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COMMENTARY

Excitatory P2-receptors at sympathetic axon terminals: role in temperature control of cutaneous blood flow

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The mechanisms underlying the reduction in cutaneous blood flow in response to cooling are only partially understood. A study published in this issue of the *British Journal of Pharmacology* now provides evidence for the involvement of excitatory P2-receptors located at sympathetic axon terminals in the cooling-induced vasoconstriction in the skin. Cooling appears to cause the release of adenine nucleotides followed by the activation of excitatory presynaptic P2-receptors at noradrenergic axon terminals. Activation of these excitatory P2-receptors induces the release of noradrenaline, which subsequently causes constriction of blood vessels in the skin by action on smooth muscle α_1- and α_2-adrenoceptors. The commentary discusses the implication of the results and remaining questions. *British Journal of Pharmacology* (2006) **148,** 561–562. doi:10.1038/sj.bjp.0706767; published online 15 May 2006

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The paper of Koganezawa *et al*. (2006), in this issue of the *British Journal of Pharmacology*, provides evidence for the involvement of excitatory P2-receptors located at sympathetic axon terminals in the cooling-induced constriction of cutaneous blood vessel. The published data indicate that a decrease in temperature causes a release of adenine nucleotides from unknown sources followed by an activation of presynaptic excitatory P2-receptors and a subsequent release of noradrenaline from sympathetic axon terminals. Finally, noradrenaline elicits vasoconstriction *via* smooth muscle α_1- and α_2-adrenoceptors (Koganezawa *et al*., 2006).

Cooling causes constriction of cutaneous blood vessels reducing the loss of body heat. The underlying mechanisms are only partially understood. The responses of cutaneous blood vessels to sympathetic nerve stimulation are increased at low temperature (e.g. Flavahan & Vanhoutte, 1986). Recent findings indicate an involvement of α_2-receptors of the α_{2c}-subtype in addition to α_{2a}-receptors in vasoconstriction at low temperature. The α_{2c}-receptors appear to be silent at higher temperatures (e.g. 37°C; see Chotani *et al*., 2000; Bailey *et al*., 2004). There is also evidence for an augmented contribution of purinergic components in vasoconstriction at lower temperature (Flavahan & Vanhoutte, 1986). In their present study, Koganezawa *et al*. (2006) now studied the involvement of α-adrenoceptors and P2-receptors in the local regulation of the cutaneous microcirculation during cooling *in vivo*. For this purpose, blood flow in the plantar skin of anaesthetized rats was measured by a laser Doppler flow probe; the propagation of action potentials was blocked by tetrodotoxin. Cooling of the left foot now caused a reduction in local blood flow. This response was sensitive to treatment with guanethidine and bretylium, drugs known to block transmitter release from postganglionic sympathetic nerves. The vasoconstrictor response to cooling was also diminished by α_1- as well as α_2-adrenoceptor antagonists, indicating that released noradrenaline acting on smooth muscle α_1- and α_2-adrenoceptors mediated the response (Koganezawa *et al*., 2006). Interestingly, the P2-receptor antagonists suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonate (PPADS) also reduced the responses to cooling without any effects on the vasoconstrictor responses to the α-adrenoceptor agonist phenylephrine. A number of points now suggest the involvement of excitatory presynaptic P2-receptors located at postganglionic sympathetic axon terminals in these responses: pretreatment with the α-adrenoceptor antagonist phentolamine abolished the effect of PPADS and diminished the vasoconstriction induced by the P2-receptor agonist ATPγS. Moreover, the response to ATPγS was also affected by the treatment with guanethidine. Hence, the data shown in the paper by Koganezawa *et al*. (2006) provide evidence for a novel mechanism involved in the local control of cutaneous blood flow *in vivo*: presynaptic excitatory P2-receptors located on sympathetic axon terminals in the skin.

Postganglionic sympathetic nerve fibres are known to possess inhibitory as well as excitatory P2-receptors modulating the release of noradrenaline (von Kügelgen *et al*., 1989, 1993; Sperlagh & Vizi, 1991; Sperlagh *et al*., 2000). In many sympathetically innervated tissues, adenine nucleotides cause an inhibition of calcium influx in axons and an inhibition of evoked transmitter release by activation of release-inhibiting P2Y-receptors most likely of the $P2Y_{12}$- or $P2Y_{13}$-subtype (von Kügelgen *et al*., 1989; 1993; Kulick & von Kügelgen, 2002; Kubista *et al*., 2003; Queiroz *et al*., 2003). In the ear artery, the ileum and the heart atrium (Miyahara & Suzuki, 1987; Sperlagh & Vizi, 1991; Sperlagh *et al*., 2000) as well as in preparations of cultured sympathetic neurones (Allgaier *et al*., 1994; Boehm, 1994; 1999; von Kügelgen *et al*., 1997; 1999), adenine nucleotides have previously been shown to induce the release of noradrenaline or to cause an increase in the action potential-evoked transmitter release. Most of these studies indicated the involvement of P2X-receptors of the $P2X_2$-subtype in the excitatory effects of adenine nucleotides (Sperlagh & Vizi, 1991; Boehm, 1994; 1999; von Kügelgen *et al*., 1997; 1999; Sperlagh *et al*., 2000; see also Nörenberg & Illes, 2000; North, 2002). The study by Koganezawa *et al*. (2006) now provides experimental *in vivo* evidence for a physiological role of these excitatory presynaptic P2-receptors.

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Remaining questions

A number of questions remain. The cellular sources of the adenine nucleotides involved in the response to cooling as well as the mechanisms of nucleotide release in the skin are unknown. Neuronal cells including postganglionic sympathetic neurons (e.g. von Kügelgen *et al*., 1994) as well as non-neuronal cells (e.g. endothelial cells; Bodin & Burnstock, 2001) have previously been shown to release adenine nucleotides in response to various stimuli. The subtype of the neuronal P2-receptor involved in the cooling-induced vasoconstriction also remains unknown. Koganezawa *et al*. (2006) used P2-receptor ligands without subtype selectivity (for a blockade of various P2X- and P2Y-receptor-subytpes by suramin and PPADS, see Nörenberg & Illes (2000), Khakh *et al*. (2001), von Kügelgen (2006); similarly, ATPγS activates several P2X- and P2Y-receptor-subtypes). The results of most studies analysing excitatory presynaptic P2-receptors located at sympathetic axon terminals indicate the involvement of $P2X_2$-receptors or $P2X_2/P2X_3$-receptor heteromers (see above). However, postganglionic sympathetic neurones have been shown to express additional P2X-receptor subtypes including $P2X_1$-receptors (Calvert & Evans, 2004; see also Schädlich *et al*., 2001). Moreover, sympathetic neurones also possess excitatory P2Y-receptors (e.g. Boehm, 1994; von Kügelgen *et al*., 1997). The identification of the subtype of P2-receptor may be important for the attempt to modify the cutaneous blood flow in pharmacotherapy. In this regard, one might ask whether excitatory P2-receptors at sympathetic axon terminals innervating cutaneous blood vessels play a role in the markedly increased vasoconstrictor responses at low temperatures observed in some patients suffering from Raynaud's disease.

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