The influence of topical capsaicin on the local thermal control of skin blood flow in humans

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The control of the human cutaneous circulation includes both neural and local elements. In nonglabrous skin, neural elements are comprised of sympathetic noradrenergic vasoconstrictor nerves and active vasodilator nerves (17). These neural systems are responsible for the efferent portion of the reflex control of skin blood flow, and both are known to participate in a variety of homeostatic reflexes. Local factors also affect control of the cutaneous circulation. For example, the local temperature of the skin is one of the determinants of skin blood flow (16, 35). Local cooling can reduce skin blood flow essentially to zero, and local warming can maximally vasodilate the region being warmed (1, 16, 35, 36). Hence, the full range of skin blood flow can be achieved by local temperature changes. How local cooling or warming causes vasoconstriction or vasodilation, respectively, is not fully understood. Local effects of temperature are known to affect the responsiveness of vascular smooth muscle to sympathetic stimulation during both local warming and local cooling (38). There is strong evidence favoring an alteration in postsynaptic α-adrenergic receptor function with local cooling (10, 38). It also appears that either an axon reflex from cold receptors or more direct effects of local cooling on sympathetic vasoconstrictor nerves causes the release of neurotransmitter from noradrenergic nerve terminals, resulting in vasoconstriction (9, 23, 30, 31).

The mechanisms of the vasodilator response to direct local warming of the skin are not as well understood. During local warming, it is likely that heat-sensitive sensory nerves are involved in an axon reflex such that warming of the skin initiates the vasomotor response through activation of afferent nerves (2, 7, 22). The vasodilation induced by direct local warming is nitric oxide dependent but is not dependent on vasoconstrictor or active vasodilator nerves (20, 21, 31). Also, the effects of direct local warming have been demonstrated to spread beyond the area heated (7, 26). Rather than this vasodilation being due to any direct effects of local heating on the vascular smooth muscle per se, a significant body of evidence supports the hypothesis that local warming stimulates heat-sensitive afferent nerves, which then cause vasodilation through an axon reflex (26).

The sensation of heat in human forearm skin is detected by two populations of afferent nerves: warm receptors and mechano-heat-sensitive nociceptors (3, 11, 12, 26). Hallin et al. (12) and Green and Cruz (11) found that warm receptors are sparse in the human forearm and that areas as large as 4.8 cm² were without warm receptor innervation. Thus the dominant sensory modality for heat in the human forearm is...
likely the heat-sensitive nociceptor. Heat-sensitive nociceptors transduce sensation by the vanilloid receptor (VR-1) (4), which can be activated by capsaicin. Thus capsaicin can serve as a means of stimulating heat-sensitive nociceptors without direct heating.

We questioned whether heat-sensitive nociceptors might play a role in the cutaneous vasodilator response to local skin heating. This question arose from the temporal association between warmth perception and vasodilation during local warming. This possibility was further supported by preliminary observations we made with applications of topical capsaicin. Capsaicin stimulates heat-sensitive nociceptor afferent neurons (3, 13, 14) but has no effects on warm receptor activity (3). In preliminary studies, we noted similarities in the cutaneous vascular responses to topical capsaicin and direct local warming in that both caused the sensation of heat, albeit via separate modes of stimulation. Also, capsaicin treatment and local warming of the skin were both associated with cutaneous vasodilation at the site of treatment. Lastly, neither the responses to local heating nor to capsaicin appear to involve the sympathetic active vasodilator system (5, 20, 31). These similarities and observations led us to test the hypothesis that activation of heat-sensitive nociceptors was involved in the cutaneous vascular responses to local warming of the skin. We took advantage of the ability to stimulate heat-sensitive nociceptors chemically, with topical capsaicin cream, or thermally, with direct local heating.

METHODS

This study was approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio. The study was conducted in three parts. In part I, we examined how topical capsaicin affected the relationship between local skin temperature and skin blood flow. In part II, we tested whether the degree of vasodilation accompanying chemical stimulation of warmth perception differed from that accompanying thermal stimulation at the same perceived temperature. In part III, we tested whether blood flow was significantly different at untreated sites perceived to be the same temperature. A total of 15 subjects was involved, aged 21–51 yr, all of normal height, weight, and health. All were nonsmokers. After being interviewed and briefed about the protocol, each subject provided voluntary written consent to participate.

Part I. This portion included five healthy subjects (3 females, 2 males). One hour before the study, capsaicin cream (0.075%; CapzasinHP, Thompson Medical) was applied to a 12-cm² area of forearm skin. At the time of the study, the subject lay supine on an examination table with the arm supported at the wrist and elbow. A combination Peltier cooling/heating device with a recessed area for a laser Doppler blood flow probe was applied to the capsaicin-treated area (29, 30). A second, identical device was applied to a control site 10 cm from the capsaicin-treated site. These combination probe holder/temperature controllers cover exactly the area of capsaicin treatment except for a 0.16-cm² center area through which skin blood flow was monitored.

Skin blood flow was measured continuously by laser-Doppler flowmetry (Laser Flo, Vasamedics, St. Paul, MN) and expressed as laser Doppler blood flow (LDF) (15, 29, 34).

Blood pressure was measured by the Penaz method via a finger cuff (Finapres, Ohmeda, CO). Mean arterial pressure, obtained by electrical integration of the continuous blood pressure signal, was used with LDF to calculate cutaneous vascular conductance (CVC).

The protocol for part I, illustrated by Fig. 1, began with 15–20 min at a local temperature of 30°C at the two sites. The sites were then cooled in 2°C steps to 19–20°C over 15–20 min. For 5 min, both sites were maintained at that minimum temperature. Thereafter, the sites were simultaneously warmed in 2°C steps until achieving a local temperature of 42°C. Local temperature was held at each temperature for 5 min. In preliminary studies, skin blood flow reached a steady state in ~3 min to small changes in local temperatures below 42°C. Maximal CVC was taken as the level of blood flow achieved at 42°C local temperature (16, 36).

Part I included a second series of experiments in which capsaicin of two concentrations was applied to different areas of forearm skin [0.075 (as above) and 0.025%; Zostrix, Gen-derm]. The protocol and measurements were the same as those described above, except that the control site was replaced by a site at which 0.025% capsaicin was applied on one forearm site at the time the 0.075% capsaicin cream was applied to the other forearm site.

Data analysis for part I involved comparison of the relationship of LDF to local temperature between untreated and...
capsaicin-pretreated sites. This comparison was by paired analysis of the local temperatures at which LDF achieved 50% of its maximal response to local heating. Effects of the concentration of capsaicin were similarly analyzed.

**Part II.** We noted in part I that local cooling suppressed both vasodilation and the sensation of warmth at capsaicin-treated sites. Furthermore, on rewarming, it was uniformly seen that the onset of marked vasodilation occurred simultaneously with the first perception of local warmth, suggesting an association between warmth sensation and the vascular effects of warming.

To test this question further, capsaicin (0.025%) was applied to a 12-cm² area of forearm skin 1 h before the study in each of seven healthy subjects (4 females, 3 males). At the time of the study, subjects were instrumented, as in part I, with laser Doppler probe holders/heater-cooler units applied to the capsaicin-treated site and to a corresponding untreated site on the other forearm. It is important to note that as in part I, the area of local temperature control corresponded to the area of capsaicin application. Blood pressure, skin blood flow, and local temperatures were monitored as in part I.

The protocol for part II, illustrated by Fig. 2, involved matching the perception of temperature, our index of heat-sensitive nociceptor activity, between capsaicin-treated and untreated sites. To do this, both sites were cooled initially to 19–21°C, which removed any perception of warmth at both sites. After 5–10 min, both sites were warmed to 29°C, which was just below the threshold for perception of warmth at either the control or the capsaicin-treated site. The capsaicin-treated site was then slowly warmed until the subject noted the sensation of warmth. That temperature was held while the control site was slowly warmed until the subject considered the two sites to have the same temperature. CVC at each of the two sites was noted at that time. The capsaicin-treated site was then warmed by an additional 2°C, and the control site was then warmed until, again, the subject perceived the two sites as having the same temperature. This procedure was repeated for a total of three to four local temperatures per experiment. At no time did any of the subjects report that the direct heat stimulus was painful. First, analysis consisted of normalization of blood flow at each site to the maximum blood flow at that site achieved by local warming to 42°C (16, 36). We then compared blood flow between capsaicin-treated and untreated sites at equal actual temperatures and at equal perceived temperatures. Statistical analysis of these comparisons was made by repeated-measures ANOVA with planned contrasts.

**Part III.** Five male subjects were instrumented as described above for control of whole body and local skin temperature. Skin blood flow and blood pressure were measured as described above. This protocol was identical to that in part II, except that neither site of blood flow measurement was treated with capsaicin. Thus part III was designed to find whether CVC from untreated sites on different arms was similar at temperatures that were perceived to be the same. CVC values from this study were analyzed by ANOVA.

**RESULTS**

**Part I.** Figure 1 shows the results from part I for one subject. Initially, the blood flow at capsaicin-treated sites was higher than at control sites. With local cooling, blood flow at both sites fell and was not distinguishable statistically at the lowest local temperature (P > 0.05). As the sites were rewarmed, blood flow at both sites increased slowly at first, then a temperature was reached at which blood flow began to increase sharply, which always occurred at the capsaicin-treated site at a lower temperature than that at the control site. As shown in Fig. 3, the relationship of LDF to local temperature was shifted to the left and lower temperatures by capsaicin pretreatment. The local temperature at which LDF achieved 50% of maximal averaged 6.0 ± 0.8°C lower at capsaicin-treated sites than at control sites (P < 0.05).

The effect of capsaicin on the relationship of LDF to local temperature was related to the concentration of the capsaicin in the cream. The local temperature required for vasodilation to 50% of maximal averaged 3.1 ± 0.7°C lower at the site with the higher (0.075%) than at the site with the lower (0.025%) capsaicin concentration (P < 0.05).

Invariably, LDF and CVC at capsaicin-treated sites followed a different relationship to local temperature during the initial cooling than during the subsequent rewarming. This hysteresis in the relationship is shown in Fig. 4. It was uniformly the case that the initial cooling from normothermia was associated with higher levels of CVC at a given level of local temperature than during the subsequent rewarming (P < 0.05).
and that such a hysteresis was not seen at control sites ($P > 0.05$). At a local temperature of 29°C, CVC at the capsaicin-treated site was 44.3 ± 8.4% maximum during cooling and 8.4 ± 2.0% maximum during the subsequent rewarming. A significant difference in CVC values at the capsaicin-treated sites was detected at local temperatures of 27 and 25°C with CVC values being 27.9 ± 5.3 and 17.0 ± 2.9% during cooling and 5.5 ± 2.0 and 4.5 ± 1.6 during rewarming, respectively. A significant difference in CVC values was not detected between local cooling and subsequent rewarming at local temperatures of 23 and 21°C ($P < 0.05$). CVC values were also significantly different between capsaicin-treated and control sites during the initial cooling phase ($P < 0.05$). For example, at local temperatures of 29°C, CVC values averaged 8.6 ± 1.8% maximum at control sites with capsaicin-treated sites averaging 44.3 ± 8.4% ($P < 0.05$ between sites). During rewarming at 29°C, CVC values at control and capsaicin-treated sites were 5.8 ± 1.8 and 8.4 ± 2.0% maximum, respectively ($P < 0.05$ between sites).

We also noted in part I that the local temperature at which warmth was first perceived (as reported by the subject) was also the local temperature at which CVC began to rise sharply. This was true both for the capsaicin-treated and control sites and led to part II.

Part II. Results for part II are illustrated in Fig. 5. The data in Fig. 5 were taken from times when temperature at the two sites was perceived by the subject to be equal (see Fig. 2). Because they were at the same perceived local temperatures, they were necessarily at different actual temperatures, with the untreated sites being at temperatures averaging 2.2 ± 0.4°C greater than at capsaicin-treated sites ($P < 0.01$). This difference is shown by the CVC-local temperature relationships indicated by lines A and C in Fig. 5. When the data from the capsaicin-pretreated sites are plotted as a function of their perceived temperature, i.e., the simultaneous temperature at the untreated sites (Fig. 5, line B), the difference between the CVC-local temperature relationships for the untreated and capsaicin-treated sites diminishes (lines B and C). At none of the times when the perceived temperatures were the same...
did the values for CVC, expressed as a percent of the maximum, differ statistically between untreated and capsaicin-treated sites ($P > 0.1$).

**Part III.** Figure 6 illustrates the response in CVC from sites perceived to be the same local temperature. ANOVA did not detect a significant difference in CVC at untreated sites when they were perceived to be the same ($P > 0.05$). Regression of the local temperature data when the sites were perceived to be the same found a slope of $1.002 (\pm 0.005)$ and $R^2 = 0.95$ (see Fig. 6).

**DISCUSSION**

The major new finding from this study is that cutaneous vasodilation accompanying direct local skin warming correlates better with the sensation of heat than it does with actual skin temperature. The suggestion from this study is that local warming-induced vasodilation is mediated by heat-sensitive nociceptors. In part I, we observed that pretreatment with capsaicin shifted the relationship of vasodilation to local warming and that this effect is dose dependent. Furthermore, in part I, we noted that a correlation existed between the first sensation of nonnoxious heat and the onset of cutaneous vasodilation. In part II, we observed the same degree of vasodilation (similar blood flow) at sites with different actual temperatures but similar heat sensation. Cutaneous blood flow increases induced by combined chemical and thermal stimulation were not significantly different from those induced through only thermal stimulation, when the sensation of heat was the same. In part III, we found no significant difference in CVC values or local temperatures from untreated sites on different arms warmed to the same perceived temperature. These findings led us to the conclusion that an important part of the mechanism for the vasodilator response to local warming includes activation of heat-sensitive nociceptors.

In general, neural and local mechanisms are considered as independent means of control in the circulation. That the local environment of the nerve-vascular smooth muscle junction can influence efferent neural function is now well accepted in a variety of tissues and organ systems (6, 33, 37). More recently, it has been found that responses to local stimuli by the human cutaneous circulation are dependent on neural elements. That is, the relationship between local and neural control mechanisms is more than simply modulatory. For example, the cutaneous vasoconstrictor response to local cooling is dependent on intact sympathetic nerves (30, 31) and on the availability of $\alpha$-2 adrenoceptors (9, 24). Currently, available findings indicate that local cooling of the skin both stimulates transmitter release from vasoconstrictor nerve terminals (30, 31) and increases postsynaptic $\alpha$-2 receptor affinity for norepinephrine (10, 27, 38). Both of these elements of the remote sympathetic control of the cutaneous circulation appear to be required for the response to local skin cooling.

The response of the cutaneous circulation to local warming does not appear to require elements of sympathetic vasoconstrictor or vasodilator nerves. Local heating of the skin can cause a marked increase in blood flow with the potential for complete relaxation of cutaneous resistance vessels and a maximal vasodilation (1, 16, 36). This degree of vasodilation is not achievable by modulation of vasoconstrictor nerve...
function. Neither sympathectomy (32), nerve block (8), stellate ganglion block (39), nor inhibition of neurotransmitter release from adrenergic nerve terminals (19), all of which raise skin blood flow by inhibiting or eliminating vasoconstrictor nerve function, will cause this degree of cutaneous vasodilation. Furthermore, presynaptic blockade of the cutaneous neurogenic active vasodilator system does not eliminate or obviously affect the vasodilator response to local heating (21). These observations effectively eliminate any major role for sympathetic efferent nerves in the cutaneous vasodilator response to local heating. Similarly, the dilator effects of topical capsaicin do not appear to involve sympathetic nerves (5).

Several have noted that the vasodilator response to direct skin heating extends beyond the heated area, suggesting a neurogenic component (6, 25). Brief heat stimuli were found to cause vasodilation at an 8-mm distance from the heated site, but only at temperatures considered to be nociceptive, whereas nociceptive sensations and vasodilation were not as closely correlated if the warm stimulus was more sustained (26). In either case, the data clearly support the role of an axon reflex from heat-sensitive nociceptors in mediating increases in blood flow up to 8 mm from the site of heating. However, information is lacking regarding the mechanisms of vasodilation at the site of heating during nonnociceptive stimulation. Lynn et al. (25) demonstrated that thermally induced vasodilation of porcine skin is mediated by a distinctive type of nociceptor that is sensitive both to heat and to capsaicin, but not to pressure. In part I, the sites of blood flow measurement, measured on the same arm, were separated by at least 10 cm. The distance of separation in our experiment is larger than the distance of axon reflex vasodilation conduction observed by Magerl and Treede (26) at noxious local temperatures. Furthermore, the local temperatures during parts I, II, and III were lower than the local temperature used by Magerl and Treede (26) to induce vasodilation 8 cm distant from the site of local warming. Crockford et al. (7) showed that local warming-induced vasodilation of forearm blood flow was conducted at 10 cm. However, Crockford et al. sprayed warm water over a large area of the forearm and upper arm. The results reported in this study were collected from sites where the changes in local temperature were confined to a smaller area (12 cm²), presumably stimulating a more discrete axon reflex. Thus, given the reports in the literature and the distance of separation, an axon reflex at the capsaicin-treated site is unlikely to have importantly influenced the cutaneous vascular response to local warming observed at the control site on the same arm.

A possible limitation in part I is that 5 min may not be long enough for the cutaneous vasomotor response to reach steady state at the higher local temperatures. During local temperature changes at relatively low temperatures (e.g., <40°C), vasomotor responses reach a steady state typically in 3 min. However, at higher temperatures, the time constant to reach a steady-state blood flow response in response to local temper-
mm from the site of local warming during heating from 30 to 35°C, a range of temperatures chosen for non-no- ciceptive stimulation of warm receptors. This was true for either brief, pulsatile heating or sustained heating. On the contrary, in the present study, we found significant vasodilation between local temperatures of 30 and 35°C at the heated sites (see Fig. 2). This is in keeping with earlier reports (1, 16, 36). Taken together, these observations suggest that heat-sensitive afferents, likely C fibers, mediate vasomotor responses to direct heating and that these responses are active at temperatures below those perceived to be painful.

Is the vasodilation with local heating a direct function of the temperature of the resistance vessels, or does that response require a neural element? Part II of this study supports the latter possibility. The vasodilator response to direct heating corresponded better to the perception of temperature than to the temperature itself (see Fig. 5). Our assumption was that a given level of heat-sensitive nociceptor stimulation, be it by physical heating or by chemical stimulation, would be perceived as a particular temperature. Blood flow did not differ significantly between untreated and capsai- cin-treated sites when perceived as being at equal temperatures although actually being several degrees different. This suggests the major local stimulus for vasodilation to involve activity of heat-sensitive nociceptors and to be less dependent on the physical temperature of the resistance vessels. Furthermore, the VR is capable of mediating cationic currents that are induced by heat. Capsaicin sensitizes the VR to heat and thus amplifies the response to the heat stimulus. Caterina et al. (4) noted that VRs were only active at noxious temperatures (48°C). However, methodological differences with the present study are significant in that we tested intact human sensory function that likely includes endogenous physiological signaling mechanisms absent in transfected cell preparations.

To complete our investigation of the role of heat-sensitive nociceptors and cutaneous vasodilation, we tested whether subjects could accurately match heat sensation at untreated sites and whether blood flow differed significantly at untreated sites perceived to be the same temperature. We found that blood flow was not significantly different at untreated sites perceived to be the same (Fig. 6A) and that subjects could accurately match the local temperatures by heat sensation on their arms (Fig. 6B). The local temperatures in part III were similar to the local temperatures from the untreated sites in part II.

In summary, we have shown that chemical stimulation of heat-sensitive nociceptors shifts the vasodilator response to local warming to lower temperatures. Furthermore, these data indicate that similar levels of heat-sensitive nociceptor stimulation, whether by thermal or chemical means, cause similar levels of skin blood flow. Taken together, the findings strongly sug- gest that the cutaneous vasodilator response to local warming of the skin is dependent on the activation of sensory nerves (heat-sensitive nociceptors) in the area being warmed.

Perspectives

We propose a model for direct local heating-induced vasodilation whereby application of heat activates an axon reflex from heat-sensitive nociceptors, which then secrete a neurotransmitter that increases skin blood flow. In our model, local warming stimulates the heat-sensitive VR-1 receptor as described by Caterina et al. (4). Capsaicin sensitizes a population of heat-sensitive nociceptors such that the response to a given level of thermal stimulation is amplified. The stimulated heat-sensitive nociceptor then releases a vasodilatory neuro- transmitter, likely CGRP (40). Minson et al. (28) and Kellogg et al. (20) found that local warming-induced vasodilation is mediated largely by nitric oxide. Furthermore, Kellogg et al. (21) found that the local warm- ing-induced vasodilation is not mediated by the same population of nerves that mediate active vasodilation in response to whole body warming. In our model, the vasodilating neurotransmitter would stimulate nitric oxide secretion causing vasodilation. Thus at sites pre- treated with capsaicin, the response to heat is ampli- fied at the level of the afferent receptor such that more sensory neurotransmitter is released for a given thermal stimulus leading to a shifted relationship between blood flow and local temperature. Our contribution to this model is in demonstrating the physiological con- nexion between heat sensation and vasodilation, thereby linking the in vitro studies of Caterina et al. (4) and Zygmunt et al. (40), which demonstrate the effects of capsaicin on afferent nerves, with the functional studies of Kellogg et al. (20, 21) and Magerl and Treede (26).

The results from these studies further illuminate the mechanisms involved in the cutaneous vascular re- sponse to local heating. We have demonstrated that capsaicin pretreatment sensitizes the cutaneous vaso- motor response to local heating. Furthermore, results from part II demonstrate that the response in CVC to local heating is closely associated with the perceived temperature. These studies add to the model of the cutaneous vasomotor response to local heating, linking published in vitro and in vivo studies. Remaining ques- tions include: 1) Are both CGRP and substance P involved in the vasodilatory response to local heating? 2) What is the stimulus intensity dependence for re- lease of the vasodilatory transmitters?

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Perspectives

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