntary contraction) and constant maximum effort (resulting in decreasing force secondary to fatigue). They noted that the rapid early decrease in FVR was related to initiation of somatomotor activity, whereas continued muscle contraction produces increases in vasoconstrictor activity via the somatic pressor reflex. Of interest is the fact that other interventions that involve conscious behavior on the part of the subject, such as mental stress, resistance breathing, coughing, and the Valsalva maneuver, have been reported to result in similar vasodilatory responses as those which are induced by exercise. Therefore, central command appears to play an important role in initiating the vasodilator responses during isometric exercise.

**Cholinergic Vasodilation During Exercise**

The present study is the first, to our knowledge, to demonstrate a role for active cholinergic vasodilation in the vascular response to isometric exercise in humans. Evidence for active neurogenic vasodilation has been well documented in animals where the existence of inhibitory prejunctural cholinergic receptors on the adrenergic nerve ending in blood vessel walls has been demonstrated. Previous investigations in humans have shown that cholinergically mediated vasodilation occurs in response to various interventions. Blair et al. showed that the vasodilatory response to mental stress was abolished or markedly attenuated by either sympathetic nerve block or by intra-arterial infusion of atropine. Abdoud and Eckstein found that after intra-arterial guanethidine and phentolamine, the application of ice to the forehead, or the Valsalva maneuver, caused vasodilation in the forearm and that these responses were attenuated by intra-arterial atropine. These studies were important in that they showed that stimuli that cause reflex vasoconstriction (similar to SHG) may also activate reflex vasodilatory pathways.

Prejunctional inhibition of adrenergic neurotransmission during isometric exercise is an attractive explanation for the responses observed in the present studies. At the initiation of handgrip, when MSNA is at moderate levels, prejunctional inhibition of norepinephrine release would be expected to result in a decrease in FVR. As contraction continues, this inhibitory modulation would be overwhelmed by the increase in efferent neural activity, resulting in increased norepinephrine release and a subsequent increase in FVR. Consistent with this hypothesis are results of previous studies that show that the magnitude of SHG-induced vasodilation is reduced when sympathetic activity is either lower (head-down tilt or volume infusion) or higher (upright position or lower-body negative pressure) than normal. This pattern of response would be expected if vasodilation during exercise is dependent on modulation of sympathetic neurotransmission.

The results of the present series of investigations suggest that cholinergic vasodilation may be an important, and previously poorly appreciated, component of the net reflex response to exercise. With the onset of muscle contraction, central command results in relative tachycardia (via parasympathetic withdrawal) and vasodilation in some muscle beds (via activation of sympathetic cholinergic mechanisms). As muscle contraction continues, or during postexercise muscle ischemia, ischemic metabolites accumulate and the muscle chemoreflex is invoked. Stimulation of muscle afferents leads to an increase in efferent sympathetic vasoconstrictor tone, which appears to predominate as muscle contraction continues, resulting in a return of vascular resistance to, or above, baseline levels. Cholinergic vasodilation, as well as metabolic vasodilation, modulate vasoconstriction during these latter stages of exercise. It may also be possible that constrictor and dilator fibers act differently on various segments in the same vascular bed, resulting in a more efficient net vascular response.

**Potential Limitations of Studies**

We recognize several potential limitations in the design and interpretation of the present studies. First, as mentioned previously, the conclusion that forearm vascular and MSNA responses are dissociated during SHG is based on experimental techniques using measurements of MSNA from the peroneal nerve of the leg and calculations of FVR based on measurements of blood flow in the forearm. Unfortunately, the technical requirements for plethysmographic measurements of FBF preclude
the simultaneous measurement of MSNA from the same arm. However, previous studies from our laboratory have revealed that efferent sympathetic responses in the resting arm are the same as MSNA responses measured in the resting leg during static exercise. Therefore, we believe the dissociation between MSNA and FVR found in the present studies is a valid observation.

A second caution relates to the possibility that the decreased vascular resistance in the forearm during SHG could be attributable to an increase in blood flow to the skin, rather than an increase in muscle blood flow. Several previous studies suggest this is not the case. During performance of contra-lateral handgrip, measurements of oxygen saturation of venous blood draining forearm muscle indicate that vasodilation was occurring in muscle resistance vessels. In addition, Rusch and colleagues and Taylor and colleagues (personal communication) have shown that the decrease in FVR during exercise is not associated with vasodilation in skin. Therefore, we believe that the FVR changes noted in our studies predominantly reflect the vasculature supplying forearm muscle beds.

A third consideration is that these studies examined vascular responses only in the arm and that our conclusions should be limited to the upper extremity vascular beds. Previous investigations have shown that vascular resistance in the leg does not decrease in concert with FVR changes during exercise. A similar pattern of responses in the arm and calf is seen during mental stress, supporting the concept that the volitional component of exercise invokes central neural mechanisms that result in vasodilation.

In our studies, vasoconstriction during PHMI and during the cold pressor test appeared to be blunted after intra-arterial atropine, although not to a significant degree in the case of PHMI. Therefore, as a fourth concern, we considered the possibility that atropine had in some way produced a nonspecific neurogenic blockade or a generalized reduction in vascular reactivity that could account for the attenuation of vascular responses. To test this possibility, we examined forearm vasoconstrictor responses of eight protocol 2 subjects to intra-arterial administration of isoproterenol and norepinephrine. Atropine had no apparent effect on these responses as discussed in “Results.” These vascular responses to isoproterenol and norepinephrine were of a greater magnitude than the relatively modest changes in forearm vascular resistance observed during the other interventions used in these studies, so we cannot completely rule out the possibility that a nonspecific attenuation of vascular responses by atropine contributed in some measure to our findings. However, we believe it is unlikely that such an effect accounts for the dramatic differences in pre-atropine and post-atropine responses to SHG.

Finally, all studies were performed with subjects in the supine position, due to constraints of the experimental techniques. It is possible that interactions between arterial baroreflex, cardiopulmonary baroreflex, and the reflex pathways activated during isometric exercise could be different during upright exercise than during supine handgrip. However, with regard to the cardiopulmonary baroreflex, recent studies from our laboratory and others have clearly demonstrated that the cardiopulmonary baroreflexes fail to modulate sympathetic responses during isometric handgrip exercise in humans.

**Summary**

In summary, we have demonstrated a significant dissociation between the efferent sympathetic neural and the resting forearm vascular responses to contralateral isometric handgrip in normal human subjects. The early vasodilatory response, occurring in the presence of efferent sympathoexcitation, appears to be related primarily to cholinergic vasodilatory influences.

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