Exercise-induced attenuation of alpha-adrenoceptor mediated vasoconstriction in humans: evidence from phase-contrast MRI

Jim Hansen\textsuperscript{a,b}, Dany Sayad\textsuperscript{b}, Gail D. Thomas\textsuperscript{b}, Geoffrey D. Clarke\textsuperscript{b}, Ronald M. Peshock\textsuperscript{b}, Ronald G. Victor\textsuperscript{b}

\textsuperscript{a}Copenhagen Muscle Research Center, Rigshospitalet, 20 Tagensvej, DK-2200 Copenhagen, Denmark
\textsuperscript{b}Division of Cardiology, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235, USA

Received 27 March 1998; accepted 26 June 1998

Abstract

Objective: We recently provided evidence for contraction-induced attenuation of reflex sympathetic vasoconstriction in human skeletal muscle microcirculation. We now asked whether contraction-induced modulation of alpha-adrenoceptor mediated vasoconstriction in the human forearm (a) is evident in a large artery supplying the contracting skeletal muscle and (b) implicates a post-junctional site of action.

Methods and Results: To address these questions in humans, we used phase-contrast magnetic resonance imaging to measure blood flow velocity and cross-sectional area of the brachial artery during brachial-artery infusion of the alpha-adrenoceptor agonist norepinephrine (NE) (1.1 g/min for 5 min) at rest and during mild ipsilateral rhythmic handgrip (20% of maximum). At rest, brachial artery conductance decreased progressively during the entire 5 min period of infusion (baseline to first half to second half of infusion: 0.421 ± 0.157 to 0.255 ± 0.127 to 0.330 ± 0.097 ml/min/mmHg, \(P < 0.05\)). When NE was superimposed on handgrip, conductance at first decreased sharply (1.205 ± 0.127 to 0.330 ± 0.097 ml/min/mmHg, \(P < 0.05\)). However, during the second half of the infusion, conductance did not decrease further but rather returned progressively toward baseline (0.476 ± 0.199 ml/min/mmHg at the end of the exercise, \(P < 0.05\) vs. NE alone).

Conclusion: These data provide new evidence in humans that alpha-adrenoceptor mediated vasoconstriction is sensitive to modulation by skeletal muscle contraction. Such modulation is evident at the level of a large conduit artery and it involves a post-junctional mechanism of action. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Regional blood flow; Vasoconstriction/dilation; Adrenoceptor agonists; NMR; Exercise

1. Introduction

Many of the cardiovascular adjustments during exercise are produced by local metabolic products of contraction acting on thin fiber afferents arising in the contracting skeletal muscles [1–3]. This reflex mechanism, termed the muscle metaboreflex, triggers parallel sympathetic neural activation in both exercising and non-exercising human skeletal muscle [4,5]. In resting muscle, sympathetic activation causes vasoconstriction, thereby redistributing cardiac output to the exercising muscles [6,7]. However, the functional consequence of sympathetic activation in the exercising muscle has been a subject of considerable debate.

Previous studies in both animals and humans have provided conflicting evidence as to whether sympathetic vasoconstriction in contracting muscle is (a) well preserved, thereby partially offsetting metabolic vasodilation to maintain blood pressure, or (b) largely negated by metabolic vasodilation thereby optimizing muscle perfusion (for review see [8,9]). One explanation for these apparent contradictions is that the functional consequences of sympathetic discharge to contracting skeletal muscle likely depend on physiologic factors (such as the mode and intensity of muscle contraction, fiber type composition of the muscle and the nature and intensity of the sympathetic stimulus), while the quantification of these consequences depend on technical factors (such as the experimental

---

*Corresponding author. Tel.: +45-35-457-610; fax: +45-35-457-634; e-mail: j.hansen@post2.tele.dk

0008-6363/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0008-6363(98)00226-0

Time for primary review 26 days.
model and the methods used to measure and quantify vasomotor responses). Regardless of these considerations, concept (b) above, initially termed functional sympatholysis [10], was recently shown to be operative in the microcirculation of rhythmically contracting forearm skeletal muscle of conscious humans [11]. Using near infrared spectroscopy which measures oxygen availability at the level of the skeletal muscle microcirculation, we demonstrated that reflex sympathetic activation, which consistently decreased oxygenation in resting forearm muscles, had no effect on microcirculatory oxygenation when the muscles were exercised.

Much less is known about vasomotor responses in more proximal segments of the vasculature supplying contracting human skeletal muscle. Animal and microcirculatory studies have advanced the concept that muscle contraction gives rise to an ‘ascending’ vasodilation, where a vasodilator stimulus primarily occurring in the microcirculation is conducted to more proximal vascular segments [12–16]. The ability of such conducted vasodilation to attenuate sympathetic vasoconstriction in upstream arterioles has been demonstrated in hamster cremaster muscle [17]. In humans, the brachial artery was recently shown to dilate during ipsilateral handgrip exercise [18] and it has become clear that large ‘conduit’ arteries constrict in response to physiologic stimuli to reflex sympathetic activation [19,20], but the interaction between such opposing large-artery vasomotor forces during muscle contraction has not been studied.

In the present study we asked whether contraction-induced modulation of norepinephrine-induced vasoconstriction in the human forearm is (a) evident in a large artery supplying the contracting skeletal muscle and (b) evident in response to exogenously administered norepinephrine, thereby indicating a post-junctional mechanism of action. To address these issues, we used phase-contrast magnetic resonance imaging (MRI) to measure the cross-sectional area of, and blood flow in, the brachial artery during brachial artery infusion of norepinephrine both at rest and during rhythmic ipsilateral forearm muscle contraction.

2. Methods

The study protocols were approved by the Institutional Review Board at University of Texas Southwestern Medical Center, and written informed consent was obtained from each subject prior to the study. The investigation conforms with the principles outlined in the Declaration of Helsinki. We studied 5 healthy volunteer subjects, all males 23 to 27 years of age (mean age of 26). Subjects were not taking any medications, were non-smokers and had been instructed to abstain from caffeine on the day of the study. Subjects were studied in the supine position placed in a whole body NMR scanner. Prior to the study a 5.0 french double lumen polyurethane catheter (Cook, Bloomington, IN) was placed retrograde in the brachial artery under local anesthesia. One port was connected to a Statham pressure transducer (Gould, Oxnard, CA) for direct measurement of arterial pressure, the other port was used for drug infusion. A recovery period of a minimum of 30 min was allowed between catheter placement and start of the study. Handgrip exercise was performed with a custom-made Plexiglas handgrip dynamometer connected to a force transducer (Interface, MFG, Scottsdale, AZ). Force output from the handgrip device was recorded on paper and displayed on an LED display to provide the subject with visual feedback. Prior to the experiment, each subject’s maximal voluntary contraction (MVC) was determined as the best of 4–5 trials with verbal encouragement to improve at each trial. The exercise protocols consisted of intermittent isometric exercise where subjects matched force production to a visual target, to the rhythm of a metronome (40 beats/min) with a 50% duty cycle. Blood pressure and handgrip force were recorded continuously on an electrostatic chart recorder.

2.2. Magnetic resonance imaging

Phase contrast magnetic resonance imaging to measure vessel area and blood flow velocity [21,22] in the brachial artery was performed with a 1.5 Tesla magnetic resonance imaging system (Vistar HPQ, Picker International, Highland Heights, OH), which has a high performance gradient system capable of 15 mT/m. After placement of the arterial catheter and other physiologic monitoring equipment, a 20×15 cm saddle surface coil was placed over the region of the brachial artery just proximal to the antecubital fossa. The position of the surface coil and the handgrip device was stabilized with the use of deflatable vacuum pillows to secure optimal stability of the arm during exercise. Axial field echo scout images was used to locate the position of the catheter in the brachial artery. The plane of measurement was either at or just distal to the catheter. The tip of the catheter (and thus drug delivery) was always proximal to the plane of imaging, even if this included the catheter. Quantitative flow measurements were made using a phase contrast field echo sequence with interleaved reference and velocity sensitized acquisitions with a velocity encoding parameter of 134 cm/s. Acquisition parameters were: TR:20 ms, TE:9 ms, Flip angle: 40, Field of View: 17 cm, Matrix 241 (phase encoding)×256 (read out), slice thickness: 8 mm, Voxel size: 0.71×0.66×8.00 mm. In order to minimize the acquisition time, centrally ordered k-space segmentation [23,24] was used which allowed 24 phase-encoding views to be acquired for each...
heartbeat. An NSA (number of signals averaged) of one was used, however oversampling in the phase encode direction was implemented (407 views sampled) to improve signal-to-noise ratio while reducing wrap around. Data acquisition was triggered using prospective cardiac gating so that the entire scan required $407/24 = 17$ heartbeats for completion.

Blood flow measurement by velocity encoded MRI has been validated against independent reference methods in phantoms [25], animal models [26,27] and patients [28]. These studies have involved vessels as large as the aorta and as small as the coronary arteries with excellent agreement with invasive measures both at rest and during pharmacologically-induced increases in blood flow. Work by Li et al. [25] and Enzman et al. [29] demonstrates that the agreement is excellent for pulsatile flow over a wide range of vessel diameters, including vessels the size of the brachial artery.

The raw data were transferred to a DEC Alpha workstation where processing was performed using software written in AVS (Advanced Visualization System). Phase maps were reconstructed, and following background phase correction a phase difference map was generated. Data were then transferred over the network to PC workstations.

![Fig. 1](https://example.com/f1a.png)  
(a) Magnitude image (left) and corresponding velocity map (right) acquired for measurement of brachial artery dimensions and blood flow velocity in one subject. Brachial artery and veins are marked. (b,c) Magnified view of the region of interest in the same image, with the outline of the brachial artery (white circle) superimposed on the magnitude image (b) and the velocity map (c) for data analysis.
where a locally developed Windows tool was used to measure vessel cross-sectional area and mean velocity. If the plane of imaging was at the level of the catheter, the area of the catheter was not included in the region of interest used for the measurement of vessel cross-sectional area and mean velocity. Flow was calculated as follows:

\[
\text{Flow (ml/min)} = \text{mean velocity (cm/s)} \times \text{cross-sectional area (cm}^2) \times 60.
\]

Mean arterial pressure was calculated from the arterial pressure tracings as diastolic pressure + 1/3 pulse pressure. Brachial artery conductance was calculated based on volumetric flow: brachial artery conductance (ml/min/mmHg) = flow(ml/min)/mean arterial pressure (mmHg).

Vasomotor responses are expressed as vascular conductance, which has been proposed as the most accurate reflection of the change in blood flow and vascular tone over a wide range of blood flow values, such as during transitions from rest to exercise [30–32].

Using this approach we determined in pilot studies that images without motion artifact could be obtained during rhythmic handgrip exercise at intensities up to 25%–30% of maximal voluntary contraction. At higher intensities, a proportion of the images displayed motion artifacts, which were easily identified based on the ability to define the vessel margin on the magnified image.

Exemplary images and corresponding velocity-encoded flow maps are shown in Fig. 1. The intra-subject coefficient of variation on measurements of volumetric flow at rest in one subject was 13%, which in part reflect the expected physiologic variability. Group means for resting values (measured at the beginning of each of the 3 protocols) were reproducible (mean ± SE, n = 5): brachial artery blood flow: 42.7 ± 14.2, 51.3 ± 12.9 and 55.3 ± 12.5 ml/min. These values for volumetric flow at rest are in good agreement with those obtained by other methods [33–35].

2.3. Experimental protocol

Three protocols were performed in the sequence depicted in Fig. 2. The interval between each scan was 1 min. At least 15 min of recovery was allowed between each protocol.

2.3.1. Protocol (1): effects of intraarterial norepinephrine at rest

Measurements were obtained at rest, during 5 min of an intraarterial infusion of NE (1.1 μg/min dissolved in 5% dextrose, infusion rate 2 ml/min), and following the offset of infusion.

2.3.2. Protocol (2): effects of handgrip exercise

Measurements were obtained at rest, during 5 min of rhythmic handgrip at 20% of MVC, and during recovery.

2.3.3. Protocol (3): effects of intraarterial norepinephrine during handgrip exercise

Measurements were obtained at rest, during the first minute of handgrip exercise alone, during 4 min of NE infusion in combination with handgrip, during 1 min of continued NE infusion immediately following exercise, and after the end of infusion.

2.4. Statistical analysis

Changes from control values were assessed by analysis of variance for repeated measures. In case of significant effect of the intervention, Dunnett’s post hoc test was used to identify the data points that were different from control. Responses to norepinephrine at rest and during handgrip were evaluated using two-factor analysis of variance for repeated measures. Single comparisons were performed using a t-test for paired comparisons. A P value less than 0.05 was considered significant. All data are expressed as mean values ± standard error.

3. Results

3.1. Alpha-adrenoceptor stimulation markedly decreases brachial artery area and blood flow velocity at rest

With the arm at rest, brachial artery infusion of norepinephrine produced progressive decreases in artery cross-sectional area throughout the 5 min infusion period (from 0.099 ± 0.006 to 0.017 ± 0.007 cm², P < 0.05) and brachial area remained decreased during recovery (Figs. 3 and 4). Norepinephrine infusion virtually eliminated brachial artery blood flow: blood flow velocity decreased (from 7.0 ± 2.1 to 0.0 ± 1.3 cm/s, P < 0.05), volumetric flow
Fig. 3. Original magnitude images from one subject showing the left brachial artery (arrow) in response to intra-arterial infusion of norepinephrine at rest (left panels, a–c) and during ipsilateral rhythmic handgrip exercise at 20% of maximum (right panels, d–f). The images were acquired under control conditions (top), during the first half of norepinephrine infusion (center) and during the second half of norepinephrine infusion (bottom). The dark area in the artery is the indwelling catheter. In this subject, norepinephrine infusion at rest produced a progressive constriction of the brachial artery. Handgrip alone produced a small dilation of the brachial artery. Norepinephrine infusion during handgrip produced an initial vasoconstriction which during the last half of the infusion returned towards baseline values, resulting in a marked attenuation of the vasoconstrictor response by the end of the exercise.
decreased (from 42.7±14.2 to 1.2±1.5 ml/min, \( P<0.05 \))
and a vascular conductance decreased (from 0.421±0.157 to
0.012±0.014 ml/min/mmHg, \( P<0.05 \)) (Fig. 4). Mean
arterial pressure was unchanged (106±4 vs. to 108±4
mmHg, n.s.).

3.2. Alpha-adrenoceptor mediated vasoconstriction of
the brachial artery is attenuated during rhythmic
handgrip

Rhythmic handgrip at 20% MVC alone produced sig-
nificant increases in mean arterial pressure (from 105±3 to
116±4 mmHg, \( P<0.05 \)), brachial artery area (from
0.100±0.005 to 0.123±0.009 cm\(^2\), \( P<0.05 \)), brachial
blood velocity (from 8.3±1.8 to 19.2±2.2 cm/s, \( P<0.05 \)),
brachial blood flow (51.3±12.9 to 141.6±18.1 ml/min,
\( P<0.05 \)) and brachial artery conductance (0.503±0.141 to
1.205±0.127 ml/min/mmHg, \( P<0.05 \)) (Fig. 4).

When norepinephrine was infused during handgrip, the
norepinephrine-induced decreases in brachial artery area,
blood flow and conductance were partially attenuated and
decreases in blood flow velocity were virtually abolished
(Figs. 3 and 4). During the first half of norepinephrine
infusion superimposed on handgrip, brachial artery diam-
eter, blood flow and vascular conductance decreased to
values which were similar to those produced by norepi-
nephrine infusion at rest (Figs. 3 and 4). During the second
half of the infusion, however, these responses returned
progressively towards baseline values, resulting in an
attenuated alpha-adrenoceptor mediated constriction by the
end of the exercise (brachial area: 0.050±0.012 cm\(^2\),
blood flow: 61.6±28.4 ml/min, brachial artery conduct-
ance: 0.476±0.199 ml/min/mmHg, all \( P<0.05 \) vs. norepi-
nephrine at rest) (Figs. 3 and 4) and immediately following
cessation of exercise. Blood flow velocity decreased only
transiently with the onset of the norepinephrine infusion,
then returned to values indistinguishable from those seen
with handgrip alone (18.2±6.2 cm/s at end exercise, n.s.)
vs. 19.2±2.2 cm/s at the end of handgrip alone). In the recovery period blood flow velocity was increased compared to recovery from handgrip alone (Fig. 4). Mean arterial pressure increased similarly during handgrip plus norepinephrine as during handgrip alone (ΔMAP: 12±6 vs. 14±5 mmHg, handgrip alone vs. handgrip plus NE, n.s.).

4. Discussion

Using direct measurements of arterial dimensions we provide new evidence in humans for the concept of contraction-induced attenuation of sympathetic vasoconstriction. The data extend this concept in two ways. First, by focusing on the brachial artery, we demonstrated that such attenuation is evident, not only in the microcirculation as previously described [11], but even in a large conduit artery. Second, by infusing norepinephrine directly into the arterial supply of human forearm muscle, we demonstrated that muscle contraction can attenuate alpha-adrenoceptor mediated vasoconstriction by interfering with the postjunctional effects of norepinephrine.

The present MRI experiments provide a third line of evidence from our laboratory for modulation of sympathetic vasoconstriction by muscle contraction, which we previously demonstrated by using Doppler velocimetry to estimate changes in femoral artery blood flow in rat hindlimb muscle in response to sympathetic nerve stimulation and intraarterial infusion of alpha-adrenoceptor agonists [36–38] and by using NIR spectroscopy to measure changes in muscle tissue oxygenation in human forearm muscle in response to reflex sympathetic neural activation [11]. The methods used in those previous studies (Doppler velocimetry and near infrared spectroscopy) primarily reflected vasoconstrictor responses at the level of the microcirculation, whereas the measurements in the present study reflects the vasoconstrictor response not only in the downstream vessels (blood flow velocity) but also at the level of a large conduit artery (brachial artery cross sectional area). When we use MRI measurements of blood flow velocity in the human brachial artery to parallel our prior Doppler measurements of blood flow velocity in rat femoral artery, we find that muscle contraction virtually eliminates norepinephrine-mediated decreases in blood flow velocity in both models. When vascular conductance in the present experiments is calculated from volumetric flow, the attenuation in norepinephrine-induced vasoconstriction during handgrip was partial rather than complete. This is not surprising since we used a dose of norepinephrine which produced near maximal vasoconstriction when infused into the brachial artery of a resting arm. That we could demonstrate any attenuation of this large vasoconstrictor response constitutes new evidence that contraction-induced vasodilation can override alpha-adrenoceptor mediated vasoconstriction, at least in part, at the level of a large artery.

The importance of such an interaction at the large artery level is suggested by a number of recent studies in humans indicating that large arteries actively participate in vasoconstrictor responses to reflex sympathetic activation during the cold pressor test and orthostatic maneuvers [19] or exercise of a contralateral limb [20]. Whereas large-artery vasodilation was not present in the common femoral artery during dynamic knee extensor exercise [39], an increase of about 5% in brachial artery diameter during ipsilateral rhythmic handgrip at 10% MVC was recently demonstrated by Shoeemaker et al using ultrasound [18]. This magnitude of brachial artery dilation corresponds well with the increase of about 20% in brachial artery cross sectional area or the increase of about 10% in brachial artery diameter that we observe during rhythmic handgrip at 20% MVC alone.

During handgrip, the partial attenuation in the norepinephrine-induced decrease in brachial artery area and conductance may have been caused by (a) partial inhibition of alpha-adrenoceptors located in the brachial artery by metabolites whose concentration decreases progressively from the most distal microvessels in the muscle interstitium to the most proximal conduit vessel (i.e., brachial artery) and/or (b) propagated or conducted vasodilation from a primary vasodilator response occurring mainly at the level of the downstream microvessels which secondarily attenuates adrenergic vasoconstriction in the upstream brachial artery. The present study is not designed to distinguish between these different possibilities, which are not mutually exclusive. The precise metabolic consequences of contraction mediating functional sympatholysis remain unknown but may include tissue acidosis, hypoxia and release of other vasodilator substances such as potassium, adenosine and nitric oxide [36–40,46–48]. Several mechanisms, which could be operative in these human experiments, have been suggested to explain propagated or conducted vasodilation in large arteries including (1) flow-induced release of endothelial cell nitric oxide [47–49], and (2) release of acetylcholine from the neuromuscular junction causing spreading hyperpolarization in the vessel wall [14,16,17].

The temporal pattern of the vasomotor response to norepinephrine during handgrip suggests that the metabolic events mediating contraction-induced attenuation in alpha-adrenoceptor mediated vasoconstriction differ both from those mediating functional hyperemia and from those mediating contraction-induced large artery dilation. The key observation is that the vasoconstrictor response to norepinephrine initially was well preserved during exercise, with the attenuated vasoconstriction occurring only during the latter half of the exercise period. That norepinephrine evoked marked vasoconstriction at the onset of exercise, when functional hyperemia and large-artery dilation had already peaked (Fig. 3), demonstrates that
functional sympatholysis is not explained merely by exercise-induced hyperemia overwhelming alpha-adrenoceptor mediated vasoconstriction. Rather, we suggest that functional sympatholysis is mediated by some as yet unidentified ischemic metabolites released when a mismatch exists between muscle blood flow and metabolic demand. In the present experiments, such a mismatch was produced during the first half of the exercise when norepinephrine returned blood flow to pre-exercise values.

Because the contraction-induced attenuation of vasoconstriction was demonstrated with exogenous norepinephrine, the present data implicate a post-junctional mechanism of functional sympatholysis. While this is consistent with previous studies in anesthetized rats, the present findings by no means exclude any contribution from pre-junctional mechanisms. In humans, however, norepinephrine spillover from exercising skeletal muscle is clearly not inhibited during contraction; during unilateral quadriceps exercise, norepinephrine spillover is even greater from the exercising than from the non-exercising muscle [4].

In conclusion, the present study provides new evidence in humans that alpha-adrenoceptor mediated vasoconstriction is sensitive to modulation by skeletal muscle contraction. This modulation is evident in a large artery and it involves a post-junctional mechanism of action. The underlying events are unknown, but seem to relate to a mismatch between blood flow and muscle metabolism as seen with ischemic or intense forms of exercise. If operative during sympathetic neural activation with intense whole-body exercise, modulation of sympathetic vasoconstriction in large arteries provides a potentially important mechanism for regulation of regional vascular conductance.

Acknowledgements

Dr. Hansen is the recipient of a Fogarty International Research Fellowship (NIH-1-F05-TW04949-01) and were supported by grants from the Danish Heart Foundation, the Simonsen and Weel Foundation and the Danish Research Academy. Dr. Thomas was supported by a National Institutes of Health training grant (T32-HL-07360) and a Muscular Dystrophy Association Postdoctoral Fellowship. Dr. Victor is an Established Investigator of the American Heart Association. This research was supported by a grant to Dr. Victor from the National Institutes of Health (PO1-HL-06296).

References


