The regulation of oxygen supply to contracting skeletal muscle is tightly coupled to metabolic demand such that any change in metabolism is rapidly met with the appropriate rise or fall in blood flow. The red blood cell (RBC), in addition to being the primary oxygen carrier in the human body, functions as an oxygen sensor capable of releasing vasodilatory substances in response to deoxygenation, thereby facilitating vasodilation in response to increased metabolic demand. In addition to the formation of nitric oxide (NO) via deoxyhaemoglobin, RBCs release the potent vasodilator ATP which is directly coupled to the formation of deoxyhaemoglobin and an important regulator of vascular tone. Notably, the vasomotor properties of ATP mimic many of the properties of exercise hyperaemia, and are distinct from other vasodilators such as NO and adenosine. Specifically, intra-arterial infusion of ATP elicits robust vasodilation without developing tachyphylaxis during prolonged infusions; relies heavily on endothelium-dependent hyperpolarization (EDH) and the activation of KIR channels to elicit its vasodilatory effects; and is capable of attenuating sympathetic α-adrenergic vasoconstriction (sympatholysis). Thus, there is considerable interest in understanding the signaling pathways through which ATP elicits vasodilation and attenuates sympathetic vasoconstriction in humans.

Classically, exercise hyperemia is described as a balance between local vasodilation and sympathetic vasoconstriction, whereby vasodilatory signaling attenuates sympathetic vasoconstriction which facilitates tissue blood flow and oxygen delivery. However, our laboratory recently showed that blockade of multiple vasodilatory pathways including KIR channels, Na\(^+/K^+\)-ATPase, NO, and prostaglandins (PGs) reduces the vasodilation during handgrip exercise by more than 40%, and that despite the significant reduction in vasodilation, there was no effect on the ability of exercise to attenuate sympathetic vasoconstriction. Further, direct activation of KIR channels via intra-arterial infusion of KCl does not attenuate sympathetic vasoconstriction. Interestingly, these findings seemingly dissociate the primary pathways through which ATP elicits vasodilation and the ability to cause sympatholysis. Therefore, we performed similar studies to determine if ATP is capable of modulating sympathetic vasoconstriction, similarly to exercise, after blocking KIR channels, Na\(^+/K^+\)-ATPase, NO, and PGs. In this investigation, combined blockade of KIR channels, Na\(^+/K^+\)-ATPase, NO, and PGs reduced the vasodilation to ATP by 60%, and in some individuals the vasodilation was nearly abolished (≥90%). Under control conditions (adenosine-mediated vasodilation) vasoconstriction in response to phenylephrine was increased after combined blockade. In contrast, the very low levels of ATP-mediated signaling that remained after blockade maintained the ability to attenuate α\(_1\)-adrenergic vasoconstriction. These results demonstrate that the primary pathways responsible for ATP-mediated vasodilation are independent of those which cause sympatholysis, and reinforce the concept that control of vascular tone is more than a balance of vasodilation and constriction, rather distinct pathways may exist for eliciting vasodilation and attenuating vasoconstriction in humans.

While the ability to elicit vasodilation and the ability to attenuate sympathetic vasoconstriction may rely
on distinct downstream mechanisms, evidence suggests that EDH may be the common underlying signal responsible for coordinating both vasodilation and sympatholysis. In this context, endothelium-dependent agonists such as ATP cause an increase in intracellular Ca\(^{2+}\) which in turn activates small or intermediate conductance Ca\(^{2+}\)-activated K\(^{+}\) (SK\(_{ca}\) and IK\(_{ca}\)) channels. Efflux of K\(^{+}\) from SK\(_{ca}\) and IK\(_{ca}\) channels results in hyperpolarization of endothelial cells that travels along the endothelial cell layer, as well as from the endothelium to vascular smooth muscle via direct cellular connections known as myoendothelial gap junctions (MEGJ). Efflux of K\(^{+}\) and/or hyperpolarization per se activates K\(_{IR}\) channels, resulting in further efflux of K\(^{+}\), and amplification of EDH into a robust vasodilatory response.\(^5\)\(^,\)\(^6\) In addition to eliciting vasodilation through the activation of K\(_{IR}\) channels, EDH has been shown to directly modulate \(\alpha\)-adrenergic vasoconstriction.\(^7\) While this has long been known to occur in animal models,\(^7\) our laboratory recently demonstrated that during mild handgrip exercise, augmentation of endothelium-dependent signaling related to EDH, via intra-arterial infusion of ATP or acetylcholine, significantly increased the ability of contracting skeletal muscle to attenuate \(\alpha\_1\)-adrenergic vasoconstriction.\(^3\) Similarly, in animal models EDH mediated by activation of IK\(_{ca}\) channels within the MEGJ is a critical component of feedback regulation of \(\alpha\)-adrenergic vasoconstriction.\(^8\) Whether endothelium dependent activation of SK\(_{ca}\) or IK\(_{ca}\) channels is directly responsible for sympatholysis, and what potential downstream mechanisms regulate sympatholysis remains unknown.

Collectively, investigations in animal models and recently in humans have identified EDH as a key mechanism regulating both vasodilation and sympathetic \(\alpha\)-adrenergic vasoconstriction. Studies from our laboratory and others establish K\(_{IR}\) channel activation as a critical vascular mechanism capable of amplifying EDH-like signaling to elicit vasodilation, which underlies the vasodilatory response to fundamental physiologic stimuli including ischemia and skeletal muscle contraction. While recent studies in humans demonstrate an important role for EDH-like signaling in regulating sympathetic vasoconstriction, it seems that activation of K\(_{IR}\) channels is not obligatory to observe sympatholysis during exercise or intraluminal ATP infusion. Future studies focused on SK\(_{ca}\) and IK\(_{ca}\) channels and their downstream signaling mechanisms as potential contributors to EDH-signaling mediated attenuation of sympathetic vasoconstriction represents an important area of future investigation for pathophysiologic states characterized by reduced endothelial function and elevated sympathetic nervous system activity including aging, hypertension, and heart failure.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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