PERSPECTIVES

In vivo vasodilating mechanisms: who's NOS involved?

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The human skin circulation has a key role in thermoregulation via responses to both reflex whole-body and local thermal stimuli (Kellogg, 2006). During whole-body heat stress, a great increase in skin blood flow is necessary to dissipate body heat (Johnson & Proppe, 1996). This active vasodilatation is due to neurotransmitters such as acetylcholine, vasoactive intestinal peptide and others released from sympathetic cholinergic nerves. In addition, several studies have demonstrated that nitric oxide (NO) participated significantly in skin blood flow increase induced by whole-body heat stress (Kellogg *et al.* 2003; Kellogg, 2006). During local warming, increases in temperature cause localized cutaneous vasodilation via two independent mechanisms: an initial rapid peak followed by a slower vasodilator response that reaches a plateau after around 30 min of heating. Local sensory nerves are primarily responsible for the vasodilatation causing the initial rapid peak, whereas the plateau phase includes a substantial NO component (Kellogg *et al.* 1999). Therefore, human skin blood flow increases in response to increased body core and local skin temperature via distinct reflex and local mechanisms requiring NO production. NO generation is expressed by different NO synthase (NOS) isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). However, the mechanistic involvement of the different NOS isoforms in the vasodilatory response to heat stress, *in vivo*, remains still unknown.

In this issue of *The Journal of Physiology*, Kellogg *et al.* (2007) reported that cutaneous vasodilatation induced by both local and whole-body heating was (at least in part) a NO-dependent process that could involve two distinct NOS isoforms (eNOS and nNOS). This paper is very interesting since the authors demonstrated for the first time the involvement of nNOS in the active vasodilatation induced by whole-body heating in contrast to local body heating in healthy people. The protocol was designed to examine the effects of the nNOS inhibitor 7-nitroindazole (7-NI) on skin vasodilatation induced by both whole-body heating (38–39°C) and local skin heating (41.5°C). In this paper, readers will appreciate the importance and difficulty of identifying cellular mechanisms in physiological conditions. Indeed, 7-NI was administrated by intradermal microdialysis (2 mM) allowing large local drug concentration with local effects at the site of administration and thus avoiding systemic effects. Data were normalized to the SNP-induced maximal vasodilatation performed at the end of each protocol. Changes in skin blood flow were monitored by laser–Doppler flowmetry (LDF) that gives continuous beat-to-beat information specific to cutaneous microvascular perfusion in a non-invasive manner. LDF measurements provide a robust approach to evaluate mechanisms of microvascular dilatation and their modification by physiological perturbations. The study reported that during nNOS inhibition, the skin vasodilatation due to whole-body heating was attenuated (∼30%) in contrast to local skin heating. The authors demonstrated that, *in vivo*, the nNOS isoform is responsible for NO generation during centrally mediated, reflex cutaneous active vasodilatation in whole-body heating, but is not involved in increasing skin blood flow in response to axon reflex-mediated, local skin heating. This study also showed that the nNOS isoform generates NO from the very initial phase of cutaneous active vasodilatation and continues during prolonged whole-body heat stress. However, Kellogg *et al.* (2008) strongly suggest that NO acts to increase synergistically the vasodilating effects of other neurotransmitters rather than acting as a direct mediator of vasodilatation. Interestingly, they excluded any participation of eNOS in centrally mediated reflex cutaneous active vasodilatation since studies using L-NAME, a non-selective isoform NOS antagonist, reported approximately an identical attenuation of 30% of skin blood flow increases to that of 7-NI, a selective nNOS antagonist, highlighting the possibility that nNOS is the sole NOS isoform involved in NO generation during whole-body heat stress. Conversely, since previous studies reported an inhibitory effect of a non-selective isoform of the NOS antagonist, L-NAME, on skin vasodilatation induced by local skin heating whereas a selective nNOS antagonist, 7-NI had no effect (Kellogg *et al.* 2007), and given that iNOS is not expressed in healthy skin, the authors strongly suggest that eNOS is the isoform that mediates peripheral, axon reflex vasodilatation during local skin heating.

The alteration of the microvasculature to dilate has significant deleterious effects leading to cardiovascular diseases occurring in ageing and several pathologies (Charkoudian, 2003). In type 2 diabetes mellitus, the ability of skin blood vessels to dilate is impaired, probably contributing to the increased risk of internal hyperthermia during exposure to elevated ambient temperatures. Raynaud phenomenon and erythromelalgia appear to relate to disorders of local and/or reflex thermoregulatory control of the skin circulation. In addition, changes in reproductive hormone levels during menopause substantially alter thermoregulatory control of skin blood flow contributing to the occurrence of hot flashes (Sokolnicki *et al.* 2007).

Collectively, all these findings from Kellogg *et al.* (2008) and others are very important to further our understanding of vascular physiology under thermal stress. These studies also highlight the important role of *in vivo* investigations in advances of physiological research. Skin microcirculation is easily accessible for non-invasive measurements that allow a unique insight to detect endothelial dysfunction.

Kellogg's research group have largely contributed to the subject of how the human skin adapts under thermal stress. Several questions still remain and further research is needed to clarify and better understand the whole mechanism. The more we identify the underlying mechanisms, the more we will be able to prevent or reverse any alterations.

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