

## HIGHLIGHTED TOPIC | *A Physiological Systems Approach to Human and Mammalian Thermoregulation*

### In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges

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**Kellogg, D. L., Jr.** In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *J Appl Physiol* 100: 1709–1718, 2006; doi:10.1152/jappphysiol.01071.2005.—This review focuses on the neural and local mechanisms that have been demonstrated to effect cutaneous vasodilation and vasoconstriction in response to heat and cold stress in vivo in humans. First, our present understanding of the mechanisms by which sympathetic cholinergic nerves mediate cutaneous active vasodilation during reflex responses to whole body heating is discussed. These mechanisms include roles for cotransmission as well as nitric oxide (NO). Next, the mechanisms by which sympathetic noradrenergic nerves mediate cutaneous active vasoconstriction during whole body cooling are reviewed, including cotransmission by neuropeptide Y (NPY) acting through NPY Y1 receptors. Subsequently, current concepts for the mechanisms that effect local cutaneous vascular responses to direct skin warming are examined. These mechanisms include the roles of temperature-sensitive afferent neurons as well as NO in causing vasodilation during local heating of skin. This section is followed by a review of the mechanisms that cause local cutaneous vasoconstriction in response to direct cooling of the skin, including the dependence of these responses on intact sensory and sympathetic, noradrenergic innervation as well as roles for nonneural mechanisms. Finally, unresolved issues that warrant further research on mechanisms that control cutaneous vascular responses to heating and cooling are discussed.

thermoregulation; heat stress; cold stress; skin blood flow; cotransmission

#### MECHANISMS OF CUTANEOUS VASCULAR CONTROL DURING HEAT STRESS AND COLD STRESS IN HUMANS

HUMAN THERMOREGULATION OCCURS in response to changes in internal temperature [or core temperature ( $T_c$ )] and skin temperature ( $T_{sk}$ ). The cutaneous circulation is a major effector of human thermoregulation. During heat stress, elevated  $T_c$  and  $T_{sk}$  lead to cutaneous vasodilation through combinations of neural mechanisms and the local effects of higher temperatures on the skin vessels themselves. Conversely, during cold stress, reduced temperatures lead to cutaneous vasoconstriction through combined neural and local mechanisms. Under normothermic conditions, skin blood flow (SkBF) averages ~5% of cardiac output; however, the absolute amount of blood in the skin can vary from nearly zero during periods of maximal vasoconstriction as in cold stress to as much 60% of cardiac output distributed over the body surface during maximal vasodilation in heat stress (65).

In glabrous, or nonhairy, regions of skin (palms, plantar aspect of feet, and lips), cutaneous arterioles are innervated solely by noradrenergic sympathetic vasoconstrictor nerves (18, 35–37, 67). All thermoregulatory reflexes in these regions are mediated by changes in noradrenergic vasoconstrictor tone and the effects of local temperatures on skin (35–37, 67). In nonglabrous, or hairy, areas of skin (limbs, head, and trunk), reflex changes in SkBF are mediated by two branches of the sympathetic nervous system (noradrenergic vasoconstrictor nerves and cholinergic active vasodilator nerves, which are unique to humans) in addition to the local effects of temperature (10, 37, 67). These dual sympathetic neural control mechanisms effect the major aspects of thermoregulatory responses over most of the human body's surface.

Under resting, normothermic conditions, cutaneous arterioles in supine humans receive little neural stimulation; hence the smooth muscle cells of the cutaneous arterioles are near basal tone. With cold stress, falling  $T_{sk}$  and  $T_c$  initiate a thermoregulatory reflex to conserve body heat. This reflex is mediated by increased noradrenergic vasoconstrictor tone that causes an arteriolar vasoconstriction and a decrease in SkBF. Conversely, during heat stress, rising  $T_{sk}$

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and  $T_c$  initiate a thermoregulatory reflex to facilitate body cooling (see Fig. 1).

With the induction of mild heat stress, as  $T_{sk}$  increases, SkBF is controlled by small variations in both cutaneous vasoconstrictor and vasodilator nerve activities (39, 68). As heat stress progresses and  $T_c$  begins to rise with  $T_{sk}$ , there is the abolition of any extant vasoconstrictor tone. As  $T_c$  continues to rise, it reaches a threshold value at which the cutaneous active vasodilator system becomes fully activated. Sweating also begins at the same  $T_c$  threshold under resting conditions; however, the vasomotor and sweating thresholds can be dissimilar during exercise or baroreceptor challenges (55, 73). As whole body heat load increases, active vasodilator tone to the cutaneous arterioles is increased. This increase in neural activity mediates a decrease in smooth muscle tone, an arteriolar vasodilation, and effects an increase in SkBF. High SkBF delivers heat to the body surface where it is dissipated to the environment in conjunction with the evaporation of sweat. Overall, the active vasodilator system is responsible for 80–95% of the elevation in SkBF that accompanies heat stress (37, 66).

#### Neural Control Mechanisms of the Cutaneous Vasculature

Dual vasoconstrictor nerves and vasodilator nerves in skin were first suggested in 1931 by Lewis and Pickering (51); however, the first definitive evidence came from work by Grant and Holling (24). They measured  $T_{sk}$  as an index of blood flow in the human forearm and found large increases in response to heat stress that could be abolished by sympathectomy or nerve blockade. They noted that whereas sympathectomy or nerve blockade caused only a slight cutaneous vasodilation during normothermia, heat stress elicited a much greater increase in SkBF. In addition, nerve blockade during established hyper-

thermia and vasodilation abolished the increase in SkBF. These results suggested that cutaneous vessels in nonglabrous skin received sympathetic active vasodilator as well as sympathetic vasoconstrictor innervation.

In the 1950s, the findings of Grant and Holling (24) were confirmed by Edholm et al. (14) and by Roddie et al. (64). More recently, studies found that bretylium tosylate (a prejunctional noradrenergic neuronal blocking agent) abolishes the cutaneous vasoconstriction induced by cold stress, but it does not alter the vasodilator responses induced by heat stress (41). This confirmed that dual efferent neural systems control the cutaneous arterioles: a noradrenergic vasoconstrictor system and a nonadrenergic active vasodilator system.

**Cutaneous active vasodilator mechanisms.** Despite the fact that the cutaneous active vasodilator system is the most studied of the mechanisms that control SkBF, the precise mechanisms by which the cutaneous active vasodilator system functions remain somewhat enigmatic (24, 51, 63). Several hypotheses for how this system works have been proposed; however, none has been clearly proven.

**SUDOMOTOR ACTIVITY AND ACTIVE VASODILATION.** In the initial descriptions of cutaneous active vasodilation, it was noted that sweating and active vasodilation began at approximately the same time in resting, heat-stressed persons (24). This observation led to a proposal that the mechanism of cutaneous active vasodilation involved cholinergic sudomotor nerve activity (9, 19, 21, 24). Additional evidence favoring the possible association of active vasodilation with sweating included the observation that persons with anhidrotic ectodermal dysplasia, the congenital absence of sweat glands, also lack a cutaneous active vasodilator response to heat stress (9).

A long-held theory of the relationship between sudomotor activity and active vasodilation was drawn from work done with salivary glands (19). Fox and Hilton (19) hypothesized that during heat stress the activation of sweat glands by cholinergic sudomotor nerves caused release of an enzyme into the interstitial space that cleaved bradykinin from interstitial globulins near cutaneous resistance vessels, leading to vasodilation. Such an indirect relationship could explain how cholinergic-receptor blockade with atropine could abolish sweating but only delay and slightly reduce increases in SkBF during hyperthermia (63, 64). This hypothesized mechanism was refuted by the finding that complete blockade of bradykinin  $B_2$  receptors in skin with the specific receptor antagonist HOE-140, had no effect on the extent of cutaneous active vasodilation during heat stress. Given these findings and the fact that only bradykinin  $B_2$  receptors are present in human skin, the “bradykinin Hypothesis” cannot be correct (44).

The precise relationship between sudomotor activity and active vasodilation remains uncertain. Indeed, although it is clear that cholinergic sudomotor nerves innervate sweat glands, whether the sudomotor and vasodilator nerves are one and the same or separate nerves has not been determined; however, the observation that patients with anhidrotic ectodermal dysplasia, who lack sweat glands, also fail to actively dilate skin vessels during heat stress and the close relationship between sweat production and vasodilator skin sympathetic nerve activity (SSNA) suggests that the sudomotor nerves and vasodilator nerves could well be one and the same (9, 39, 80, 81).

**COTRANSMISSION AND CUTANEOUS ACTIVE VASODILATION.** As illustrated in Fig. 2, an alternative to the bradykinin hypothesis

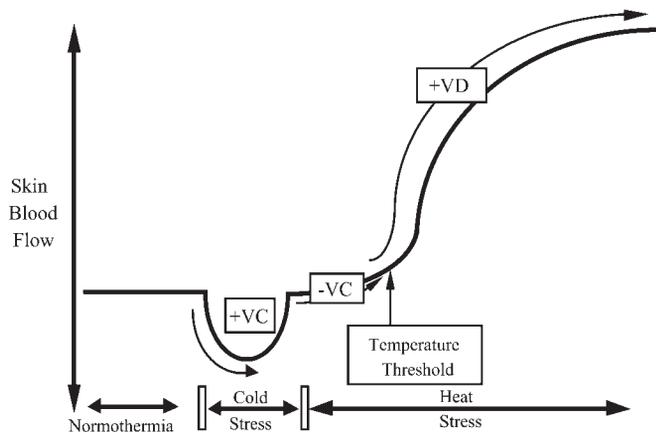


Fig. 1. Skin blood flow responses to cold stress and heat stress. During periods of normothermia, when skin blood flow represents  $\sim 5\%$  of cardiac output, cutaneous vessels receive variable, yet relatively minor neural inputs from active vasoconstrictor and vasodilator nerves. During periods of hypothermia, falling core and skin temperatures lead to reflex increases in sympathetic active vasoconstrictor nerve activity to reduce skin blood flow and conserve body heat. During periods of heat stress, increasing core and skin temperatures lead to reflex increases in sympathetic active vasodilator nerve activity to increase skin blood flow. Increases in skin blood flow can reach 60% of cardiac output during severe heat stress. High skin blood flow acts in conjunction with evaporation of sweat to reduce body heat. +VC, increased active vasoconstrictor nerve activity; -VC, decreased active vasoconstrictor nerve activity; +VD, increased active vasodilator activity; Threshold, coincident core temperature at which cutaneous active vasodilation and sweating begin under resting conditions.

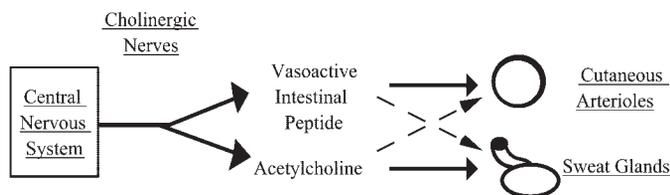


Fig. 2. Sympathetic cholinergic nerve cotransmission and cutaneous active vasodilation. Initially based on work done in the cat paw, it has been found that cutaneous active vasodilation is mediated by cholinergic nerve cotransmission (46). Acetylcholine and other cotransmitters released from cholinergic sympathetic nerves dilate cutaneous vessels and activate sweating during heat stress. The exact roles and interactions of acetylcholine and the cotransmitters that effect active vasodilation and sweating during thermoregulatory reflexes remain incompletely defined. At present, evidence suggests that vasoactive intestinal peptide may be a cotransmitter in this system.

was proposed by Hökfelt (32) that cutaneous active vasodilation relies on a cotransmitter system and was based on studies of atropine-resistant, cholinergic cotransmitter systems in the cat paw that relied on corelease of acetylcholine (ACh) and vasoactive intestinal peptide (VIP). According to this hypothesis, a single set of neurons could control both active vasodilation of cutaneous arterioles and sweating by releasing both ACh and the neuropeptide cotransmitter VIP. ACh would cause sweating, and VIP would effect active vasodilation (32). This atropine-resistant cotransmitter mechanism could explain why atropine abolishes sweating but not active vasodilation (46, 64).

The Hökfelt hypothesis has been attractive for several reasons: 1) VIP is a vasodilator (via cAMP), 2) it is found in human nerve endings associated with sweat glands (88) and blood vessels (28), and 3) it is colocalized with ACh (88). VIP has also been implicated in the control of sweat glands (83, 96). For example, exogenous VIP appears to increase muscarinic-receptor affinity for methacholine (a muscarinic-receptor agonist) and may thus promote sweat production as well as active vasodilator (83, 96).

The initial test of the Hökfelt hypothesis in human active cutaneous vasodilation was reported by Savage et al. (69). Nerves from patients with cystic fibrosis (CF) were known to have little or no VIP (31). Savage et al. reasoned that if VIP were the cotransmitter for active vasodilator, CF patients would have reduced cutaneous vasodilation in response to heat stress; however, they found that patients with CF had normal active vasodilator responses to body heating. Skin biopsies revealed very sparse levels of VIP in their patients. They concluded that VIP probably was not the elusive transmitter that effected cutaneous active vasodilator (69). This work left the issue of cotransmission in human skin unresolved.

Subsequent studies showed conclusively that the cutaneous active vasodilator system did involve cotransmission: specifically, a cholinergic cotransmitter system (46). The first of a series of studies confirmed the classic results of Roddie et al. (64) that local application of atropine to skin abolished sweating completely, but it did not abolish active vasodilation during heat stress. In addition, iontophoretic pretreatment of skin with atropine blocked all vasomotor responses to exogenously applied ACh, demonstrating that all cutaneous vascular responses to ACh are mediated by muscarinic receptors. These two studies ruled out ACh as the sole neurotransmitter of the active vasodilator system (46). A third finding was that an intradermal

dose of botulinum toxin, taken up specifically by cholinergic nerve terminals and interrupting the release of all neurotransmitters from those terminals, completely abolished both cutaneous active vasodilation and sweating in the treated area of skin. This series of three observations led to the following conclusions: 1) the only functionally important cutaneous vascular cholinergic receptors are muscarinic, 2) active vasodilation is effected by cholinergic nerves, and 3) the substances causing vasodilation must include at least one neurotransmitter coreleased with ACh from cholinergic nerves (46).

In contrast to the findings of Savage et al. (69), in CF patients, recent work by Bennett et al. (5, 6) supports the involvement of VIP as a cotransmitter in active vasodilator. This work tested whether active vasodilator is a redundant system in which ACh and VIP are coreleased from cholinergic nerves. The neuropeptide fragment VIP<sub>10–28</sub> was used to block the effects of VIP at VIP type 1 and VIP type 2 receptors. VIP<sub>10–28</sub> was chosen for these studies because it not only blocked the two major receptors for VIP but it also blocked the effects of peptide histidine-methionine (PHM). PHM and VIP are structurally related neuropeptides formed from the same prepropeptide, and both are reported to be present in human skin (33). VIP<sub>10–28</sub> was given alone and in combination with atropine. VIP<sub>10–28</sub>, alone or in combination with atropine, attenuated (but did not abolish) the rate of rise of SkBF during heat stress. This finding supported a role for VIP in active vasodilation (5, 6); however, recent work with VIP<sub>10–28</sub> has failed to replicate the antagonist effect of the agent during heat stress, and thus decisive conclusions about the role of VIP remain problematic (94).

**NITRIC OXIDE AND ACTIVE VASODILATION.** In the 1990s, work by Taylor et al. (84, 85) showed a role for nitric oxide (NO) in thermoregulatory reflex-mediated active vasodilation of the rabbit ear. This work provided the rationale to study and clarify roles for the NO system in cutaneous active vasodilation in humans (13, 40, 72). Although initial work based on intra-arterial infusions of the NOS inhibitor *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA) was unable to establish such a role (13), subsequent studies (40, 42) based on intradermal administration of NO synthase (NOS) inhibitors [*N*<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME and L-NMMA)] via microdialysis probes during hyperthermia proved such a role to be extant. These studies found that the increase in SkBF mediated by active vasodilation during heat stress was significantly attenuated by NOS inhibition (40, 42). Shastry et al. (72) also investigated this issue with laser-Doppler flowmetry from forearm skin in combination with intra-arterial infusions of L-NMMA after a marked cutaneous vasodilation effected by whole body heating. They found that NOS blockade reduced SkBF modestly. The results of the studies by Kellogg et al. (40) and Shastry et al. (72) show that an active vasodilator requires functional NOS to achieve full expression.

The foregoing studies might lead one to assume that the “functional” NOS required for an active vasodilator meant that NO levels in skin increased during active cutaneous vasodilation; however, a novel alternative was proposed by Farrell and Bishop (15). Their proposal was based on their studies of how NO functions as a vasodilator in the rabbit ear during hyperthermia (15). They noted that nitroprusside could restore the vasodilation inhibited by prior NOS blockade during hyperthermia, but the same dose of nitroprusside infused into the ear

circulation in normothermia did not raise ear blood flow. The implication was that an active vasodilator in the rabbit ear required both NO production in addition to activation of the vasodilator nerves and that these two elements were not arranged in series (vasodilator activity did not increase NO production) as had been supposed. Instead, their studies led them to the novel conclusion that NO served a "permissive" role in the active vasodilation in the rabbit ear. They proposed that NO had to be present for vasodilation to be effected by another neurotransmitter but that the absolute level of NO did not increase in heat stress.

An initial test of the Farrell and Bishop (15) hypothesis in human skin examined whether increased levels of NO breakdown products could be found in skin during hyperthermia, but no such increases were found. This suggested that NO acted as a "permissive factor" rather than as an effector of cutaneous active vasodilation. Kellogg et al. (47) repeated this study to examine NO levels in hyperthermia by measuring bioavailable NO in vivo as detected by NO-selective amperometric electrodes (47). In contrast to the initial study based on NO breakdown products, this study found that, during hyperthermia, both SkBF and bioavailable NO concentrations began to increase at the same  $T_c$ . In addition, both SkBF and bioavailable NO concentrations increased during heat stress, demonstrating that bioavailable NO does increase in skin during heat stress in humans, attendant to active vasodilation. This result suggests that NO has a role beyond that of a permissive factor in the process; rather, NO could well be an effector of cutaneous vasodilation during heat stress (43, 45, 47, 48).

Additional evidence in favor of NO as an active effector of cutaneous active vasodilation came from recent work by Wilkins et al. (93). These authors used intradermal microdialysis to deliver the NOS inhibitor L-NAME during heat stress and low doses of the NO donor nitroprusside to skin during both normothermia and heat stress. They found that, during heat stress, NOS inhibition alone attenuated cutaneous vasodilation and that administration of low doses of nitroprusside to restore NO levels during continued NOS inhibition failed to overcome the attenuation. They concluded that during heat stress, NO did not act "permissively" but rather "directly causes a portion of vasodilation" during heat stress. In addition, they found that low doses of nitroprusside caused a greater cutaneous vasodilation during heat stress than in normothermia. This suggests that NO may have a synergistic vasodilatory relationship with the neurotransmitters that effect cutaneous active vasodilation.

Another question about the role(s) of NO in active vasodilator involves the factor(s) that mediate the NO increase occurring in heat stress. This issue was recently addressed by two studies. Shastry et al. (71) investigated the hypothesis that ACh from cholinergic nerves increased NO via muscarinic-receptor stimulation. They combined muscarinic-receptor blockade by atropine with NOS blockade by L-NAME. These agents were given after activation of the active vasodilator system in prolonged heat stress, when SkBF had already risen significantly. These authors found that atropine had little effect on established active vasodilator but that L-NAME reduced SkBF during established active vasodilator in prolonged heat stress. They concluded that, although production of NO was required, neither sweating nor muscarinic-receptor-mediated

NO production was needed to sustain active vasodilator during the late, established phase of heat stress (71).

Subsequently, Shibasaki et al. (74) published work addressing whether muscarinic receptor activation leads to NO production early in heat stress. They postulated that ACh contributed to active vasodilator because ACh is released from cholinergic nerves during heat stress and ACh vasodilates through muscarinic-receptor-mediated NO production. To test this hypothesis, they combined acetylcholinesterase inhibition with neostigmine to magnify the agonist effects of ACh and NOS blockade with L-NAME to abolish NO effects. In contrast to Shastry et al. (71), Shibasaki et al. (74) gave the drugs before initiation of body heating, when active vasodilation was not activated, and continued throughout a period of hyperthermia. Shibasaki et al. found that early in body heating (when  $T_{sk}$  was increased but  $T_c$  was not), SkBF increased at sites treated with neostigmine (with presumably augmented ACh levels) before SkBF increased at untreated sites. They found that augmenting effects of acetylcholinesterase inhibition was abolished by NOS inhibition. Late in heat stress, when active vasodilation was well established, SkBF at neostigmine-treated sites did not differ from SkBF at untreated sites, but SkBF at L-NAME treated sites was lower than at untreated sites. Their results suggested that ACh-mediated NO production was possible early in heat stress but not after substantial cutaneous vasodilation had occurred (74). Shibasaki et al. found that "at the neostigmine treated sites sweating and cutaneous vasodilation occurred with the elevation in skin temperature but before measurable changes in internal temperature." This finding limited their overall conclusions about mechanisms of active vasodilator in late heat stress (74); however, the important finding by Kamijo et al. (39) that active vasodilator tone can be increased by elevation of  $T_{sk}$  alone during whole body heating lends strength to the argument that ACh mediates NO production early in heat stress.

Recently Wong et al. (95) reported evidence that  $H_1$  histamine receptors may play a role in the generation of NO during cutaneous active vasodilation in hyperthermia. They found that the first-generation antihistamine pyrilamine attenuated the rise of SkBF during heat stress. They also found that part of the NO generated during active vasodilation was mediated by histamine type 1 receptors. The source of histamine remains to be identified; however, recent work by Wilkins et al. (92) suggests that 1) VIP-induced release of histamine from mast cells could be involved, and 2) VIP and histamine both have NO-dependent components to their effects. Thus there appear to be several pathways that may generate NO in the skin during hyperthermia (39, 74, 92, 95).

*Cutaneous active vasoconstrictor mechanisms.* Early evidence for the control of SkBF by sympathetic active vasoconstrictor nerves came from studies of the effects of peripheral sympathectomies in human patients (1, 18, 67). These studies revealed that interruption of sympathetic neural activity by surgical or pharmacological means lead to increases in SkBF when done in a cool environment, an observation consistent with the release of active vasoconstrictor tone.

Unlike the rather enigmatic active vasodilator system, the cutaneous sympathetic vasoconstrictor system has been assumed to be a comparatively well-understood, simple system that effects vasoconstriction through classical noradrenergic mechanisms involving the action of norepinephrine on  $\alpha_1$ - and

$\alpha_2$ -receptors (7, 14, 18, 24, 26, 34, 49, 50, 67). This has been clearly demonstrated by studies using prejunctional sympathetic nerve blockade with bretylium and postjunctional blockade with  $\alpha$ -receptor antagonists (7, 27, 41, 52); however, recent work has shown that the cutaneous vasoconstrictor system is not so simple.

Cutaneous vasoconstrictor nerves have been known to contain several neurotransmitters colocalized with norepinephrine, including neuropeptide Y (NPY) and ATP, which have been shown to participate in noradrenergic vasoconstriction in animal models (8, 29, 30, 57, 58, 90). Similar results have been reported from in vitro studies with isolated human tissues and vascular smooth muscle cells (61, 62). In vivo work by Taddei et al. (82) showed that such systems were extant in human forearm circulation and mediated the vasoconstriction induced by baroreflex unloading. These studies provided the rationale for examining the role of cotransmission in the control of the human cutaneous circulation.

In 2001, Stephens et al. (76) demonstrated that human cutaneous active vasoconstrictor nerves, like human active vasodilator nerves, represent a cotransmitter system. These authors compared the effects of differing combinations of  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -receptor antagonists on the SkBF responses to hypothermia. Their results confirmed a role for postjunctional excitation of  $\alpha_1$ - and  $\alpha_2$ -receptors in reducing SkBF during hypothermia as induced by whole body cooling. In addition, they found that the pretreatment of skin with the  $\beta$ -receptor antagonist propranolol produced a more consistent vasoconstriction during hyperthermia. This suggests that a  $\beta$ -receptor-mediated vasodilation may modulate  $\alpha$ -receptor-mediated vasoconstriction during hypothermia. The most significant result of their work was the finding that simultaneous and complete blockade of  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -receptors failed to abolish the cutaneous vasoconstriction induced by hypothermia. Combined with their confirmation that bretylium blockade of all neurotransmitter release from cutaneous noradrenergic nerves totally abolished reductions in SkBF during body cooling, Stephens et al. concluded that nonnoradrenergic, cotransmitter mechanisms effect reductions of SkBF during activation of the cutaneous active vasoconstrictor system in hypothermia.

Subsequent work by Thompson and Kenney (87) confirmed the foregoing results and further characterized the importance of cotransmission in active vasoconstriction. These authors found that combined blockade of  $\alpha$ - and  $\beta$ -receptors in forearm skin attenuated the maximal vasoconstriction of forearm skin effected by whole body cooling by 40% in young subjects. Combined blockade completely abolished the vasoconstriction to cold stress in subjects 61 yr and older. Prejunctional blockade of all sympathetic neurotransmitter release with bretylium tosylate completely abolished vasoconstriction in both groups. These findings revealed the great contribution that cotransmission makes to cutaneous active vasoconstriction in youth and how this contribution is lost with advancing age.

A recent study by Stephens et al. (78) has further explored the role of cotransmission in active cutaneous vasoconstriction. They tested the hypothesis that NPY acted as a cotransmitter along with norepinephrine to mediate reductions in SkBF during hypothermic periods in humans. They further hypothesized that NPY Y1 receptors mediated the effects of NPY. They found that the NPY Y1 antagonist BIBP-3226 significantly attenuated reductions in SkBF during whole body cold

stress. Furthermore, the combination of NPY Y1 receptor antagonism with complete  $\alpha$ - and  $\beta$ -receptor blockade abolished the cutaneous vasoconstriction attendant to hypothermia. As illustrated in Fig. 3, these results clearly show that the cutaneous active vasoconstrictor system is a cotransmitter system that effects reductions in SkBF via the release of NPY and norepinephrine and the postjunctional activation of NPY Y1,  $\alpha_1$ -,  $\alpha_2$ -, and perhaps  $\beta$ -receptors (78).

#### Local Temperature Control Mechanisms of the Cutaneous Vasculature

**Local warming of the skin and vasodilation.** In response to increases in local  $T_{sk}$  as occurs with hyperthermia, cutaneous blood vessels dilate by local temperature-dependent mechanisms in addition to the previously discussed neural mechanisms. With local warming of skin, local SkBF increases in direct proportion to the temperature achieved, with maximal local SkBF reached when local  $T_{sk}$  is held at 42°C for 35–55 min (86). The local vasodilation is biphasic with an initial brisk vasodilation followed by a prolonged plateau phase (see Fig. 4).

The mechanisms that effect the local, temperature-dependent cutaneous vasodilation involve both local neural mechanisms as well as local generation of NO. These two mechanisms appear to be independent of each other (56). The previously discussed neural cutaneous active vasodilator system does not appear to be involved in the cutaneous vascular response to local skin warming because neither botulinum toxin-induced abolition of active vasodilation nor muscarinic-receptor blockade alters the vasodilator response (23, 46). Prostanoids do not appear to be involved either, because cyclooxygenase inhibition fails to alter local temperature-dependent vasodilation (23).

The initial phase of the local warming response has been found to be mediated by local activation of afferent cutaneous sensory nerves. This portion of the vasodilation can be greatly attenuated by topical anesthesia directly at the locally warmed site but not by cutaneous nerve blockade at points distant from the heated site (56, 60).

Recent work by Stephens et al. (77) has further characterized the role of sensory afferents in the vasodilation caused by local skin warming. On the basis of studies done with topical application of the vanilloid type 1-receptor activator capsaicin, they proposed that local increases in skin temperature stimulates heat-sensitive vanilloid type 1 receptors on afferent nerves. This activates a local axon reflex to cause the antidromic release of a vasodilatory neurotransmitter (or neurotransmitters) that effect local skin blood flow increases (77).

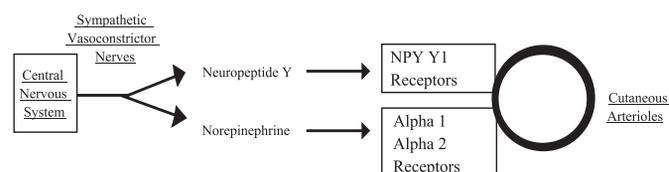


Fig. 3. Sympathetic noradrenergic nerve cotransmission and cutaneous active vasoconstriction. Noradrenergic cutaneous active vasoconstriction has long been known to be mediated by norepinephrine (NE) release acting on  $\alpha_1$ - and  $\alpha_2$ -receptors on the vascular smooth muscle of cutaneous arterioles. Stephens et al. (76, 78) have recently demonstrated that the neuropeptide cotransmitter neuropeptide Y (NPY) is also involved in active vasoconstriction and works through postjunctional NPY Y1 receptors.

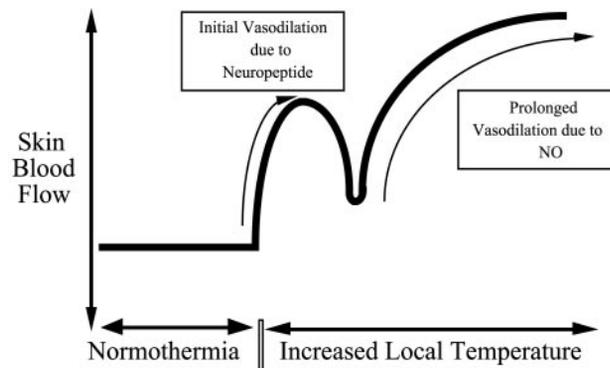
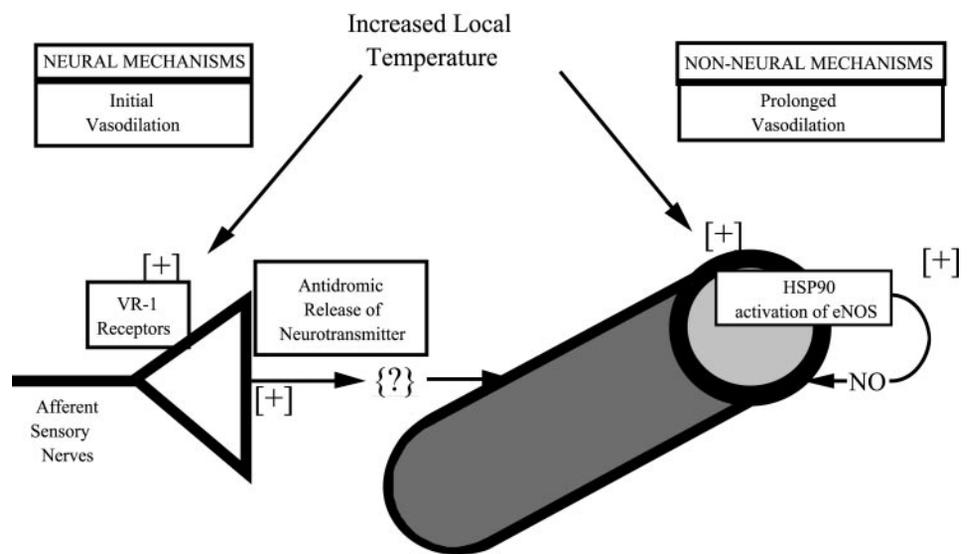


Fig. 4. Local warming responses and vasodilator mechanisms in human skin. Increases in the local temperature of cutaneous blood vessels increase skin blood flow in a biphasic response: an initial rapid increase to a peak followed by a prolonged plateau phase. The initial phase is mediated by antidromic neurotransmitter release from sensory afferents mediated by temperature-sensitive vanilloid type 1 receptors (VR-1). Sympathetic active vasodilator nerves are not involved. The plateau phase that is observed with prolonged local warming of the skin is mediated by nitric oxide (NO) generation. The source of this NO appears to be at least partially through heat shock protein 90 (HSP90) activation of endothelial NO synthase (eNOS). None of the responses to increased local skin temperature depend on the presence of intact cutaneous active vasodilator nerves (+ = augmented effects due to increased temperature; ? = unknown neurotransmitter).



The nature of the vasodilatory neurotransmitter of the initial phase of local temperature-dependent vasodilation remains uncertain.

The prolonged plateau phase of local temperature-dependent vasodilation has been shown to be mediated by local generation of NO. This phase can be greatly attenuated by pretreatment of the locally warmed area of skin with the NOS inhibitor L-NAME (43, 56). Shastry et al. (70) used the heat shock protein 90 (HSP90) inhibitor geldanamycin to further examine the mechanisms involved. HSP90 has been shown to bind endothelial NOS (eNOS; type III NOS) and enhance eNOS activation and NO generation (22). They found that geldanamycin attenuated increases in SkBF caused by local skin warming by ~20% and thus suggest that eNOS may be the NOS isoform that mediates the prolonged plateau phase of the cutaneous vascular response to local hyperthermia (70) (see Fig. 5).

*Local cooling of the skin and vasoconstriction.* As with local increases in  $T_{sk}$ , decreases in local  $T_{sk}$  with local cooling of the skin causes a local, temperature-dependent vasoconstriction. In contrast to the local warming vasodilatory response that is independent of cholinergic cutaneous active vasodilator mechanisms, the local cooling vasoconstrictor response is dependent on intact noradrenergic cutaneous active vasoconstrictor nerves

(38, 59, 60). The requirement for intact noradrenergic neurons was made evident from studies that used bretylium to block neurotransmitter release. With intact noradrenergic nerves, local cooling causes a progressive reduction in SkBF with falling local temperature. Pretreatment of skin with bretylium, which blocks prejunctional release of neurotransmitters from sympathetic vasoconstrictor nerves, reverses the initial portion of the local cooling-induced vasoconstriction into a local cooling-induced vasodilation. It is only with continued cooling that bretylium-treated sites show a reduction in SkBF (27, 59, 60).

Recent work by Johnson et al. (38) has further defined how the cutaneous active vasoconstrictor system participates in the vasoconstrictor response to local skin cooling in the absence of  $T_c$  changes by examining the roles of noradrenergic receptors, NPY Y1 receptors, and afferent sensory nerves. They found that blockade of  $\alpha$ - and  $\beta$ - receptors altered the response as did bretylium; i.e., combined  $\alpha$ - and  $\beta$ -receptor blockade reversed the initial phase of vasoconstriction was reversed to a vasodilation followed by vasoconstriction as cooling continued. NPY Y1-receptor blockade with BIBP-3226 did not alter the response at all. Finally, topical anesthesia with EMLA cream also reversed the initial vasoconstriction to a dilation followed by constriction with prolonged cooling. Johnson et al. concluded that local cooling of skin occurs in two phases: an initial

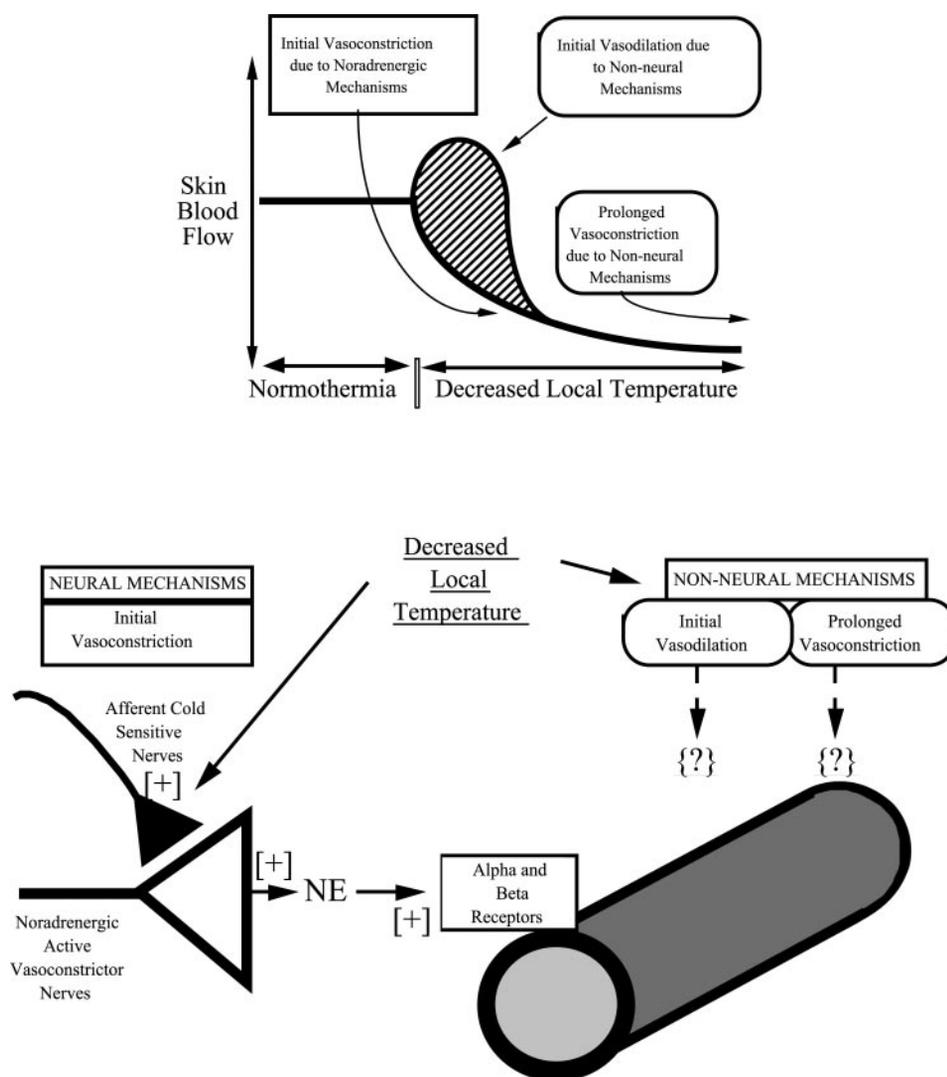


Fig. 5. Local cooling responses and vasoconstrictor mechanisms in human skin. Decreases in the local temperature of cutaneous blood vessels reduces skin blood flow through mechanisms that require intact sympathetic noradrenergic innervation. Effects of local temperature decreases are independent of any change in core temperature. Local temperature reductions are sensed by cold-sensitive afferent nerves. These nerves effect the release of norepinephrine from sympathetic active vasoconstrictor nerves. Norepinephrine acts through  $\alpha$ - and  $\beta$ -receptors to produce an initial vasoconstriction. Local cooling also mediates an initial vasodilation that competes with the initial noradrenergically mediated vasoconstriction. This dilation is mediated through nonneural mechanisms and is observed when release or postjunctional effects of norepinephrine are blocked. Finally, continued local cooling effects a prolonged vasoconstriction through nonneural mechanisms. The nature of the nonneural mechanisms is unknown in humans.

phase lasting a few minutes, and a prolonged phase. The initial phase is mediated by activation of cold-sensitive afferent neurons that effect the release of norepinephrine from sympathetic cutaneous vasoconstrictor nerves. Norepinephrine then vasoconstricts skin vessels through postjunctional  $\alpha$ -receptors. NPY and NPY Y1 receptors appear not to be involved in either the initial or prolonged phases. In addition, the mechanisms for the prolonged phase appear to be nonneurogenic because no manipulations altered the prolonged cutaneous vasoconstriction during local cooling; however, these mechanisms have not been defined further (38).

Work done over the last several decades has suggested a role for  $\alpha_2$ -adrenoreceptors in causing the cutaneous vasoconstriction induced by local skin cooling (2, 3, 11, 16, 17, 89). This work was based on the *in vivo* observation that local skin cooling augmented  $\alpha_2$ -adrenergic vasoconstriction, but attenuated  $\alpha_1$ -vasoconstriction in humans (20). In particular, Flavahan and colleagues have extensively explored the role of  $\alpha$ -adrenergic receptors. Their studies have used the mouse tail as well as isolated human tissues and cells. With these models, they have shown that local cooling causes augmented adrenergic vasoconstriction through  $\alpha_{2C}$ -adrenoreceptors (11). These receptors have been found not to be directly thermore-

active; rather they are translocated from the trans-Golgi apparatus of vascular smooth muscle cells to the plasma membrane through the cold-induced activation of RhoA and Rho kinase (2, 3). RhoA and Rho kinase activation also appears to enhance the calcium sensitivity of the vascular smooth muscle contractile apparatus (2).

Recently, Flavahan (17) published work that the generation of reactive oxygen species (ROS) from mitochondria in vascular smooth muscle may mediate the vasoconstriction induced by local cooling. Baily et al. (3) found that cooling of mouse tail arteries led to an increase in ROS in vascular smooth muscle cells. Manipulation of ROS level by application of rotenone, *N*-acetylcysteine, or a superoxide dismutase mimetic abolished the vasoconstrictor response to  $\alpha_{2C}$ -adrenoreceptor activation. These manipulations of ROS levels also abolished the activation of RhoA in cultured human vascular smooth muscle cells. These results suggest that the vasoconstriction caused by local cooling is effected by increased ROS generation in mitochondria in vascular smooth muscle cells. Increased ROS activates RhoA/Rho kinase activation and consequent mobilization of  $\alpha_{2C}$ -adrenoreceptors to the cell membrane where they can be activated by catecholamines from sympathetic neurons (3). Whether this elegant model partici-

pates in the in vivo human cutaneous response to local cooling is unproven.

#### FUTURE RESEARCH DIRECTIONS

##### *Cutaneous Active Vasodilator Mechanisms*

An alternative explanation for the finding by Savage et al. (69) that the vasodilation induced by hyperthermia is preserved in CF despite sparse cutaneous levels of VIP is based on the possibility of redundancy between ACh and VIP in cutaneous active vasodilator control, as occurs in other cotransmitter systems (4, 53). According to this explanation, in CF patients, an apparently normal increase in SkBF during heat stress could be caused by ACh release. The active vasodilator system in CF patients would be highly dependent on ACh-mediated vasodilation and hence highly sensitive to muscarinic-receptor blockade. Similarly, in healthy, non-CF persons, coreleased ACh and VIP each contribute to cutaneous active vasodilator during heat stress. Blockade of postjunctional muscarinic receptors with atropine in non-CF persons would leave the VIP portion of active vasodilator intact and give the impression that ACh was not involved. Furthermore, blockade of prejunctional muscarinic receptors with atropine would reduce prejunctional inhibition of neuropeptide release, produce an enhanced release of VIP, and further mask the role of ACh (4, 53). Conversely, in non-CF persons, blockade of VIP receptors would leave the ACh-mediated portion unaltered, suggesting that VIP was not important to active vasodilator. Cotransmitter systems are known to have redundancies whereby lack of one neurotransmitter can be compensated for by another (4, 53). Whether this occurs in the cutaneous active vasodilator system is unknown.

An additional complexity of cotransmitter systems is that the release of the different neurotransmitters depends on the frequency of nerve firing (4, 54). In nerves with colocalized ACh and VIP, ACh is preferentially released at low firing frequencies and VIP at high firing frequencies (4, 54). Such differential release of "classical" neurotransmitters such as ACh at low frequencies and neuropeptide release at higher nerve firing frequencies are well documented (4, 54). Given that SSNA increases progressively with  $T_c$  during heat stress (79), it would not be surprising to find that ACh plays a role early in heat stress (when SSNA is low) and VIP and/or other neuropeptides play roles late in heat stress (when SSNA is high).

The studies by Shibasaki et al. (74) and Shastry et al. (71) are consistent with the differential release of neurotransmitters mentioned in the foregoing section and suggest the possibility of different mechanisms of NO generation within the active vasodilator system. Given that bioavailable NO increases in the interstitial space during heat stress (47) and the results of Shibasaki et al. (74) and Shastry et al. (71), it may be that different mechanisms are involved in the early and late phases of active vasodilation in general and in NO production in particular.

A related and unanswered question about the role of NO in the active vasodilator system pertains to which isoforms of NOS participate in active vasodilator. Both neuronal NOS and eNOS have been detected in normal human skin (75, 91). Although it is clear that NOS generation of NO mediates part of active vasodilator system, it is unclear which NOS isoform(s) produce the increased NO required for active vasodilator.

##### *Cutaneous Active Vasoconstrictor Mechanisms*

NPY can effect vasoconstriction either directly or through the potentiation of noradrenergic  $\alpha$ -receptor activation. Based on the work by Stephens et al. (76, 78), it is clear that both NPY and norepinephrine act together to reduce SkBF during hypothermia, but whether NPY acted directly on cutaneous vascular smooth muscle or through ostentation of noradrenergic mechanisms was unclear. They did note that, during normothermia, exogenous norepinephrine caused small and transient vasoconstriction at sites with complete  $\alpha$ - and  $\beta$ -receptor blockade. Furthermore, the addition of NPY Y1-receptor blockade abolished this vasoconstriction. However, because of the transient nature of the response and because of the requirement for tonic NPY release under normothermic conditions for their observation to be true, the authors were reluctant to conclude whether NPY acts as a direct cutaneous vasoconstrictor or whether it potentiates the effects of noradrenergic receptor activation. This remains an unresolved issue.

##### *Local Warming of the Skin and Vasodilation*

The major area of uncertainty regarding the mechanisms by which local hyperemia effects vasodilation is what neurotransmitter or neurotransmitters are released by afferent nerves to cause local SkBF increases. The C-fiber afferents in skin that are likely involved in the process are known to contain substance P and calcitonin gene-related peptide (CGRP). Whether substance P and/or CGRP are the actual neurotransmitters involved remains to be proven in humans.

Another unanswered question relates to the generation of NO that mediates the prolonged plateau phase of the cutaneous vascular response to local skin hyperemia. It has been suggested that HSP90 causes calmodulin to displace eNOS from caveolin-1. This leads to eNOS activation and potentially increased NO generation (25). The observation that HSP90 is necessary for full expression of the NO-dependent, prolonged plateau phase of the local warming response suggests that eNOS is involved in the process and that calcium and calcium-dependent proteins are involved. These mechanisms remain to be studied.

##### *Local Cooling of the Skin and Vasoconstriction*

The role of sympathetic vasoconstrictor nerves in the initial phase of vasoconstriction effected by local cooling of the skin is well characterized; however, the nonneural mechanisms that mediate the prolonged response to local cooling are unknown. The intricate model of cold-induced ROS generation leading to RhoA/Rho kinase activation and  $\alpha_{2C}$ -adrenoreceptor translocation proposed by Flavahan and colleagues (2, 3, 11) must be verified in vivo in humans. Other mechanisms that may be involved include alterations of endothelial function, blood viscosity, receptor affinity, and/or cutaneous vascular smooth muscle function. None of these mechanisms have been investigated at present in humans.

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