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Sympathetic Activation Markedly Reduces Endothelium-Dependent, Flow-Mediated Vasodilation

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OBJECTIVES We sought to evaluate whether increased sympathetic outflow may interfere with flow-mediated dilation (FMD).

BACKGROUND Endothelial function, assessed as FMD, is frequently used as an intermediate end point in intervention studies. Many disease states with increased sympathetic tone are also characterized by endothelial dysfunction.

METHODS Sixteen healthy volunteers underwent FMD studies with and without concomitant sympathetic stimulation. Intra-arterial nitroglycerin (NTG) infusion was used to assess endothelium-independent vasodilation. Pathophysiologically relevant sympathetic stimulation was achieved by baroreceptor unloading, using a lower body negative pressure box. In a subset of eight volunteers, this protocol was repeated during loco-regional alpha-adrenergic blockade by intra-arterial infusion of phentolamine (PE). Reactive hyperemic flow was assessed with strain-gauge phlethysmography.

RESULTS Overall, FMD responses (8.3 ± 3.4%) were significantly attenuated by concomitant sympathetic stimulation (3.6 ± 3.4%, $p < 0.01$). Loco-regional alpha-adrenergic blockade had no effect on baseline FMD responses (10.7 ± 4.7%), whereas the attenuation by sympathetic stimulation was abolished completely during PE co-infusion (11.5 ± 3.3%). During intra-arterial NTG infusions, arterial diameters relative to baseline were not significantly different between the four possible stages.

CONCLUSIONS Sympathetic stimulation, at a clinically relevant range, significantly impairs the FMD response by an alpha-adrenergic mechanism. (J Am Coll Cardiol 2002;39:683–8) © 2002 by the American College of Cardiology

It has been generally accepted that endothelial dysfunction represents a hallmark in the development of atherosclerosis. All major risk factors that have been identified in epidemiologic studies have been shown to be associated with impaired endothelial function. Reduced endothelial responses can be observed early in the course of atherogenesis (1), before structural lesions can be found (2). More recently, endothelial dysfunction has been shown to have a clear predictive value for future cardiovascular disease (3,4). Although several invasive tests have been employed successfully for the assessment of endothelial function over the last two decades, attention has now focused predominantly on noninvasive techniques. Thus, assessment of flow-mediated dilation (FMD) of the brachial artery, using an ultrasound device, has received much attention as a tool to evaluate endothelial function noninvasively. Using this technique, numerous studies have shown that impaired FMD responses are associated with a wide range of cardiovascular risk factors (1,4–6), although the impairment is reversible with specific drug interventions (7,8).

Over the past few decades, the importance of the sympathetic nervous system as a determinant of vascular tone and as an indicator of adverse cardiovascular outcomes has been generally accepted (7–9). Interestingly, many of the risk factors associated with impaired FMD responses are also characterized by increased sympathetic activity (10,11). In addition, many of the drug interventions associated with improvement of FMD responses may also result in sympathicolysis. For example, heart failure is clearly associated with impaired FMD responses, as well as activation of sympathetic activity (12). Consequently, all effective therapeutic interventions for this condition will invariably result in sympathicolysis. Moreover, we recently demonstrated that specific agents, such as angiotensin-converting enzyme inhibitors, also have direct symphathicolytic effects (13,14). Endothelial function, as measured with FMD, has been shown to have a diurnal effect (i.e., FMD is low when sympathetic activation is considered to be high) (15–17). Therefore, if sympathetic activation results in a reduced FMD response, this may have direct consequences for the interpretation of an impaired FMD response as an indicator of endothelial dysfunction.

To evaluate whether and to what extent increased sympathetic activity interferes with FMD, we studied FMD in healthy volunteers at baseline and during sympathetic activation induced by baroreceptor unloading. In a subset of

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Abbreviations and Acronyms

ANOVA = analysis of variance
FBF = forearm blood flow
FMD = flow-mediated dilation
LBNP = lower body negative pressure
MSNA = muscle sympathetic nerve activity
NO = nitric oxide
NTG = nitroglycerin
PE = phentolamine

subjects, the experiments were repeated during local intra-arterial alpha-adrenergic blockade by phentolamine (PE) infusion.

METHODS

This study was designed to evaluate the interaction between endothelium-dependent vasodilation and the sympathetic system at the level of the conduit arteries.

For stimulation of the sympathetic nervous system, lower body negative pressure (LBNP) was selected, as it is a widely accepted, well-validated model for sympathetic activation that allows careful standardization (18). All measurements were performed in a quiet, temperature-controlled room. The study was approved by the Institutional Review Committee of the University Medical Center of Utrecht. All subjects gave written, informed consent. The procedures followed were in accordance with institutional guidelines.

Study group. The studies were performed in 16 healthy, nonsmoking volunteers (Table 1). None of the volunteers used medication. All of the volunteers were free of signs indicative of atherosclerosis on physical examination and had a normal electrocardiogram. Blood testing excluded any individual with: 1) cholesterol over 6.0 mmol/l; 2) triglycerides over 2.0 mmol/l; 3) glycosylated hemoglobin over 6.0%; and 4) renal function outside of the laboratory's standard values. Urine samples from all volunteers were tested for drug abuse. Caffeine-containing beverages were not allowed within 12 h before the measurements took place.

Table 1. Volunteer Characteristics

Age (yrs)	27.5 ± 5.1
Blood pressure (mm Hg)	
Systolic	112 ± 4.7
Diastolic	69 ± 2.8
Creatinine (μmol/l)	72.6 ± 9.3
Urea (mmol/l)	5.0 ± 1.7
Total cholesterol (mmol/l)	5.0 ± 0.7
HDL cholesterol (mmol/l)	1.7 ± 0.5
Triglycerides (mmol/l)	1.2 ± 0.6
HbA_{1c} (%)	5.3 ± 0.43
BMI (kg/m^2)	26.5 ± 3.2
Gender (male/female)	12/4

Data are presented as the mean value ± SD, except for gender (n).
BMI = body mass index; HbA_{1c} = glycosylated hemoglobin; HDL = high-density lipoprotein.

Protocol outline. In the first eight volunteers, the effect of LBNP on FMD and forearm blood flow (FBF) was assessed in a two-day experiment with a one-week interval. Volunteers were asked to lie down in the LBNP box (see subsequent paragraph), and the protocol was re-explained to them. Next, at baseline, the FMD of their right brachial artery (dominant arm) was measured (see subsequent paragraph). After a 1-h rest period, negative pressure was introduced gradually until the heart rate had increased 15% relative to baseline values, or until LBNP reached −20 mm Hg. After obtaining a stable heart rate increase for 10 min, the second FMD measurement was performed, after which the study day ended. After one week, a similar experiment was repeated by applying the same LBNP steps, substituting FMD for FBF measurements to obtain reactive hyperemia flow rates using strain-gauge plethysmography (see subsequent paragraph).

In the second eight volunteers, the effect of LBNP on FMD was repeated with and without loco-regional alpha-adrenergic blockade in a single-day experiment. The volunteers were placed in the LBNP box, and a cannula was inserted in their dominant (i.e., right brachial) artery. A period of 45 min was allowed to regain a stable baseline condition. All volunteers underwent, in random order, FMD measurement alone and FMD measurement with LBNP, or FMD with PE and FMD with PE plus LBNP. At the end of each period, nitroglycerin (NTG) was infused at 20 μg/min for 5 min, after which the brachial diameter was measured. Phentolamine was administered locally in the brachial artery at a rate of 100 μg in 5 min, followed by a maintenance infusion of 25 μg in 5 min (19). In between the measurement blocks, a period of 60 min of rest was allowed to re-establish baseline conditions. During this period, volunteers remained supine.

FMD. Flow-mediated dilation was assessed using a Wall-tracking system (WTS2, PIE Medical, Maastricht, The Netherlands), as published previously (8,20,21). In short, the brachial artery diameter at the level of the antecubital crease was measured before and 4 min after forearm occlusion. To minimize mental stress, care was taken to make the volunteers as comfortable as possible. The object arm was stabilized with cushions, and the ultrasound probe was fixated in the three-dimensional space using a custom-built probe holder. The brachial artery was scanned in a longitudinal window, according to previously described criteria (20). Before occluding the forearm with a standard blood pressure cuff, at least four serial measurements were taken. Next, the blood pressure cuff was inflated for 4 min at 200 mm Hg and released abruptly. Post-occlusion diameters were obtained at 45, 75, 105, 135 and 165 s after deflation. Flow-mediated dilation was calculated as the maximal post-occlusion diameter relative to the averaged pre-occlusion diameters. Of note, the reproducibility of the brachial artery diameter during the session has a coefficient of variation of 1.1% (20).

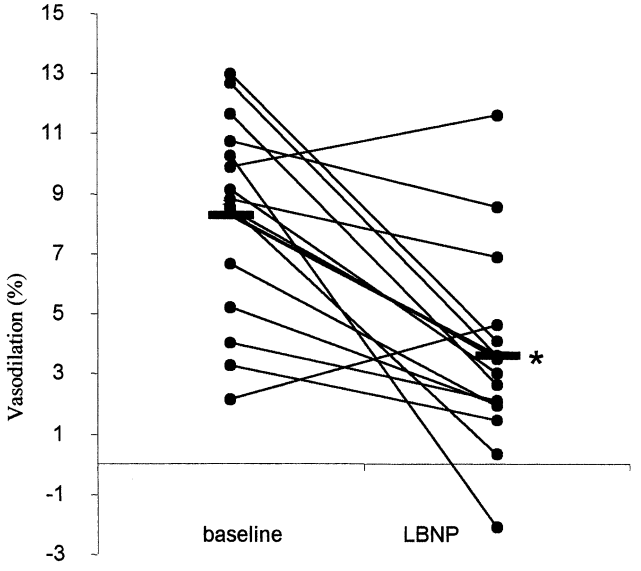

Figure 1. Effect of lower body negative pressure (LBNP) on flow-mediated dilation. Flow-mediated dilation decreased significantly during LBNP (−20 mm Hg) in healthy volunteers (*p = 0.0008 by the paired *t* test). **Bold line** = average value of all observations.

Sympathetic activation. Sympathetic activation was elicited using a LBNP box, as described previously by us and other groups (14,18,22,23). In short, volunteers were supine in this airtight box from the level of the iliac crest. Suction was applied gradually with increments of 5 mm Hg, until the heart rate increased by at least 15% or a level of −20 mm Hg of LBNP was reached. At each level, there was a pause of at least 10 min before applying the next level. The FMD and FBF studies were performed at least 5 min after stabilization of heart rate and blood pressure during LBNP.

FBF measurements. Forearm blood flow was measured with venous occlusion phlethysmography using a fully automated R-wave–triggered system, as published previously (24). Mercury in Silastic strain gauges was placed at the widest part of the forearm. Flow was registered at 10-s intervals. The occlusion cuff for reactive hyperemia was placed at the same level as used for FMD measurements, just distal to the strain gauges. The upper arm-collecting cuffs were inflated intermittently to 40 mm Hg by the system.

Statistical analysis. Flow-mediated dilation was calculated as the difference between the maximal post-occlusive diameter and the average baseline diameter, relative to the average baseline diameter, and expressed as a percentage. All results are expressed as the mean value ± SD.

Differences in baseline FMD, blood pressure, heart rate and brachial artery diameter at baseline were tested with the paired *t* test. The effect of LBNP on FMD (Fig. 1) and heart rate (Fig. 2) was tested using one-way repeated measures analysis of variance (ANOVA). Differences in reactive hyperemic blood flow during rest and LBNP (Fig. 2A), as well as differences in the FMD/NTG response

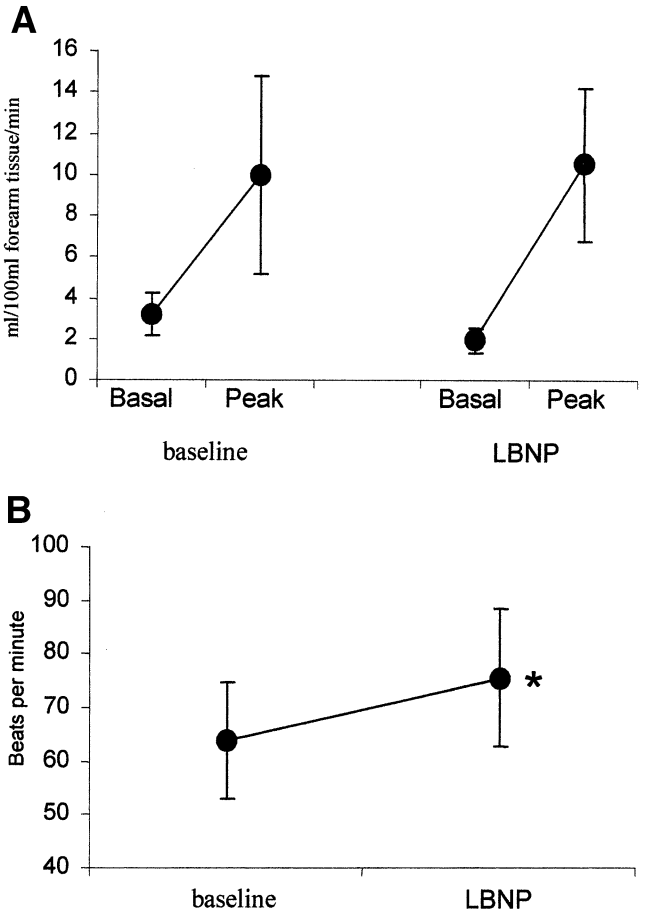

Figure 2. Effect of lower body negative pressure (LBNP) on reactive hyperemic flow and heart rate. **(A, left)** Basal and maximal post-ischemic flow at baseline. **(A, right)** Basal flow and maximal post-ischemic flow during LBNP. The increase in blood flow during hyperemia was not significantly different between baseline and LBNP (p = 0.099 by two-way repeated measures analysis of variance). **(B)** Lower body negative pressure significantly increased the heart rate (*p = 0.0013 by the paired *t* test).

without and with PE (Fig. 3), were evaluated using two-way repeated measures ANOVA (using SAS proc mixed assuming unstructured covariance matrices) (SAS Institute, Cary, North Carolina). If the *F* test reached statistical significance, differences between the mean values were analyzed with a one degree-of-freedom test for $p < 0.05$.

RESULTS

Effect of LBNP on FMD. Both the first and second cohorts of eight volunteers underwent FMD testing with and without LBNP at −20 mm Hg. In these 16 volunteers, FMD without LBNP was 8.3 ± 3.4%, and with concomitant LBNP, 3.6 ± 3.4% (p = 0.0008) (Fig. 1). During LBNP, the mean heart rate increased from 64.4 ± 10.8 beats/min to 75.8 ± 12.6 beats/min (p = 0.0013) (Fig. 2B). In the subgroup that underwent FBF measurements, baseline blood flow during LBNP was reduced from 3.2 ± 1.0 to 2.0 ± 0.6 ml/100 ml forearm tissue per min (Fig. 2A). Using two-way repeated measures ANOVA, we have shown that the statistically significant increase in FBF

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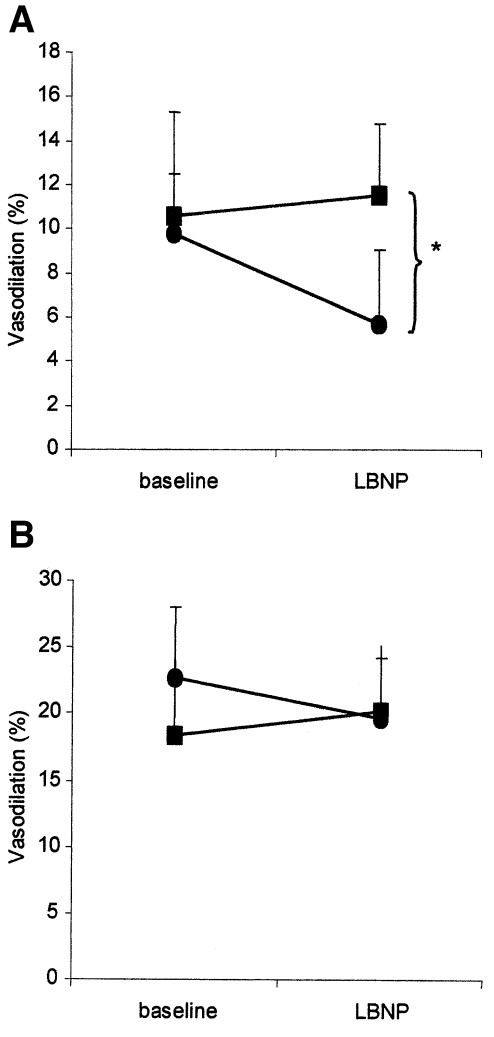

Figure 3. Effect of alpha-receptor blockade on the flow-mediated dilation (FMD)/nitroglycerin (NTG) response with and without lower body negative pressure (LBNP). **(A)** During phentolamine (PE) **(solid squares)**, the LBNP-induced impairment in FMD **(solid circles)** could no longer be demonstrated (two-way repeated measures analysis of variance: *p = 0.046 control vs. PE). **(B)** Phentolamine **(solid squares)** did not change the NTG response compared with baseline **(solid circles)** (p = NS).

during reactive hyperemia (i.e., the stimulus for the FMD response) was not significantly different during LBNP, as compared with that in the control experiments (p = 0.099) (Fig. 2A). Blood pressures remained stable throughout the experiments (mean arterial pressure [MAP] 106.6 ± 2.8 mm Hg at baseline vs. 108.3 ± 5.2 mm Hg during LBNP; p = NS).

Effect of PE on LBNP effects. The baseline FMD response did not change significantly during PE infusion (9.8 ± 2.7% vs. 10.7 ± 4.7%; p = NS by the paired *t* test). In contrast, the decrease in FMD during LBNP could no longer be demonstrated during PE co-infusion (control vs. PE: p = 0.046 by two-way repeated measures ANOVA) (Fig. 3A). Endothelium-independent vasodilation with NTG infusion was 22.7 ± 5.3% at baseline. Lower body negative pressure did not significantly change the NTG vasodilator response. Phentolamine infusion had no significant effect on the NTG response (Fig. 3B).

Local infusion of intra-arterial PE and NTG had no effect on heart rate and/or systemic MAP. Baseline arterial diameters did not change significantly (2.834 ± 0.351 mm at baseline, 2.846 ± 0.374 mm with LBNP, 2.951 ± 0.335 mm with PE and 2.984 ± 0.317 mm with both LBNP and PE).

DISCUSSION

The present study shows that sympathetic stimulation evoked by baroreceptor unloading markedly reduces flow-mediated vasodilation, whereas the response to exogenous nitric oxide (NO) remains unaffected during LBNP. Local alpha-adrenergic receptor blockade prevents the LBNP-induced reduction in FMD.

LBNP as a stimulus for sympathetic activation. Lower body negative pressure has been extensively validated as a model to elicit sympathetic stimulation. Thus, muscle sympathetic nerve activity (MSNA) recorded in either the peroneal nerve or radial nerve has been shown to increase similarly at −20 mm Hg of LBNP (25). At a level of −20 mm Hg, we and others (26) have shown a significant rise in MSNA, without significant changes in cardiac output or blood pressure (25–28). Noradrenalin spillover studies (23) have also shown a significant increase in noradrenalin production already at a level of −15 mm Hg of LBNP. Healthy volunteers exposed to −20 mm Hg of LBNP have been shown to have a mean heart rate increase of 15%, as well as an increase in MSNA (19 ± 8 bursts/100 heartbeats at rest vs. 58 ± 13 bursts/100 heart beats during −20 mm Hg of LBNP [22]). These MSNA values during LBNP are comparable to those in patient groups characterized by increased endogenous sympathetic outflow (e.g., patients with renal failure) (53 ± 8 bursts/100 heart beats) (14). Based on these data, we can safely assume that we have elicited sympathetic activation at clinically relevant levels, using a well-validated method.

Interaction of sympathetic nervous system with endothelial function. Several reports have presented data on the interaction between the sympathetic nervous system and endothelial function. Thus, increased NO activity has been observed during increased sympathetic tone. Sympathetic activation elicited by mental stress results in a basal, NO-mediated increase in both forearm (29) and coronary blood flow (30). In agreement with this, cold pressor testing, another frequently used sympathetic stress test, also results in increased basal blood flow in the forearm (31) as well as coronary arteries (32). The latter effects have been attributed to NO synthase activation through $beta_2$ (33,34) and/or $alpha_2$ receptor activation (35). Of note, these observations describe sympathetic effects on basal NO availability in microvascular resistance vessels. In contrast, in the present study, we evaluated the effect of sympathetic activation through baroreceptor unloading with LBNP on shear-

stimulated NO release, assessed as FMD. We observed a significant impairment in FMD during LBNP. These data are in accordance with previous findings showing significant FMD impairment during mental stress (29,36–38). The discrepancy between shear-induced (macrovascular) and agonist-induced (microvascular) NO synthase activation is underscored by Engelke et al. (19), who demonstrated that LBNP-induced sympathetic activation has no significant effect on acetylcholine-induced microvascular vasodilation.

The impaired FMD response during LBNP can relate to either increased vasoconstrictor tone, decreased shear stimulus or decreased NO availability. The response to exogenous NO remains intact, which argues against increased nonspecific vasoconstrictor tone being responsible for the impaired FMD response. Accordingly, it has been shown that local noradrenalin infusion does not impair receptor-mediated NO activity by metacholine infusion (39). The second option—that is, decreased shear stimulus during LBNP—is less likely, because the main determinants of shear stress (i.e., basal brachial diameter, *reactive* hyperemic blood flow and hematocrit) are comparable in the small time frame before and after LBNP. Of note, one report (40) has also suggested an association between basal shear stress levels (in contrast to reactive hyperemia shear stress levels) and FMD. However, it has recently been emphasized that for assessment of NO-dependent endothelial function, FMD responses to early flow, rather than sustained flow changes, should be evaluated (41). Taken together, the current data imply a specific inhibitory effect of sympathetic activation on shear-mediated NO release. Few reports have addressed this issue, although an inhibitory effect of exercise-induced sympathetic stimulation on NO metabolites has been reported in rat heart tissue (42). Further research is needed to elucidate the exact interaction between sympathetic activation and shear-stimulated NO production by endothelial cells.

Study limitations. In this study, we assessed the heart rate increase, rather than directly evaluating MSNA and/or noradrenalin spillover as index of sympathetic activation. However, we have previously demonstrated that MSNA at a level of −20 mm Hg induces a mean increase in heart rate of 15%, in addition to an increase in MSNA activity, within a clinically relevant range, in healthy volunteers (14,22). Hence, it is safe to assume that in the present study, the LBNP of −20 mm Hg, which resulted in a mean 15% heart rate increase and a slight decrease in FBF, indeed produced sympathetic stimulation in the volunteers.

Relevance for use of FMD in clinical trials. Flow-mediated dilation has emerged as an attractive tool to estimate endothelial function in patients. Over the last decade, FMD as variable for endothelial dysfunction has been successfully used to further characterize high-risk groups, and the reversibility of impaired FMD responses has been used to optimize pharmaceutical interventions in patients at increased cardiovascular risk. The use of FMD has increased because recent studies have shown the predictive value of both coronary and brachial endothelial function for future cardiovascular morbidity and mortality (3,4). In the present report, we show that sympathetic activation has a profound effect on the FMD response. This observation may have direct consequences for the use of FMD for patients with increased sympathetic outflow, such as in those with heart failure, renal failure and hypertension. In these patient groups, symptomatic treatment modalities, which affect sympathetic outflow (e.g., diuretics, beta-blockers), may result in improvement in FMD due to changes in sympathetic activity. Hence, in studies evaluating FMD as an indicator of endothelial dysfunction, the strong effect of sympathetic activity on FMD should be taken into account.

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