A cholinergic contribution to the circulatory responses evoked at the onset of handgrip exercise in humans

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1Faculty of Physical Education, University of Brasília, Brasília, Brazil; 2Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri; 3Department of Anaesthesia, The Copenhagen Muscle Research Centre, Rigshospitalet, University of Copenhagen, Denmark; and 4School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, United Kingdom

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Vianna LC, Fadel PJ, Secher NH, Fisher JP. A cholinergic contribution to the circulatory responses evoked at the onset of handgrip exercise in humans. Am J Physiol Regul Integr Comp Physiol 308: R597–R604, 2015. First published January 14, 2015; doi:10.1152/ajpregu.00236.2014.—A cholinergic (muscarinic) contribution to the initial circulatory response to exercise in humans remains controversial. Herein, we posit that this may be due to exercise mode with a cholinergic contribution being important during isometric handgrip exercise, where the hyperemic response of the muscle is relatively small compared with the onset of leg cycling, where a marked increase in muscle blood flow rapidly occurs as a consequence of multiple redundant mechanisms. We recorded blood pressure (BP; brachial artery), stroke volume (pulse contour analysis), cardiac output, and systemic vascular resistance (SVR) in young healthy males, while performing either 20 s of isometric handgrip contraction at 40% maximum voluntary contraction (protocol 1; n = 9) or 20 s of low-intensity leg cycling exercise (protocol 2; n = 8, 42 ± 8 W). Exercise trials were conducted under control (no drug) conditions and following cholinergic blockade (glycopyrrolate). Under control conditions, isometric handgrip elicited an initial increase in BP (+5 ± 2 mmHg at 3 s and +3 ± 1 mmHg at 10 s, P < 0.05), while SVR dropped after 3 s (-27 ± 6% at 20 s; P < 0.05). Cholinergic blockade abolished the isometric handgrip-induced fall in SVR and, thereby, augmented the pressor response (+13 ± 3 mmHg at 10 s; P < 0.05 vs. control). In contrast, cholinergic blockade had a nonsignificant effect on changes in BP and SVR at the onset of leg cycling exercise. These findings suggest that a cholinergic mechanism is important for the BP and SVR responses at the onset of isometric handgrip exercise in humans.

The first 20 s of dynamic exercise (e.g., cycling) typically evokes a biphasic blood pressure (BP) response, including an initial pressor response followed by a fall in BP despite a rise in cardiac output and is, thus, secondary to a fall in systemic vascular resistance (SVR) (42, 48). Multiple reflex and hemodynamic factors may contribute to this fall in SVR at the onset of exercise. Important are the skeletal muscle pump (46), which by facilitating venous return can enhance cardiopulmonary afferent activation, leading to withdrawal of sympathetic vasoconstrictor activity (12, 36, 40, 48), and vasodilatory substances released within active skeletal muscles (e.g., prostaglandins). A contribution of a cholinergic mediator to regulating late BP at the onset of exercise has also been posited (31, 48), but that proposal remains controversial.

In several species (e.g., cat and dog) skeletal muscle vasculature and several other regions (e.g., skin and the mesentery circulation) are innervated by sympathetic cholinergic vasodilator nerves (6). Stimulation of the defense areas of the midbrain and hypothalamus in anesthetized or decerebrate cats elicits skeletal muscle vasodilatation, which is abolished by cholinergic blockade with atropine (2, 14, 32). In addition, intravenous administration of atropine attenuates the increase in BP, brachial blood flow, and brachial vascular conductance in the exercising forelimb during voluntary isometric exercise in the conscious cat (24, 31). Muscarinic blockade does not, however, appear to affect the hyperemic response of exercising skeletal muscles in canines running on a treadmill (10, 13). It may be that these conflicting findings are attributable to differences in exercise modality. In other words, a multitude of redundant factors contribute to the large increases in blood flow observed at the onset of dynamic exercise with a large muscle mass that may mask the effects of blocking the muscarinic cholinergic contribution. In contrast, the circulatory effects of muscarinic blockade may be more evident during isometric exercise because of the smaller hyperemic response that can be further blunted as the contracting muscles compress the blood vessels, particularly, at higher exercise intensities (4, 7, 17, 24).

The effect of cholinergic blockade on the circulatory response to exercise in humans is also conflicting. Atropine blocks the exercise-induced decrease in vascular resistance of the nonexercising forearm (41), abolishes the exercise-induced increase in tooth pulp blood flow (1), and middle cerebral artery mean blood velocity (45). Furthermore, atropine blunts the normal increase in oxygenated hemoglobin within the nonexercising musculus vastus lateralis at the onset of one-legged cycling (21). In contrast, muscarinic blockade does not affect the forearm blood flow response to ipsilateral handgrip exercise (8, 23) or exercising limb blood flow during one-legged knee extensor exercise (18). These observations cast doubt upon the importance of a sympathetically mediated cholinergic vasodilatation within exercising skeletal muscles and, therefore, its importance to the BP response to dynamic exercise in humans. In this regard, the effect of cholinergic mediated dilation on the BP response to exercise in humans remains unclear (18, 21, 41, 45).

Here, we investigate the impact of cholinergic blockade on the immediate BP and circulatory response to the onset of isometric handgrip (protocol 1), not considered to be associated with a large increase in muscle blood flow, and cycling exercise (protocol 2), considered to be associated with an
immediate and dramatic increase in muscle blood flow. We tested the hypotheses that muscarinic cholinergic receptor blockade (with glycopyrrolate) would abolish the fall in SVR and augment the pressor response during the first few seconds of exercise in humans and that this effect would be particularly evident during isometric handgrip exercise.

METHODS

Seventeen men participated in this investigation (age, height, and weight 23 ± 4 years, 181 ± 7 cm, and 76 ± 6 kg; mean ± SD) comprising two separate protocols. The first protocol involved isometric handgrip (n = 9) and the second leg cycling exercise (n = 8). Records were analyzed from previous studies (15, 16) testing different hypotheses for which experimental protocols and procedures were approved by the local ethics committee (H-B-2009-024, H-3-2011-101) and undertaken in accordance with the Declaration of Helsinki. In these studies, the responses to the onset of exercise were not analyzed. Participants were provided with written and verbal explanation of the experiment following which they gave their written consent for participation. Participants had no significant medical history and were not using prescribed or over-the-counter medications. The ambient laboratory temperature was maintained between 23°C and 24°C for all studies, and external stimuli were minimized. Participants arrived at the laboratory having abstained from eating for ≥2 h, caffeine for ≥12 h, and strenuous physical activity and alcohol for ≥24 h prior to experimentation.

Measurements

Heart rate (HR) was monitored using a lead II ECG, and arterial BP was measured by a catheter (1.1 mm ID, 20-gauge) inserted into the left brachial artery and connected to a transducer (Baxter, Uden, Netherlands) adjusted to the level of the right atrium. ECG and BP signals were obtained through a Dialogue 2000 monitor (IBC-Danica, Copenhagen, Denmark) interfaced with an analog-to-digital converter (Powerlab, AD Instruments) and personal computer equipped with data acquisition software (AD Instruments). Cardiovascular variables were sampled at 1 kHz, and beat-to-beat values for HR and BP were stored for offline analysis. Stroke volume (SV) was calculated by analysis of the blood pressure waveform using pulse contour analysis (protocol 1; Beatscope, FMS, Amsterdam, Netherlands; protocol 2; Nefxim, Bmeye, Amsterdam, Netherlands) (49). Mean arterial pressure (MAP) was determined on a beat-by-beat basis by integration of the BP waveform. Cardiac output (CO) was calculated as SV × HR and SVR as MAP/CO. A strain-gauge pneumograph was placed around the participants’ abdomen (Pneumotrace; UFI, Morro Bay, CA) to monitor ventilatory movements and ensure that no inadvertent Valsalva maneuvers were performed during handgrip exercise.

Experimental Protocol

Trials of handgrip and leg cycling were performed under control conditions (no drug) and following cholinergic blockade with glycopyrrolate. In protocol 1, glycopyrrolate was administered via a catheter (2.4 mm; REF 681698 BD Medical Systems, Singapore) inserted retrograde into the right internal jugular vein under local anesthesia (Lidocaine, 2%) and guided by ultrasound. In protocol 2, glycopyrrolate was administered via a left forearm vein. Following instrumentation and catheterization, subjects rested for a minimum of 30 min. Complete blockade with glycopyrrolate was considered achieved when no further increase in resting HR was elicited by consecutive 0.2-mg doses (group average dose of 25.2 ± 1.6 μg/kg, including the initial and supplementary doses, equating to a total infused volume of 9.5 ± 0.6 ml).

 Protocol 1. Isometric Handgrip. A handgrip dynamometer was held in the participants’ right hand, while they were seated in a semirecumbent position on a hospital bed. A display was positioned at eye level to provide feedback regarding the force exerted (Chart and Powerlab, AD Instruments). Participants performed 3–5 maximal efforts separated by ~1 min with the highest designated as the maximum voluntary contraction (MVC). Following 3 min of rest, participants were instructed to briskly commence a handgrip contraction at 40% MVC, while avoiding straining maneuvers.

 Protocol 2. Leg Cycling. Participants rested in a semirecumbent position for 3 min with their feet secured to the pedals of an electrically braked cycle ergometer (25). Then, they were instructed to perform leg cycling exercise, while a metronome-guided pedal cadence at 60 revolutions per minute. The workload (42 ± 8 W) was predetermined as being sufficient to raise HR to a target of ~100 beats/min within 2–4 min under control (no drug) conditions.

Data and Statistical Analysis

Beat-to-beat data were recorded continuously at rest (3 min) and during 20-s bouts of isometric handgrip and leg cycling under control (no drug) conditions and following administration of cholinergic blockade by glycopyrrolate. Resting variables are compared using paired t-test (control vs. cholinergic blockade) for each protocol. Beat-to-beat MAP, HR, SV, CO, and SVR during exercise were linearly interpolated at 1 Hz and expressed as a change from rest on a second-by-second basis. The area under the curve (AUC) was then calculated to summarize the hemodynamic response to exercise, and comparisons were made between the control and cholinergic blockade conditions for each protocol using paired t-tests. In addition, repeated-measures ANOVA was employed to evaluate the main effects of time (3, 10, and 20 s exercise) and condition (control, cholinergic blockade) on cardiovascular variables during exercise. Student-Newman-Keuls’ post hoc tests were used to evaluate significant main effects and interactions. Data are presented as means ± SE, and statistical significance was set at P < 0.05. Analyses were conducted using SigmaStat for Windows (Jandel Scientific Software, SPSS, Chicago, IL).

RESULTS

Protocol 1. Isometric Handgrip

For both control conditions and with cholinergic blockade, resting hemodynamic variables are shown in Table 1. As intended, HR increased with cholinergic blockade (+52 ± 5 beats·min⁻¹) and despite a fall in SV, CO was elevated (all P < 0.05). No differences in resting SVR or MAP were noted between the control and cholinergic blockade trials. With cholinergic blockade, the pressor response to isometric handgrip exercise was augmented (AUC: 32 ± 13 vs. 136 ± 22 mmHg/s for control and cholinergic blockade trials, respectively: P < 0.05) with the progressive increase in MAP over the 20 s of handgrip being significantly enhanced (Fig. 1A). Under control conditions isometric handgrip produced an exponential decrease in SVR (AUC: −28 ± 5 mmHg·l·min⁻¹·s⁻¹) that was abolished with cholinergic blockade (AUC: −2 ± 5 mmHg·l·min⁻¹·s⁻¹; P < 0.05) (Fig. 1B). Handgrip evoked a progressive increase in HR in both conditions, but the magnitude was attenuated with cholinergic blockade (AUC: 172 ± 26 vs. 94 ± 8 beats·min⁻¹·s⁻¹; P < 0.05) (Fig. 2A). SV and CO responses to handgrip were not different between conditions, and both increased similarly over the 20-s handgrip bout (AUC: 34 ± 33 vs. 55 ± 18 ml/s and 20 ± 4 vs. 13 ± 2 l·min⁻¹·s⁻¹ for control and cholinergic blockade trials, respectively: both P > 0.05) (Fig. 2, B and C).

Protocol 2. Leg Cycling

Prior to leg cycling, cholinergic blockade increased resting HR, CO, and MAP (P < 0.05), while SV was reduced and
SVR remained unchanged. In both the control and cholinergic blockade conditions, MAP fell to a nadir at 10 s of leg cycling and then returned to baseline levels 20 s after exercise onset (AUC: 232 ± 20 vs. 70 ± 10 beats-min⁻¹·s for control and cholinergic blockade trials, respectively; *P < 0.05) (Fig. 4A). Conversely, the increase in SV during leg cycling was greater with cholinergic blockade (AUC: 150 ± 20 mmHg/s) than in the control condition (AUC: 83 ± 8 mmHg/s; *P < 0.05). Thus, CO responses to leg cycling were not different between conditions (AUC: 31 ± 3 vs. 24 ± 3 l·min⁻¹·s for control and cholinergic blockade trials, respectively; *P > 0.05) (Fig. 4C).

DISCUSSION

The aim of this investigation was to determine whether a cholinergic mechanism is involved in the initial circulatory

### Table 1. Resting hemodynamics prior to isometric handgrip and leg cycling under control (no drug) conditions and following cholinergic blockade

<table>
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<tr>
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<th>Isometric Handgrip</th>
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<th>Leg Cycling</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cholinergic Blockade</td>
<td>Control</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91 ± 2</td>
<td>90 ± 3</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>HR, beats-min⁻¹</td>
<td>67 ± 3</td>
<td>119 ± 3*</td>
<td>67 ± 4</td>
</tr>
<tr>
<td>SV, ml</td>
<td>95 ± 5</td>
<td>62 ± 3*</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>6.3 ± 0.3</td>
<td>7.3 ± 0.4*</td>
<td>6.8 ± 0.5</td>
</tr>
<tr>
<td>SVR, mmHg·l⁻¹·min</td>
<td>15 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
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Values are expressed as means ± SE. MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; SVR, systemic vascular resistance. *Significantly different from control (*P < 0.05).
responses to exercise in humans. The major findings of the study are twofold. First, cholinergic blockade abolished the initial drop in SVR and, therefore, augmented the pressor response at the onset of isometric handgrip exercise. Secondly, cholinergic blockade had a modest effect on the changes in SVR and BP at the onset of leg cycling exercise. These findings highlight an underappreciated influence of cholinergic vasodilatation on BP and SVR at the onset of isometric handgrip exercise.

Acetylcholine is a potent vasodilator, and there are multiple mechanisms by which it regulates systemic vascular resistance. Activation of cholinergic receptors on endothelial cells within the skeletal muscle vasculature releases a multitude of vasodilators (e.g., nitric oxide and prostanoids) and initiates
rapid conducted ascending and descending vasodilation (9, 11, 43). One potential source of ACh is its synthesis and release from endothelial cells due to a flow-mediated mechanism (30, 34). Another possibility is that ACh spills over from the neuromuscular junctions within the exercising skeletal muscle and diffuses into the microcirculation, where it may act upon the endothelium (44). In addition, neurogenic release of ACh within exercising skeletal muscle has been described. Komine et al. (24) reported that intravenous administration of atropine blunts the HR, MAP, brachial blood flow, and conductance responses to voluntary isometric contraction of the forelimb in trained cats. A similar blunting effect was noted following administration of the ganglionic blocker hexamethonium (24). In addition, Ishii et al. (21) reported that mental imagery of cycling exercise evoked an increase in femoral artery blood flow, indicative of a centrally induced vasodilatation in humans (21). Thus, it has been suggested that central command-mediated activation of a sympathetic cholinergic nervous pathways may contribute to the hyperemic responses at the onset of exercise (31).

Despite the multiple potential pathways by which ACh may influence perfusion of skeletal muscles, the evidence for such a cholinergic contribution has been largely negative in humans. Cholinergic blockade has no effect on the skeletal muscle blood flow response to a single 1-s forearm contraction (8), rhythmic forearm contractions (47), or one-legged knee extensor exercise (18). In light of these observations, it is unlikely that elimination of the fall in SVR at the onset of isometric handgrip with glycopyrrolate depends on an increase in active muscle blood flow. Indeed, the hyperemic response to exercise is minimal when an isometric contraction exceeds ~30% MVC (7, 17). In contrast, during dynamic exercise, there is a notable increase in perfusion of the active muscles, which greatly impacts SVR as a consequence of multiple redundant mechanisms with the potential to mask any effect of cholinergic blockade (8, 18, 47). In the present study, although there was a tendency for a blunted fall in SVR and BP at the onset of leg cycling exercise with glycopyrrolate, the lack of a strong effect perhaps signifies an effect of exercise modality on the mechanisms involved in control of circulatory responses. For example, a reduction in sympathetic vasoconstrictor neural outflow is documented at the onset of seated isometric quadriceps exercise (37) and low-intensity leg cycling (20) that may be secondary to activation of the cardiopulmonary baroreceptors resulting from an increase in venous return and right atrial pressure by the skeletal muscle pump (46, 48). In contrast, sympathetic vasoconstrictor activity is typically unchanged at the onset of moderate intensity isometric handgrip and in-
creases at higher handgrip intensities (26). In addition, dy-
namic exercise is accompanied by an increase in a plethora of
released vasodilatory substances with the capacity to reduce
SVR (27). Thus, at the onset of leg cycling exercise, these
mechanisms may mask any cholinergic contribution to the
initial fall in SVR and consequently its effect on BP.

The concomitant increase in BP and fall in SVR at the onset
of isometric handgrip is well established (29), although the
existence of individual differences has been reported (51). A
cholinergic contribution to the SVR and BP responses to the
onset of isometric handgrip may be explained by events in
vascular beds other than that of the exercising skeletal muscle.

Fig. 4. Heart rate (HR; A), stroke volume (SV; B), and cardiac output (CO; C) over the first 20 s
of leg cycling exercise for the control (solid symbols) and cholinergic blockade (open sym-
 bols) conditions. Arrows indicate the 3-, 10-, and
20-s time points used for ANOVA analyses (B). P
values are result of ANOVA. *P < 0.05 vs. 3
s; §P < 0.05 vs. 10 s; #P < 0.05 vs. cholinergic
blockade. Values are expressed as means ± SE.
involved and given the rapidity of the response, a neural mechanism might be implicated (31). Atropine abolishes vasodilation in the resting forearm during contralateral isometric handgrip exercise (21, 41), possibly implying the contribution of a sympathetic cholinergic mechanism (31, 41). In contrast, Reed et al. (38) demonstrated that the forearm vasodilation accompanying contralateral handgrip to fatigue persisted despite stellate ganglion blockade to anesthetize the sympathetic nerves of the quiescent forearm. These observations seemingly rule out a neurally mediated vasodilation of resting muscles, and as L-NMMA and propranolol partially blocked this vasodilatory response, a role for nitric oxide and circulating catecholamines was suggested as a likely alternative (38). In humans, mental stress evokes an increase in forearm blood flow that is blocked by atropine (3, 5, 39), and while a local cholinergic mechanism cannot be excluded, nitric oxide and adrenaline rather than a neurally released cholinergic mediator are proposed to underlie this observation (22). Cholinergic blockade also abolishes the exercise-induced increase in tooth pulp blood flow (1) and middle cerebral artery blood velocity (45), but the extent to which these circulations contribute to the SVR changes observed is likely minimal. A rise in parasympathetic activity increases mesenteric artery blood flow via a cholinergic mechanism (50), and cholinergic blockade increases mesenteric vascular resistance during head-up tilt (35). Thus, cholinergic blockade may abolish the initial drop in SVR and augments the pressor response at the onset of isometric handgrip exercise by an effect on circulations other than skeletal muscle with the mesentery likely involved. However, no regional flow measurements (e.g., active or inactive skeletal muscles, skin, or the mesentery) were included in this study but warrants future consideration.

Since subjects undertook dynamic exercise with a large muscle mass and performed isometric exercise with a small muscle mass, additional studies are required to elucidate the independent effects of muscle mass vs. exercise mode (dynamic vs. isometric) on the contribution of a cholinergic mechanism to the circulatory responses at exercise onset. Although, separate groups of subjects performed isometric handgrip (protocol 1) and leg cycling (protocol 2), within each exercise modality, both a control (no drug) and a cholinergic blockade trial was incorporated, thus permitting us to establish the cholinergic contribution to the initial circulatory response evoked within each paradigm. Furthermore, pharmacological antagonism of nitric oxide was not undertaken either with or without cholinergic blockade; thus, we are unable to determine whether the cholinergic effects we observed were operating via an interaction with nitric oxide (8, 24, 47). Administration of the exogenous nitric oxide synthase inhibitor L-NMMA to young healthy individuals evokes a greater pressor response (by ∼300%) with atropine (28), suggesting that impaired cholinergic vasodilation precipitates a heightened BP response to exercise. Concomitant reductions in nitric oxide bioavailability and cholinergic signaling as a consequence of endogenous or exogenous factors (e.g., anticholinergic medication use) could exaggerate the BP response to exercise, which may be of clinical significance as an augmented BP response to exercise is associated with cardiovascular risk (e.g., stroke and myocardial ischemia) (19, 33).

**Perspectives and Significance**

The role of cholinergic vasodilatation in modulating blood pressure and SVR at the onset of exercise in humans is controversial. We observed that cholinergic blockade abolished the drop in SVR and, thereby, augmented the pressor response to the onset of isometric handgrip exercise. In contrast, cholinergic blockade had a nonsignificant effect on the circulatory responses to the onset of leg-cycling exercise. In summary, a cholinergic mechanism is important for arterial blood pressure and SVR responses at the immediate onset of isometric exercise in humans.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

L.C.V. was involved in the analysis and interpretation of data, and revising the article critically for important intellectual content. P.J.F. and N.H.S. were involved in conception and design of the experiments, collection and interpretation of data, and revising the article critically for important intellectual content. J.P.F. was involved with the conception and design of the experiments, the collection, analysis and interpretation of data and drafting the first version of the article.

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BLOOD PRESSURE RESPONSES TO THE ONSET OF EXERCISE