Effects of chronic sympathetic denervation on endothelium-dependent cutaneous vasodilation in humans

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Charkoudian, Nisha, John H. Eisenach, John L. D. Atkinson, Robert D. Fealey, and Michael J. Joyner. Effects of chronic sympathetic denervation on endothelium-dependent cutaneous vasodilation in humans. J Appl Physiol 92: 685–690, 2002. First published October 5, 2001; 10.1152/japplphysiol.00758.2001.—In human skin, the vasodilator response to local heating includes a sensory nerve-dependent peak followed by a nadir and then a slower, nitric oxide-mediated, endothelium-dependent vasodilation. To investigate whether chronic sympathetic denervation diminishes this endothelium-dependent vasodilation, we studied individuals who had previously undergone surgical T2 sympathectomy (n = 9) and a group of healthy controls (n = 8). We assessed the cutaneous vascular response (laser-Doppler) to 30 min of local warming to 42.5°C on the ventral forearm (no sympathetic innervation) and the lower legs (sympathetic nerves intact). Lower body negative pressure (LBNP) was measured to confirm sympathetic denervation. During local warming in sympathectomized individuals, vascular conductance reached an initial peak at both sites [achieving 1.73 ± 0.22 laser-Doppler units (LDU)/mmHg in the forearm and 1.92 ± 0.21 LDU/mmHg in the leg]. It then decreased to a nadir in the innervated leg [to 1.77 ± 0.23 LDU/mmHg (P < 0.05)] but not in the sympathetomized arm (1.69 ± 0.21 LDU/mmHg; P > 0.10). The maximal vasodilation seen during the slower phase was not different between limbs or between groups. Furthermore, LBNP caused a 44% reduction in forearm vascular conductance (FVC) in control subjects, but FVC did not decrease significantly in sympathectomized individuals, confirming sympathetic denervation. These data indicate that endothelial function in human skin is largely preserved after sympathectomy. The altered pattern of the response suggests that the nitric oxide-dependent portion may be accelerated in sympathectomized limbs.

METHODS

Subjects

The protocol for this study was approved by the Institutional Review Board of the Mayo Clinic. Subjects were either individuals who had undergone upper extremity surgical sympathectomy (n = 9; 8 men, 1 woman) or healthy controls (n = 9; 8 men, 1 woman) (see Table 1 for subject characteristics). All sympathectomized individuals had undergone transthoracic endoscopic T2 sympathectomy for the treatment of palmar hyperhidrosis within 6 mo to 5 yr of the experiments (15). This resulted in interruption of sympathetic innervation to the upper thorax, arms, and hands. Sympathetic denervation was documented by the demonstration of anhidrosis of the upper extremities on a thermoregulatory sweat test done postoperatively (2). These individuals were otherwise healthy, although three were taking medications (amitriptyline, atenolol, and Claritin). These individual...
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sympathectomized</th>
<th>Control</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>32.7 ± 3.0</td>
<td>32.5 ± 2.8</td>
<td>0.97</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.7 ± 2.6</td>
<td>179.4 ± 2.8</td>
<td>0.94</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88.6 ± 5.2</td>
<td>75.7 ± 1.8</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE for sympathectomized individuals (n = 9) and control subjects (n = 8). The groups were similar in age and height; however, the sympathectomized group had significantly higher body weight than their control counterparts.

als’ cutaneous vasodilator responses did not appear different from those of the rest of the sympathectomized group and were therefore included in our analysis. Control subjects were healthy individuals who were age matched with sympathectomized individuals. Subjects gave written informed consent before participating.

Procedures

**Subject monitoring.** During the study, heart rate was monitored using a five-lead electrocardiogram. Arterial pressure was monitored noninvasively on a beat-to-beat basis by using a Finapres blood pressure monitor (model 2300, Ohmeda, Englewood, CO).

**Skin blood flow and local temperature.** Skin blood flow was measured as laser-Doppler flow (LDF) by using integrating LDF probes (Perimed Periflux System 5000, Stockholm, Sweden) on the ventral forearm and lower leg. The LDF probes were positioned on the skin in specialized holders that measured and control local temperature (Perimed).

**Lower body negative pressure.** We used lower body negative pressure (LBNP) to confirm interruption of reflex sympathetic innervation of the forearms in sympathectomized individuals. Subjects were in the supine position and sealed at the iliac crest level into an airtight box. Subjects were monitored using a Finapres blood pressure monitor (model 2300, Ohmeda, Englewood, CO).

**Forearm blood flow.** Forearm blood flow (FBF) was measured by using venous occlusion plethysmography with mercury-in-Silastic strain gauges and is expressed as milliliters per 100 milliliters of tissue volume per minute. During measurement of FBF, blood flow to the hand was excluded by inflation of a wrist cuff to 250 mmHg. The upper arm cuff collecting pressure was 50 mmHg, and both cuffs were inflated for 7.5 of 15-s intervals (4 flows/min).

**Protocol.**

The study was conducted in two parts. First, to assess cutaneous vasodilator responses to local warming in an innervated and a denervated limb, subjects were instrumented for local warming and measurement of LDF on the ventral forearm and the lower leg. Because all of the sympathectomies were bilateral, it was not possible to use a contralateral forearm for control measurements in the sympathectomized individuals. However, T2 sympathectomy only affects the upper extremities. Thus, in the sympathectomized individuals, the leg served as a control site with intact sympathetic innervation. Comparison of vasodilator responses between the arm and the leg of healthy controls allowed us to rule out any inherent differences between the arm and leg in the cutaneous vascular response to local warming. Local temperature at both sites was held at 33°C for a 10-min baseline period. Local temperature was then increased at both sites to 42.5°C over 1.5 min and held there for 30 min. Care was taken to always increase local temperature at the same slow rate, because rapid increases can engender a pain sensation that alters the mechanisms involved in the vasodilator response (8, 9). LDF and mean arterial pressure were sampled at 250 Hz and subsequently analyzed off-line (WinDaq, Dataq Instruments, Akron, OH).

Second, to confirm interruption of reflex sympathetic innervation of the arms, subjects were positioned in the LBNP chamber and were instrumented for the measurement of FBF as described above. After baseline measurements of FBF, the pressure in the box was decreased in 2-min steps to −10, −20, and −30 mmHg. FBF was measured during the last minute of each level of LBNP.

**Data Analysis.**

Cutaneous vascular conductance (CVC) was calculated as LDF/mean arterial pressure. Maximum CVC was assessed as the average of the last 3 min of the local warming period. CVC is expressed both as laser-Doppler units (LDU)/mmHg and as a percentage of the maximum value (% of maximum).

To analyze the apparent acceleration of the NO-dependent phase of the vasodilation in the sympathectomized group, the initial rapid vasodilation was compared with the lowest CVC value in the subsequent 5 min for each limb. This latter value was defined as the “postpeak” value, and it usually referred to the nadir after the initial peak. In the sympathectomized limbs, when there was no nadir, the lowest value during that time period was included, which was usually close to the peak value. Peak and postpeak values were 30-s averages and were compared by paired t-test. Within each group, baseline and maximal CVC was compared between the arm and leg by paired t-test. These values and responses to local warming were compared between groups by unpaired t-test and ANOVA, respectively.

FBF was divided by mean arterial pressure to derive an index of forearm vascular conductance (FVC). FVC values were averaged over the last minute of each level of LBNP. FVC responses to progressive LBNP were analyzed by one-way ANOVA with repeated measures. Statistical significance was accepted for P < 0.05.

**RESULTS.**

**Cutaneous Vascular Responses to Local Warming.**

No subject reported any sensation of pain at any time during local warming. In control subjects, CVC in both the arm and the leg showed a typical biphasic response to the 30-min local warming protocol. Figure 1A shows the response to local warming at the two sites in a representative individual from the control group. In sympathectomized individuals, however, the cutaneous vascular response to local warming was substantially altered. Figure 1B shows the responses in the arm and leg of a representative sympathectomized individual to local warming. As can be seen in the figure, the response in the sympathectomized arm was monophasic. The initial rapid rise in CVC was
followed immediately by the slower phase vasodilation, and no nadir was observed.

Maximum CVC was not significantly different between the forearm and leg in control subjects (forearm: 2.14 ± 0.23 LDU/mmHg vs. leg: 2.23 ± 0.22 LDU/mmHg; P > 0.05). In sympathectomized individuals, there was a trend for maximal vasodilation to be less in the sympathectomized arm (2.14 ± 0.24 LDU/mmHg) compared with the innervated leg (2.48 ± 0.21 LDU/mmHg), but this trend did not reach statistical significance (P = 0.09). Therefore, although our conclusions are not altered whether absolute or relative (% of maximum) units are used, the averaged data below are expressed both ways.

Baseline CVC was not significantly different between limbs or between groups (P > 0.05 for all comparisons). In the control group, baseline CVC in the forearm was 0.20 ± 0.03 LDU/mmHg, which corresponded to 10.24 ± 1.91% of maximum. In the leg, baseline CVC was 0.26 ± 0.06 LDU/mmHg (12.16 ± 2.56% of maximum). In the sympathectomy group, baseline CVC was 0.24 ± 0.04 LDU/mmHg (13.53 ± 3.80% of maximum) in the forearm and 0.30 ± 0.06 LDU/mmHg (12.71 ± 3.06% of maximum) in the leg.

Figure 2A shows the average peak and postpeak (nadir) values during the initial phase of the response to local warming in control subjects in the arm and the leg. These values are shown in absolute terms (LDU/mmHg). Corresponding values expressed in % of maximum are as follows. CVC decreased from initial peaks of 77.62 ± 3.05% of maximum (arm) and 73.46 ± 2.25% of maximum (leg) to postpeak values of 71.44 ± 2.97% of maximum (arm) and 65.00 ± 3.45% of maximum (leg) (P < 0.05 for both). Figure 2B shows the same values for sympathectomized subjects. As can be seen in the figure, in the sympathectomized arm, there was no difference between peak and postpeak CVC values (P = 0.18). In the leg, where sympathetic innervation was intact, the response was not different from that in control subjects, and a significant decrease in CVC followed the initial peak. Corresponding values for sympathectomized individuals expressed as % of maximum are as follows. CVC initial peak values were 80.91 ± 1.63% of maximum in the forearm and 77.46 ± 3.42% of maximum in the leg, and CVC postpeak values were 79.16 ± 1.25% of maximum in the forearm and 70.67 ± 4.62% of maximum in the leg.

**FVC Responses to LBNP**

In control subjects, FVC decreased progressively with each level of LBNP (P < 0.01; see Table 2). In sympathectomized individuals, there was no significant change in FVC at any level of LBNP, confirming effective interruption of reflex sympathetic control of blood flow in the forearms of these individuals (Table 2).

**DISCUSSION**

The main new findings from this study are that 1) endothelium-dependent cutaneous vasodilation to local warming was preserved after surgical sympathectomy in humans, and 2) this vasodilation appeared to be accelerated in sympathectomized limbs.

Minson et al. (9) recently demonstrated that the vasodilation to local warming of the skin in humans can be divided into two phases. The first phase is the initial peak, which is rapid and occurs within the first few minutes of local warming. This phase reaches ~80% of the maximal vasodilation to local warming, and it appears to be mediated by sensory nerves, probably via local release of vasodilator neuropeptides (9).

![Diagram](http://jap.physiology.org/)
This initial burst of sensory nerve activity and vasodilation is followed by a nadir in the vascular conductance response. The second phase of the vasodilation is slower, largely dependent on endothelial NO (8, 9), and usually plateaus around 20–30 min of heating (3, 8, 9).

The existence and activity of perivascular autonomic nerves appear to exert important trophic influences on vascular endothelial function. For example, chronic sympathectomy in rats decreased endothelial NO synthase expression in both aortic endothelial cells (1) and in the tail vasculature (17). Studies in animals also demonstrate diminished endothelium-dependent vasodilation after chronic sympathectomy. Endothelium-dependent relaxations to acetylcholine (5) and substance P (6) were inhibited in rabbit carotid artery rings after sympathectomy. However, non-endothelium-dependent vasodilation was not altered in this model (5, 6).

Table 2. Average forearm vascular conductance responses to LBNP (% of baseline)

<table>
<thead>
<tr>
<th>LBNP Level</th>
<th>Sympathectomized</th>
<th>Control</th>
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<tr>
<td>−10 mmHg</td>
<td>100.2 ± 3.7</td>
<td>77.6 ± 6.4*</td>
</tr>
<tr>
<td>−20 mmHg</td>
<td>99.3 ± 6.4</td>
<td>65.7 ± 5.3*</td>
</tr>
<tr>
<td>−30 mmHg</td>
<td>96.2 ± 7.4</td>
<td>56.2 ± 6.3*</td>
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Values are means ± SE for 9 sympathectomized individuals and 8 control subjects. LBNP, lower body negative pressure. Note that in control subjects all levels of LBNP resulted in significant vasoconstriction of the forearm, whereas in sympathectomized individuals there was no significant decrease in forearm vascular conductance at any level of LBNP. *P < 0.05 vs. baseline.

On the basis of this evidence, we hypothesized that the NO-dependent cutaneous vasodilator response to local warming would be diminished after chronic sympathectomy. Although there was a trend for decreased vasodilation in the sympathectomized limb, the skin of the sympathectomized limb still exhibited substantial vasodilation in response to local warming in all subjects. This suggests that endothelium-dependent vasodilation was preserved in sympathectomized skin. A major difference between the present study and the above-mentioned animal studies demonstrating altered endothelium-dependent vasodilation in vascular rings after sympathectomy (5, 6) is that we studied vasodilation in the microcirculation. Laser-Doppler measurement of skin blood flow is limited to the microcirculation within ~1-mm depth at the site of measurement (11). Taken together, these observations suggest that the interactions between autonomic nerves and the vascular endothelium differ between conduit arteries and the microcirculation. In this way, our study is consistent with previous work (10, 13) indicating differences in endothelium-dependent responses in resistance vessels compared with large conduit arteries.

As mentioned above, two major mechanisms have been identified in the cutaneous vasodilator response to prolonged local warming (7, 9): an initial, sensory nerve-dependent peak, followed by a slower vasodilation that is dependent on endothelial NO. It is possible that either or both of these mechanisms were altered to effect the shift from a biphasic to a monophasic vasodilation in the sympathectomized limbs (see Fig. 2). For example, sympathetic activity might have an
hibitory effect on the sensory nerve-mediated initial peak so that this initial vasodilation is prolonged when sympathetic activity is absent. Data from Pér- göla et al. (12) may provide some insight on this point. These investigators studied cutaneous vascular responses to local warming after two types of acute sympathectomy: antebrachial cutaneous nerve block or local administration of bretylium. Although those investigators did not specifically study the biphasic nature of the vasodilator response, inspection of the figures from that study shows that the initial peak, nadir, and slower second phase were all intact after both methods of acute sympathectomy (see Ref. 12, Figs. 3 and 6). This suggests that the altered pattern of response seen in the present study requires chronic sympathetic denervation. Furthermore, in the present study, the extent of the initial rapid vasodilation was not significantly different between limbs or between groups (see Fig. 2) and was comparable to previously reported values in healthy subjects [i.e., ~80% of maximum (9)]. Additionally, there was no apparent decrement in sensation after sympathectomy, indicating that sensory nerve function was probably unaltered. Therefore, it seems most likely that, in the present study, the NO-dependent phase was accelerated. This then probably evoked a faster increase in CVC such that the nadir after the initial peak was not seen.

The idea that NO-dependent vasodilation was accelerated by sympathectomy in the present study is consistent with the findings of Sartori et al. (16), who studied whole limb vasodilator responses to insulin infusion in individuals who had previously undergone surgical T2 sympathectomy. They reported that the NO-dependent vasodilation to insulin was accelerated in sympathectomized limbs, although the absolute extent of the vasodilation was not different between innervated and denervated limbs (16). Thus the existence of intact sympathetic innervation may have an inhibitory effect on NO-dependent vasodilation in the whole limb (16) as well as specifically in the skin (present study).

Potential Limitations

It is possible that individuals with palmar hyperhidrosis have microcirculatory control mechanisms that are fundamentally different from control individuals and that our findings of altered vasodilator responses in the sympathectomized limb were not due to a lack of sympathetic innervation per se. We addressed any such potential differences between the two groups by including a within-group comparison. The comparison of innervated vs. denervated limbs in the sympathectomized group strongly suggests that it was the lack of sympathetic innervation of the arm that altered the vasodilator response.

In the present study, laser Doppler values are presented both as absolute units (i.e., LDU/mmHg) and relative to the maximum attained during local warming (% of maximum). Although the trend for a decreased maximum CVC in sympathectomized limbs was not statistically significant, we analyzed the data both ways to ensure that no differences in interpretation would result with one or the other method. Importantly, the conclusions regarding the altered pattern of vasodilation are not different whether absolute or relative units are used.

In summary, surgical sympathectomy in humans resulted in important alterations in local mechanisms of cutaneous vasodilation during direct local warming of the skin. The absolute extent of the vasodilation was largely preserved, although there was a trend for this vasodilation to be decreased slightly. The NO-mediated portion of this vasodilation appears to have been accelerated after sympathectomy, which is consistent with previous studies of insulin-mediated, NO-dependent limb vasodilation after sympathectomy in humans (16). These interactions between reflex control and local vasodilator mechanisms in the human cutaneous circulation may have important implications for pathophysiological conditions in which the perivascular neural environment is altered.

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REFERENCES


