Dissociation of muscle sympathetic nerve activity and leg vascular resistance in humans

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Shoemaker, J. Kevin, Michael D. Herr, and Lawrence I. Sinoway. Dissociation of muscle sympathetic nerve activity and leg vascular resistance in humans. Am J Physiol Heart Circ Physiol 279: H1215–H1219, 2000.—We examined the hypothesis that the increase in inactive leg vascular resistance during forearm metaboreflex activation is dissociated from muscle sympathetic nerve activity (MSNA). MSNA (microneurography), femoral artery mean blood velocity (FAMBV, Doppler), mean arterial pressure (MAP), and heart rate (HR) were assessed during fatiguing static handgrip exercise (SHG, 2 min) followed by posthandgrip ischemia (PHI, 2 min). Whereas both MAP and MSNA increase during SHG, the transition from SHG to PHI is characterized by a transient reduction in MAP but sustained elevation in MSNA, facilitating separation of these factors in vivo. Femoral artery vascular resistance (FAVR) was calculated (MAP/MBV). MSNA increased by 59 ± 20% above baseline during SHG (P < 0.05) and was 58 ± 18 and 78 ± 18% above baseline at 10 and 20 s of PHI, respectively (P < 0.05 vs. baseline). Compared with baseline, FAVR increased 51 ± 22% during SHG (P < 0.0001) but returned to baseline levels during the first 30 s of PHI, reflecting the changes in MAP (P < 0.005) and not MSNA. It was concluded that control of leg muscle vascular resistance is sensitive to changes in arterial pressure and can be dissociated from sympathetic factors.

metaboreflex; Doppler ultrasound; isometric handgrip exercise

BLOOD PRESSURE IS MAINTAINED during postural stress and is increased during exercise. The large increase in peroneal nerve muscle sympathetic nerve activity (MSNA) during exercise (16, 23, 24) and upright posture (3) has led to the assumption that centrally mediated neurogenic vasoconstriction in skeletal muscle is a critical component of increasing vascular resistance in these conditions.

Reports that leg vascular resistance and MSNA are highly correlated during fatiguing exercise (23, 24) support the concept that, in contrast to arms (22, 27, 31), sympathetic engagement is a major, direct vasoconstrictor mechanism in the lower limb. However, muscle vasculature may also be subject to pressure-dependent mechanisms (14, 21). MSNA and blood pressure increase with similar time courses under conditions of fatiguing exercise. In situ preparations indicate that muscle vasculature is highly responsive to changes in pressure with smaller contributions from sympathetic activation (14, 21). In humans, changes in leg vascular tone that were independent of sympathetic innervation have been observed during postural stress (10), supporting the hypothesis of myogenic vascular control.

In the current study we addressed the question of whether the increase in leg vascular resistance during a metaboreflex-induced sympathoexcitation is related to MSNA or blood pressure. We used Doppler ultrasound measures of femoral artery blood flow velocity (FAMBV), together with microneurographic measures of MSNA, to examine the dynamic short-term responses of human limb vascular resistance (LVR) during rapid changes in systemic blood pressure that occur at the transition from static handgrip (SHG) to posthandgrip ischemia (PHI) and from PHI to recovery phases. With these procedures we tested the hypothesis that the increase in inactive leg vascular resistance during forearm metaboreflex activation is dissociated from MSNA and is more closely related to changes in blood pressure.

METHODS

A total of 9 healthy subjects (8 males and 1 female) volunteered for the study. The subjects were 28.4 ± 9.3 yr (mean ± SD) (range = 21–48 yr) with average heights and weights of 176 ± 5 cm and 77 ± 8 kg, respectively. Each subject provided signed consent to the experimental procedures that had been approved by the Institutional Review Board at the Milton S. Hershey Medical Center.

Experimental Design

The study was designed to obtain short-term vascular responses in a resting muscle vascular bed during rapid changes in systemic blood pressure under conditions of elevated MSNA. In preliminary studies we have observed such changes in systemic pressure during the transition from a

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period of fatiguing SHG exercise to a period of PHI. We reasoned that a pressure-dependent vascular response would result in a time course of LVR that matches that of arterial blood pressure rather than MSNA.

Each subject lay supine with the right leg lowered 30°. The leg was lowered below horizontal because sympathetic constriction is reported to be enhanced in vessels with myogenic tone (10, 18). After a 5-min period of baseline measures, the subject performed 2 min of SHG exercise with their nondominant forearm at 40% of their predetermined maximal voluntary contraction force. Approximately 3 s prior to the end of the handgrip period, a blood pressure cuff placed around their upper arm was inflated to ~200 mmHg to produce PHI for 2 min. This ischemia was used to trap muscle metabolites and continue stimulation of sympathoexcitatory muscle afferents (1). A 2-min recovery period followed the forearm ischemia.

Data Acquisition

Continuous measures of blood pressure, blood flow, and MSNA were made in order to observe transient changes in systemic and local vascular hemodynamics. Mean arterial pressure (MAP) was measured using the photoplethysmographic finger cuff method (Finapres; Ohmeda, Madison, WI). The hand from which MAP recordings were made was always held at the level of the heart. The mean blood flow velocity (MBV) in the femoral artery of the right leg was examined continuously using a 4-MHz pulsed-wave Doppler ultrasound probe (model 500M; Multigoni, Yonkers, NY) that insonated the vessel at 45°.

To further examine the mechanisms regulating lower limb circulation in humans, we also measured MBV in the tibialis posterior artery of the right leg simultaneously with the other variables. For these measures an 8-MHz pulsed-wave Doppler ultrasound probe was fixed to the skin over the tibialis posterior artery. When measured at the level of the medial malleolus, tibialis posterior flow is predominantly reflective of vascular responses in the foot tissues (20) with a proportionately high level of acral skin perfusion. Thus we reasoned that measures of vascular resistance in this vessel would allow us to separate the effects of changes in skin vascular tone from those of the total leg measured at the common femoral artery. The data were collected according to the description outlined above for the femoral artery.

MSNA was recorded from the peroneal nerve of the right leg using the microneurographic technique (28) as described previously for our laboratory (25). A 200-μm-diameter, 35-mm-long tungsten microelectrode that was tapered to an uninsulated 1- to 5-μm tip was inserted transcutaneously into the peroneal nerve just posterior to the fibular head. A reference electrode was positioned subcutaneously 1–3 cm from the recording site. Neuronal activity was amplified 1,000 times by a preamplifier and 50–100 times by a variable gain isolated amplifier. The signal was band-pass filtered with a bandwidth of 700–2,000 Hz and then was rectified and integrated to obtain a mean voltage neurogram. An MSNA site was confirmed by manually manipulating the microelectrode until the characteristic pulse-synchronous burst pattern was observed that increased in frequency during a voluntary apnea but did not change in response to arousal or produce skin paresthesias (5). The analog signals of all data were sampled at 100 Hz by a dedicated computerized data acquisition system (MacLab; ADInstruments, Castle Hill, NSW, Australia) and stored on hard disk.

Data Analysis

Signal averaging of MSNA. The traditional methods of visually identifying and quantifying MSNA are limited in their ability to derive quantitative measures of sympathetic outflow with high temporal resolution. However, signal averaging of the MSNA data (2, 12) uses signal summation to progressively increase signal events while decreasing the amplitude of random background noise and uncorrelated bursts. The improved signal-to-noise ratio produced by signal averaging permits the detection of small, correlated MSNA bursts that would be overlooked by traditional visual analysis. Signal averaging also eliminates the subjectivity involved in deciding what is and is not an MSNA burst. The signal averaging approach allowed determination of total MSNA energy produced during 10-s segments to correspond with the hemodynamic data. For each subject, the integrated MSNA response was segmented into 10-s periods during the 2 min of each phase. The MSNA signal for each cardiac cycle was processed into an averaged MSNA response for each 10-s period. An average of 12 such segments provided the mean baseline MSNA.

Mean blood velocity. The quadrature output of the ultrasound spectrum of velocities was demodulated to provide a continuous analog signal of the instantaneous MBV. The average MBV in the femoral (FAMBV) and tibialis posterior (TPMBV) arteries was quantified by integrating the area under the mean velocity curve for each cardiac cycle over the course of the experimental trial. The average beat-by-beat MBV was then determined by averaging over 60 s at rest and over 10-s intervals at the end of the SHG, PHI, and recovery phases. Importantly, 10-s averages of MAP, heart rate (HR), and MBV were also obtained during the first minute of the PHI and recovery phases. Averaging the MBV data over specific time periods was used to account for the effect that changes in HR have on total limb flow. In a separate control study of six volunteers, we measured the femoral artery diameter during the SHG and PHI ischemia protocol with an echo Doppler imaging system (Toshiba model SSH-140A) and a 7.5-MHz transducer placed over the femoral artery ~2–3 cm below the inguinal ligament. Compared with baseline (9.5 ± 0.5 mm; means ± SE), femoral artery diameter was not different at the end of SHG (9.8 ± 0.5 mm), 30 s into PHI (9.6 ± 0.5 mm), at the end of PHI (9.5 ± 0.4 mm), or after 2 min of recovery (9.6 ± 0.4 mm). Therefore, it was assumed that dimensions of the femoral and tibialis posterior vessels remained constant throughout the protocol in the current study so that MBV reflects leg blood flow. Indices of vascular resistance in the femoral (FAVR) and tibialis posterior (TPVR) vessels were calculated as the quotient of MAP and the respective arterial flow velocity.

Statistics. All variables were examined using a repeated measures one-way ANOVA procedure with a mixed-effects linear model (SAS) and Tukey's post hoc analysis. To assess more directly the relationship between MAP and MSNA on FAVR and TPVR, the percent changes in these variables were calculated and compared using a repeated measures one-way ANOVA. In addition, multiple regression analysis was used to assess the contributions of %ΔMAP and %ΔMSNA on the %ΔFAVR. A random coefficients model was used to account for longitudinal data (15) in the multiple regression analysis. The level of probability was set at P < 0.05. All data are described as means ± SE.
RESULTS

Effect of Lowering the Leg

Tibialis posterior MBV was 152 ± 48 cm/min with the leg level to the heart and 120 ± 27 cm/min with the leg lowered 30° [not significant (NS)]. The unchanged flow in conjunction with the −34 mmHg increase in hydrostatic pressure with this maneuver resulted in a 62 ± 13% increase in TPVR (P < 0.005). It is noteworthy that lowering the leg did not alter TPVR in the contralateral leg. FAMBV was not changed from baseline (time = 0) (P < 0.05).

Effect of SHG and PHI

Figure 1 shows the absolute responses for each variable during the protocol. MSNA data were successfully collected from seven individuals, whereas the hemodynamic responses were obtained from all 9 volunteers. The ANOVA results indicated a statistically significant main effect of time for HR (P < 0.0001), MAP (P < 0.0001), FAMBV (P < 0.04), TPMBV (P < 0.0001), FAVR (P < 0.0001), and MSNA (P < 0.009) during the protocol.

Compared with baseline, the signal-averaged MSNA integrals increased from 2.9 ± 0.5 units at baseline to 4.13 ± 0.54 units at end SHG (P < 0.02). After SHG, the MSNA continued to increase transiently during the first 30 s of PHI and thereafter was maintained above baseline levels for the remainder of PHI and for the first 20 s of recovery. MAP, averaged over 10-s periods, was greater than rest at the measured time points of 1.5 and 2 min of SHG (P < 0.0001). In contrast to the MSNA response, there was a rapid reduction in MAP during the initial 10 s of PHI (P < 0.0001) when compared with the end SHG MAP. FAMBV was unchanged during each phase of the protocol but tended to increase during the transitions from SHG to PHI and from PHI to recovery (P < 0.09 at 10 s of PHI). FAVR increased above baseline at 1.5 and 2 min of SHG (P < 0.0001) but returned towards baseline levels during the first 30 s of PHI. FAVR was increased above baseline levels at 40 s of PHI (P < 0.05). Although a significant main effect of time was observed for TPVR, post hoc analysis did not reveal statistically significant point-wise differences during the protocol.

The percent change in each measure is plotted in Fig. 2 to more directly compare the temporal relationship between the variables. Significant main effects of time during the protocol were observed for %ΔMAP (P < 0.001), %ΔFAVR (P < 0.0001), %ΔTPVR (P < 0.009), and %ΔMSNA (P < 0.008). Compared with baseline, the signal-averaged MSNA increased by 59 ± 21% at the end of SHG (P < 0.02). The %ΔMSNA continued to increase transiently during the first 30 s of PHI (NS) and remained above baseline levels until 10 s of recovery. Compared with baseline, MAP increased by 35 ± 4% by end SHG (P < 0.0001) but was reduced to a new level by 10 s of PHI that was greater than baseline (20 ± 3%; P < 0.0001) but less than end SHG (P < 0.0001). FAVR increased by 51 ± 22% (P < 0.05) during SHG but returned to baseline levels during 10 and 20 s of PHI (11 ± 18 and 4 ± 11%, respectively). However, FAVR increased temporarily above baseline levels again at 40 s of PHI (P < 0.05). Again, TPVR was not changed during the entire protocol.

Multiple regression analysis was performed in two stages. First, the entire data set for each subject was used in the random coefficient model. Second, a piecewise multiple regression was performed by separating the data according to the protocol phase. Because of the small subject number, this latter approach must be viewed conservatively. In the first approach the %ΔFAVR was significantly related to %ΔMAP (P < 0.005, slope = 1.7, r = 0.61) but not %ΔMSNA (P > 0.89, slope = 0.007, r = 0.28). In the second approach the contribution of %ΔMAP to %ΔFAVR during SHG was slope = 1.1 and r = 0.26 (P < 0.04), and the contribution of %ΔMSNA to %ΔFAVR was slope = 0.6 and r = 0.29 (P < 0.06). However, during PHI and recovery the %ΔFAVR was significantly related to
Previously, elevated MSNA directed to leg muscle vasculature has been highly correlated with the increase in calf vascular resistance during fatiguing exercise (23, 24). However, these studies (23, 24) focused on the MSNA and calf vascular resistance responses during the SHG period and did not account for concurrent changes in MAP. Moreover, transient changes in limb perfusion were not investigated. As such, the dynamics of human leg vascular resistance under conditions of changing transmural pressure have not been examined. In addition to potential sympathetic constrictor mechanisms, a potent pressure-sensitive constrictor response in muscle vasculature has been demonstrated (7, 10, 13, 14).

The dissociation of leg vascular resistance and MSNA in the current study was observed during PHI, suggesting that a portion of the leg vasoconstriction during SHG was related to the concurrent change in intraluminal pressure. The separation of the MSNA and FAVR responses was most apparent during the dynamic phase at the onset of PHI. It may be that when both MSNA and MAP act in the same direction, the effect on vascular resistance is interactive (17, 18), whereas when they act in different directions, the overall effect may be based on the magnitude of each separate input.

It was also clear that while MAP and MSNA remained elevated during the final minute of PHI, FAVR was reduced to levels that were not statistically different from rest. These steady-state data also argue for a dissociation between leg vascular resistance and MSNA. In addition, this latter observation suggests that a threshold of arterial transmural pressure may exist in human muscle vasculature below which the myogenic constriction is not manifest. This concept has been proposed for adipose tissue (11) but to our knowledge has not been considered for muscle blood vessels.

An interesting observation of the current study was the tendency for FAMBV to increase above baseline levels during the first 20–30 s of PHI despite sustained elevations in MSNA. This phenomenon, observed previously (13, 19, 21), has been termed “superregulation” of flow, to differentiate it from “autoregulation,” a term that defines the maintenance of baseline flows in the presence of changing pressures. It is not known how leg vasculature responds to similar reductions in MAP without previous “conditioning” of the vessels with elevated MSNA. In exposed arterioles, the magnitude (21) and rate (26) of vascular relaxation with pressure reduction is much greater when sympathetic tone is high. Other studies have also demonstrated important interactions between sympathetic outflow and myogenic constriction (4, 6, 17). Therefore, it is possible that the close relationship between MAP and FAVR in the current study was enhanced by the concurrent increases in MSNA.

On the basis of the tissues supplied by the tibialis posterior artery, changes in blood flow in this vessel probably reflect control of skin and foot vascular tone, which demonstrate variable vasoconstrictor responses to the increased skin sympathetic nerve activity during

DISCUSSION

Examination of constrictor mechanisms in human legs has been difficult to perform because of limitations in methods used to measure blood flow in dependent limbs. The use of Doppler ultrasound in the current study provided a noninvasive method with beat-by-beat resolution that facilitated measurement of transient yet marked changes in vascular resistance. Moreover, the signal-averaging approach to analyzing MSNA, used previously in our laboratory (12), together with Doppler measures of flow, allowed temporal alignment of the neural and hemodynamic responses during rapid changes in arterial pressure. In this study, the temporal adjustments in leg vascular resistance were examined both during SHG when MAP and MSNA both increased and during the transition from SHG to PHI when rapid reductions in MAP occurred but MSNA remained elevated. The primary finding of this study was that control of leg muscle vascular resistance is sensitive to changes in arterial pressure and can be dissociated from sympathetic factors. This finding extends the earlier reports by Hassan and Tooke (8) and by Henriksen and Sejersen (10), who reported a significant myogenic constrictor effect in human muscle vasculature during postural changes. This is in contrast to the TPVR, which, although increased by dropping the foot below heart level, was not sensitive to further increases in either MSNA or MAP. Thus the changes in FAVR primarily reflect changes in leg skeletal muscle.

Fig. 2. The percent changes in FAVR, TPVR, MSNA, and MAP are plotted together to facilitate direct comparison of these variables during a period of SHG, PHI, and recovery. Statistics of the responses are described in the text. Statistical notation is not included here, to enhance clarity. The standard error of the mean ranged from 3.3 to 21.6% for FAVR, from 1.3 to 4.2% for MAP, from 11 to 30% for MSNA, and from 7 to 14% for TPVR. Although FAVR, MAP, and MSNA increased together during SHG, the FAVR response tracked MAP during the period of postcontraction ischemia and recovery. TPVR was not changed by the protocol.

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\%\Delta MAP \quad (P < 0.001; \text{ slopes } = 1.7 \text{ for both PHI } (r = 0.46) \text{ and recovery } (r = 0.47)) \text{ but not } \%\Delta MSNA \quad (P > 0.95, \text{ slope } = 0.006, r = 0.14 \text{ for PHI}; \text{ and } P > 0.79, \text{ slope } = -0.02, r = 0.13 \text{ for recovery}).
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SHG (29). However, TPVR was increased above supine levels when the hydrostatic blood pressure was increased by \(\sim 34 \text{ mmHg}\) by lowering the foot, a finding that is consistent with important pressure-dependent responses in these vessels (8, 9, 30). Whether this constrictor response is myogenic in nature or represents local vasoarteriolar neural responses (8, 30) cannot be determined from the current data.

**Summary**

We have obtained time-course information of FAMBV, MSNA, and MAP during static isometric handgrip contraction and during a period of PHI to assess whether leg vascular resistance is related to changes in MSNA or to changes in pressure. The data argue that the control of leg muscle vascular resistance is sensitive to changes in intraluminal arterial pressure and can be dissociated from sympathetic nerve activity.

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