Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery

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Dyson, Kenneth S., J. Kevin Shoemaker, and Richard L. Hughson. Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery. Am J Physiol Heart Circ Physiol 290: H1446–H1453, 2006. First published November 11, 2005; doi:10.1152/ajpheart.00771.2005.—We tested the hypothesis that flow-mediated dilation (FMD) of the brachial artery would be impaired by acute increases in sympathetic nervous system activity (SNA) in models where similar peak shear stress stimulus was achieved by varying the duration of forearm muscle ischemia. Eleven healthy young men were studied under four different conditions, each with its own control: lower body suction (LBS), cold pressor test (CPT), mental arithmetic task (MAT), and activation of muscle chemoreflex (MCR). The duration of ischemia before observation of FMD by ultrasound imaging was 5 min each for control, LBS, and CPT; 3 min for MAT; and 2-min for MCR. Peak shear rate was not different between control and any of the SNA conditions, although total shear in the first minute was reduced in MAT. MCR was the only condition in which brachial artery vasoconstriction was observed before forearm occlusion (4.38 (SD 0.53) mm vs. control 4.60 (SD 0.53) mm, P < 0.05); however, diameter increased to the same absolute value as that of the control, so the percent FMD was greater for MCR [9.85 (SD 2.33) vs. control 5.29 (SD 1.50)%]. Blunting of the FMD response occurred only in the CPT model [1.51 (SD 1.20)%]. During SNA, the increase in plasma cortisol from baseline was significant only for MCR; the increase in plasma norepinephrine was significant for MCR, LBS, and CPT; and the increase in epinephrine was significant only for MCR. These results showed that the four models employed to achieve increases in SNA had different effects on baseline brachial artery diameter and that blunted FMD is not a general response to increased SNA.

endothelium; shear stress; muscle chemoreflex; cold pressor test; mental stress

FLOW-MEDIATED DILATION (FMD) is studied by high-resolution ultrasound imaging of the change in conduit artery vessel dimension in response to a sudden increase in blood flow after release of circulatory occlusion. When compared with that in healthy, young subjects, FMD is impaired in patients with coronary artery disease (27, 40), as well as essential (11, 28) and white-coat (13) hypertension. Reduced FMD reflects impaired endothelial function, which has been found to be an independent risk factor of coronary artery disease (6, 12). Some evidence suggests that FMD might also be impaired in apparently healthy subjects during an acute stress that elevates sympathetic nervous system activity (SNA) (18, 24, 35). However, these findings are not universal (17) and might depend on the nature of the stress and the experimental design. Lind et al. (24) concluded that acute increases in SNA with mental arith-
Quantified by using a Coat-a-Count solid-phase 125I radioimmunoassay (2690 Separations Module, Waters). Serum cortisol was separated (2690 Separations Module, Waters) and washed alumina and released into a 0.1 M perchloric acid solution and electrochemical detection (2465 Electro Chemical Detector, Waters, supernatant was transferred to microtubes and frozen at -80°C. The remaining 3 ml of whole blood was transferred to tubes containing 75 µL of EGTA-glutathione anticoagulant and were centrifuged at 4°C for 10 min at 2,500 g. The plasma supernatant was transferred to microtubes and frozen at -80°C.

Epinephrine and norepinephrine were determined by HPLC with electrochemical detection (2465 Electro Chemical Detector, Waters, Milford, CT) after being extracted from plasma samples by acid-washed alumina and released into a 0.1 M perchloric acid solution and separated (2690 Separations Module, Waters). Serum cortisol was quantified by using a Coat-a-Count solid-phase 125I radioimmunoassay kit (Diagnostic Products, Los Angeles, CA) and gamma counter (Beckman Gamma 5500) according to the catalog No. TKCO2 procedure. Analysis was performed in duplicate.

**Blood Collection and Analysis**

Subjects rested supine with a catheter (20 gauge, BD Insyte, BD Medical Systems, Sandy, UT) inserted in the left antecubital vein. Blood samples (4 ml) were obtained at rest and 1 min after release of the occlusion cuff. Blood was divided into two tubes. One milliliter was transferred to tubes containing no anticoagulant and left to stand for 10 min on ice, rimmed, and centrifuged (Beckman GS-150, Irvine, CA) at 4°C for 10 min at 2,500 g. The serum supernatant was transferred to microtubes and frozen at -80°C. The remaining 3 ml of whole blood was transferred to tubes containing 75 µL of EGTA-glutathione anticoagulant and were centrifuged at 4°C for 10 min at 2,500 g. The plasma supernatant was transferred to microtubes and frozen at -80°C.

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**Experimental Design**

Subjects reported to the laboratory for familiarization with the test protocols, and at least 7 days later they then returned on four separate occasions, each separated by at least 2 days, for the data collection sessions. Tests were conducted at the same time of day to limit possible circadian effects. Each testing session consisted of a control trial, followed by 10 min of supine rest, and then one of four experimental condition trials was administered in random order. Data were collected continuously during 1 min of baseline, the full occlusion period, and 3 min after release of occlusion. The duration of forearm occlusion varied between trials to normalize the peak shear stimulus after occlusion cuff release. In each control trial, occlusion was maintained for 5 min, as it was during lower body suction and the cold pressor test. Preliminary testing revealed that the 3-min occlusion for the mental arithmetic test and the 2-min occlusion for the muscle chemoreflex test achieved similar peak shear on cuff release.

**Acute Sympathetic Activation Protocols**

**Muscle chemoreflex activation.** Before the trial, both calf muscles were placed under circulatory arrest just below the knees by inflating standard blood pressure cuffs to 250 mmHg. The muscle chemoreflex was activated by having the subjects perform rhythmic plantar flexion exercise until enough muscle activity had been completed to cause MAP to remain at least 20 mmHg above baseline during sustained muscle ischemia. The experimental protocol was completed during this postexercise ischemic period.

**Lower body suction.** Subjects were sealed into an airtight, lower body suction box at the level of the iliac crest by a neoprene kayak skirt. Suction was gradually applied with increments of -5 mmHg every 2 min, until a level of -30 mmHg of lower body suction was reached. The trial began 5 min later with suction maintained constant throughout the trial.

**Cold pressor test.** Before the trial, subjects were supine with knees bent over the edge of the table. Both feet were submerged in ice-water slurry (5°C). Forearm cuff inflation was initiated 2 min after submersion, and the feet remained in the slurry for the duration of the trial.

**Mental arithmetic task.** Based on the protocol described by Lind et al. (24), subjects were asked to subtract 13 or 17 from a three digit number called out by an investigator. The subjects were instructed that they would be required to announce the correct answer within 5 s or would be interrupted by a buzzer. In fact, the buzzer was initiated at random intervals and did not discriminate between correct or incorrect answers. The trial began after 1 min of questioning, and questioning continued until the FMD response was observed.

**Data Analysis**

Beat-to-beat brachial artery MBV was obtained as the average between consecutive R waves from the ECG. Brachial artery diameter was measured from the videotaped images at intervals of 30 s during the baseline and 60 s during occlusion periods, at 5 and 10 s after occlusion cuff release, and at 10-s intervals through the first 2 min after occlusion cuff release and every 30 s until the end of the trial. All measurements were taken during the diastolic phase of the cardiac cycle and were reported as the average of four measurements taken at each time point with the electronic calipers of the ultrasound unit. All data sets were coded without indication of subject or test condition, thus preventing any technician bias. Results from each test were compared against data from an independent observer, and any deviations >0.2 mm were resolved. To further minimize the effect of random error in the estimation of peak changes in diameter and to get a continuous estimate of diameter for calculation of forearm blood flow from the product of MBV and cross-sectional area, the entire time series data set of diameter values was fit by an exponential curve as previously described (1). This approach with least squares analysis prevents assignment of peak FMD to what could potentially be a point of measurement error. Shear rate was calculated as 8 × MBV/ diameter. Baseline values were determined from averages over the last 30 s of baseline. Peak forearm blood flow and shear rate were determined from 10-s averages beginning 5 s after occlusion cuff release. Total flow and shear rate were reported as the time-weighted sum of values during the first minute after occlusion cuff release to give an index of potential stimulus before achieving peak FMD.

**Statistical Analysis**

Statistical analysis software (SAS Institute, Cary, IN) was used to examine the effect of each condition relative to its own control by repeated-measures ANOVA with one within-subject factor. A probability of P < 0.05 was accepted as statistically significant, and any differences were subsequently analyzed with the least squares difference post hoc test. Data are presented as means (SD). For the purpose of clarity, the four different control tests have been pooled into a single control value for visual presentation.

**RESULTS**

**Arterial Pressure and Heart Rate**

Significant elevations in systolic and diastolic arterial blood pressure were achieved during the muscle chemoreflex, cold pressor, and mental arithmetic protocols (Table 1). There was no change in blood pressure during the lower body suction protocol (Table 1). Heart rate was significantly elevated throughout the muscle chemoreflex and mental arithmetic protocols but was unaffected by the lower body suction and cold pressor test protocols (Table 1).

**Biochemical Indicators of Sympathetic Activation and Stress**

From the pretest baseline levels of plasma norepinephrine [161 (SD 54) pg/ml] and plasma epinephrine [20 (SD 9)
Fig. 1. Change in serum cortisol (top) and plasma epinephrine (bottom) and norepinephrine (middle) values from baseline (BL) to 1 min after release of the forearm circulatory occlusion cuff are shown for control (CON), muscle chemoreflex (MCR), lower body suction (LBS), cold pressor test (CPT), and mental arithmetic task (MAT). Values are means (SD) and represent change from baseline, except for control, which are means of all 4 control trials; n = 11 subjects. *P < 0.05, significantly different from control.

DISCUSSION

In this study, we used four different protocols to acutely elevate SNA while studying the FMD response to standardized peak shear stress stimuli. Our results revealed that the FMD response was dependent on the protocol to elevate SNA. FMD...
Fig. 2. Shear rate measured in brachial artery during flow-mediated dilation (FMD) protocol. Solid symbols are control tests; open symbols are during sympathetic activation. OC, forearm occlusion. There was no difference in peak shear rates; however, shear rate decreased more rapidly in mental arithmetic tests, resulting in less total shear in first minute after cuff release. Values are means (SD).

Fig. 3. Brachial artery diameter expressed as percentage of BL over course of each experimental model. Brachial artery FMD was significantly enhanced during muscle chemoreflex activation and reduced during cold pressor test. Symbols are as in Fig. 2. Values are means (SD).
ACUTE SYMPATHETIC ACTIVATION AND FLOW-MEDIATED DILATION

Fig. 4. Brachial artery diameter in BL before forearm circulatory occlusion (open bars) and peak diameter during FMD (solid bars), with percent change indicated above bars. Control values are means of all 4 control trials. Values are means (SD). *P < 0.05, significant difference from baseline; †P < 0.05, significant difference from control; #P < 0.05, significant difference from percent FMD in control.

was impaired only in the cold pressor test, was enhanced when expressed as a percent change in the muscle chemoreflex condition, and was unchanged for lower body suction and mental arithmetic tests. These results were contrary to the hypothesis that all models of elevated SNA would impair FMD. The results might help to explain some of the discrepancy in the current literature, where acute elevations in SNA have been shown to impair (18, 24, 35) or enhance (17) FMD.

Methodological Considerations

The models used to achieve increased SNA caused variable degrees of sympathetic activation with an increase in MAP during muscle chemoreflex activation, the cold pressor test, and with mental arithmetic, but there was no elevation with lower body suction. Elevated MAP in combination with potential changes in brachial artery diameter could dramatically alter the shear stimulus that is linked to the FMD response (20). Therefore, we adjusted the duration of forearm occlusion in these conditions to achieve similar peak shear stimuli. It was more difficult to achieve the same shear stimulus during the first minute after occlusion cuff release. However, the only condition in which FMD was impaired was the cold pressor test where the total shear stimulus in the first minute after 5-min circulatory occlusion was not different from control or from the muscle chemoreflex and lower body suction tests. The shear stimulus was reduced in the mental arithmetic tests, but there was no impairment of FMD. At present, there is debate concerning the specific stimulus to FMD, whether it is the peak shear or the sustained shear (1, 30), because some studies (4) have shown the rate of onset of shear to be important. In this study, we have direct measurements of blood flow velocity and diameter that allowed us to explore the relationship between shear stress and FMD, and we have indicators of sympathetic and psychological stress from direct measurements of norepinephrine, epinephrine, and cortisol.

In this study, we evaluated the FMD response by placing markers on videotaped images of vessel walls and taking the average of four measurements at each time point. The estimates of two trained observers were compared and resolved by consensus if differences were >0.2 mm. This approach is consistent with published recommendations (7). Although this is not as objective as new edge detection software, our approach was without bias because all records were coded to prevent identification of subjects and conditions. We further utilized a regression analysis of all data points (1), so that peak FMD was not biased by a single value that had the potential to be a measurement error.

Sympathetic Activation and Stress Response

After we took into account all the physiological and biochemical evidence collected in this investigation, it appeared that the muscle chemoreflex had the greatest sympathetic response and was also the only model that displayed a marked stress response. Significant elevations were observed in plasma norepinephrine in the muscle chemoreflex, cold pressor, and lower body suction tests. These data are consistent with observations from direct measurement of muscle SNA (MSNA) during muscle ischemia (39), cold pressor test (33), and lower body suction (23, 31). The effects of mental stress are equivocal, with some studies showing a small increase in MSNA (5), whereas with others there was a decrease (16).

Serum cortisol levels decreased slightly over the course of the test session, except in the muscle chemoreflex test. This suggests that the 10 min of quiet, supine rest used in this investigation might not have been sufficient to achieve a stable baseline value of circulating cortisol. However, useful information can still be gleaned from the observation that cortisol had a similar small decrease in each of the lower body suction, cold pressor, and mental arithmetic tests and that this response did not differ from the control experiments; that is, the muscle chemoreflex test was considerably more stressful than any of the other models examined.

Muscle chemoreflex. To the best of our knowledge, this is the first study to examine the impact of activating the muscle chemoreflex on the FMD response. We observed a greater percent dilation as a consequence of an initial constriction of the brachial artery during the period of muscle ischemia followed by FMD to the same diameter as observed in the control tests; that is, it was only the initial constriction that made the percent change in FMD appear greater than that in the control. The muscle chemoreflex activates a general sympathetic response with increased total peripheral resistance and SNA (39) and, as we observed, elevated norepinephrine, epinephrine, and cortisol. It has been proposed that the magnitude of FMD is influenced by the initial vessel size (32), although this effect is likely due mainly to a greater hyperemic shear stress stimulus in small arteries (19). In this study, we attempted to control shear stress so that the peak and the total shears in the first minute of reactive hyperemia were not different between the chemoreflex and control tests. In the current study, it could only be speculated whether there was more nitric oxide (NO) and/or other vasodilator released in response to the shear stimulus to cause the greater percent dilation or whether the vascular smooth muscle was more responsive to the vasodilators. The elevated circulating catecholamines may have played a role in augmenting NO release from the endothelium. α-Adrenergic receptors on the endothelium have been shown to facilitate the release of NO from the endothelium during
hemorrhage (8), and β2-receptor stimulation by salbutamol has been shown to mask hyperglycemia-induced endothelial dysfunction (21). It is unlikely that reaching the same peak dilation was a physiologically defined upper limit because many studies have shown greater dilation with nitroglycerin (18, 35) or with greater shear stimulus as after ischemic exercise (1).

Lower body suction. Reactive hyperemia and FMD were not significantly affected by lower body suction that was sufficient to cause a significant elevation in plasma norepinephrine. This finding contrasted with results from Hijmering et al. (18) that indicated a >50% reduction in FMD with no change in the forearm muscle blood flow as assessed by strain-gauge plethysmography during lower body suction. The reason for the discrepancy with the results of Hijmering et al. (18) is not obvious. There were some differences in methodology that might have influenced the results, including the duration of supine posture as well as the measurement of total forearm blood flow by strain gauge rather than flow through the brachial artery. In the study of Hijmering et al., subjects were supine for at least 90 min before the application of the final level of lower body suction, and this might have accounted for the >15% elevation in heart rate (18), whereas heart rate in the current study and other research (3, 31) showed little or no increase until lower body suction exceeded ~20 mmHg. Even though heart rate increased very little with ~20 to ~30 mmHg lower body negative pressure, MSNA is increased by lower body suction (23, 31, 38). It is possible that prolonged supine posture might have interacted with the lower body suction to modify the cardiovascular response, resulting in elevated heart rate, as has been observed after very short durations of slight head-down bed rest (2, 25).

Cold pressor test. The cold pressor test was the only condition that we examined where FMD was reduced. This finding is consistent with the results of Lind et al. (24) when they expressed their data relative to the blood flow stimulus. The cold pressor test elevates arterial blood pressure primarily by a sympathetic vasoconstrictor response, as evidenced by the 34% increase in MAP and the elevated plasma norepinephrine in the current study. Even though MAP remained high throughout the cold pressor test, subjective data indicated that for most subjects the painful nature of the stimulus subsided and gave way to numbness in the feet 2-2 min after immersion in the cold-water slurry. From the current data where we observed some of the anticipated signs of elevated SNA, it is not clear whether other unmeasured factors might have been responsible for the impairment of FMD. Spieker et al. (35) implicated endothelin as a cause of reduced FMD after mental stress, but it is not clear whether this could partially explain our findings with the cold pressor test and, if so, why it would happen only with this test condition.

Mental arithmetic task. In this study, FMD was not changed from control by the mental arithmetic task. Several different investigations (9, 14, 24, 35) have reported impaired FMD in response to an acute increase in SNA with mental stress, whereas one study (17) observed enhanced FMD with mental stress. A parallel between impaired FMD with acute mental stress and that with white-coat hypertension has been made (13), but not all investigations (29) find impaired FMD in white-coat hypertension in contrast to consistent findings with essential hypertension. In our study, where we used a mental arithmetic task very similar to that employed by Lind et al. (24), we observed a small increase in MAP. The other markers of elevated SNA and stress taken from norepinephrine, epinephrine, and cortisol were not elevated by the mental arithmetic task. Our results, coupled with the findings that MSNA is variably affected by mental stress (5, 16), lead us to question the validity of mental arithmetic as a model of sympathoexcitatory reflex activation. The slightly elevated heart rate observed in this condition might have been due to parasympathetic nervous system withdrawal.

An important factor that might influence FMD with acute mental stress is the magnitude of shear stress as determined by the peak and/or total reactive hyperemia. Lind et al. (24) noted that, although the magnitude of FMD with acute mental stress was not different from control, the ratio of FMD to blood flow was significantly reduced. Many of the studies (9, 13, 14, 17, 29, 35) cited here either did not measure blood flow during hyperemia or did not report the data. To achieve the same peak shear stress in our mental arithmetic test as in the control, we reduced the duration of the period of occlusion. However, shear stress declined more rapidly during the mental arithmetic task so that total shear stress over the first minute after occlusion cuff release was significantly reduced; that is, in contrast with Lind et al. (24), we might have concluded relatively enhanced FMD from the ratio of FMD to shear rate. This conclusion would be consistent with enhanced FMD reported by Harris et al. (17), but it is worth noting that they actually found reduced baseline diameter in the mental stress test with dilation to the same absolute end value, a finding similar to what we reported for muscle chemoreflex.

In summary and in conclusion, we have demonstrated that there is not a generalized impairment of endothelial FMD in a conduit artery caused by elevated SNA. The muscle chemoreflex test induced the greatest overall stress and sympathetic activation, and it was the only condition in which a baseline constriction of the conduit brachial artery was observed. However, FMD occurred to the same end diameter as in the control conditions so that the percent FMD response was greater than that in any other model. No impairment of FMD was observed in the lower body suction experiments where plasma norepinephrine was elevated nor in the mental arithmetic tests where blood pressure was elevated without an increase in norepinephrine. Impaired endothelial function was found only in the cold pressor test. In this test, blood pressure and plasma norepinephrine were elevated, but in contrast to the muscle chemoreflex test, there was no elevation in epinephrine or cortisol. Thus it does not seem that the sympathetic stress was as great in this condition, yet there was impaired FMD. The mechanism(s) responsible for the differences in FMD between the muscle chemoreflex and the cold pressor test remain to be identified. These data collectively indicate that FMD does not appear to be impaired by an acute increase in SNA. The data are consistent with the no impairment of FMD in one study of white-coat hypertension (29), but this finding is controversial (13, 22) and awaits further explanation of the link to the consistent finding of impaired endothelial function with essential hypertension (10, 26, 28). There are important implications from the current study. First, it is essential to monitor the shear stimulus as well as the FMD response.
Second, the presence of any stressor may modify the FMD response, but the direction of change is dependent on complex interactions that appear to go beyond simple activation of the sympathetic nervous system. Thus we agree with one aspect of the conclusion of Tschakovsky and Pyke (37) in a recent point-counterpoint (15, 37) that FMD might be due to multiple cascade pathways with different mediators, but we do not concur with the statement that there is “unquestionable evidence that sympathetic activation influences the FMD response.” We observed that FMD is not always reduced by every means of activating the sympathetic system.

REFERENCES


