Mechanisms of vasoconstriction with direct skin cooling in humans

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DIRECT SKIN COOLING CAUSES a vasoconstriction partly dependent on α2-adrenergic function. In in vitro studies, this is a manifestation of reactive oxygen species stimulating Rho and Rho kinase to cause translocation of α2C-receptors from the Golgi to the plasma membrane. In this issue of *American Journal of Physiology-Heart and Circulatory Physiology*, Thompson-Torgerson et al. (24) show the Rho-Rho kinase system to be importantly involved in cold-induced vasoconstriction in humans in vivo, accounting for over 60% of the response.

Direct cooling of hairy (nonglabrous) skin causes a vasoconstriction, related to the extent and duration of cooling, that follows a deceivingly simple pattern of an immediate vasoconstriction followed by a slow further reduction in blood flow, disguising the multiplicity of mechanisms acting to bring it about. Recent studies regarding vascular responses to local cooling go beyond the description of the phenomenon, bridge studies from intact humans to isolated vascular muscle to cellular preparations, and strike at the mechanisms responsible for this fundamental cardiovascular adjustment.

Although the cutaneous vasoconstriction accompanying local cooling is part of local control, intrinsic to the tissue, important contributions arise from sensory and autonomic nerves (19). Indeed, local cooling in the presence of blockade of either sensory nerves or sympathetic vasoconstrictor function reverses the vasoconstriction seen at the onset of local cooling to a transient vasodilation (8, 19, 20, 27). The mechanism for this vasodilation is not known. It is not adrenergic, not nitric oxide dependent (16, 27), not due to substance P or CGRP (19), and not likely to be prostaglandin dependent because it is unaffected by aspirin (Alvarez GE, unpublished observations). Because it is transient and dependent on the rate of cooling (27), a depletable substance rather than biophysical effects of the lower temperature is suggested. It can be provoked in neurally intact tissue with sufficient rate and extent of cooling (20). The data imply that sensory nerves, likely originating from cold receptors, inhibit an intrinsic vasodilator response to cooling. Unknowns about this vasodilator phenomenon include whether its origin is in the vascular smooth muscle, the endothelium, or within the surrounding tissue.

The above vasodilator phenomenon is replaced by a net vasoconstriction. Inhibition of sympathetic adrenergic function significantly reduces this longer-term cutaneous vasoconstrictor response to direct local cooling (16, 27). Thus there is an important role for adrenergics in this locally induced response, and it is within that system that the current report by Thompson-Torgerson et al. (24) makes an important contribution.

Local cooling inhibits norepinephrine synthesis and release in vitro and in isolated systems (4, 9, 25, 26). Although reuptake mechanisms are also depressed (17), it seems unlikely that the adrenergic component of the cutaneous vasoconstriction is due to a net increase in the concentration of norepinephrine in the subsynaptic cleft. The more probable explanation develops from a series of important in vitro and in vivo studies of postsynaptic adrenergic receptors. Studies in isolated veins indicated that the apparent sensitivity of α-receptors for norepinephrine is enhanced by local cooling (17, 21). That enhancement depends on α-adrenergic receptor subtypes. Cooling reduces adrenergic α1-receptor responsiveness in the face of a large receptor reserve while enhancing α2-mediated vasoconstriction in vitro (8–11). What was originally taken as enhanced adrenergic α2-receptor affinity proved to be more involved, as revealed in a series of enlightening studies by Flavahan and colleagues (1, 2, 5, 6, 18), who showed that the apparent increase in α2-sensitivity was due to cold-induced translocation of α2C-receptors from the Golgi to the plasma membrane (5, 6, 18). That translocation is dependent on RhoA/Rho kinase (1), which, in turn, is activated by mitochondrial reactive oxygen species (2).

This series required a broad use of in vitro preparations and species, begging the question regarding the general applicability of the findings. While the role of α2C-receptor translocation, Rho-Rho kinase activation, and mitochondria-derived reactive oxygen species may be a general phenomenon, it is also possible that the mechanisms are species dependent. For example, rabbit vessels do not show a dependence on α2-adrenoceptors for cold-induced vasoconstriction (14, 15). There is also the possibility that tissue factors contribute to the vasoconstrictor response in a way not apparent in isolated vessels or that mechanisms might vary between arteries and veins or among vessel sizes. In that light, Faber (8) found the sensitization of α2-adrenoceptor responsiveness by cooling to extend to arterioles in situ.

Ekenvall et al. (7) made important contributions to the resolution of the above problem with α1- or α2-adrenoceptor antagonists applied to dorsal finger skin of healthy humans. α2-Blockade abolished cold-induced vasoconstriction, in keeping with the findings in isolated vessels (11). Similarly, Freedman et al. (13) found cooling of the fingers to enhance α2-mediated vasoconstriction but to markedly inhibit the vasoconstrictor response to α1-agonists, entirely consistent with in vitro findings.

As reported in this issue, Thompson-Torgerson et al. (24) tested the Rho-Rho kinase involvement in the cutaneous vasoconstrictor response to local cooling in vivo in humans. The investigators used intradermal microdialysis to deliver the Rho kinase inhibitor fasudil to a limited area of forearm skin, with and without yohimbine and propranolol to antagonize α- and β-adrenoceptors (23). Although this combination has some redundancy in that either would inhibit α2C-function, Rho-Rho kinase also sensitizes the vascular smooth muscle to calcium (1). Hence those two roles might be discriminated through the combinations of antagonists.

The authors found that 1) fasudil inhibits the cutaneous vasoconstrictor response to local cooling; 2) this inhibition was more marked by fasudil than by adrenoceptor antagonism; and...
3) fasudil alone provided the same inhibition of cold-induced vasoconstriction as fasudil plus α- and β-adrenoceptor inhibition. These results applied both early during local cooling as well as later. The only notable difference was that, early, adrenergic receptor blockade alone was associated with the vasodilatation noted earlier (19, 20), but treatment with fasudil (with or without adrenergic receptor blockade) was associated with no response to local cooling. Later in local cooling, all treatments were associated with significant vasoconstriction, but that differed in degree among the sites. Adrenergic blockade inhibited the vasoconstriction after 30 min of cooling, as seen earlier (16). When fasudil was added, the vasoconstrictor response was further inhibited. Early or late, these findings strongly indicate that the Rho-Rho kinase system is involved in cold-induced vasoconstriction in human skin in vivo and that part of that effect is through adrenoceptors, part is not. The portion of the vasoconstrictor response blocked by fasudil, but not by adrenergic receptor antagonism, is probably through increased sensitivity to calcium (1). That unblocked by fasudil, amounting to nearly one-half of the control response, most likely reflects inhibition of nitric oxide synthase (NOS) and steps downstream of NOS, because combined blockade of adrenergic function and NOS eliminates cutaneous vasoconstriction with cooling (16, 27). This implies that, in the current study (24), the vasoconstriction in the presence of postsynaptic adrenoceptor antagonism is through inhibition of the NOS system, some of which involves Rho kinase (the difference in the response to cooling between fasudil treatment and adrenoceptor blockade) and some of which does not (the persistent vasoconstriction with fasudil).

The novelty of the present study by Thompson-Torgerson et al. (24) is that it confirms in vivo the importance of the in vitro findings by Flavahan and colleagues (1, 2, 5, 6, 18). Furthermore, as the findings apply to humans, Rho kinase inhibition represents a potential therapeutic target in pathophysiological circumstances, possibly in this case for cold-induced vasospasms or Raynaud’s disease (3, 12, 22).

GRANTS
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REFERENCES