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Sympathectomy potentiates the vasoconstrictor response to nitric oxide synthase inhibition in humans[1]

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Abstract

Objective: Nitric oxide exerts its cardiovascular actions at least in part by modulation of the sympathetic vasoconstrictor tone. There is increasing evidence that nitric oxide inhibits central neural sympathetic outflow, and preliminary evidence suggests that it may also modulate peripheral sympathetic vasoconstrictor tone. **Methods:** To test this latter concept, in six subjects having undergone thoracic sympathectomy for hyperhydrosis, we compared the vascular responses to systemic L-NMMA infusion (1mg/kg/min over 10 min) in the innervated and the denervated limb. We also studied vascular responses to the infusion of the non-nitric-oxide-dependent vasoconstrictor phenylephrine. **Results:** L-NMMA infusion evoked a roughly 3-fold larger increase in vascular resistance in the denervated forearm than in the innervated calf. In the denervated forearm, vascular resistance increased by 58±10 percent (X±SE), whereas in the innervated calf it increased only by 21±6 percent ($P<0.01$, forearm vs. calf). This augmented vasoconstrictor response was specific for L-NMMA, and not related to augmented non-specific vasoconstrictor responsiveness secondary to sympathectomy, because phenylephrine infusion increased vascular resistance similarly in the denervated forearm and the innervated calf (by 24±7, and 29±8 percent, respectively). The augmented vasoconstrictor response was related specifically to denervation, because in control subjects, the vasoconstrictor responses to L-NMMA were comparable in the forearm and the calf. **Conclusions:** These findings indicate that in the absence of sympathetic innervation, the vasoconstrictor responses to nitric oxide synthase inhibition are augmented.

Keywords: Autonomic nervous system; Nitric oxide; Vasoconstriction/dilation; Regional blood flow; Vasoactive agents

1. Introduction

Nitric oxide which is synthesized by the endothelium from the amino acid L-arginine by the ubiquitous enzyme nitric oxide-synthase [1], has emerged as a key regulatory mechanism of blood pressure and vasomotor tone in both animals and humans [2,3]. While there is abundant evidence that nitric oxide exerts its vasodilator action at least in part by a direct effect at the vascular smooth muscle cell [4], more recent evidence suggests that nitric oxide which is also synthesized in the central nervous system [5], exerts its cardiovascular actions at least in part by modulating the sympathetic vasoconstrictor tone [6–8]. Regarding the latter, two concepts have been proposed. First, in both animals [6–8], and humans [9], nitric oxide inhibits central neural vasoconstrictor outflow. More recently, it has been suggested that nitric oxide also may modulate sympathetic tone peripherally [10], as evidenced by the suppression of the L-NMMA induced pressor effect by sympathectomy in anesthetized, paralyzed, baroreflex-denervated cats [11]. To test this latter concept, we examined vasoconstrictor responses to systemic L-NMMA infusion in the innervated calf and the denervated forearm in subjects having undergone thoracic sympathectomy for hyperhydrosis. We compared these responses with those evoked by the non-nitric-oxide-dependent vasoconstrictor phenylephrine.

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2. Methods

2.1. Subjects

We studied six healthy subjects (2 men and 4 women, mean [±SD] weight 64±3 kg, height 167±4 cm, body mass index 22.8±1.1 kg/m^2, age 34±6 years) having undergone thoracic sympathectomy for hyperhydrosis (mean [±SD] time from surgery at the time of the study was 40±7 months), and five age- and sex-matched control subjects (weight 70±3 kg, height 176±4 cm, body mass index 22.4±1.1 kg/m^2, age 30±5 years).

All subjects were normotensive, were taking no medications, and had no evidence of metabolic or cardiovascular disease. The studies were performed in the morning after an overnight fast. The subjects were asked to abstain from alcohol, caffeine and tobacco for at least 24 h before each study. The investigation conforms with the principles outlined in the Declaration of Helsinki. The experimental protocol was approved by the Institutional Review Board on Human Investigation, and all subjects provided written informed consent.

2.2. General procedures

The subjects were studied in the supine position. Heart rate (electrocardiogram) and blood pressure (Finapres) were measured continuously. For drug infusion, an intravenous catheter was inserted in an antecubital vein.

2.3. Measurement of muscle blood flow

Limb blood flow was measured by venous occlusion plethysmography, using mercury-in-Silastic strain gauges. The limbs were elevated 10 to 15 cm above the level of the right atrium to collapse the veins. The circulation to the hand and the foot was arrested by inflating a cuff around the wrist and the ankle during blood flow determinations, which were performed at 15-second intervals over a 5-min period. [12]

2.4. Drugs

Drugs were dissolved in physiological saline immediately before use. L-NMMA, L-arginine, and D-arginine were obtained from Clinalfa (Läufelfingen, Switzerland), and phenylephrine from Winthrop Pharmaceuticals (Zurich, Switzerland).

2.5. Experimental protocols

Protocol 1: Cardiovascular effects of L-NMMA infusion. After instrumentation the subjects rested quietly for 30 min. They then received sequential infusions of normal saline (1 ml/min for 20 min), L-NMMA (1mg/kg/min for 10 min), D-arginine (100 mg/kg over 10 min), and L-arginine (100 mg/kg over 10 min). Hemodynamic measurements were recorded during two 5 min periods of the saline infusion, and 5 min after the end of each of the drug infusions. The present dose of L-NMMA was chosen, because it has been shown to consistently increase calf vascular resistance. [13]

To demonstrate the efficacy of thoracic sympathectomy, we measured blood pressure and limb blood flow responses to 2 min immersion of the hand in ice water [14], and compared vasoconstrictor responses in the denervated and innervated limbs during the second minute of this test. Vasoconstrictor responses to immersion of the hand in ice water in the sympathetically denervated forearm were abolished; during the second minute of the cold pressor test, vascular resistance had increased by 48±20 percent in the innervated limb, whereas it had decreased by 17±5 percent in the denervated limb ($P<0.02$ innervated vs. denervated limbs).

Protocol 2: Cardiovascular effects of equipressive dose of phenylephrine infusion. Four of the subjects having undergone thoracic sympathectomy returned for this study during which, after 30 min of normal saline infusion (1 ml/min), they received a phenylephrine infusion titrated at a dose to match the increase in arterial pressure observed during the L-NMMA infusion. Hemodynamic measurements were recorded during two 5 min periods of the saline and the phenylephrine infusion.

3. Data analysis

Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure. Vascular resistance was determined as mean arterial pressure in mmHg divided by blood flow in ml/min per 100 ml of tissue. The measurements of limb blood flow, arterial pressure, and heart rate that were collected over 5 min periods were averaged to a single value.

Statistical analysis was performed using paired and unpaired two-tailed *t*-tests, as appropriate. A P value <0.05 was considered to indicate significance. Unless stated otherwise, data are expressed as means±SE.

4. Results

In the subjects having undergone thoracic sympathectomy, the base-line vascular resistance (Table 1) in the denervated forearm and the innervated calf was comparable ($P=0.9$). The base-line vascular resistance in the denervated forearm was also not different ($P=0.3$) from the baseline vascular resistance in the control subjects.

Figs. 1 and 2 show that L-NMMA infusion evoked a roughly 3-fold larger increase in vascular resistance in the denervated forearm than in the innervated calf. In the forearm, vascular resistance increased by 58±10 percent

Table 1
Hemodynamic responses to L-NMMA and phenylephrine infusion[a]

	Subjects with sympathectomy						Control subjects	
	Basal	L-NMMA infusion	D-arginine infusion	L-arginine infusion	Basal	Phenylephrine infusion	Basal	L-NMMA infusion
Mean arterial pressure (mm/Hg)	79±5	94±4[b]	89±4[b]	80±5	78±4	94±2[b]	78±4	94±2[c]
Heart rate (beats/min)	62±2	52±2[c]	51±3[b]	61±2	63±2	53±3[c]	57±2	48±2[b]
Forearm blood flow (ml/min/100 ml)	1.9±0.1	1.4±0.1[c]	1.4±0.2[b]	1.8±0.2	1.7±0.2	1.6±0.2	1.6±0.2	1.6±0.1
Calf blood flow (ml/min/100 ml)	1.9±0.3	1.9±0.3	1.8±0.4	2.0±0.1	1.7±0.3	1.6±0.3	1.7±0.2	1.6±0.1
Forearm vascular resistance (Units)	43±5	68±6[c]	64±4[b]	44±2	47±6	59±5[b]	50±4	59±3[b]
Calf vascular resistance (Units)	42±9	51±12[b]	49±4[b]	41±7	48±7	60±5[b]	47±5	60±4[b]

[a] Values are means±SE for six subjects with sympathectomy (except for phenylephrine and D-arginine infusion; $n=4$), and five control subjects.
[b] $P<0.05$ vs. corresponding base-line.
[c] $P<0.01$ vs. corresponding base-line.

during L-NMMA infusion whereas in the calf, it increased only by 21±6 percent ($P<0.001$, forearm vs. calf). The L-NMMA induced vasoconstriction was reversed by L-arginine, but not by D-arginine, infusion (Table 1). In contrast to L-NMMA, infusion of an equipressive dose of phenylephrine increased the vascular resistance comparably in the denervated and the innervated limb (by 24±3 and 26±7 percent, respectively; $P>0.5$, forearm vs. calf).

In the control subjects, the vascular responses to L-NMMA infusion in the forearm and the calf were comparable (Table 1).

5. Discussion

Nitric oxide has emerged as a key regulatory mechanism of vasomotor tone and arterial pressure [2,3]. Recent studies suggest that it does so, at least in part, by modulating sympathetic vasoconstrictor outflow [15]. Here we used subjects having undergone thoracic sympathectomy for hyperhydrosis, to probe the role of the peripheral sympathetic nervous system in the modulation of the vascular responsiveness to nitric oxide synthase inhibition. We found that sympathectomy markedly potentiated the vasoconstrictor effect of L-NMMA infusion. The L-NMMA induced vasoconstrictor effect was almost three times larger in the denervated than in the innervated limb. These findings provide the first evidence for an important interplay between the peripheral sympathetic nervous system and the L-arginine–nitric-oxide system in the regulation of the vascular tone in humans, and indicate that sympathetic innervation attenuates the vasoconstrictor effect of nitric oxide synthase inhibition.

The augmented L-NMMA-induced vasoconstrictor effect

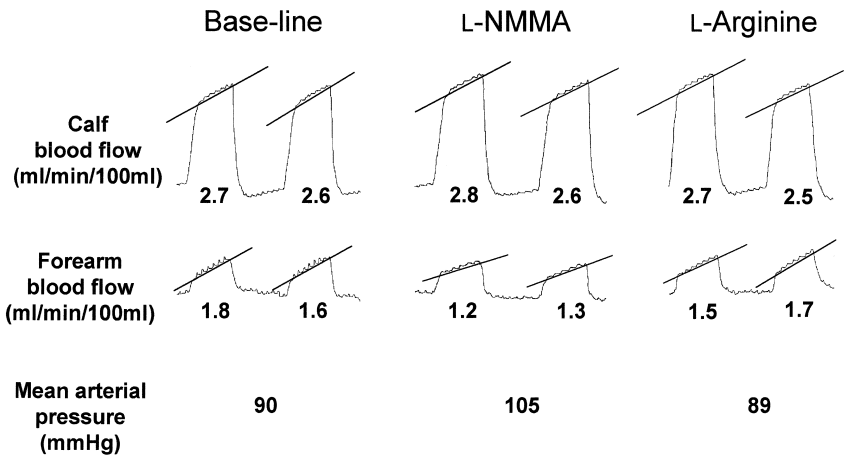

Fig. 1. Segments of simultaneous recordings of forearm and calf blood flow in a subject with thoracic sympathectomy obtained at base-line, and during L-NMMA and L-arginine infusion. L-NMMA infusion decreased blood flow in the denervated forarm but not in the innervated calf. This effect was reversed by L-arginine infusion.

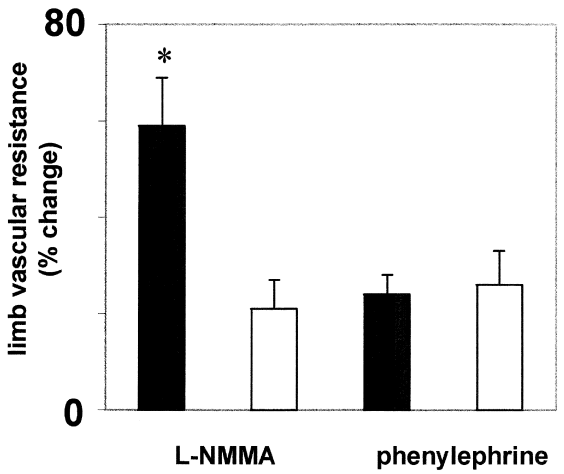

Fig. 2. Effects of L-NMMA and phenylephrine infusion on vascular resistance in the denervated forearm (closed bars) and the innervated calf (open bars) in four subjects having undergone thoracic sympathectomy for hyperhydrosis. L-NMMA infusion caused an almost 3-fold larger (*$P<0.01$) vasoconstriction in the denervated than in the innervated limb. This augmented vasoconstrictor effect in the denervated limb is specific for nitric oxide-dependent vasoconstriction, because the nitric oxide-independent vasoconstrictor phenylephrine had similar effects in the denervated forearm and the innervated calf.

in the denervated forearm is related specifically to denervation, and not to differential vascular effects of nitric oxide synthase inhibition in the forearm and the calf, as evidenced by the comparable vasoconstrictor effects of L-NMMA infusion in the control subjects. Moreover, the L-NMMA induced vasoconstrictor effects are related specifically to nitric oxide synthase inhibition, because these hemodynamic effects were promptly reversed by L-, but not by D-arginine infusion. Finally, the augmented vasoconstriction in the denervated forearm is specific for nitric oxide synthase inhibition, because the non-nitric-oxide-dependent vasoconstrictor phenylephrine caused comparable, not differential, vasoconstriction in the innervated and the denervated limb. These findings also indicate that the augmented L-NMMA-induced vasoconstriction in the denervated forearm can not be attributed to an augmented nonspecific vasoconstrictor responsiveness secondary to sympathectomy.

Previous findings in anesthetized, paralyzed animal preparations have shown that peripheral sympathectomy attenuates the pressor response to nitric oxide synthase inhibition [11], suggesting that inhibition of sympathetic vasoconstriction plays an important part in the regulation of vascular tone by nitric oxide. Our present findings challenge this concept, and indicate that one has to be cautious when extrapolating from findings in anesthetized, paralyzed, baroreflex-denervated animal preparations to conscious humans. Further studies are needed to examine the underlying mechanism(s) explaining these divergent findings.

In summary, this study provides the first evidence that sympathetic denervation potentiates the vasoconstrictor effects of nitric oxide synthase inhibition. We do not know yet the underlying mechanism(s) by which sympathetic innervation attenuates the L-NMMA induced vasoconstriction. It is possible that in denervated limbs, the contribution of nitric oxide to local vascular resistance regulation may be augmented, leading to augmented vasoconstrictor responses during L-NMMA infusion. Alternatively, in innervated limbs, neuronal, non-nitric-oxide-dependent vasodilator mechanisms may attenuate the vasoconstrictor effects of nitric oxide synthase inhibition.

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