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Thermoregulatory and thermal control in the human cutaneous circulation

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1. ABSTRACT

The past 10-15 years has been a time of focus on the mechanisms of control in the human cutaneous circulation. Methodological developments have provided powerful means for resolving the important contributors to the reflex control of skin blood flow (thermoregulatory control) and also for the equally impressive effects of direct heating and cooling of the skin (thermal control). This review is devoted largely to that recent literature. We treat the sympathetic vasoconstrictor system and its transmitters and modulatory factors and the sympathetic active vasodilator system and its abundant mysteries, with focus on the putative transmitters and cotransmitters, the involvement of nitric oxide and the relationship to sweating and modulatory factors. We also deal with the current understanding of the mechanisms of vasoconstriction and vasodilation that accompany direct skin cooling and heating, noting that adrenergic function, afferent nerve function and the nitric oxide system are involved in the vascular responses to both thermal stimuli.

2. INTRODUCTION

The cardiovascular system undergoes a collection of coordinated adjustments when a human is exposed to changes in environmental temperature or changes in heat production (1-6). Those cardiovascular responses represent major changes in cardiac output and in the distribution of blood flow among the various regional circulations. The principal cardiovascular adjustment to such thermal challenges is increases and decreases in blood flow to the skin. Over the past ten to fifteen years, considerable attention has been paid to improving our understanding of the mechanisms of control of the cutaneous circulation. That is the subject of this review, in which we consider the evidence explaining how skin blood rises when the skin is locally warmed and how it rises as a reflex when body temperature increases. We also consider the evidence regarding how skin blood flow falls when the skin is directly cooled and how it falls as a reflex when the entire body is subjected to cooling. It is these areas that have benefitted considerably from the development of methods

to address such questions. Our focus is largely on the human cutaneous circulation; more is known about the control of skin blood flow in humans than in any other species. We do rely on those valuable studies in animals and *in vitro* when they help answer basic questions that arise in humans. Our focus also limits us from considerations of all the reflexes that impinge on the skin circulation and from the studies of the influence of disease. Those are important considerations, but lie outside the bounds of this review.

3. METHODOLOGY

Our understanding of the mechanisms of reflex control of the skin circulation has been markedly enhanced over the past twenty years. This knowledge has been gained, in part, because of the development of new methods and in part because the skin circulation has become the focus of a number of very active laboratories.

3.1. Laser Doppler flowmetry

A valuable method refined over the past thirty years or so is laser Doppler flowmetry (7, 8). This method relies on the Doppler shift of laser light reflected from the moving cells in the tissue (skin), as well as the number of Doppler shifted photons relative to the entire intensity of reflected light. The physical basis and instrument development for laser Doppler flowmetry are beyond the scope of this review. We limit our comments to noting that the measurement agrees well with other blood flow measurement methods, has the advantages of being limited to the skin (9), delivering a continuous signal with excellent characteristics in terms of frequency response and sensitivity, and typically interrogating a small area (1mm2) of skin (7, 10, 11).

The above characteristics make the method nearly ideal to combine with pharmacological interventions to elucidate mechanisms of control. Small areas of skin can be treated with high local levels of, for example, antagonists, but the amount being trivial relative to the body size. Investigators exploring control mechanisms have found that this combination of discrete blood flow measurement with local pharmacologic treatment can be applied at multiple sites on the same limb, simultaneously allowing measurements from control sites and sites with various kinds of intervention.

As with any method, laser Doppler flowmetry has caveats. One is motion sensitivity. It is blood cell motion that is the basis for the method. Stabilization to avoid artifact generation is not a problem in resting individuals, but exercise is a challenge. Measurement of skin blood flow from an exercising limb by laser Doppler flowmetry is probably not possible. A second caveat relates to the quantification of blood flow in traditional units of blood flow. Although some instruments display the blood flow in such units, they are probably not correct. Indeed, they probably differ from actual blood flow by a factor of 4-6. This is a conclusion that arises, in part, from the realization that skin blood flow in the resting forearm averages around 2 ml per 100g of forearm, or about 2 ml per 8g of skin (2, 12). The resting skin blood flow is therefore well above the 2-5 ml per 100g · min-1 commonly reported by these instruments.

That laser Doppler flowmetry does not provide blood flow in conventional or absolute units at first glance is disappointing. However, it is not generally a limitation to the use of the method, especially for the mechanistic studies reviewed here. This is because there is a relatively wide heterogeneity in the level of skin blood flow from 1 mm2 of skin to the next, even across the forearm (11). That being the case, the level of blood flow at one measurement site can hardly reflect, on an absolute basis, the level of skin blood flow to the entire body surface, or even in a relatively defined area such as a limb. Thus, the high regional specificity of the method limits its ability to make estimates of more global absolute levels of blood flow.

Fortunately, despite differences in the absolute levels of blood flow among different sites, the pattern of the response to given local or reflex perturbations is quite similar among sites (10, 11). For this reason, it is common to normalize the measurement from a given site to some characteristic for that site. In our hands, we have found that normalization to maximal blood flow at that site to be analytically valuable (13), especially during studies in which skin blood flow reaches high levels. The rationale for this normalization scheme is that, at maximal blood flow (conductance), all sites have zero vascular smooth muscle activity. This is a solid reference point and can be achieved with local skin heating to 42°C (14, 15) or by application of high levels of sodium nitroprusside if a microdialysis route is available (16, 17). Because the vasodilator response to skin heating is largely nitric oxide dependent (16, 17), local warming may not deliver full vascular smooth muscle relaxation if the nitric oxide system has been experimentally inhibited or affected by pathology.

In cases of low blood flow, we find comparison of responses among sites to be more analytically revealing if the normalization is to baseline. This is largely because the levels of blood flow being analyzed are near the normalization standard. This does not resolve question of a role of potential baseline differences and whether that might affect the analysis (18). For example, differing pharmacological interventions might generate differing baseline levels of blood flow. This is an unanswered problem, and the best approach is to establish original baselines before any intervention

and base the analysis on that characteristic (e.g., see (19)).

3.2. Pharmacological approaches

Although normalization remains an important consideration, it has not proved to be limiting in reaching firm conclusions based on the interventions applied. The application of pharmacological probes to small areas of skin, coupled with laser Doppler flowmetry, has enabled important advances over the past decade or so. One approach is the iontophoresis of pharmacologically active drugs into the skin. Lindblad and colleagues first used this as an approach, coupled with laser Doppler flowmetry, to understand the makeup of alpha adrenergic receptor subtypes in dorsal finger skin and to then apply that knowledge and approach to the discrimination of the involvement of those subtypes in the vasomotor responses to local skin cooling (20-22). Their use of a mild current to force alpha1 and alpha2 adrenergic agonists and antagonists into dorsal finger skin provided important information relative to the role of that system in humans and served as a stimulus to use that approach to address other questions. With this technology, bretylium, atropine, acetylcholine, nitroprusside, among others, have been iontophoretically applied to treat small areas of skin in order to address questions about vasomotor control.

Key to the choice of molecules for iontophoresis is solubility in the particular solvent chosen, the ability to ionize and the electrical mobility such that they carry part of the current. The above agents are included as having those characteristics, whereas larger and/or uncharged molecules do not. The choice of solvent is important, as well. In our laboratories, we use almost exclusively propylene glycol (e.g., (23) because it is an organic solvent, but does not itself ionize.

Ionized water is sometimes used as the solvent, as is saline. Two considerations arise with such choices: First, the electrical mobility and concentration of the other ions (protons, sodium, anions) must be considered relative to those of the agents of interest because the amount of charge carried by the latter, and therefore its tissue deposition, will depend on that ratio. Second, there may be effects of H+ or OH- on the vasomotor state. Indeed, there is a vasodilation even with water iontophoresis, and the degree of that vasodilation is significantly greater at the anodal site (24). This suggests an effect of something other than current *per se* and probably relates to the local pH. It is also the case that the passage of current through the tissues can cause vasodilation (25). This latter effect depends on the duration and intensity of the current. For example, to apply enough bretylium to assure presynaptic vasoconstrictor nerve block requires a combination of current strength and duration that itself causes a significant vasodilator effect that lasts for up to one hour (23). Such a vasodilator response to the application of the drug has the potential for interfering with the analysis of the drug actions. In the case of bretylium, the drug actions last at least several hours (23), well beyond the duration of the current-induced hyperemia. Nevertheless, it is important to recognize the possible encroachment of the hyperemia from the iontophoretic procedure on the duration of action of the agent of interest, potentially limiting the useful period.

The limitation imposed by iontophoresis to the useful period in drug delivery can be overcome by a second approach to drug delivery--intradermal microdialysis. The method (16, 26) involves placement of a single dialysis fiber intradermally, through which pharmacological agents with known actions are perfused. This approach has many of the same advantages as iontophoresis: small area of skin treated, systemic dose too small to measure, monitoring of skin blood flow by laser Doppler flowmetry over the treated areas, multiple individual measurement sites allowing for comparison among treatments. The microdialysis method also allows for continuous perfusion with the active agent so that washout and duration of action are no longer issues. For this latter reason, we replaced intradermal injections (27, 28) with microdialysis in our studies of sympathetic co-transmitters (29). In particular, intradermal injection causes an initial hyperemia seen with needle injection (30) that lasts about an hour. During that and the subsequent hour or so, the depot of active drug was slowly washed out, ultimately reaching ineffective levels and limiting significantly the experimental period. Use of a constant perfusion with microdialysis allows the needle trauma and associated hyperemia to resolve without depleting the active drug to suboptimal levels.

Microdialysis not only increases the available experimental period, but also permits the administration of non-ionized substances. This would include low molecular weight compounds such as BIBP3226, VIP10-28, antagonists for nitric oxide synthase, endothelin, angiotensin, norepinephrine, etc. Many of the above could not be administered by iontophoresis, and their duration of action following intradermal injection is potentially too short to allow meaningful experimental opportunity. Further, intradermal microdialysis is minimally invasive; no untoward effects have been experienced in our laboratories, nor have any been reported.

Some variation exists among laboratories in the use of microdialysis. These variations include the area of skin involved (e.g., arm vs. leg) and the nominal molecular weight cutoff of the membrane. In some laboratories, the skin is numbed with a cold pack. In others, no anesthetic is used. There is no clear difference among these different approaches, although there is clear evidence that presence of a microdialysis fiber has some effect on the vasodilator

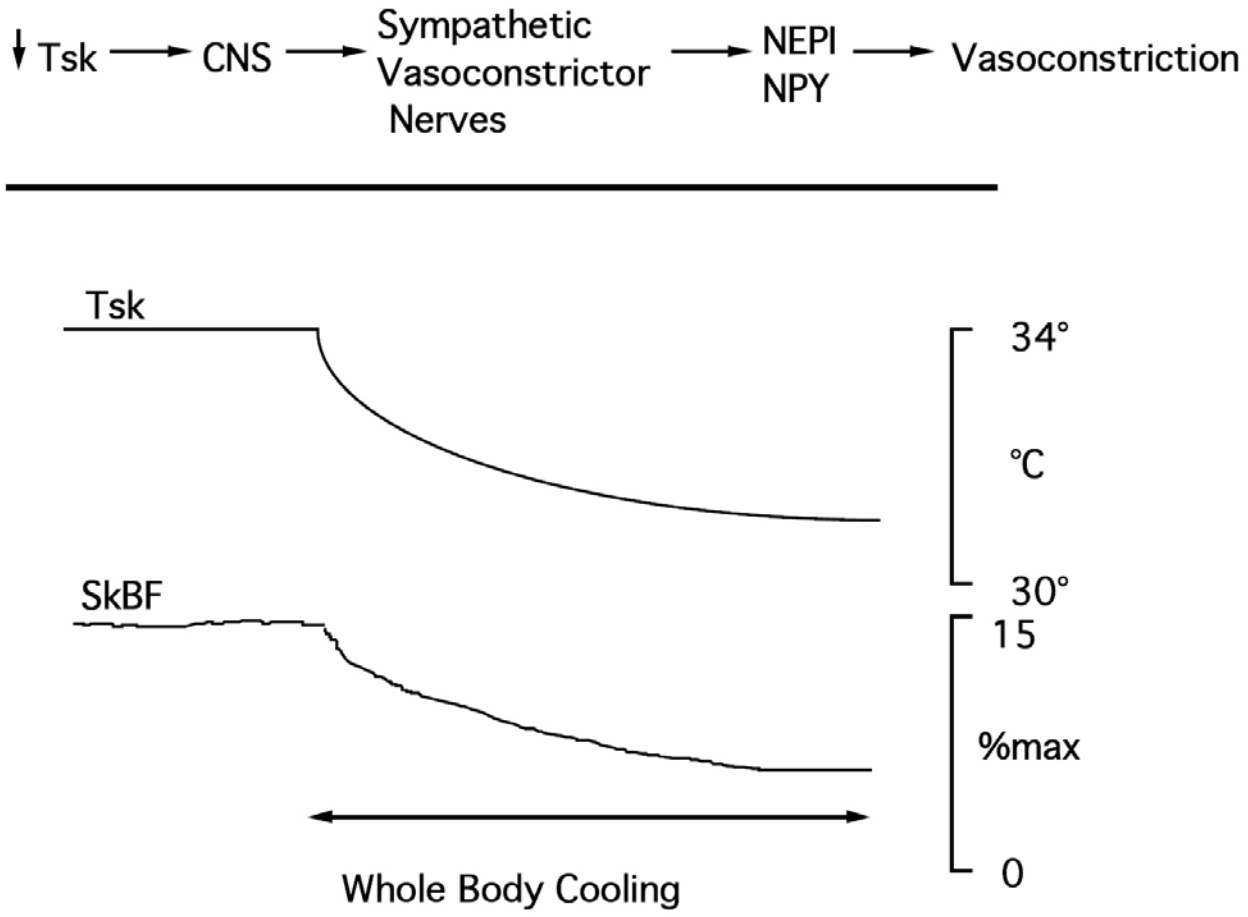

Figure 1. Reflex response in skin blood to whole body cooling. Below: Pattern of blood flow accompanying a reduction in skin temperature (Tsk). Above: Reflex arc accomplishing that pattern. Lower skin and/or internal temperature is sensed, leading to an increased sympathetic vasoconstrictor outflow directed to the skin. Sympathetic nerves release norepinephrine and NPY, each of which acts on cutaneous vascular smooth muscle to cause vasoconstriction.

potential in that area of skin (31). At this stage, it is highly recommended that these characteristics (membrane material, molecular weight cutoff, skin pretreatment) be reported to allow clear comparison among studies.

The effects of any procedure should be minimized and accounted for. In the case of microdialysis, there is the initial hyperemia from fiber placement, which is dealt with adequately by waiting 60-90 minutes before beginning any experimental protocols (32). On the other hand, the agents themselves, or their solvents, may affect baseline blood flow. Isotonic saline or Ringers solution are common carriers, but not all of the pharmacological agents of interest are sufficiently water soluble for them to carry an effective dose. Dimethyl sulfoxide (DMSO) is a very good solvent, but it can have vasodilator properties, itself (33, 34). While this is not necessarily limiting, it does require finding the lowest level of solvent adequate to dissolve the agent. The agent, itself can also cause background vasodilation. Depending on the question, control studies may require an alternate vasodilator to mimic the background effects of the primary agent (e.g. (35-37). Bearing these caveats in mind, microdialysis promises to continue to be a very powerful approach tofurther clarification of our understanding of the mechanisms of control in the cutaneous circulation.

4. REFLEX CONTROL OF THE CUTANEOUS CIRCULATION

The effects of direct heating and cooling on the skin circulation are potentially quite large, having the capacity for maximal vasoconstriction (and the risk of frostbite) as well as maximal vasodilation (15, 38). Reflex mechanisms in the skin circulation have a similarly broad range. Extreme whole body cooling can reduce skin blood flow to very low levels, although perhaps not to zero. Body heating and the associated increases in skin and internal

temperatures can, in resting supine individuals, force skin blood flow to maximal levels, even when the elevated body temperatures are within tolerable limits (1, 15). This ability of reflex control to cause such large changes in blood flow is of particular importance to cardiovascular control generally because the effects are not isolated to small areas of skin, but are broadcast over the entire body surface area. Pressor effects from cutaneous vasoconstrictor responses to body cooling are to be expected; conversely, the demands for increased cardiac output during body heating challenge the heart to pump a high minute volume of blood without the advantage of the muscle pump supplying energy for the return of blood to the heart (1, 5, 6).

4.1. Vasoconstrictor mechanisms

In nonglabrous (hairy) and glabrous (nonhairy) skin, vasoconstrictor mechanisms are tonically active; small increases or reductions in skin blood flow over the body surface provide the changes in heat elimination sufficient to control body temperature within narrow limits without a requirement for either sweating or shivering (3, 39-41). Indeed, the regulation of body temperature in humans is accomplished by those subtle changes most of the time, most of our lives. Only when the challenges of the environment or of increased heat production as in exercise are significant, do other mechanisms become involved in the regulation of body temperature. In fact, unmeasurably subtle changes in skin blood flow, carried out over an extended period of time, can cause measurable changes in internal temperature (42). For example, a reduction in the blood flow over the entire body by 2% can cause internal temperature to increase by an estimated 0.5°C over the period of 24 hours.

The level of tonic activity of sympathetic vasoconstrictor fibers is dependent on the environmental conditions. If the environment is warm, there may be little or no vasoconstrictor activity, whereas a cooler environment will be associated with significant tonic activity. One consequence of this is that cutaneous nerve block will cause an increased skin blood flow in the latter case, but not in the former, providing a probable explanation for the modest differences among laboratories in response to such blockade (1, 43, 44).

The current working model for the mechanisms involved in reflex cutaneous vasoconstriction are developed below and are outlined in Figure 1. This development includes our current understanding of the transmitters involved and of the factors that serve to modulate vasoconstrictor activity.

4.1.1. Transmitters

The primary transmitter for reflex cutaneous vasoconstriction is norepinephrine. Both $alpha_1$, and $alpha_2$ receptor functions are demonstrable in human nonglabrous skin through locally applied selective blocking and agonist studies (21), as well as through nonselective blockade (27, 45-48). Systemic alpha adrenergic blockade causes skin temperature to increase while core temperature falls in a dose-dependent fashion (47), indicating inhibition of tonic adrenergically mediated vasoconstriction in the skin.

The question arises of whether there are other vasoconstricting transmitters released from sympathetic nerves to the skin. This originates from conclusions derived from other species and other regions, as well as from experiments in humans. Neuropeptide Y (NPY) was found to participate in sympathetically mediated vasoconstriction in a variety of tissues and species (49-51) and was also found to have a variety of other functions relative to energy balance and feeding behavior (52, 53). With respect to a peripheral vasoconstricting role, the consensus is that it is colocalized in sympathetic nerve terminals with norepinephrine and participates in the vasoconstriction that follows nerve activation, e.g.(51, 54, 55).

Given the history, it was important to find whether NPY might act as a vasoconstricting co-transmitter from sympathetic adrenergic nerves in human skin. The fact that locally applied bretylium completely blocks reflex vasoconstriction during whole body cooling indicates that such vasoconstriction is carried out entirely by adrenergic nerves (23). Bretylium acts presynaptically to block all transmitter release from synaptic nerves, so its inhibition of vasoconstriction does not discriminate between or among the possible transmitters involved (56). Stephens and colleagues (27-29) tested the notion of a participation by something other than norepinephrine in the reflex cutaneous vasoconstrictor response to whole body cooling by blocking adrenergic receptors (rather than transmitter release) through the intradermal injection of yohimbine at a level that probably did not distinguish between alpha1 and alpha2 receptor subtypes. In this original attempt, the vasoconstriction to body cooling was reduced but not eliminated. The test for blocking efficacy, iontophoretic application of norepinephrine, showed the normal vasoconstrictor response to be reversed to vasodilation by yohimbine, signaling an activation of beta adrenergic receptors by the norepinephrine (26) and possibly obscuring the results from reflex-mediated vasoconstriction. The addition of the beta adrenergic antagonist propranolol to yohimbine successfully inhibited both vasoconstrictor and vasodilator responses to exogenous norepinephrine and, importantly, made the persistent reflex vasoconstriction in the areas subjected to dual alpha- and beta- block much clearer, indicating the participation by a vasoconstricting co-transmitter in the vasoconstrictor response to body cooling (27).

The above results serve as good evidence for participation by something other than norepinephrine in sympathetically mediated reflex vasoconstriction,

but do not identify that co-transmitter. That identification came from a subsequent study in which the NPY Y1 antagonist BIBP3226, applied by microdialysis, caused a significant attenuation of the reflex vasoconstriction (29). The combination of BIBP3226 with yohimbine and propranolol delivered to a single site further strengthened the conclusion that NPY played a significant role in reflex cutaneous vasoconstriction because that combination appeared to block completely the reflex cutaneous vascular response to body cold stress (29).

NPY is generally described as having three roles in sympathetically mediated vasoconstriction: a direct postsynaptic vasoconstriction, a postsynaptic potentiation of the response to norepinephrine and a presynaptic inhibition of norepinephrine release (57). The above studies do not permit a clear discrimination among those three actions, but the evidence does support the first possibility. The persistent vasoconstriction in the presence of adrenergic blockade and its disappearance with Y1 blockade is strong evidence that NPY plays a direct vasoconstricting function and that is its major role in the human cutaneous circulation (29).

Similarly designed studies found that the role for vasoconstricting sympathetic co-transmitters in reflex cutaneous vasomotor control was subject to modification by age and by gender. Stephens *et al.* (28)found that the persistent reflex vasoconstriction in the presence of adrenergic receptor blockade in response to body cooling varied between menstrual phases in young women. With elevated estrogen and progesterone levels, the reflex cutaneous vasoconstrictor response to body cooling is much the same as with young men, about 30-40% of the vasoconstriction is non-adrenergic. When female steroid levels are low, however, there was no measurable reflex vasoconstriction in the presence of adrenergic receptor blockade.

This observation is at first enigmatic, as most studies involving gender comparison opt to study women in the follicular phase, based on the reasoning that any gender differences due to acute effects of estrogen and progesterone would be avoided. However, estrogen priming followed by progesterone does increase mRNA for NPY in the hypothalamus and pituitary in rats (58). It is not possible to say whether the same is operative in human sympathetic cutaneous nerves, but it would explain the greater non-adrenergic reflex vasoconstriction in women when hormone levels are elevated. Nevertheless, it does appear that female steroids are stimulatory in some part of the NPY regulatory scheme. The possibility of either adrenergic affects of progestins or the very low levels of estrogen and progesterone in men might also contribute to an explanation for this somewhat unexpected parallel between the responses in women and men (28).

Aging also acts to modify the balance between the roles for norepinephrine and nonadrenergic co-transmitters in reflex cutaneous sympathetic vasoconstriction (59). Thompson *et al.* (60) verified the above non-adrenergic reflex vasoconstrictor response to body cooling in young men (18-25 years) but failed to find evidence for such an occurrence in older subjects (63-78 years). In that study, bretylium blocked completely the reflex cutaneous vasoconstrictor response to body cooling in both age groups, whereas the combination of yohimbine and propranolol also completely inhibited that vasoconstrictor response in the older group, but only reduced it in the younger group; the persistent vasoconstriction in the latter case indicating a role for sympathetic co-transmitters only in the younger group (60).

In the cases of gender and aging influences above, there was no specific demonstration that NPY is the co-transmitter involved. It seems likely, however, that this was the case. In the case of age differences, it is known that in young men YI antagonism reduces the vasoconstrictor response to one dependent solely on adrenergic mechanisms (29). It stands to reason that aging (or the menstrual cycle) leads to a loss of that co-transmitter (NPY), mimicking the effects of pharmacological removal.

4.1.2. Modulation of cutaneous vasoconstrictor function

The most robust activation of reflex vasoconstriction in the skin occurs with body cooling. That response, however, is subject to modification by influences that are not primarily thermoregulatory in nature. For example, the phase of the menstrual cycle has a role in determining cutaneous vasoconstrictor function (61). In this case, the pattern of the response to body cooling does not differ between high and low hormone levels of the menstrual cycle (as established through oral contraceptives) with respect to the time of cooling, but the vasoconstriction acts to protect the prevailing body temperature. Thus, the higher body temperatures of the luteal (high hormone) phase are associated with the same degree of vasoconstriction during cooling as are the lower body temperatures of the follicular (low hormone) phase but do so around a higher internal temperature; vasoconstrictor functions ‘reset’ to a higher internal temperature.

Diurnal control of the skin circulation shows a similar pattern of modification of the control of cutaneous vasoconstrictor activity (62). In this case, the reflex vasoconstrictor response to whole body skin cooling was more sensitive in the early morning (when internal temperature is lower) than in the evening. This indicates that the control of cutaneous vasoconstriction is modified in the afternoon to support the shift in internal temperatures to higher levels. How that happens is less clear; however, there is a possible role for melatonin, a hormone that has a diurnal secretion profile (63). Further, the

administration of melatonin usually leads to a reduction in internal temperature under resting conditions (63-66), suggesting reduced cutaneous sympathetic vasoconstrictor activity and increased skin blood flow. For the most part, differences in the vasomotor control of skin between late afternoon and early morning are mimicked by the ingestion of melatonin in the afternoon (67). There is a downward shift in the internal temperature about which vasoconstrictor control operates and a reduced sensitivity to whole body skin cooling, both characteristics of the control of skin blood flow in the early morning (67). These observations suggest that nocturnal secretion of melatonin may have a role in the diurnal variation in body temperature through modulation of cutaneous sympathetic vasoconstrictor function.

4.2. Sympathetic active vasodilation

When environmental temperature and/or metabolic heat production exceed the ability to control body temperature through modulation of cutaneous vasoconstrictor activity, other powerful systems are brought into play. The secretion of sweat and its evaporation is an extremely efficient means of liberating heat to the environment. At the same time, neurogenic active vasodilation is initiated, allowing for the transport of heat from deeper tissues to the skin for its elimination. How this system works and how it is controlled have attracted substantial research interest, but much about the system remains controversial and conjectural, including the specific transmitters involved.

The recognition of the existence, in humans, of a neurogenic vasodilating mechanism in skin other than withdrawal of vasoconstrictor activity (passive vasodilation) is generally said to originate with observations by Lewis and Pickering (68) and Grant and Holling (69), who noted that nerve block or sympathectomy failed to raise skin blood flow to the same extent as whole body heating (free of direct influences of heating). This implied that intact sympathetic nerves were required to achieve the high levels of blood flow seen with body heating--an active system was implied. That conclusion was strengthened considerably in a classic study by Edholm *et al.* (43), who applied local anesthesia to the skin of the forearm proximal to the area of blood flow measurement. Such application prior to heating prevented the usual increase in blood flow and, when applied during heat stress, cause a large reduction in forearm skin blood flow. Again, an active system is implied. Roddie *et al.* (44, 70, 71) came to similar conclusions based on sampling of oxygen content from deep and superficial veins. Lastly, Kellogg *et al.* (3, 23) applied bretylium iontophoretically to skin and measured the reflex vasomotor responses to body cooling and heating. They found that the antiadrenergic properties of bretylium blocked the reflex vasoconstrictor response to body cooling, but had no obvious effect on the vasodilator response to body heating. These data are, collectively, essential proof of the presence of an active vasodilator system in the skin.

The vasodilator system is quite powerful. Its activation can cause skin blood flow to rise by several fold. Even within tolerable limits of hyperthermia, the active vasodilator system can cause maximal cutaneous vasodilation (15). That level of skin blood flow is potentially quite high. Rowell (5, 6) estimates that total skin blood flow can reach levels on the order of 8 L·min-1 during whole body heat stress. This would make skin second only to active skeletal muscle in the capacity for vasodilation in intact humans. The increase from values in normothermia of 300-500 ml·min-1 is accomplished almost entirely by activation of the active vasodilator system.

The cutaneous active vasodilator system is found in nonglabrous skin, whereas vasodilation in glabrous skin is achieved through withdrawal of vasoconstrictor activity (2, 4). At one time it was held that the entire hand was limited to vasoconstrictor control (2, 4, 72), despite the original observations by Lewis and Pickering (68) having been made in the fingers of patients with Raynaud's disease. However, the advent of the use of laser Doppler flowmetry and the spatial resolution it permits led to the recognition that there was important active vasodilator function in the dorsal hand and fingers (73). It is generally held that the active vasodilator control is distributed to nonglabrous (hairy) skin, although there is some presumption even to that generalization.

4.2.1. Mechanisms of active vasodilation

The potential capacity for the active vasodilator system to raise skin blood flow is unquestioned. How it does so remains a point of current research and discussion. Here, we deal with three elements of the mechanism(s) for a cutaneous active vasodilation: (a) What are the neurotransmitters? (b) What is the involvement of nitric oxide? (c) What is the linkage to sweating? These questions all have been the subject of investigation over the past decade, and they are not mutually exclusive.

The postulated mechanisms involved in the reflex responses to body heating, with emphasis on active vasodilation are outlined in Figure 2 and are developed below. This development and the outline include the role for NO and the possible cotransmitters, which are still under discussion and unproven

4.2.2. Neurotransmitters

The earliest attempts to identify the neurochemical linkage between sympathetic vasodilator nerves and cutaneous arterioles tested for the involvement of acetylcholine. Roddie *et al.* (44) found that intra-arterial

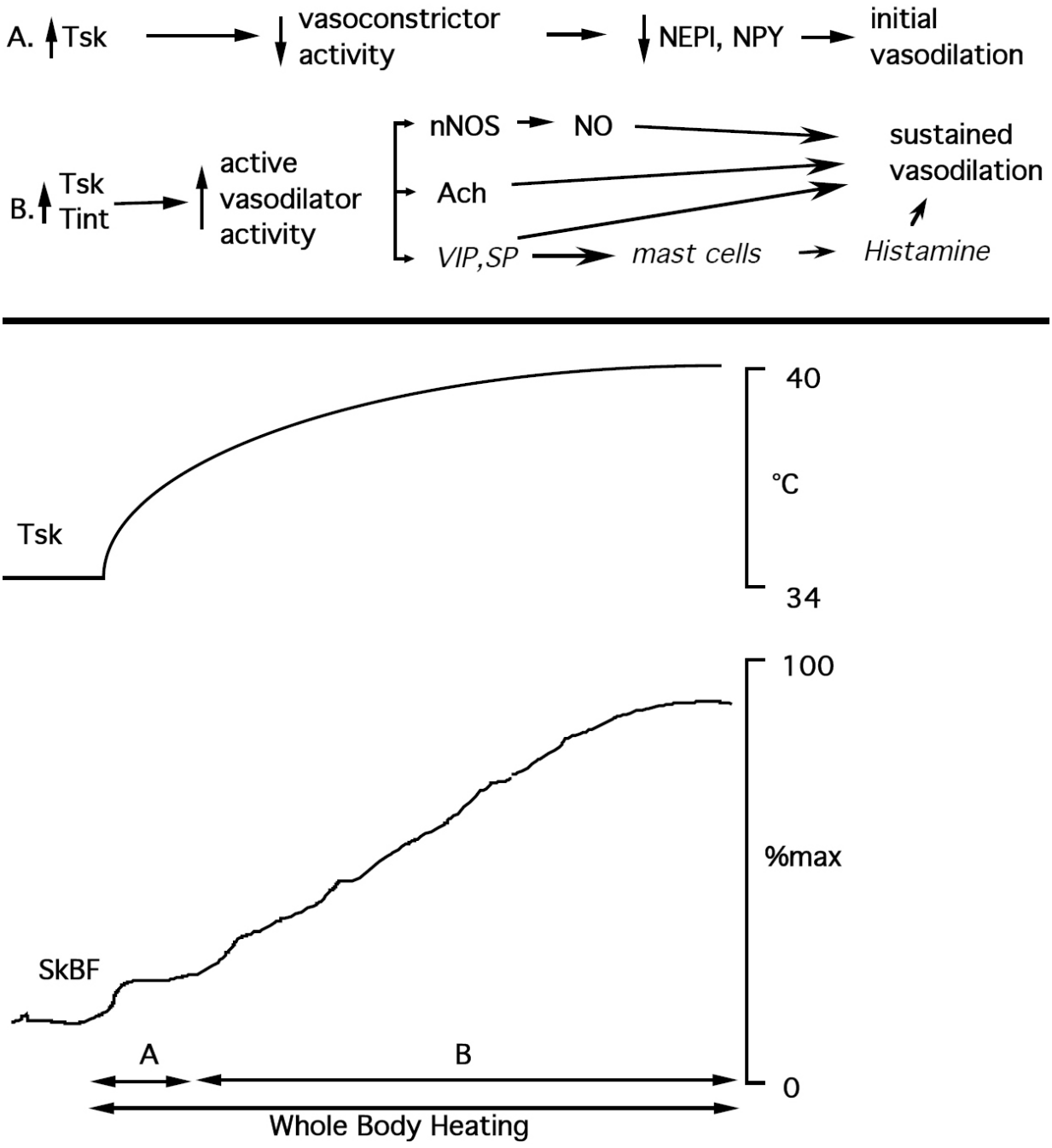

Figure 2. Reflex responses in skin blood flow to whole body heating. Below: The increase in skin blood flow occurs in two phases. The first phase (A) is associated with increased skin temperature (Tsk) and is due to withdrawal of any tonic vasoconstrictor activity, which causes a small vasodilation. The second phase (B) accompanies the subsequent increase in internal temperature (Tint) and is characterized by the initiation of sweating and of a neurogenic active vasodilator system, accounting for most of the increase in skin blood flow during body heating. Possible mechanisms for neurogenic active cutaneous vasodilation as developed in this review are shown. In both phases increased body (skin and internal) temperatures inhibit adrenergic vasoconstrictor activity. In the second phase, the active vasodilator system is responsible for the majority of the vasodilation. NO is involved, perhaps from prejunctional nerves. Acetylcholine is involved, as are one or more cholinergic cotransmitters, with evidence in support of VIP and/or a neurokinin such as Substance P (SP). Histamine from mast cells, perhaps stimulated by VIP, and prostanoids are also thought to have roles in the active vasodilator process. Terms in italics have yet to be firmly established.

atropine caused a delay in the onset of the reflex cutaneous vasodilator response to heat stress and also reduced the level that skin blood flow ultimately achieved. Given after active vasodilation was established, however, atropine was without effect on forearm skin blood flow. Sweating was blocked completely in both instances. These findings have been repeated by several groups (74, 75), but create a scenario in which the interpretation is not clear. One series that runs counter to the above found that atropine caused an increased skin blood flow during exercise, albeit sweating was significantly reduced (76, 77). A possible explanation for this observation relates to the effects of the decreased sweating on skin temperature and the role skin temperature plays in the regulation of skin blood flow. An increased skin temperature causes a central modification of the control of blood flow by reduction in the threshold for vasodilation (78, 79). Otherwise, this observation is difficult to reconcile with the inhibiting influences of cholinergic muscarinic receptor blockade in resting conditions. It may, indeed, be an important glimpse into the mechanisms of control of active cutaneous vasodilation, but awaits clarification.

The role for acetylcholine appears to be most important early in the reflex responses to body heating (3). This conclusion is based on several observations. In particular, Shastry *et al* (75) noted that atropine given after heat stress and active vasodilation were established, had little or no effect on blood flow, whereas given before heating commenced there was a delay in the onset of vasodilation. These observations reflect those made earlier by Roddie *et al* (44). Those findings found support from a differently styled study by Shibasaki *et al* (80), who gave the phosphodiesterase inhibitor neostigmine locally to skin. In this case, they found the inhibition of acetylcholinesterase had the predicted effect of enhancing skin blood flow and sweating early in heating, but not later. They also found the effect of neostigmine early in heating to be NO-dependent (80). These studies lead to the likely conclusion that there is an important time element in the roles of the transmitters involved in active vasodilation.

An important insight into the possible role of cholinergic mechanisms in cutaneous active vasodilation comes from the use of botulinum toxin A to antagonize cholinergic nerves presynaptically to prevent the release of acetylcholine and any associated co-transmitters from cholinergic nerves (81, 82). The mechanism of action of botulinum toxin, which causes botulism, appears to involve primarily its uptake into cholinergic nerve terminals, where it acts to cleave the proteins responsible for neurotransmitter exocytosis, effectively blocking synaptic function (81, 82). It is this property that confers both the toxicity of botulinum toxin as well as the growing number of therapeutic uses (83, 84). The binding to the exocytotic SNAP-25 proteins is irreversible; new synthesis and axonal sprouting are required for recovery of function, making the actions long lasting. Further, the toxin binds to and disables both cholinergic motor nerves to skeletal muscle and autonomic cholinergic nerves.

The above characteristics led to the test of the effect of botulinum toxin A on cutaneous active vasodilation (74). An intradermal injection into a small (~ 1cm2) area of skin made that area, 2-14 days later, completely unresponsive to the reflex effects of whole body hyperthermia. Both cutaneous active vasodilation and sweating were completely antagonized in the area of the botulinum toxin A injection. Importantly, the reflex vasoconstrictor response to whole body cooling was unimpaired. In the same series, atropine was found to reduce but not block active vasodilation, but to block sweating, as had been seen by others (44, 75). The interpretation of these data, based on the specificity of botulinum toxin A on presynaptic cholinergic nerves (3, 81, 82, 85, 86), is that cutaneous active vasodilation is effected by a cholinergic co-transmitter mechanism, with acetylcholine stimulating some of the vasodilation and some other substance(s), co-released from cholinergic terminals, responsible for the rest. Indeed, the co-transmitter(s) would appear to be responsible for around 80% of the active vasodilator response (74, 87).

An important assumption rests on the specificity of botulinum toxin A for cholinergic nerve terminals. That those terminals were affected is shown by the abolition of sweating response to hyperthermia in the treated areas, but that does not exclude an effect on noncholinergic neurons. Any neuron that has the appropriate receptors for uptake of the neurotoxin and utilizes SNAP-25 protein for exocytosis of the neurotransmitter(s) would be subject to inhibition by botulinum toxin A. Indeed, Morris *et al*. (87) found *in vitro* evidence that botulinum toxin A was inhibitory to contractions of guinea pig vena cava and uterine artery to direct sympathetic stimulation and that SNAP-25 proteins could be found colocalized with tyrosine hydroxylase and NPY. Furthermore, incubation with botulinum toxin revealed cleavage of SNAP-25. However, it remains quite unlikely that the results from hyperthermia in humans (74) can be explained on the basis of involvement of noncholinergic mechanisms. The major argument is that the levels of botulinum toxin were without measurable effect on adrenergically mediated vasoconstriction (74, 88, 89).

It was also observed that the *in vitro* stimulation of vasodilator nerves in guinea pig uterine arteries causes the release both of acetylcholine and of neuropeptides, notably VIP and CGRP (90), but that the inhibitory effects of

botulinum toxin A on the release of acetylcholine were more complete than on the release of the putative co-transmitters, suggesting some differential effect of the neurotoxin on the release of vesicles of different sizes with different transmitters. This might also potentially apply to *in vivo* human cutaneous neurogenic active vasodilation, but in our earlier study enough botulinum toxin was used to completely inhibit all active neurogenic vasodilator function, so such differential control of transmitter release was not seen (74).

4.2.3. Cotransmitters

The results from pre- and post- synaptic antagonism of cholinergic terminals suggesting a co-transmitter mechanism stimulated a number of studies designed to discover the identity of the co-transmitter or co-transmitters. For the most part, these studies follow a common design. Microdialysis membranes placed in forearm skin are perfused with a solution containing an antagonist to a particular hypothetical vasodilating transmitter or with a control solution. Whole body heating is then used to activate the active vasodilator system at the same time and to the same extent at both sites. Characteristics of the ensuing vasodilator responses are compared, testing for an effect of the antagonist.

4.2.3.1. VIP

VIP is known to be colocalized in cholinergic neurons, and has significant vasodilating properties (50, 91-94). The original findings were made in cat submandibular glands, and the possibility of glandular-based active vasodilation arising from sudomotor function clearly exists in the skin (95). Indeed, Hökfelt (49) raised the possibility that VIP was involved as a co-released transmitter from sympathetic cholinergic nerves in skin.

Could VIP contribute to cutaneous active vasodilation? A test of that question came from work by Bennett *et al.* (96) who applied the fragment VIP 10-28 by microdialysis. VIP 10-28 competitively antagonizes both VPAC1 and VPAC2 receptors (3, 97-99). A problem with that generation of VPAC receptor antagonist is a much lower affinity for those receptors than for VIP, itself (100). Furthermore, high concentrations of VIP 10-28 cause vasodilation (96). Consequently, the window for antagonist concentration is limited at both low and upper ends – too little there is insufficient antagonism, too much there is the problem of elevated baseline.

Bennett *et al.* (96) found a concentration of VIP (7.3 mM) that when infused via intracutaneous microdialysis, caused a vasodilator response similar to that occurring during heat stress. They also found the maximum concentration of VIP 10-28 (214 mM) that could be applied by microdialysis without causing significant vasodilation. That concentration of VIP 10-28 caused a 54% inhibition of the vasodilator response to 7.3 mm VIP. The above concentration of VIP 10-28 also significantly inhibited the reflex vasodilator response to body heating, reducing the change in cutaneous vascular conductance (CVC) relative to internal temperature by 53% and the extent of reflex vasodilation by 42%. Interestingly, atropine co-administered with the antagonist did not provide further inhibition, perhaps reflecting the temporal character of the contribution by acetylcholine mentioned earlier.

The inhibition of the reflex vasodilator response to body heating by the VPAC1/VPAC2 antagonist VIP 10-28 supports a role for VIP in active cutaneous vasodilation. There are concerns that prevent reaching a firm conclusion, however. First, the characteristics of the antagonist are not ideal. It could provide only partial inhibition of either the vasodilator response to exogenous VIP or to whole body heat stress. We cannot say from these data whether the remaining vasodilation was due to another co-released transmitter (in the case of hyperthermia) or to an incomplete blockade of VIP receptors (in both cases). Further, the VPAC2 receptor is also sensitive to PACAP (3, 101), opening the possibility that PACAP, not VIP, is the transmitter involved in active vasodilation. Some doubt was expressed from the observation by others that VIP 10-28 augmented the local vasodilator response to exogenous VIP (102). We cannot explain how the antagonist might enhance the response to the agonist, but it is of interest that the use of VIP 10-28 in that study also augmented the reflex vasodilator response to hyperthermia. This observation is in keeping with VIP acting as the transmitter for active vasodilation inasmuch as the effects of VIP 10-28 on the actions of VIP paralleled those on the response to hyperthermia..

A challenge to the idea of an important role for VIP in cutaneous active vasodilation comes from a series of studies involving patients with cystic fibrosis. The first test of a role for VIP in thermoregulatory vasodilation was reported by Savage *et al.* (103), following the report that patients with cystic fibrosis had little or no VIP in skin nerves (104). If VIP were involved mechanistically in active vasodilation, cystic fibrosis patients might have attenuated skin blood flow responses to heat stress. This was not the case; patients with cystic fibrosis had preserved vasodilator responses to hyperthermia. Those results suggested that VIP might not be involved in cutaneous active vasodilation (103).

Because of the possibility of redundant mechanisms between acetylcholine and VIP in cutaneous active vasodilation, two additional tests of the active vasodilator system in cystic fibrosis have been done (105, 106). In one of these studies, the possibility was raised that an enhanced role for acetylcholine could replace reduced VIP function in cystic fibrosis. This idea was tested by assessing the effects of atropine on active vasodilation in the

patients with cystic fibrosis. The proposal was that, since the patient group lacked a fully functional VIP system, blockade of the acetylcholine function would effectively remove the reflex cutaneous vasodilator response to body heating. However, this muscarinic receptor antagonist had the same effect in cystic fibrosis patients as in matched controls, hence arguing against such a redundant mechanism (105). In a study by Wilkins *et al*. (106) the possibility was tested that augmented contributions of the NO system compensate for reduced VIP function in cystic fibrosis. This idea was based on observations that exogenous VIP vasodilates, in part, through NO-dependent mechanisms (107). Administration of the NOS antagonist L-NAME attenuated active vasodilation in hyperthermia equally in patients with cystic fibrosis and control subjects, suggesting that stimulation of the NO-dependent processes, such as VIP-mediated vasodilation, were not altered in cystic fibrosis (106). Thus, to date, there is no clear explanation for how active vasodilation occurs in cystic fibrosis, but the collection of observations in this patient group have to be considered in any model of active cutaneous vasodilation involving VIP.

4.2.3.2. Substance P

One of the more surprising entries onto the list of proposed contributors to cutaneous active vasodilation is the neurokinin Substance P (108). The experimental design testing this possibility varied from those employed above. In this case, the authors noted that neurokinin receptors became desensitized following exposure to Substance P, there being less vasodilation in response to a second exposure to Substance P (109). The authors reasoned that, if Substance P has a role in the cutaneous active vasodilator response to body heating, then prior exposure to Substance P should reduce the vasodilator response to heat stress. That is what they observed. Prior exposure to Substance P reduced the vasodilator response to body heating by 30%, in support of a role for neurokinin receptors and perhaps Substance P as the native agonist in active vasodilation.

A difficulty in accepting Substance P, *per se*, as having a role in active cutaneous vasodilation is because Substance P is known to be localized to afferent nerves and endothelial cells (110-112). Substance P from those sources is clearly involved in inflammatory responses. However, it is not clear how central activation of sympathetic vasodilator pathways would set into motion the release of Substance P. Other tachykinins (neurokinin A, neurokinin B) might be involved. Clearly, more work is required to resolve this issue.

4.2.4. Nitric oxide

There is now very strong evidence that the nitric oxide system is involved in cutaneous active vasodilation. Simultaneous publication of studies from two laboratories using differing approaches to nitric oxide synthase (NOS) inhibition showed such inhibition to limit the reflex vasodilator response to body heating (113, 114). Administration of the nonspecific NOS inhibitors L-NAME via microdialysis (113) or L-NMMA via brachial artery infusion (114) limited the reflex vasodilator response to body heating. This observation has been repeated several times, with the general conclusion being that NOS blockade reduces the vasodilator response by over 40% (75, 108, 115, 116).

The above studies all involved non-specific NOS inhibitors. Recently, the availability of more specific antagonists has enabled exploration of which of the NOS isozymes is involved in active cutaneous vasodilation. NOS I (neuronal NOS, nNOS) inhibition with 7-NI reduced the sensitivity of the vasodilator response to rising internal temperature during body heating by 40% and the extent of the vasodilation by a similar amount (33). That antagonist had no effect on the vasodilator response to local heating. This finding is in keeping with NOS I being the source of the NO involved in active cutaneous vasodilation but not for that involved in the vasodilator response to local warming. Blockade of NOS III (endothelial NOS, eNOS) with L-NAA had no significant effect on the reflex vasodilation response to whole body heating, but did significantly inhibit the vasodilator response to local skin heating (117). These results are also consistent with NOS I having an important role in reflex vasodilation, but not in locally mediated responses.

The above requires some resolution relative to the neurotransmitter studies discussed above. In particular, where is the NO generated, and what is the relationship to the putative neurotransmitters or co-transmitters? In the studies involving Substance P (108) and prostanoids (115), the combination of nonspecific NOS inhibition (L-NAME) with the antagonists caused a greater attenuation in the vasodilator response to body heating than did the antagonist or L-NAME alone. The effects were essentially additive, indicating neither Substance P nor prostanoids relied on NO for their vasodilator effects. Similar results were obtained for VIP, although they are more difficult to interpret given the augmentation of vasodilation by the antagonist VIP 10-28 in that study (102). Nevertheless, these effects were unaffected by nonspecific NOS inhibition, indicating parallel vasodilator mechanisms for VIP and NO, rather than an interdependence.

These findings suggest the model illustrated in Figure 2 for a working hypothesis for the mechanisms of vasodilation. In the model, NO is released by nonvesicular means from presynaptic cholinergic terminals at the same time a co-transmitter or co-transmitters are released by a SNAP-25 dependent vesicular mechanism. The NO acts through a cGMP mechanism in the vascular smooth muscle, while VIP and/or other transmitters

act in parallel via CAMP and prostanoids. Mast cell release of histamine and endothelial Substance P may also be involved downstream from the peptide co-transmitters.

A critical challenge to this model is the abolition of active cutaneous vasodilation by interference with vesicular transport by botulinum toxin (74). NOS I presumably forms NO in presynaptic terminals from which it is released by non-vesicular mechanisms (90). If that is the case, how is its role in vasodilation blocked by botulinum toxin? One possibility is that the NO system works cooperatively with the system(s) downstream from VIP or other co-transmitters. Studies in the rabbit ear, a model of cutaneous vascular control having an active vasodilator system with NOS dependence (118, 119), raise this possibility. A synthesis that developed from those studies is that NO acts in a permissive way with the peptide transmitters to enhance their vasodilator effects (120, 121). Although application of this proposal to humans has been questioned (122), neither the proposal nor the counter-argument can be easily dismissed. The possible interaction among these players in cutaneous active vasodilation deserves further study as it may finally enlighten us as to the mechanisms for the powerful active vasodilator system.

4.2.5. Histamine

Wong *et al.* (116) infused pyrilamine (H1 receptor antagonist) and cimetidine (H2 antagonist) intradermally via microdialysis. The H1 antagonist reduced the peak vasodilation by 26% whereas the H2 antagonist was without significant effect. The H1 effect may involve nitric oxide (116). The authors raise the possibility that the involvement of histamine is through VIP being released from vasodilator nerves and acting on mast cells to cause the release of histamine for subsequent vasodilation via vascular H1 receptor activation. Could it be that VIP is causing active cutaneous vasodilation through approximately equal engagement of mast cells to release histamine and direct actions via VPAC receptors on the cutaneous resistance vessels? This intriguing possibility requires further study.

4.2.6. Prostaglandins

McCord *et al.* (115) tested whether prostanoids might contribute to active cutaneous vasodilation through inhibition of cyclooxygenase pathways with ketorolac, applied by microdialysis. Indeed, they found ketorolac to reduce the peak vasodilation during heat stress by about 26%. It therefore is likely that prostanoids are involved in the process, although it is also likely that it is a postsynaptic role in which a transmitter activates the cyclooxygenase enzymes. The finding of a cyclooxygenase involvement in active cutaneous vasodilation is supported by a recent study in which chronic aspirin therapy was shown to limit significantly the vasodilator response to body heating, probably through an inhibitory action on active vasodilator function (123). It is important to note that the dose (81 mg daily) is widely used as a chronic anti-inflammatory treatment.

4.2.7. Relationship to sweating

There is a long-held hypothesis that the sudomotor control of sweat glands is also the source of active cutaneous vasodilation (95, 124). There is a series of observations that support that hypothesis, as well as a series of observations that do not (1, 5). Active vasodilation and sweating are initiated at about the same time during heat stress. The congenital absence of sweat glands is associated with the lack of an active vasodilator response to body heating (125). Similar observations (see Fox and Hilton (95)) led to the 'bradykinin hypothesis,' which described how sudomotor activation might cause cutaneous active vasodilation. Activation of cholinergic sudomotor nerves supplying eccrine sweat glands would cause those glands to release an enzyme into the interstitial space. That enzyme would then cause the formation of the vasodilator bradykinin, which, in turn, would cause vasodilation in the skin.

The bradykinin hypothesis is made up of two major elements. The first is that sudomotor control and active vasodilation are functionally and causally linked. The second, related element is that bradykinin serves as the link between those two functions. The arguments around the first issue have been detailed earlier (1, 5, 95), and there has been little added to the discussion since. It remains an important unresolved question.

The involvement of bradykinin, either as a product of sweat gland activity or as a transmitter itself has seen some resolution. Kellogg *et al.* (126) blocked bradykinin B2 receptors in forearm skin with the antagonist HOE-140 delivered by microdialysis. Subsequent whole body heat stress caused equal vasodilation at those sites and at nearby control sites. The onset of vasodilation during heat stress did not differ between blocked and control sites, as well. These data effectively rule out participation in cutaneous action vasodilation by B2 receptors. A role for B1 receptors can also be dismissed. Both in the study by Kellogg *et al.* (126) and in a previous study in rabbits by Warren and Loi (127), B2 receptor blockade was sufficient to completely inhibit the vasodilator response to exogenous bradykinin. Further, B1 antagonism was without effect on bradykinin stimulated vasodilation. Although the question of a relationship between sudomotor activity and active vasodilation is unresolved, a role for bradykinin is unlikely.

4.2.8. Modulation of vasodilator function

An increase in internal temperature, especially, is the major drive for the active vasodilator system. For example, skin temperature has reflex effects, as well, but

does so largely through shifting the relationship of active vasodilator function to internal temperature (78, 79). There are also non-thermoregulatory modulators of active vasodilator function. These include exercise and blood pressure control (88, 128, 129). The effects of dynamic exercise on the cutaneous circulation have been extensively reviewed (130, 131) and will not be dealt with further here. However, the cutaneous vascular responses to isometric exercise deserve some comment because they have some bearing on control mechanisms for the cutaneous active vasodilator system. Isometric handgrip exercise does not have a measurable effect on cutaneous vascular conductance in normothermic conditions (132-134). This applies, at least, to nonglabrous skin. Palmar and plantar skin show a marked vasoconstrictor response (134). In heat stress there is a significant vasoconstrictor response by nonglabrous skin to isometric exercise (133, 135). It is this latter observation that is of particular interest relative to the unresolved debate regarding the connection between the sudomotor control of sweating and active cutaneous vasodilation. During isometric handgrip exercise, there is a significant reduction in cutaneous vascular conductance whereas there is an increased sweat rate. The reduced cutaneous vascular conductance occurs equally in skin with and without adrenergic function (the latter via bretylium blockade of transmitter release), indicating that the reduced vascular conductance is via inhibition of active vasodilator activity. This is the only example to date of sweat rate and active vasodilation being caused to change in different directions and is one piece of evidence that these thermoregulatory effectors are not causally related (133, 135).

There are also more slowly developing modulators of active vasodilator function. Similar to the modifiers of vasoconstrictor control, daily rhythms and, in women, changes through the menstrual cycle also affect active vasodilator function (42, 136-138). In the luteal phase of the menstrual cycle, when estrogen and progesterone are both elevated, the relationship of skin blood flow to internal temperature is shifted to higher temperatures relative to the follicular phase (138). This is reflected by a higher threshold internal temperature for the onset of cutaneous vasodilation during heat stress and a generally parallel shift of the relationship beyond that threshold. This shift is unaffected by removal of vasoconstrictor function with bretylium; hence, the shift to higher temperatures in the luteal phase of the menstrual cycle is through a similar shift in the control pattern of cutaneous active vasodilation (42, 137). That shift to a higher threshold for the initiation of active vasodilation during the luteal phase reflects combined central effects of the principal hormones, estrogen and progesterone. When evaluated independently, estrogen has the effect of lowering body temperature and the threshold for vasodilation, whereas progesterone has an opposite, more pronounced effect. It is likely that these hormones affect active cutaneous vasodilator function in a similar way, with the net increase in vasodilator threshold and body temperature reflecting the greater effect of progesterone (42).

There is also a daily fluctuation in internal temperature. This diurnal rhythm in body temperature occurs in everyone; in women it sums with that for the menstrual cycle (139). The diurnal cycle in body temperature is accompanied by a similar cyclic variation in the control pattern for skin blood flow (136). Unlike the change in pattern with the menstrual cycle phases, diurnal changes in the relationship of skin blood flow to internal temperature show changes both in the threshold for the onset of vasodilation (higher in the late afternoon) and in the sensitivity (also higher in the late afternoon). Aoki and colleagues (136) tested for the roles of the cutaneous vasoconstrictor and vasodilator systems in these diurnal changes, finding that blockade of vasoconstrictor function did not affect the increase in vasodilator threshold seen in the late afternoon, showing that diurnal effect to be brought about through inhibition of active vasodilation. However, blockade of adrenergic function did remove the difference in sensitivity between early morning and late afternoon, showing that diurnal effect to be a function of vasoconstrictor system activity.

The possibility that the diurnal modulation of active vasodilator function was through similar fluctuations in melatonin levels represented an interesting possible link between the time of day and thermoregulatory control of skin blood flow. Evidence supportive of that possibility was gained from daytime ingestion of melatonin, which reduced baseline internal temperature and the threshold for vasodilation (140). This effect of melatonin on vasodilator threshold was not changed by removal of vasoconstrictor system function with locally applied bretylium, showing that melatonin lowered the threshold through the active vasodilator system. These observations are supportive of the notion that melatonin, acting centrally, brings about the diurnal changes in the control of the active vasodilator system, although some differences in pattern exist. Also, the levels of melatonin were probably significantly higher than those likely from the diurnal secretory pattern. Nevertheless, it appears likely that melatonin has an important role in the diurnal shifts in body temperature and its regulation, including effects on the reflex control of skin blood flow through the active vasodilator system.

The influence of healthy aging on temperature regulation and the control of the cutaneous circulation are covered in detail elsewhere in this series. It bears mentioning here that older healthy subjects show, relative to younger subjects, a delay in the initiation of the active vasodilator

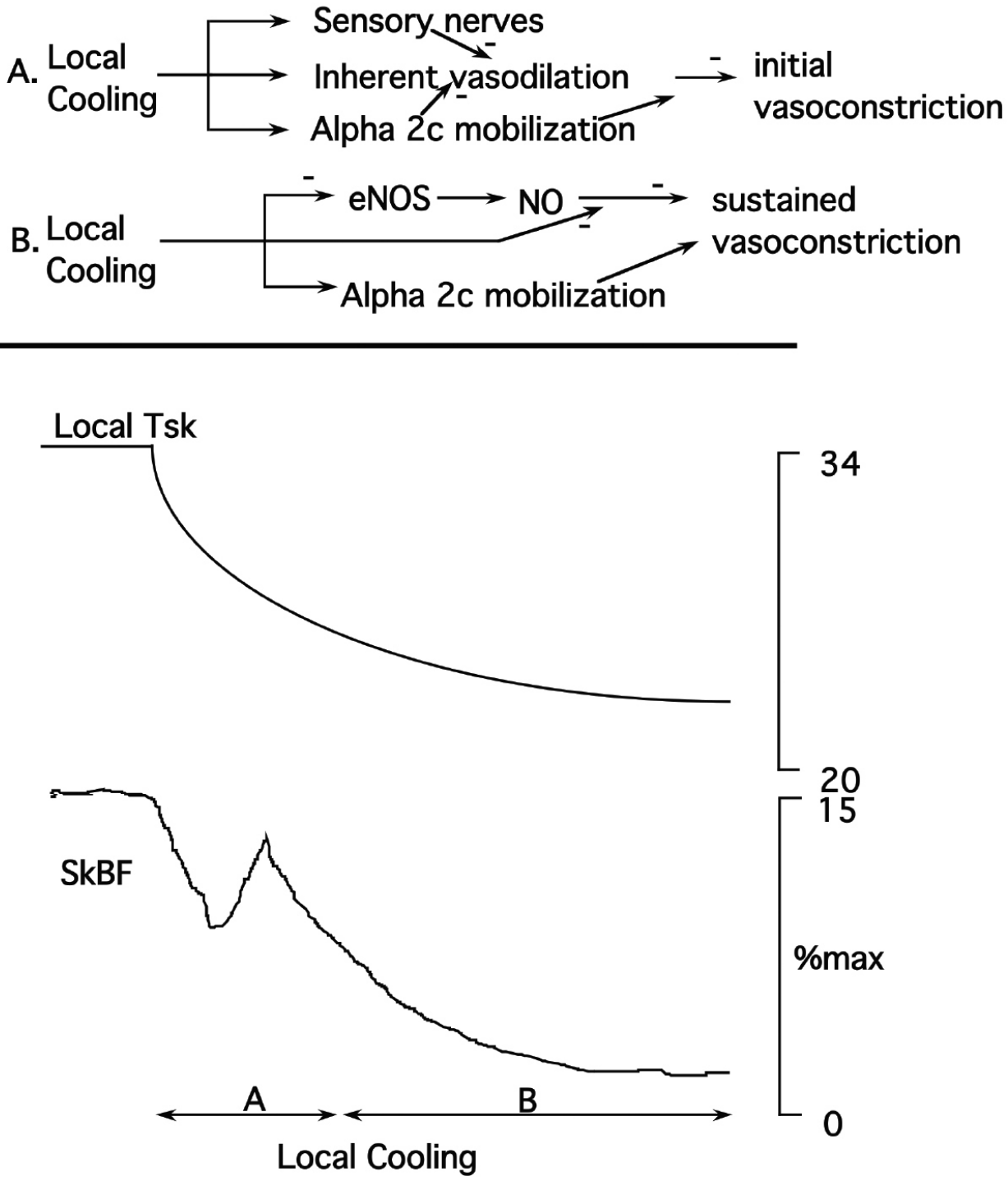

Figure 3. Cutaneous vasoconstrictor response to local skin cooling: Below: Pattern of response in skin blood flow to a rapid reduction in local skin temperature falls into 2 phases. (A) The initial response is a sharp drop in blood flow, often interrupted by a transient vasodilation that appears to be inherent to the vasculature but is normally inhibited by adrenergic function and by sensory nerve function. (B) A sustained vasoconstriction is achieved by the combination of increased adrenergic function via translocation of alpha2c receptors to the vascular cell surface and reduced NO system function via eNOS inhibition and inhibition of steps downstream from NO formation. Above: Mechanisms involved in the steps of cutaneous vasoconstriction stimulated by local cooling involve sensory nerves, adrenergic receptor mobilization, NO system inhibition and an inherent vasodilator response.

response to body heating and a reduced net vasodilation (141). This difference disappears with supplementation of arginine or with arginase inhibition, both supplied by microdialysis (142). Similarly, acute local treatment with ascorbate normalizes the active vasodilator response in older subjects to equal that in younger subjects whose response to body heating was unaffected by such antioxidant treatment (143). The suggestion from these important studies is that oxidant stress is a major player in the impaired active vasodilator function with aging and that this impairment is through limiting NOS function (144). It is impressive that this limitation can be reversed by acute treatment with antioxidants or inhibition of arginase, suggesting a tonic interference with NOS function by reactive oxygen species. This is in addition to reducedmaximal vasodilator capacity with age, which theoretically cannot be reversed by such acute treatments (145, 146).

5. LOCAL THERMAL CONTROL

Cooling or heating the skin directly can have major effects on the level of blood flow, with the ability to reduce skin blood flow to zero and the risk of frostbite by intense local cooling, or to maximally vasodilate the skin with local heating within tolerable limits (147). Over the past decade or so great strides have been made in improving our understanding of how the local temperature can have such large effects on the cutaneous circulation. Those advances have been made possible through the development of methodology described earlier and by application to the study of the cutaneous circulation of the large amount of knowledge of cardiovascular control derived from many laboratories, species, and approaches. Local thermal control in humans by both heating and cooling is rarely treated in a single study; hence they are treated separately here. However, certain common themes do arise. It is noteworthy that both local cooling and local heating exert some of their effects on the cutaneous vasculature through the nitric oxide system, one inhibitory and the other stimulatory. Both involve sensory nerve function, although the sensory modalities and the specific afferents involved probably differ. Both local cooling and local heating involve the adrenergic system in exerting their effects on the cutaneous vasculature, although in a counter-intuitive way. Local cooling inhibits and local warming stimulates neurotransmitter release from adrenergic nerves, but both require intact adrenergic function to achieve the full vasoconstrictor and vasodilator expressions, respectively. In both local cooling and local warming, it is the composite of the roles of nitric oxide system function, adrenergic function and afferent nerves that accounts for almost the entire vasomotor response.

5.1. Local cooling

Direct local cooling of the skin has long been known as a stimulus for vasoconstriction. The vasoconstriction induced by local cooling is graded with local temperature and can reduce skin blood flow to essentially zero (147). The mechanisms for the vascular response to local cooling involve the adrenergic system, although the effect is largely post-synaptic. Local cooling involves the nitric oxide system at more than one site, and involves cold-sensitive afferent nerves. For our purposes here, we will define local cooling as a reduction in skin temperatures below 33°C. The cutaneous vascular responses to local cooling are outlined in Figure 3 and described in detail below.

5.1.1. Adrenergic contributions

That multiple mechanisms are involved in the vascular response to local cooling is now quite clear. It is also clear that a separation of the neural control elements of the cutaneous vasculature from the mechanisms of local control is not only arbitrary, it is wrong. These conclusions are illustrated from the pretreatment of a small area of skin with bretylium, a presynaptic antagonist for transmitter release from adrenergic nerve terminals (23, 56). Rapid cooling of untreated skin shows an equally rapid vasoconstrictor response at the control site, briefly interrupted by a transient vasodilator phase (see Figure 3). At the site pretreated with bretylium, presumably with no adrenergic nerve function because of the blockade of transmitter release, there is a substantial initial vasodilator response, a lack of an obvious initial vasoconstriction and a more slowly developing final vasoconstriction. These findings show an involvement of adrenergic function in the vascular response to local cooling. The data also show adrenergic function is not an explanation for the entire response (see below).

Potential points for the adrenergic contribution to the vasoconstrictor response to local cooling include the synthesis of norepinephrine, its release from the nerve terminals, and the sensitivity or number of postsynaptic receptors. Further, a role for sympathetic non-adrenergic co-transmitters must be considered. An examination of *in vitro* and *in vivo* findings narrows the list of possibilities considerably.

First, a series of studies by Vanhoutte and colleagues found that direct cooling (148) reduced the release of norepinephrine from electrically stimulated sympathetic nerves. A component of that reduced transmitter release is likely from an inhibition of the synthesis of norepinephrine by cooling (149). Clearly, these effects of local cooling could not account for the above adrenergic component of the vasoconstrictor response to local cooling.

A series of *in vitro* studies, begun almost thirty years ago and continuing to the present, offers a good explanation for the adrenergic contribution to the vasoconstriction induced by local cooling. The original observation was that cooling enhanced postsynaptic receptor sensitivity, in particular by

alpha2 receptors (150-154). This effect, which would serve as an explanation for the adrenergic component, was demonstrated by showing an increased contractile responsiveness to exogenous agonists specific to the alpha2 subtype (150-152). The observation suggested an increased sensitivity of alpha2 receptors to norepinephrine, but that proved not to be the case. In an important series of *in vitro* studies, the Flavahan laboratory provided evidence strongly supportive of a scenario in which cooling stimulated the mitochondrial production of reactive oxygen species which, in turn, activated rho kinase. The activated enzyme caused the translocation of alpha 2c receptors, normally silent and associated with the Golgi apparatus, to the plasma membrane (155-158). This series of events raises the alpha2 sensitivity to norepinephrine through an increased receptor number rather than through a change in the individual receptor function. Also, the net contractile response to cooling implies that the heightened sensitivity accompanying the additional alpha 2c receptors overcomes the effects of a reduction in the synthesis and release of norepinephrine. However, it also implies that transmitter release is, indeed, required for this adrenergic portion of the vascular response to cooling.

Verification of the above *in vivo* in humans was recently accomplished by Thompson-Torgerson and colleagues in a study in which the expertise of the human-oriented Kenney laboratory group was combined with the *in vitro* background of the Flavahan laboratory (159). The investigators applied the rho kinase inhibitor fasudil to a limited area of skin by microdialysis. Local cooling of the skin had a significantly reduced vasoconstrictor response at sites treated with fasudil, and this was true for both the immediate response to cooling as well as for the more slowly developing vasoconstriction. Importantly, the combination of fasudil with the post-synaptic adrenergic receptor antagonists yohimbine and propranolol (27) did not inhibit the cold-induced vasoconstriction any more than did fasudil alone. This collection of observations strongly supports the conclusion that, *in vivo*, the adrenergic portion of cold-induced vasoconstriction is linked to the rho kinase system and, further, that the *in vitro* findings involving increased mitochondrial production of reactive oxygen species most likely applies in the *in vivo* setting, as well.

5.1.2. The nitric oxide system and local cooling

The nitric oxide system plays an important role in determining the vasoconstrictor response to local skin cooling. In general, cooling has effects on the functioning of enzymes and the NOS family of enzymes is no exception. This point is dealt with more specifically below, but serves here as a theoretical basis, at least, for expecting cooling to affect NOS function. That expectation is satisfied by several key findings.

Yamazaki *et al.* (160) applied the NOS inhibitor L-NAME via microdialysis to areas of skin, some of which had been pretreated with bretylium. This provided a design with selective blockade of NOS, blockade of adrenergic function, as well as combined blockade of both NOS and adrenergic function. Local skin cooling caused different patterns of response among those four sites. Bretylium and L-NAME alone caused a reduction in the net vasoconstrictor response after forty minutes of cooling. At the site lacking intact adrenergic function (bretylium treatment), there was a pronounced vasodilation at the beginning of local cooling. At the site with both bretylium and L-NAME (neither intact adrenergic function nor intact NOS function), that early vasodilator response was also seen but, importantly, there was no net vasoconstriction after forty minutes---skin blood flow (or cutaneous vascular conductance) did not fall below the level that existed prior to local cooling. The importance of this observation is that it suggests that the vasoconstrictor response to local cooling can be accounted for by the contribution of enhanced adrenergic function and the inhibition of NOS function. These observations also address, to some extent, the transient vasodilator response seen early in local cooling, indicating it to be normally inhibited by adrenergic function (161) and that NOS function has no important role in its creation or inhibition.

That transient vasodilation at the beginning of rapid local cooling remains enigmatic as to the mechanisms producing it. There is an antagonistic role for the adrenergic system, but how that works is not known. It is sensitive to the rate of local cooling (160), being almost absent when local cooling proceeds at 0.33°C min-1, but is quite evident at rates of cooling of 4°C min-1.

Also, Hodges *et al.* (19) followed the studies by Yamazaki *et al.* (160) by focusing on slow local cooling but again employing separate and combined blockade of NOS and adrenergic systems. The absence of the transient vasodilation provided a clear stable background level for demonstrating that the combination of adrenergic and NOS blockades also eliminates the vasoconstrictor response to local cooling.

The interpretation of the above findings relative to the NO system involvement in responses to local cooling does not necessarily show NOS, per se, to be the thermally sensitive point in the NO system. That notion was tested directly by Hodges *et al.* (19), who blocked both adrenergic function with iontophoretically administered bretylium and NOS function with L-NAME administered to those and to adjacent areas by microdialysis, again showing that the response to local cooling was a combination of increased adrenergic function and inhibited NO system function. Unique to Hodges *et al.* (19) was the restoration of NO to the double-blocked sites by

administering sodium nitroprusside to those sites. In that case, the level of cutaneous vascular conductance was restored to baseline levels by replacing the NO normally produced by NOS. The vasoconstrictor response to local skin cooling was also largely restored. Overall, these data suggest direct cooling to suppress both NOS function and a process, or processes, downstream from NOS. The latter involvement is clear from the restored vasoconstrictor response following NO replacement. The conclusion of a role for NOS, per se, comes from the greater effect of local cooling on sites pretreated with bretylium (without a functional adrenergic system, but with a functional NO system) than on sites with bretylium, L-NAME and NO restoration (relying solely on post-NOS inhibition). Taken together, these data indicate that the cutaneous vasoconstrictor response to a lowering of skin temperature by 10°C has approximately equal dependence on increased adrenergic function, NOS-inhibition and effects of lowered temperature at steps after the NOS enzyme.

The component due to NOS inhibition of the response to local cooling is slightly at odds with knowledge of the *in vitro* thermal sensitivities of the NOS isozymes (162). In particular, nNOS and iNOS show significant inhibition by levels of cooling similar to those above, whereas eNOS had the least sensitivity to its temperature. At this stage, it is not possible to conclude from the foregoing *in vivo* and *in vitro* findings which of the NOS isoforms are involved in the vasoconstrictor response to local cooling. If eNOS is involved, the mechanism by which the enzyme is inhibited *in vivo* may not be via the lower temperature, per se. For example, the reduction in blood flow via either the adrenergic component or via the NOS independent inhibition of the NO system would also reduce any flow-mediated stimulation of NOS.

5.1.3. Afferent nerves

There is a role for sensory nerves in the vasoconstrictor responses to local skin cooling (163, 164). This conclusion is made clear by the influence of topical anesthesia on that response. What is most obvious is the unmasking of an initial vasodilation at the beginning of local cooling, similar to the vasodilation seen with pre- or post- synaptic interference with adrenergic function (164). It was this similarity that led to the speculation that local cooling stimulated norepinephrine release from adrenergic nerves through cold receptors acting through axon reflexes (164). We now know that scenario to be incorrect. Hodges *et al.* (163) directly tested that notion by combining afferent nerve block (topical anesthesia) and presynaptic adrenergic blockade (iontophoretic bretylium), reasoning that such double blockade would have the same effects on the response to local cooling as either adrenergic blockade or sensory nerve blockade alone if those systems were acting in series. This did not prove to be the case. With slow local cooling there was a clear dissociation of the effects of local anesthesia from the effects of adrenergic inhibition. Bretylium reduced the net vasoconstriction after thirty minutes of local cooling, whether alone or combined with local anesthesia. Local anesthesia did not affect the ultimate vasoconstriction to slow local cooling but was associated with an early, transient vasodilator response as usually seen with more rapid cooling in untreated skin (160, 161). Bretylium pretreatment by itself did not have that effect with slow local cooling. This dissociation shows the adrenergic effects to be independent of sensory nerve function and, further, that different mechanisms are in play at the beginning of local cooling relative to those involved in the later stages (163).

The working model for the mechanisms involved in the vasoconstrictor response to local cooling, summarized in Figure 3, supports a scenario in which there is an early vasodilator phenomenon working in the background. This is not normally seen, except in rapid local cooling (160, 161) because a function of sensory nerves is to suppress that vasodilation (163). That is also a function of adrenergic nerves (160, 161, 163). How those very different kinds of nerve bring that inhibition about is not clear. It is clear that removal of sensory nerves exposes that latent vasodilation more dramatically than does adrenergic blockade. The usual vasoconstriction at the onset of local cooling appears largely to be a function of the adrenergic system, in which incorporation of new alpha 2c receptors into the plasma membrane is stimulated by a cascade in which mitochondrial reactive oxygen species stimulate the rho kinase system. As local cooling proceeds, the nitric oxide synthase system (or systems) is inhibited, removing that tonic vasodilator function.

Lastly, the origin of the latent vasodilator process, noted above, is unknown. It appears to be neither adrenergic nor sensory, making participation by Substance P or CGRP unlikely. It may be a phenomenon of smooth muscle energetics. It is tempting to ally the phenomenon to the cold-induced vasodilation seen in the digits (2), although it has generally been held that this latter vasodilation is a property of arteriovenous anastomoses, which are common in glabrous skin but not in the nonglabrous skin of the forearm, where the former phenomenon was observed.

5.2. Local warming

Direct warming of the skin has a vasodilator effect (147) which goes through several phases and which bears several mechanistic similarities to the responses to local cooling. However, it is not clear where the effects of local cooling and warming are through actions on the same continuum versus being independent. That uncertainty and the fact that very few studies have included both local cooling and local warming of the skin lead to a separate development here.

The onset of local warming follows a pattern of a sharp initial transient increase in forearm skin blood flow, a return toward baseline levels, an increase to a near steady state plateau and, finally, a slow downward drift ("die away") relative to that. The mechanisms for these characteristics have received recent attention through which

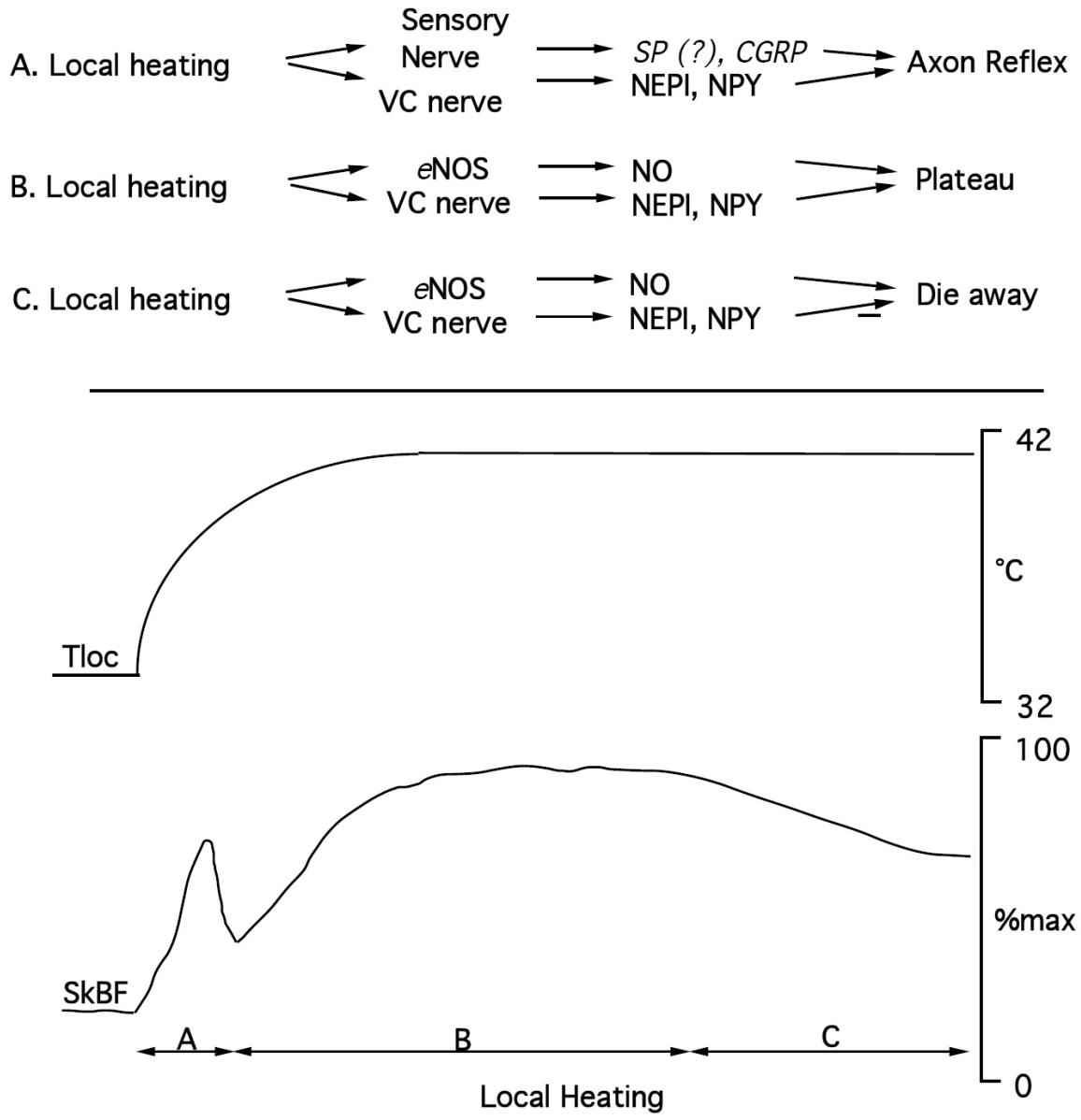

Figure 4. Cutaneous vascular responses to local skin warming: Below: The vasodilator response to local heating is characterized by 3 phases. (A) There is an initial transient vasodilation due to sensory nerves and an axon reflex, followed by (B) a plateau phase which is largely due to stimulation of NOS (evidence is mixed regarding eNOS and nNOS) supported by norepinephrine and NPY. The plateau phase is succeeded by (C) a 'die away' phase in which skin blood flow falls from the plateau level and is due to norepinephrine and/or NPY. Above: Mechanisms involved in the 3 phases of the vasodilator response to local skin warming include an axon reflex, increased NO system activity and stimulated release of norepinephrine and NPY, which support the axon reflex and plateau vasodilation, while acting to reduce skin blood flow during the die away phase. Terms in italics have yet to be firmly established.

substantial understanding has been gained. Figure 4 shows the outline of the current working model of the cutaneous vascular response to local skin warming.

5.2.1. Sensory nerves

Minson *et al.* (17) applied local anesthetic cream to the skin surface and found that the initial transient vasodilator response to local heating was significantly attenuated, whereas the latter plateau was not remarkably affected, leading to the conclusion that the early abrupt vasodilation is an axon reflex, likely mediated by heat-sensitive nociceptors. That conclusion was also reached by Stephens *et al.* (165), who treated skin with capsaicin and compared the vasodilation to local skin warming there against that at untreated sites both on the basis of equal thermal stimulation and on the basis of equal perceived thermal stimulation. Perceived thermal stimulation was assumed to reflect equal excitation of

warm sensitive nociceptors-one by the combination of chemical and thermal activation, and the other through strictly thermal stimulation. An equal perception of heating gave rise to an essentially equal increase in skin blood flow, despite an 8°C difference in the actual temperature. The result indicates the vasodilation was more dependent on thermal receptor activation than on the heating of the vascular smooth muscle.

The above results (17, 165) strongly indicate that most, if not all, of the early transient vasodilator response to local warming is due to activation of an axon reflex. This allows an interesting comparison with the role of sensory nerves in the response to local cooling because, in cooling, sensory nerves inhibit a vasodilation whereas in warming, sensory nerves provide the stimulus for vasodilation.

The extent to which sensory nerve activation extends beyond the initial few minutes of local warming is unknown. However, the available data (17, 31) suggest that the involvement is only transient, lasting only about five minutes. That duration of action corresponds to some extent to the sensitivity to the rate of change of local temperature (31); i.e., during the transient in local temperature the axon reflex is activated, but its level of activity soon falls to low levels as the local temperature reaches an elevated steady state and is no longer changing. Rate dependency also occurs with local cooling (160), but is apparently independent of sensory nerves.

These data do not specify what kind of warm sensitive receptors mediate the axon reflex, but the likelihood is that most or all of the effect is mediated by heat-sensitive nociceptors, rather than by warm receptors. This speculation is because the representation of the former mixed modality receptor is much greater in human hairy skin than is the representation of purely warm-sensitive receptors (166).

5.2.2. Nitric oxide function

After the initial transient vasodilation, local warming causes skin blood flow to rise markedly to a plateau, dependent on the level and rate of local warming. Kellogg *et al.* (16) and Minson *et al.* (17) tested whether this steady-state vasodilator response to local warming is dependent on the nitric oxide system. In both cases, a NOS inhibitor was used and, in both cases, the level of skin blood flow reached during the plateau phase was significantly reduced. Administration of the nonspecific NOS antagonist L-NAME by microdialysis when the skin was already vasodilated by submaximal heating to 40°C, caused an approximate 47% reduction in cutaneous vascular conductance and in skin blood flow (16). This reduction was restored by local administration of sodium nitroprusside, supporting the conclusion that NOS activation was a key part of the vasodilator response to local skin warming. Minson *et al.* (17), who applied L-NAME by microdialysis before local heating began, also supported that conclusion. This inhibited the vasodilator response by about 50%, in keeping with the conclusions from the study by Kellogg *et al.* (16), but did not affect the initial transient vasodilation (17).

Gooding *et al* (167) also provided evidence for an important role for NO in the cutaneous vasodilator response to local skin warming through microdialysis of L-NAME. They also provided evidence against roles for either prostaglandins or histamine in that vasodilation because neither aspirin nor cetirizine (histamine H1 receptor blocker) ingestion had a significant effect on the vasodilator response to local heating to 42°C.

The use of L-NAME, a nonspecific NOS antagonist showed an involvement of the NO system in the response to local skin warming, but did not show which of the NOS isoforms was responsible. That question has recently become testable through the use of more specific inhibitors, generating a pair of independent studies that, unfortunately, gave quite different answers (117, 168). Kellogg *et al.* (117) applied NG-amino-L-arginine (L-NAA) to skin via microdialysis. Both *in vitro* and *in vivo* studies found L-NAA to have a reasonably high selectivity for eNOS over nNOS (169-172); probably more selective *in vivo* than with isolated NOS enzymes because access to the nNOS enzyme is more limited in the *in vivo* setting. Treatment with L-NAA significantly reduced the vasodilator response to local skin warming (117). The same concentration of L-NAA was without significant effect on the reflex vasoconstrictor response to body cooling or, importantly, on the reflex vasodilator response to body heating. The reverse was seen for nNOS blockade (117). These data suggest that different NOS isoforms have different roles in the vasodilator responses to local skin heating vs. that involved in the reflex vasodilation to whole body heating and that that division of responsibility is characterized by an eNOS participation in the locally-mediated vasodilation, whereas nNOS generates the NO involved in active cutaneous vasodilation, described in more detail earlier in this review.

The above construct is internally consistent, but is counter to conclusions drawn from studies by Stewart *et al.* (168). In those studies, the nNOS antagonist Nω was administered by microdialysis to the skin of the legs of both healthy subjects and patients with orthostatic intolerance in a protocol that involved sequential local skin heating-once before the antagonist and, following recovery, again in the presence of Nω. The investigators found Nω did not affect the early phase of the response to local heating, but significantly reduced (by about 50%) the later plateau phase (168). Nω is reported to be an inhibitor specific to nNOS (173, 174), which is why these results differ importantly from those of Kellogg *et al.* (117).

At this stage, it is not possible to conclude which of those scenarios is correct. Differences in protocol (sequential heating vs. use of separate sites), area of skin (leg vs. arm), and NOS antagonists all invite speculation, but none stands out as a compelling difference. Without isoform–specific agonists, it is also difficult to test the adequacy and the specificity of the NOS blockade, so this important question will have to remain on the table for the present.

An interesting and potentially important role for NO is its antagonism of adrenergic function (35-37, 89, 175, 176). This conclusion arises from a series of important studies by Crandall and colleagues and arose from the demonstration that NO is a participant in the vasodilation with local heating as well as involved in the reflex vasodilator responses to whole body heating. In testing for an inhibition by NO of adrenergic function, the Crandall group found that (a) the vasoconstrictor response to norepinephrine (applied by microdialysis) was significantly inhibited by either local or whole body heating (176) (b) the elevation of tissue NO levels through the application of sodium nitroprusside significantly inhibited the adrenergically-mediated vasoconstrictor response to whole body cooling (175) (c) NOS inhibition by intradermal L-NAME enhances the vasoconstrictor response either to an orthostatic stimulus during body heating or to whole body cooling (89) (d) exogenous NO inhibits the cutaneous vasoconstrictor response to exogenous norepinephrine (36) and (e) removal of NOS function through intradermal L-NAME also removes the inhibitory effect of local warming on the vasoconstrictor response both to exogenous norepinephrine and its stimulated release by tyramine (37).

The above findings show that there is an inhibition of adrenergic function by local or reflex responses to heating and that inhibition is largely or entirely manifest through NO. The studies help explain earlier observations of inhibited vasoconstrictor responses to exercise initiation by local warming (177) or to local cooling by whole body warming (161) and also show that the NOS system has both the functions of vasodilation and of inhibition of vasoconstriction in the skin.

5.2.3. Adrenergic function

Sympathetic release of norepinephrine and/or NPY has roles in both the reflex vasoconstrictor response to whole body cooling and in the vasoconstrictor response to local cooling. Reduced sympathetic vasoconstrictor nerve activity and transmitter release is the first step in the reflex vasodilator response to whole body heating (passive vasodilation). It has recently been shown that adrenergic function also plays roles in the vasodilator response to local skin heating (31, 178, 179). Surprisingly, these roles are largely in support of the vasodilator response.

Houghton *et al.* (179) found that blockade of transmitter release from vasoconstrictor nerves with locally applied bretylium abolished the early axon reflex portion of the response to local skin heating. NOS blockade also either abolished or delayed the axon reflex. They also found bretylium to reduce the level of vasodilation during the subsequent plateau phase of the vasodilator response to local skin warming. Hodges *et al.* (31) verified the existence of a role for norepinephrine in the response to local skin warming by showing that combined blockade of post synaptic alpha and beta adrenergic receptors delayed the axon reflex portion of the vasodilator response to slow local heating and reduced the level of skin blood flow during the subsequent plateau phase. Hodges *et al.* (31) also found that blockade of Y1 receptors for NPY also delayed the axon reflex and reduced the vasodilation during the plateau phase. They also noted that either adrenergic blockade with bretylium or NOS blockade with L-NAME abolished the axon reflex and lowered the plateau level for vasodilation. It was noted that the lowering of the plateau vasodilation by bretylium was not further reduced when combined with NOS blockade, which provides possibly an important clue for the mechanism for adrenergic support of vasodilator responses.

The above observations are strong evidence for an involvement of sympathetic transmitters in local heat stimulated vasodilation. Both norepinephrine and NPY appear to be involved and each of the transmitters has a similar support for both the axon reflex and the succeeding plateau phase. How do traditional vasoconstrictor transmitters support vasodilation? The mechanism is not revealed by the above studies and it remains unclear. The apparent involvement of NO in the adrenergic support of vasodilation may, however, be an important signal. It may be that the transmitters are stimulating endothelial alpha2 and Y1 receptors to initiate NO synthesis via eNOS (154, 180-182). Such would serve as a vasodilator stimulus. Coupled with the elevated NO production with local skin warming (16, 17) expected to inhibit adrenergic vasoconstrictor function (175, 176), such stimulated release of NO could lead to a net vasodilator function for the adrenergic transmitters.

Adrenergic function also plays a role in the classical ‘die away’ phenomenon with local skin heating (147, 178). Normally, after the plateau phase of the response to local skin heating, a slow downward drift in skin blood flow is seen: This phenomenon begins after about thirty minutes of submaximal local heating and proceeds for at least the next hour, with an approximate 40% fall in blood flow from that achieved during the preceding plateau. In areas pretreated with bretylium, however, there is no ‘die away’ phase (178). Instead, the plateau phase is sustained, with the blood flow in untreated warmed skin falling to the same level as that seen in the skin without adrenergic function (bretylium treated). At

this point it is unclear whether the 'die away' phenomenon in untreated skin is because a dilator function is reduced or a constrictor function is being enhanced. Nevertheless, it is another example of adrenergic function having a necessary role in the characteristic responses of the skin to direct local warming.

An interesting discovery was that the local heating stimulates the release of the norepinephrine and NPY apparently involved in the cutaneous vascular responses. This was shown by Hodges *et al* (178), who found that at a whole body skin temperature of 34° C there is essentially no tonic vasoconstrictor activity in resting humans. This was shown by the failure for bretylium, delivered to the skin by microdialysis, to cause skin blood flow to increase. Under those same conditions there was a significant role for the vasoconstrictor nerve transmitters in axon reflex vasodilation, the plateau level of blood flow and the die away, indicating that the heating itself served to stimulate their release. This observation is consistent with the finding that local cooling reduces transmitter release from sympathetic vasoconstrictor nerves (149).

Finally, these locally stimulated responses are modified in the elderly (144). This topic is covered by Kenney elsewhere in this series. In brief, the local vasodilation stimulated by sensory nerves is reduced in older healthy subjects as assessed by a significantly reduced response to the acute application of capsaicin (183), suggesting the axon reflex portion of the response to local warming would be similarly affected by age. Indeed, this is apparently the case both for the initial axon reflex and the ensuing NOS-dependent vasodilation (184). Local administration of a NOS antagonist by microdialysis either before or during local skin warming yielded a smaller inhibition of the response to local warming, indicating a smaller role for NOS in the elderly. Similarly, the initial peak response to local heating, thought to be largely an axon reflex, was reduced in the older subjects (> 70 years). It is of some interest to note that the data analysis is based on values normalized to maximal cutaneous vascular conductance, as is often required for comparison across the small areas of skin under inspection by laser Doppler flowmetry. The smaller fractional vasodilator responses by the older subjects to local skin heating takes on additional importance in the context of lower maximal skin blood flow in that group (145, 146). Together, these findings imply an even greater attenuation in the response to local heating when considered on the basis of changes in skin blood flow in absolute terms.

6. CONCLUSIONS

Over the past 20 years there has been extraordinary progress in our understanding of the mechanisms that operate to control the human cutaneous circulation in health, which has been the focus of this development. In this review we have not gone the next step – the incorporation of this knowledge into a rational approach to how circulatory function might be altered in certain disease states. Some groups are already doing that, and we apologize to them that we could not broaden the scope of this review to include those studies, but we do acknowledge that they are and will continue to be important. The potential capacity for vasodilation of the skin, when considered over the whole body surface, is so large, that it has to be a player in the challenges to the circulation of orthostatic dysfunction (168, 185), heart failure (186-188) or other pathologies that place demands on the level and distribution of blood flow. The cutaneous circulation is compromised in diabetes (189-191), and understanding how altered mechanisms of control might be part of the problem is a challenge as well as the means to reducing the incidence of amputation.

Our basic understanding of the mechanisms of control in health is far from complete. A consensus regarding the transmitters and cotransmitters for active vasodilation awaits new experimental approaches to complement those presented here. How NO is involved in that process and in local thermal control is not satisfactorily answered, nor is the answer to whether the sudomotor system is important in blood flow control. The perfect antagonists to address these questions have yet to appear. The metaphor of peeling the onion is appropriate – as we learn more about the mechanisms of control at one level, we discover there is always another level to explore. It is the challenge and the excitement of our area of research to devise ways to carry out that exploration.

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