Role of sensory nerves in the cutaneous vasoconstrictor response to local cooling in humans

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Hodges GJ, Traeger JA 3rd, Tang T, Kosiba WA, Zhao K, Johnson JM. Role of sensory nerves in the cutaneous vasoconstrictor response to local cooling in humans. Am J Physiol Heart Circ Physiol 293: H784–H789, 2007. First published April 27, 2007; doi:10.1152/ajpheart.00323.2007.—Local cooling (LC) causes a cutaneous vasoconstriction (VC). In this study, we tested whether there is a mechanism that links LC to VC nerve function via sensory nerves. Six subjects participated. Local skin and body temperatures were controlled with Peltier probe holders and water-perfused suits, respectively. Skin blood flow at four forearm sites was monitored by laser-Doppler flowmetry with the following treatments: untreated control, pretreatment with local anesthesia (LA) blocking sensory nerve function, pretreatment with bretylium tosylate (BT) blocking VC nerve function, and pretreatment with both LA and BT. Skin local temperature was slowly reduced from 34 to 29°C at all four sites. Both sites treated with LA produced an increase in cutaneous vascular conductance (CVC) early in the LC process (64 ± 55%, LA only; 42 ± 14% LA plus BT; P < 0.05), which was absent at the control and BT-only sites (5 ± 8 and 6 ± 8%, respectively; P > 0.05). As cooling continued, there were significant reductions in CVC at all sites (P < 0.05). At control and LA-only sites, CVC decreased by 39 ± 4 and 46 ± 8% of the original baseline values, which were significantly (P < 0.05) more than the reductions in CVC at the sites treated with BT and BT plus LA (−26 ± 8 and −22 ± 6%). Because LA affected only the short-term response to LC, either alone or in the presence of BT, we conclude that sensory nerves are involved early in the VC response to LC, but not for either adrenergic or nonadrenergic VC with longer term LC.

In the longer term following this transient vasodilation, there is a net vasoconstriction, which is dependent in part on sympathetic vasoconstrictor function (5, 7, 10, 15, 21) and in part on the nitric oxide system (7, 21). Local cooling influences not only the nitric oxide synthase enzymes but also processes downstream of these enzymes in the nitric oxide vasodilator pathway (7, 21). The dependence of cooling-induced vasoconstriction on adrenergic function could be via translocation of α2C-adrenergic receptors to the vascular smooth muscle membrane due to Rho-kinase (1, 19) or an action of sensory nerves on sympathetic nerves (10), or some combination of these. The observation that the transient vasodilation is unmasked by either sensory or sympathetic nerve blockade suggested the possibility that local cooling stimulates the vasoconstrictor nerves via a sensory nerve pathway that communicates directly to the sympathetic vasoconstrictor nerves (10). That earlier interpretation is not consistent, however, with in vitro findings of cooling causing reduced synthesis and release of norepinephrine (20). Therefore, the role of sensory nerves in longer term local cooling, and whether they play a role in the adrenergic contribution to the cutaneous vasoconstriction with local cooling, has not been established. Therefore, in this study, we directly tested whether intact sensory nerves are required for the adrenergically dependent component of cutaneous vasoconstriction with local cooling.

METHODS

Subjects. The Institutional Review Board of the University of Texas Health Science Center approved this study. Participants were informed of the methods and risks before written consent was obtained. A total of six (4 male and 2 female) subjects participated in the study. All subjects were healthy nonsmokers, taking no medications. They refrained from alcoholic and caffeinated beverages for a period of at least 12 h before the study. The menstrual status was noted for the women participants but was not considered, because previous studies have shown the vasoconstrictor response to local cooling to be unaffected by reproductive hormones (4). Measurements. Skin blood flow was monitored by laser-Doppler flowmetry (Moor Instruments, Axminster, UK) on the ventral aspect of the forearm and expressed as laser-Doppler flow (LDF) (8). LDF measurements have been shown to be exclusive to the skin and are not affected by underlying skeletal muscle blood flow (17). The LDF probes were housed in custom-built metal Peltier cooling/heating probe holders (7, 10, 21). These devices, which allow local temperature (Tloc) of the skin surrounding the LDF measurement site to be controlled independently of whole body skin temperature (Tsk), cover 6.3 cm² with the exception of a small aperture (0.28 cm²) in the center of the holder to enable blood flow measurement. Local skin temperature was assessed by a thermocouple placed between the skin and

THERMOREGULATION IN HUMANS is achieved, in part, by changes in skin blood flow. During exposure to extreme cold, skin blood flow can be almost negligible. By contrast, during hyperthermia, skin blood flow can account for upwards of 60% of cardiac output (9, 16). Both reflex responses to changing internal and skin temperatures and local responses to direct cooling or heating of the skin can contribute to these large changes in skin blood flow.

Local skin cooling causes an immediate vasoconstriction, followed by a further fall in skin blood flow as cooling continues. In the presence of sensory nerve blockade or vasoconstrictor system inhibition (either presynaptically with bretylium tosylate or postsynaptically with yohimbine and propranolol), local cooling evokes an initial transitory vasodilation during the first 5 min (10, 15, 19, 21). This vasodilation is best seen under those conditions and can be minimized by reducing the rate at which cooling is performed, showing an influence of the rate of cooling on the phenomena (21).

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probe holder. Mean arterial pressure (MAP) was measured continuously at the left middle finger by photoplethysmography (Finapres, Ohmeda, WI) (14). The ratio of LDF to MAP was calculated as an index of cutaneous vascular conductance (CVC). Water-perfused suits, which covered the entire body except the head, hands, feet, and the experimental forearm, were used to control Tsk. Tsk was calculated as the weighted average of temperature readings from thermocouples at the following sites: chest, upper back, lower back, abdomen, thigh, and calf (18). To minimize tonic vasoconstrictor activity, whole body Tsk values were kept at 36°C, except for a 3-min period of aggressive whole body cooling to 31°C to test for adequate blockade of vasoconstrictor nerve neurotransmitter release (11). Whole body Tsk was kept at the slightly warm temperature of 36°C because we wanted to remove as much of the tonic release of norepinephrine as possible, without increasing Tsk so much as to increase blood flow. Tskc for the 6.3-cm² area surrounding LDF sites was controlled at 34°C during the baseline period and was slowly reduced to 29°C (−0.33°C/min) during the local cooling protocol. All variables were sampled at 1-s intervals and stored as 20-s averages for off-line analysis.

Blockade of transmitter release from vasoconstrictor nerve terminals was achieved through iontophoresis of bretylium tosylate (BT; Schweizer Hall, South Plainfield, NJ) at a concentration of 10 mM at 250 μA for 10 min to a 0.64-cm² area of skin (11). Sensory nerve blockade was achieved through the use of a topical local anesthetic

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<th>Site 1: Control</th>
<th>Baseline 10 min</th>
<th>Slow LC 15 min</th>
<th>Tloc stabilize 15 min</th>
<th>Rewarm 15 min</th>
<th>WBC 3 min</th>
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<td>Tskin</td>
<td>36°C</td>
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<th>Site 2: BT</th>
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<th>Tloc stabilize 15 min</th>
<th>Rewarm 15 min</th>
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<th>Tloc stabilize 15 min</th>
<th>Rewarm 15 min</th>
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<td>Tloc</td>
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<th>Slow LC 15 min</th>
<th>Tloc stabilize 15 min</th>
<th>Rewarm 15 min</th>
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Fig. 1. Protocol used to verify whether sensory nerves were involved in the vasoconstrictor response to local cooling at 4 sites: 1, untreated control; 2, treated with bretylium (BT); 3, treated with local anesthesia (LA); and 4, treated with both BT and LA. After 10 min of baseline measures, local cooling commenced at a rate of −0.33°C/min, reducing local temperature (Tloc) from 34°C to 29°C over a 15-min period. Once local temperature of 29°C was reached, it was maintained at this level for a further 15 min to allow stabilization of cutaneous vascular conductance (CVC). Warming was performed slowly (1°C/min) back to 34°C and held at this temperature for 10 min. Following this, aggressive whole body cooling (WBC) from 34 to 31°C was performed for 3 min to confirm adequate vasoconstrictor nerve blockade with BT. LC, local cooling; Tskinc, whole body skin temperature.

Fig. 2. Representative responses from 1 subject to the protocol outlined in Fig. 1. Note the pronounced transitory vasodilation at sites 3 and 4, both of which were treated with LA. This was despite the slower local cooling protocol, which showed virtually no response during this time period at the BT-treated sites. Importantly, there were larger and similar degrees of vasoconstriction at the control and the LA-only sites (sites 1 and 3) compared with the BT-treated sites (sites 2 and 4) at the end of cooling. This suggests no linkage between sensory nerves and vasoconstrictor nerves.
cream (Fougera, Melville, NY) consisting of lidocaine (2.5%) and prilocaine (2.5%) (10). The cream was applied to an area of 8 cm² under an occlusive dressing, where it was left for a minimum of 2 h. The area was tested for sensory loss both before the study began and at its completion.

Protocol. The protocol was performed to test whether functional sensory nerves are required for the adrenergic portion of the vasoconstrictor response to local cooling. Four sites were randomly selected on the ventral aspect of the forearm. The treatment of those sites and the protocol are outlined in Fig. 1. Site 1 was an untreated control; site 2 was treated with BT to inhibit the release of norepinephrine from vasoconstrictor nerves; site 3 was treated with topical local anesthetic to inhibit sensory nerve function; and, finally, site 4 was treated with the combination of BT and local anesthetic to inhibit both systems. We reasoned that if a significant portion of the vasoconstrictor to local cooling was due to sensory nerve stimulation of vasoconstrictor nerves, then the degree of vasoconstriction at sites without functional adrenergic nerves, without functional sensory nerves, or lacking both would be approximately equal and all would have responses reduced from those seen at control sites. After a minimum of 2 h had elapsed, allowing maximum anesthetization, those sites (sites 3 and 4, Fig. 1) were tested by pin prick to confirm that they were anesthetized. Subjects were then instrumented as described above. After the subject had been lying quietly for a minimum of 30 min, baseline measurements were recorded for 10 min. After a 3-min period of whole body cooling to test for the adequacy of vasoconstrictor nerve blockade by BT (sites 2 and 4, Fig. 1), Tsk was returned to 36°C. This also verified whether vasoconstrictor nerve function remained intact in the presence of local anesthesia. Tloc at all sites was maintained at 34°C throughout this portion of the study. Next, Tloc was reduced to 29°C, at a rate of −0.33°C/min, for the next 15 min. This slow rate was used to lessen the early transitory vasodilation (21). Once CVC had stabilized (~20 min), Tloc was returned to 34°C. Whole body Tsk was again cooled to 31°C to confirm inhibition of vasoconstrictor nerve activity with BT treatment. A reduction in CVC of >10% at BT-treated sites was taken to indicate inadequate blockage, and data from such sites were excluded from further analysis (11). Finally, sites treated with local anesthesia were tested, by pin prick, to ensure they were still anesthetized. No sites had to be excluded on these bases in this study.

Statistical analysis. We evaluated the response to local cooling at the four sites treated as follows: untreated control, no vasoconstrictor nerve function, no sensory nerve function, and both vasoconstrictor nerve function and sensory nerves blocked. This evaluation came from the change in CVC (normalized to baseline) during the first 5 min of local cooling (early local cooling) and at the end of the local cooling protocol (5 min of data, ~15 min after the local cooling ramp had finished and CVC had stabilized). Analysis was performed using paired statistics or, when appropriate, repeated-measures ANOVA. Statistical significance was assumed when P < 0.05. Power analysis indicated that a minimum of six subjects would be required to detect a P < 0.05 with 95% power.

RESULTS

Figure 2 displays representative responses to the protocol from all four sites. Note the pronounced vasodilation early during the local cooling at both sites with local anesthetic treatment (sites 3 and 4). Importantly, after 25 min of local cooling, the site with only local anesthetic (site 2) displayed a similar degree of vasoconstriction as shown by the control site (site 1). The site treated with both local anesthesia and BT had a reduction in CVC similar to that at the BT-only sites. Those two sites failed to achieve the same degree of vasoconstriction as the other two sites. Because of this biphasic response at sites treated with local anesthesia, results are compared for the first 5 min and the final 5 min of local cooling.

The above results seen in one subject were representative for the group. Figure 3 shows the mean percentage changes in CVC in response to local cooling for all sites from all six subjects during the early cooling phase. CVC levels at the control and BT-treated sites were not significantly changed (5 ± 6 and 6 ± 8%, respectively; P > 0.05). By contrast, there were large and significant (P < 0.05) increases in CVC at sites with local anesthesia and local anesthesia plus BT, with CVC increasing by 64 ± 55 and 42 ± 14%, respectively (P < 0.05), which did not differ significantly from each other (P > 0.05).

The changes in CVC observed later during local cooling for all six subjects are shown in Fig. 4. All sites showed a
significant ($P < 0.05$) vasoconstriction from the precooling baseline, regardless of treatment; nevertheless, CVC reductions at the control and local anesthesia only treated sites were greater ($−39±4$ and $−46±8$; $P < 0.05$) at the end of local cooling than those at the BT and BT plus local anesthesia sites ($−26±8$ and $−22±6$%, respectively; $P < 0.05$). The reductions in CVC at the local anesthesia-only sites did not differ significantly from those at the untreated sites ($P > 0.05$), and the responses at the BT-only sites did not differ significantly from those at the BT plus local anesthesia sites ($P > 0.05$).

**DISCUSSION**

The major findings from this study are that 1) local anesthesia reverses the initial vasoconstrictor response with slow local cooling to one of vasodilation; 2) local anesthesia does not affect the vasoconstrictor response to longer term cooling; and 3) blockade of vasoconstrictor nerves does not affect the initial vasoconstrictor response to slow local cooling but reduces the longer term vasoconstrictor response. The implications of these findings are that sensory nerves participate in the cutaneous vasoconstrictor response to the initiation of local cooling but not in the net vasoconstriction when local cooling is sustained ($>10$ min). In addition, intact adrenergic function is required for a significant portion of the vasoconstrictor response to prolonged local cooling ($>10$ min). Thus there is a mechanism independent of sensory nerves that links local cooling to adrenergic function, at least in terms of the late vasoconstrictor response to local cooling. This conclusion is supported by results from the BT sites and local anesthesia plus BT sites having similar degrees of vasoconstriction. At BT-treated sites, only the nonadrenergic component of the local cooling response is functional (7, 21). If that portion were dependent on sensory nerve activation, one would expect a smaller vasoconstriction at sites treated with local anesthesia.

Lindblad et al. (12) also examined the influence of the infiltration of local anesthetic in the response in finger blood flow to brief (30 s) local cooling. They found such treatments block completely the vasoconstrictor response. The short duration and rapidity of the cooling in that study precluded comparison with the current study, however. Furthermore, it is not known whether dorsal finger skin and forearm skin have the same mechanisms for locally induced vasoconstrictor responses. For example, the authors did not report a vasodilator response to rapid local cooling in locally anesthetized skin, as was seen in forearm skin in the current study and earlier (10).

The rate of cooling has other important roles, as well. For example, the vasoconstriction seen early in local cooling in previous studies (10, 21) was not seen until later in the procedure in the current study, but the degree and rate of cooling were less. Also, the initial vasodilation seen at sites pretreated with BT is rate dependent, requiring rapid cooling for the vasodilator phase to be pronounced (21). It is also

![Fig. 5. Model of the mechanisms contributing to the cutaneous vascular responses to local cooling. Local cooling has an inhibitory effect on sympathetic nerve function and the nitric oxide pathway, affecting both the nitric oxide synthase enzyme activity and the utilization of nitric oxide. Local cooling also has an excitatory role in stimulating Rho-kinase (ROCK), eliciting the translocation of $\alpha_{2C}$-receptors. Under conditions of sensory nerve blockade, sympathetic nerve inhibition and/or $\alpha$- and $\beta$-receptor antagonism, a transitory dilation is apparent early in the cooling process, which is succeeded by a later nonneural vasoconstriction. The mechanism for the early vasodilation is unknown, yet it is apparent that sensory nerves play a key role. The later nonneural vasoconstriction is via the nitric oxide vasodilator pathway and the translocation of $\alpha_{2C}$-receptors, both of which are heavily dependent on ROCK activation. Not shown are the effects of local cooling on noradrenaline release and synthesis, or vascular smooth muscle function. +, Excitatory stimuli; −, inhibitory stimuli.](http://ajpheart.physiology.org/)

**A** Early-phase local cooling

- Sympathetic nerve
- $\alpha_{2C}$
- Early Dilator
- Local Cooling
- Cutaneous vessel
- Sensory nerve
- Cold receptor

**B** Late-phase local cooling

- Sympathetic nerve
- $\alpha_{2C}$
- Local Cooling
- Cutaneous vessel
- Nitric Oxide Synthase
- ROCK
important to note that in the earlier study (10), an initial vasodilation was noted if either sensory or sympathetic function was inhibited; that study involved relatively rapid cooling. With slow local cooling, a clear difference was revealed in which sites treated with local anesthesia showed a vasodilation, but sites treated with BT only did not. This observation supports our conclusion of a separation between sensory and sympathetic contributions to the responses to local cooling. Furthermore, if repeated bouts of local cooling are applied, the initial vasodilation, at least at the BT-treated sites, decreases in magnitude (21). This, plus the transient nature of the response and its rate dependency, suggests that the vasodilator phenomenon might be mediated through a depletable intermediate. Regardless of this speculation, ascertaining the basis of this response remains problematic, and its origin remains a mystery.

The vasoconstriction elicited from adrenergic-dependent mechanisms would appear most likely to be due to cooling causing a translocation of $\alpha_{2C}$-receptors from the Golgi apparatus to the vascular smooth muscle cell surface by Rho-kinase (1, 2, 19). This effect on adrenergic receptors occurs despite reduced synthesis of norepinephrine from sympathetic nerve endings and a general inhibition of the contractile process (3, 6, 20). As such, the vasoconstriction that occurs in response to the lower local temperatures might be mediated entirely by Rho-kinase through the translocation of $\alpha_{2C}$-receptors. If, indeed, local cooling decreases synthesis and release of norepinephrine in vivo as it does in vitro, then the adrenergic-dependent portion of the vasoconstriction to local cooling would be entirely due to an increased number of $\alpha_{2C}$-receptors. In that case, one might speculate that the increased number of $\alpha_{2C}$-receptors more than balances any reductions in transmitter synthesis and release and, furthermore, that tonic transmitter release is necessary to stimulate those receptors. This is, however, speculation and requires further investigation.

Figure 5 shows our current working model of how local cooling exerts its effects on the cutaneous circulation. It is based on this and previous studies from our laboratory and those of others. Early in the local cooling process (Fig. 5A), sensory and sympathetic nerves are stimulated to cause a vasoconstriction. This is reversed if either of these systems is inhibited, unmasking a transitory vasodilation. Later in the local cooling process (Fig. 5B), nitric oxide synthase activity is reduced, as is the release of transmitter from sympathetic nerves; there is also a translocation of $\alpha_{2C}$-receptors to the cell surface. The reduction in nitric oxide activity and the translocation of $\alpha_{2C}$-receptors appears to be mediated by Rho-kinase (1, 19).

The current study has furthered our knowledge specifically of the involvement of sensory and vasoconstrictor nerves in producing a vasoconstrictor response and of their roles in the both the early and late phases of vasoconstriction during local cooling. It is now obvious that for longer term cooling, the vasoconstrictor response to local cooling occurs independently of sensory nerves. It is apparent, though, that sensory nerves, and to a lesser extent, vasoconstrictor nerve function, inhibit the early vasodilation to local cooling. However, the rate of cooling influences how pronounced this early vasodilation is under conditions of vasoconstrictor nerve blockade, whereas it appears irrespective of the rate of cooling in the presence of local anesthesia.

The specificity of the local anesthesia acting only on the sensory nerves and not affecting the vasoconstrictor nerves was again confirmed (10). Intuitively, it would be expected that the combination of lidocaine and prilocaine would interfere with nerve conduction of all small myelinated and unmyelinated fibers; nevertheless, that does not appear to be the case. This might be due to sensory nerves being more superficial than the vasoconstrictor nerves. The topical application of anesthesia would require a greater distance for the diffusion of these substances to the vasoconstrictor nerves.

In summary, we have confirmed that the longer term vasoconstrictor response to local cooling can be completely achieved without functional sensory nerves. Interestingly, although intact sensory nerves are not required for a complete vasoconstrictor response to local cooling in the cutaneous circulation, sensory nerve function is required for vasoconstriction to occur early in the local cooling process. Without functional sensory nerves, a transient vasodilation is unmasked even during slow local cooling. However, the current study shows clearly that the involvement of sensory nerves does not induce an axon-reflex stimulation of release of norepinephrine from sympathetic nerves. On the basis of work by others, the adrenergic portion of the response to local cooling would appear to involve largely a postsynaptic translocation of $\alpha_{2C}$-receptors mediated by Rho-kinase (1, 19). It would be most likely that the effects of local cooling via translocation of $\alpha_{2C}$-receptors would also be dependent on the tonic release of norepinephrine. In the present study, that should have been low, because whole body $T_{sk}$ was kept at a slightly warm (36°C) temperature throughout. We do not know, however, if all norepinephrine release had been eliminated.

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REFERENCES