Bradykinin does not mediate cutaneous active vasodilation during heat stress in humans

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Kellogg, D. L., Jr., Y. Liu, K. McAllister, C. Friel, and P. E. Pérubola. Bradykinin does not mediate cutaneous active vasodilation during heat stress in humans. J Appl Physiol 93: 1215–1221, 2002; 10.1152/japplphysiol.01142.2001.—To test the hypothesis that bradykinin effects cutaneous active vasodilation during hyperthermia, we examined whether the increase in skin blood flow (SkBF) during heat stress was affected by blockade of bradykinin B2 receptors with the receptor antagonist HOE-140. Two adjacent sites on the forearm were instrumented with intradermal microdialysis probes for local delivery of drugs in eight healthy subjects. HOE-140 was dissolved in Ringer solution (40 μM) and perfused at one site, whereas the second site was perfused with Ringer alone. SkBF was monitored by laser-Doppler flowmetry at both sites. Mean arterial pressure (MAP) was monitored from a finger, and cutaneous vascular conductance (CVC) was calculated (CVC = LDF/MAP). Water-perfused suits were used to control body temperature and evoke hyperthermia. After hyperthermia, both microdialysis sites were perfused with 28 mM nitroprusside to effect maximal vasodilation. During hyperthermia, CVC increased at HOE-140 (69 ± 2% maximal CVC, P < 0.01) and untreated sites (65 ± 2% maximal CVC, P < 0.01). These responses did not differ between sites (P > 0.05). Because the bradykinin B2-receptor antagonist HOE-140 did not alter SkBF responses to heat stress, we conclude that bradykinin does not mediate cutaneous active vasodilation.

Microdialysis; laser-Doppler flowmetry

The cutaneous circulation is the major effector of human thermoregulatory reflex responses. This circulation serves a vital role in maintaining thermal homeostasis during the physiological challenges of heat and cold stress or during hyperthermia induced by prolonged dynamic exercise. During periods of hyperthermia, elevated internal temperature and skin temperature (Tsk) lead to a reflex cutaneous vasodilation (13, 18). In nonglabrous or hairy areas of skin (limbs, head, and trunk), vasomotor nerves consist of two branches of the sympathetic nervous system: noradrenergic vasoconstrictor nerves and active vasodilator nerves (13, 18, 24). Cutaneous vascular reflexes are thus controlled by dual neural systems over much of the human body.

The precise mechanism by which the cutaneous active vasodilator system functions has proven elusive, despite numerous investigations over the last 70 years (20). Although dual vasoconstrictor and vasodilator systems in skin were first suggested in 1931 by Lewis and Pickering (20), the first definitive evidence came from work by Grant and Holling in 1938 (10). In their initial descriptions of cutaneous active vasodilation, these authors noted that sweating and active vasodilation began at approximately the same time in a resting, heat-stressed person (10). This observation led to the concept that the mechanisms of cutaneous active vasodilation and sweating were mechanistically related (3, 7, 9, 10). According to this concept, sudomotor activity led to vasodilation.

In the 1950s, several studies explored mechanisms by which the sudomotor system could effect cutaneous active vasodilation. A number of these investigations were based on analogies drawn between the sudomotor system and earlier work on salivary gland control mechanisms (11). For example, in 1872, Heidenhain (11) found that atropine prevented salivary gland secretion but did not abolish the vasodilation induced by chorda tympani nerve stimulation. Based on these observations, Roddie et al. (23) examined the effects of intra-arterial atropine on sweat gland secretion and cutaneous vasodilation during heat stress in humans. These authors found that atropine treatment completely abolished sweating; however, it did not abolish cutaneous vasodilation during hyperthermia (23). They proposed several explanations for their observation, including that “the dilatation is not mediated through vasodilator nerves, but is due to increased metabolic activity of the (sweat) gland with the release of a vasodilator substance” (23).

In 1958, Fox and Hilton (7, 8) hypothesized that, during heat stress, the activation of sweat glands by cholinergic sudomotor nerves caused release of an en-
zyme into the interstitial space that cleaved bradykinin from interstitial globulins near cutaneous resistance vessels, leading to cutaneous vasodilation. This proposal was supported by their discovery of bradykinin-forming enzyme in sweat and of increased bradykinin-like activity in subdermal perfusates during heat stress (7, 8). These authors also found that intrarterial atropine “markedly delayed and reduced” the sweating induced by body heating but that cutaneous vasodilation was only slightly altered (7). They interpreted this as evidence for a vasodilator substance produced by activated sweat glands and that this substance was bradykinin (7).

More recently, work by our laboratory showed that cutaneous active vasodilation is effected by a cholinergic cotransmitter system (19). We found that intradermal injections of botulinum toxin completely abolished both sweating and cutaneous active vasodilation during heat stress. We also found that cholinergic muscarinic-receptor blockade with iontophoretically applied atropine abolished sweating, but it only caused a minor delay and attenuation of the cutaneous vasodilator response to hyperthermia. We hypothesized that “perhaps sweat gland activation by muscarinic receptors effects a local periglandular hyperemia that contributes to the vasodilation of heat stress” (19). According to this hypothesis, the major effector of cutaneous vasodilation is a still unidentified cotransmitter elaborated by cholinergic nerves. The smaller periglandular vasodilation could be effected by acetylcholine and could possibly involve bradykinin production by the activated sweat glands themselves. This work did not exclude a mechanistic role for bradykinin; however, it suggested that any such role would be relatively minor.

Suggestion of a possible role for bradykinin, and specifically the bradykinin B2 receptors in cutaneous active vasodilation, can be found in recent studies by Shastry et al. (28) and by our laboratory (14). These studies showed that production of nitric oxide by nitric oxide synthase (NOS) is necessary for full expression of cutaneous active vasodilation during heat stress in humans. As with atropine (7, 19, 23), NOS antagonists were found to attenuate, but not abolish, cutaneous active vasodilation during hyperthermia. Because bradykinin B2 receptors are known to effect vasodilation by stimulating nitric oxide or eicosanoid production (29, 31), these studies are consistent with the suggestion that bradykinin acting on B2 receptors could effect cutaneous active vasodilation by nitroxidergic mechanisms.

In contrast to the foregoing studies, other work has reported evidence that is directly contrary to the hypothesized sweat gland-bradykinin-active vasodilation relationship. In 1973, Frewin et al. (9) were unable to find any kinins or kininogenses in human sweat collected from human subjects during hyperthermia. Their results led the authors to question the role of these substances, and in particular bradykinin, in cutaneous active vasodilation.

Despite contradictory data that support varying degrees of involvement of bradykinin in cutaneous active vasodilation, the postulate persists in physiology texts that sweat gland activation mediates active vasodilation of skin vessels through a bradykinin-dependent pathway. For example, according to a current physiology textbook, “Sweat contains an enzyme that acts on a protein moiety in the tissue fluid to produce bradykinin, a polypeptide with potent vasodilator properties. Bradykinin formed in the tissue acts locally to dilate arterioles and increase blood flow to the skin” (2). Given the inconsistency between the results of Fox and Hilton (7) and Frewin et al. (9), our recent proposal of cholinergic periglandular vasodilation (19), the possibility that bradykinin B2 receptors could activate NOS (29, 31), and the persistence of this uncertain hypothesis as dogma in current physiology texts (2), we decided to closely examine bradykinin as a possible effector of cutaneous vasodilation during heat stress in humans.

The approach we took was to directly examine the effects of a bradykinin-receptor antagonist on cutaneous active vasodilation during heat stress. Although two major subtypes of bradykinin receptors have been described, only the B2 subtype has been found in normal human skin (21, 27). We, therefore, used a bradykinin B2-receptor antagonist for our studies of the role of bradykinin in cutaneous active vasodilation in humans. Our hypothesis was that treatment of the skin with a bradykinin B2-receptor antagonist would attenuate or abolish the cutaneous vasodilator response to heat stress if bradykinin were mechanistically involved in the process.

METHODS

Eight subjects (5 men and 3 women) participated in this study. Their average age, weight, and height (±SE) were 28 ± 3 yr, 84 ± 7 kg, and 177 ± 2 cm, respectively. All subjects were in good health, were nonsmokers, and were taking no medications. All subjects gave their informed consent to participate in these institutionally approved studies. There was no caffeine intake on the day of the study.

To control body temperature, subjects wore a tube-lined suit that was used to alter Tsk by perfusing the suit with water of different temperatures (12, 14, 16, 25). The water temperatures used were −18°C for cold stresses, 33–34°C for normothermia, and 48°C for heat stress periods. Subjects wore a water-impermeable plastic garment over the suit to insulate them from the room environment and prevent evaporation of sweat. The suit and garment covered the entire body except for the head, arms, and feet. The suit was perfused with warm water to raise Tsk to 39–40°C during heating periods and with cold water to lower Tsk to −32°C for cold stress.

Internal temperature was monitored with a thermocouple placed in the sublingual sulcus [sublingual temperature (Tsl)]. Tsk was recorded as the weighted electrical average of six thermocouples taped on the skin surface (12, 14, 16, 25). Mean arterial pressure (MAP) and pulse rate were recorded continuously from a finger (Finapres BP Monitor, Ohmeda, Madison, WI).

On arrival in the laboratory, subjects had two intradermal microdialysis probes placed on the ventral surface of one
forearm. The probes were of our own manufacture and were made from polyimide tubing and a 1-cm length of capillary microdialysis membrane (200-μm diameter, molecular cutoff 20 kDa) reinforced by a 51-μm-diameter coated stainless steel wire placed in the lumen of the membrane and tubing (14).

Placement of the microdialysis probe in the forearm skin was accomplished at each site, as previously described (14). Briefly, a 25-gauge needle was inserted through the dermis by using sterile technique. Entry and exit points were ~2.5 cm apart. The microdialysis probe was threaded through the internal lumen of the needle that was then withdrawn, leaving the probe in place. The microdialysis membrane was entirely within the dermis, with entry and exit through the skin via the polyimide tubing. Subjects waited 150 min or longer to allow for insertion trauma to resolve before additional instrumentation was placed (1).

Because only the B2 subtype is found in normal human skin (21, 27), we chose a bradykinin B2-receptor antagonist for our studies. The specific agent that we used was HOE-140. The agent is a potent and selective B2 antagonist with an in vivo half-life of at least 4 h (22, 32). The concentration of HOE-140 used in our experiments was based on preliminary studies that examined what concentration of HOE-140 was needed to abolish the vasodilation induced by perfusion of intradermal microdialysis probes with exogenous bradykinin. Concentrations from 4 to 400 μM HOE-140 in Ringer solution at a rate of 3 μl/min were perfused through the microdialysis fibers with and without 40 μM HOE-140. Whereas these concentrations of bradykinin could increase skin blood flow (SkBF) up to 32% of maximal levels, HOE-140 blockade was not overcome.

Approximately 150–180 min after microdialysis probe placement, subjects were placed in a supine position and instrumented to measure laser-Doppler flowmetry (LDF) from skin at the two microdialysis sites (MBF3D dual-channel flowmeter, Moor Instruments, Devon, UK). LDF measurements are specific to skin, being uninfluenced by blood flow in the underlying skeletal muscle (26). After placement of the LDF probes, both microdialysis probes were perfused with Ringer solution at a rate of 3 μl/min by using a microinfusion pump (14).

Data collection began with a 5- to 20-min control period to obtain baseline SkBF levels, followed by a 3-min application of whole body cooling. Body cooling was used to verify that active vasoconstrictor function was intact at the microdialysis sites and that the trauma of probe placement had not caused a loss of neurovascular reactivity. Subjects were then returned to normothermia, and one of the microdialysis probes was perfused with a 40 μM solution of HOE-140 (Sigma Chemical, St. Louis, MO) dissolved in Ringer solution.

After 60 min of continuous perfusion with HOE-140, 3 min of cold stress were repeated to test for any effects of the drug on vasoconstrictor function. After the cold stress, Tsk was raised to 38–39°C and maintained at that level for 40–60 min to induce heat stress and thus activate the vasodilator system. After hyperthermia, subjects were cooled and returned to normothermia. After LDF values had fallen to stable levels, both microdialysis probes were perfused with exogenous bradykinin to verify the persistence of bradykinin B2-receptor blockade by HOE-140. Finally, both microdialysis probes were perfused with 28 mM nitroprusside (Sigma Chemical) in Ringer solution for 20–40 min to effect maximal vasodilation (14, 17). This concentration of nitroprusside dilates cutaneous vessels to maximal levels no different than those achievable by raising the local temperature of the skin to 42°C (14, 17).

Data are presented as means ± SE. For data analysis, SkBF was indexed as cutaneous vascular conductance (CVC) (CVC = LDF/MAP) and normalized to the maximal levels as achieved with nitroprusside to better reflect absolute changes in SkBF (14, 15, 17). The vasomotor responses during each of the separate periods of cold stress were analyzed by comparing the precold stress levels of CVC with the levels achieved during the final minute of cold stress. The vasomotor responses to heat stress were analyzed by comparing preheat stress levels of CVC with the levels achieved during the final minute of heat stress. Responses to exogenous bradykinin were analyzed by comparing levels of CVC during the minute just before bradykinin perfusion with the level of CVC recorded over the final minute of bradykinin perfusion. All CVC responses were analyzed by repeated-measures ANOVA followed by planned contrasts to examine any attenuation in the degree of cutaneous active vasodilation by bradykinin B2-receptor blockade.

The internal temperatures (Tsl) thresholds at which CVC began to rise during whole body heating were ascertained for each subject from plots of CVC vs. Tsl for both the untreated and HOE-140-treated sites. Effects of HOE-140 on the Tsl thresholds were analyzed by comparing the Tsl thresholds for the different sites by paired t-tests to examine any delay in the onset of cutaneous active vasodilation by bradykinin B2-receptor blockade.

RESULTS

During the initial, normothermic control period, Tsk averaged 34 ± 0.3°C. During this normothermic period, when both microdialysis probes were perfused with Ringer solution, CVC at the two microdialysis sites averaged 12 ± 1 and 14 ± 2% of maximal levels (P > 0.05). During the initial cold stress, Tsk was reduced to 32 ± 0.5°C. During this cold stress, CVC fell to 8 ± 2% (P < 0.05 vs. control) and 9 ± 2% (P < 0.05 vs. control) of the maximal level at the two microdialysis sites.

During the second normothermic period, Tsk averaged 34 ± 0.5°C. Subsequent perfusion of HOE-140 at one microdialysis site during normothermia did not significantly alter CVC (14 ± 2% maximum, pre-HOE-140; and 13 ± 2% maximum, post-HOE-140; P > 0.05). When the cold stress was repeated by lowering Tsk to an average of 32 ± 0.9°C, CVC fell from 13 ± 2 to 9 ± 2% maximum (P < 0.05 vs. normothermia) at the sites treated with HOE-140. CVC at the sites still perfused with Ringer solution fell from 12 ± 1 to 8 ± 1% maximum (P < 0.05). Overall, CVC values and responses did not significantly differ between sites that received HOE-140 and untreated control sites. CVC results are summarized in Fig. 1. Taken together, these results demonstrate that neither insertion trauma nor HOE-140 affected SkBF under normothermic or hypothermic conditions.

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During whole body heating, $T_{sk}$ was increased from normothermic levels to an average of $40.110^\circ C$. Before initiation of whole body heating, $T_{sl}$ averaged $36.5410^\circ C$. During heating, the $T_{sl}$ at which cutaneous vasodilation began was $36.6710^0.07^\circ C$ at untreated sites and $36.6610.06^\circ C$ at HOE-140-treated sites ($P < 0.05$ between sites). $T_{sl}$ reached $37.6310.12^\circ C$ at the peak of heat stress.

During heat stress, CVC simultaneously rose at both untreated and HOE-140-treated sites. At the peak of heat stress, CVC at the HOE-140-treated sites reached $6912\%$ maximal CVC ($P < 0.01$ vs. preheat stress) and $6512\%$ maximal CVC at untreated sites ($P < 0.01$ vs. preheat stress). This rise did not differ between the untreated sites and the HOE-140-treated sites ($P > 0.05$ between sites). Thus HOE-140 treatment had no effect on the thermoregulatory increase in CVC during whole body heating. These results are summarized in Fig. 2.

After heat stress, subjects were cooled to normothermic levels ($T_{sk}$ averaged $3410^0^\circ C$). Blockade of bradykinin B$_2$ receptor was then tested by perfusing both microdialysis sites with exogenous bradykinin. At untreated sites, perfusion with bradykinin (10 $\mu$M) increased CVC at untreated sites from $2213$ to $3613\%$ maximal CVC ($P < 0.05$). At HOE-140-treated sites, CVC showed no such increase in response to bradykinin ($2716$ to $1917\%$ maximal CVC, $P > 0.05$), demonstrating that HOE-140 blocked the effects of bradykinin ($P < 0.05$ between sites). These results are summarized in Fig. 3.

**DISCUSSION**

A long-standing hypothesis of the mechanism of active cutaneous vasodilation is that it results from bradykinin produced by sweat glands following glandular activation by cholinergic sudomotor nerves. Bradykinin was hypothesized to be the substance elaborated by activated sweat glands and thus responsible for effecting active cutaneous vasodilation (7). The specific hypothesis proposed that, during heat stress, activation...
of sweat glands led to the release of a kallikrein (a bradykinin-forming enzyme) from the glands into the interstitial space (7). Enzymatic cleavage of bradykinin from interstitial globulins near cutaneous resistance vessels would lead to cutaneous vasodilation. Prior evidence favoring this hypothesis includes the detection of “bradykinin-like activity” in subdermal perfusates of heat-stressed subjects, as well as bradykinin-forming enzyme activity in human sweat (7). Our results show that the portion of this hypothesis that proposes bradykinin to be involved in the mechanism of cutaneous active vasodilation in not correct.

The results of the present study clearly demonstrate that cutaneous active vasodilation in heat-stressed humans does not involve activation of bradykinin B2 receptors by bradykinin. This conclusion is based on our observation of no difference in vasodilator responses to hyperthermia between areas of skin treated with the selective bradykinin B2-receptor antagonist HOE-140 and adjacent untreated sites. Neither the degree of vasodilation nor the internal temperature threshold at which active vasodilation began differed with blockade of bradykinin B2 receptors by HOE-140. If bradykinin played a role in cutaneous active vasodilation (7), cutaneous vasodilation during heat stress would have been attenuated or abolished by HOE-140 treatment. SkBF at the HOE-140-treated sites reached 69 ± 2% of maximal CVC and 65 ± 2% of maximal CVC at untreated sites; these responses were not statistically different. Our failure to see an alteration in the SkBF responses to hyperthermia at the HOE-140-treated site shows that bradykinin B2 receptors are not involved in active vasodilation and that bradykinin is not part of the vasodilator mechanism.

Our findings are not due to effects of the techniques used. During perfusion of both microdialysis probes with Ringer solution, levels of CVC in normothermia (12 ± 1 and 14 ± 2% maximum) and in response to cold stress (8 ± 2 and 9 ± 2% maximum) were not different. These results demonstrate that placement of intradermal microdialysis probes did not alter either the vasocostrctor neural control or vasomotor responsiveness of cutaneous vessels. This finding is consistent with our prior experience with microdialysis (14, 17).

With the initiation of HOE-140 perfusion, no change in CVC was observed (14 ± 2% maximum before HOE-140, and 13 ± 2% maximum after HOE-140 perfusion). During perfusion with HOE-140, levels of CVC in normothermia were no different than CVC levels at sites perfused with Ringer solution (13 ± 1 and 14 ± 2% maximum, respectively). Thus under normothermic conditions, we found no evidence of tonic vasomotor activity maintained by bradykinin B2 receptors. When a period of cold stress was repeated during HOE-140 perfusion, CVC fell to 9 ± 2% maximum at HOE-140-treated sites and to 8 ± 1% maximum at sites perfused with Ringer solution; thus no effect of the drug was observed on the vasoconstriction induced by hypothermia. This result shows that bradykinin B2 receptors play no role in cutaneous vasoconstriction during exposure to cold.

In the design of our study, we chose to block only bradykinin B2 receptors. Several lines of evidence suggested to us that, if bradykinin were to be involved in active vasodilation, the receptor for this vasoactive peptide would have to be of the B2 subtype. First, bradykinin B2 receptors are constitutively expressed, in contrast to bradykinin B1 receptors that are induced by inflammation (30). In addition, Warren and Loi (31) found that B2-receptor antagonists abolished the cutaneous vasodilation induced by intradermal injections of bradykinin, whereas B1-receptor antagonists did not alter the response in the rabbit, a species frequently used to model the human cutaneous active vasodilator system (5, 6). Bradykinin B2 receptors effect vasodilation by stimulating nitric oxide or eicosanoid production (29, 31), and cutaneous active vasodilation in humans is also known to involve nitric oxide (14, 28), thus suggesting a possible role for the B2-receptor subtype in cutaneous active vasodilation. In our preliminary studies, we found that treatment of skin with HOE-140 abolished all responses to exogenous bradykinin. Given these observations, we reasoned that only the bradykinin B2-receptor subtype was present in normal human skin. In addition, our findings reveal that control of NOS by bradykinin B2 receptors is not involved in cutaneous active vasodilation.

After heat stress, we tested the HOE-140 blockade by administering exogenous bradykinin to both microdialysis sites. CVC at the untreated site increased significantly in response to bradykinin administration, whereas CVC at the HOE-140-treated site did not. These results verify that bradykinin B2 receptors were effectively blocked by HOE-140 throughout heat stress in our protocol. In addition, the failure to observe vasodilation at the HOE-140-treated site in our preliminary as well as our final studies demonstrates that the only functional receptors for bradykinin in the skin are of the B2 subtype. If bradykinin B1 receptors were present on cutaneous resistance vessels, these unblocked receptors would have responded to exogenous bradykinin.

Whereas our study directly examined roles of bradykinin as hypothesized by Fox and Hilton (7) and found no evidence for a mechanistic role for this vasoactive protein, the present study did not address whether sweating and active vasodilation might be linked in other ways. Nonetheless, our laboratory and others have made several observations that suggest that sweating and cutaneous active vasodilation may be effected by separate mechanisms. For example, we confirmed that atropine completely abolishes sweating but only slightly attenuates active cutaneous vasodilation (19). This shows that active vasodilation is mediated in part by activation of cholinergic muscarinic receptors (19), whereas sweating is completely dependent on muscarinic-receptor activation. We also found that exercise limits active vasodilator activity during prolonged exercise in a hot environment but does not produce a similar limitation of sweating (15). In a
similar observation, the reduced active vasodilator activity during isometric handgrip exercise is accompanied by an increased sweat rate (4). Taken together, these findings show that active vasodilation does not always parallel sweat gland activity as assessed by sweat production and thus suggest that active vasodilation may not be a function of sweat gland activation. Whereas the foregoing suggests that cutaneous active vasodilation does not depend on sweat gland activity, classic work by Brengelmann et al. (3) suggests otherwise. In an innovative study of the cutaneous vasodilator system, these authors studied patients with the rare genetic syndrome of anhidrotic ectodermal dysplasia. These patients congenitally lack sweat glands. Brengelmann et al. found that these patients also lack cutaneous active vasodilation, thus inferring that sweat glands are mechanistically involved in the vasodilator response to hyperthermia. Alternatively, these patients may completely lack cholinergic, cutaneous active vasodilator nerves, as well as sweat glands themselves. Given the contradictory nature of studies of the mechanistic relationship between sweating and active vasodilation of skin blood vessels, the true relationship between sudomotor and vasodilator nerve activity in the human skin remains an open issue.

In summary, we found that blockade of bradykinin B₂ receptors did not alter any aspect of the cutaneous vasodilator responses to heat stress in healthy human volunteers. We conclude that, whereas sweat may “contain an enzyme that acts on a protein moiety in the tissue fluid to produce bradykinin, a polypeptide with potent vasodilator properties,” the postulate that “bradykinin formed in the tissue acts locally to dilate arterioles and increase blood flow to the skin” (2) is not correct. Bradykinin does not have a mechanistic role in effecting cutaneous active vasodilation during heat stress in humans.

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