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REGULATION OF VASCULAR REACTIVITY IN SCLERODERMA: NEW INSIGHTS INTO RAYNAUD'S PHENOMENON

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Cold and α_2 -ARs in Cutaneous Arteries

Cold-induced vasoconstriction in the cutaneous circulation is normally a protective response that acts to reduce heat loss¹. Cold exposure initiates cutaneous vasoconstriction by a reflex increase in sympathetic nerve activity and by a direct action on the blood vessel to enhance sympathetic constriction¹. The major sympathetic neurotransmitter, norepinephrine, initiates cutaneous vasoconstriction by activating two distinct families of receptors on vascular smooth muscle cells (VSMs), α_1 and α_2 -adrenoceptors (ARs)^{2–5}. However, cold exposure amplifies constriction mediated by only one of these subtypes, the α_2 -AR, while simultaneously reducing the activity of the other, the α_1 -ARs^{6–8}. Although α_1 -ARs are fairly ubiquitous, the functional activity of α_2 -ARs is most prominent on VSM of cutaneous blood vessels including human digital arteries^{2,4,9,10}, reflecting their important involvement in thermoregulation. The effect of cold on cutaneous arteries is dramatically increased in Raynaud's phenomenon leading to the vasospastic attacks. Local inhibition of α_2 -ARs inhibits cold-induced cutaneous vasoconstriction in healthy humans, and prevents the vasospastic attacks of Raynaud's phenomenon^{11–13}.

Although α_2 -ARs represent a family of three different subtypes (α_{2A} , α_{2B} , α_{2C}), only one subtype, the α_{2C} -AR, appears to be 'thermosensitive'^{3,8,14}. Initial studies on α_{2C} -ARs suggested that they may be "silent" or "vestigial" receptors¹⁵. Indeed, unlike α_{2A} -ARs and α_{2B} -ARs, which are directed to the plasma membrane, α_{2C} -ARs are predominantly localized to ER/Golgi compartments^{14,16–18}. Although intracellular α_{2C} -ARs are functionally competent, they do not cycle to the cell surface and are not accessible to physiological agonists^{17,18}. One exception is certain neurons and neuronal cell lines (e.g. PC12) where α_{2C} -ARs are targeted to the plasma membrane and are functional¹⁸. α_{2C} -ARs are expressed in cutaneous VSMs and are essential for the thermoregulatory function of cutaneous arteries⁸. Constriction to α_2 -AR stimulation at warm temperatures is mediated by α_{2A} -ARs, with no apparent contribution from α_{2C} -ARs⁸. However, during cold exposure, the augmented α_2 -AR response is mediated entirely by α_{2C} -ARs⁸. Cold causes a remarkable functional and spatial rescue of α_{2C} -ARs from the *trans*Golgi to the cell surface, where they can respond to stimulation^{3,14,19,20}. Although α_{2C} -ARs are important effectors in the cutaneous vascular response to cooling, they do not appear to respond directly to cold and therefore should be considered 'thermoeffectors' rather than 'thermosensors'.

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Cold Signaling in Cutaneous Arteries

Immediately upon cooling, there is increased generation of reactive oxygen species (ROS) from VSM mitochondria in cutaneous arteries from the mouse tail²⁰. Indeed, this represents the earliest detectable cold-induced response in cutaneous arteries, suggesting that the mitochondria may be the 'thermosensor' responsible for initiating cold-induced vasoconstriction in cutaneous blood vessels. These mitochondrial ROS do not appear to be pathogenic, but instead activate novel REDOX signaling through the RhoA/Rho kinase (ROCK) pathway¹⁹. A similar REDOX signaling pathway has since been linked to oxygen-induced constriction of the ductus arteriosus²¹. RhoA is a member of the Ras family of small GTP-binding proteins^{22,23}, and plays a central role in regulating actin:myosin-dependent processes in VSMs, including contractility and motility^{22,24}. Actin:myosin interaction is determined by the phosphorylation status of regulatory myosin light chains (MLCs), which reflects the relative activities of MLC kinase and MLC phosphatase²⁴. Activation of RhoA and its effector, Rho kinase (ROCK) inhibits MLC phosphatase, causing increased phosphorylation of MLC and contraction of VSMs in the absence of an increase in intracellular calcium concentration^{24,25}. This mechanism, termed "calcium sensitization" can contribute to VSM and blood vessel contraction. However, in the mouse tail artery, cold-induced activation of ROS/Rho/ROCK was not directed to generalized constriction but to selective amplification of the α_2C -AR response¹⁹. Indeed, inhibition of ROCK either by pharmacological (fasudil, Y27632 or H-1152) or molecular approaches (RNA interference to reduce protein expression) prevented the cold-induced mobilization of α_2C -ARs to the cell surface¹⁹. Therefore, under these experimental conditions, the ROS/Rho/ROCK pathway contributed to cold-induced constriction by an indirect manner to facilitate delivery of constrictor α_2C -ARs to the cell surface^{19,20}. Under normal conditions, cold exposure actually caused vasodilation in the absence of α_2 -AR stimulation and there was no evidence that cold-induced activation of ROS/Rho/ROCK initiated direct constriction of these cutaneous arteries^{8,19}. Therefore, cold-induced dilation likely negated the direct influence of ROS/RhoA/ROCK on the contractile process, ensuring that their role in facilitating α_2C -AR translocation predominated during cold exposure. Interestingly, in permeabilized cutaneous tail arteries, cold increased the "calcium sensitivity" of constriction in the absence of agonist stimulation¹⁹, confirming that under some circumstances, ROS/Rho/ROCK can contribute directly to cold-induced constriction²⁴.

The role of the Rho/ROCK pathway in mediating cold-induced vasoconstriction in the cutaneous circulation has been confirmed in several studies^{26–28}. *In vivo* analysis of the cutaneous circulation of mice and humans demonstrated an important role of ROCK activity in mediating the cold-induced increase in adrenergic and α_2C -AR vasoconstrictor activity^{26–28}. Interestingly, during prolonged exposure to local cooling in human volunteers (>30 min), a large component of the cold-induced constriction was independent of adrenergic receptors but was still dependent on ROCK^{27,28}. Therefore, in humans, cold-induced activation of the Rho/ROCK pathway may initiate constriction mediated by both indirect (likely mediated by α_2C -ARs) and direct (likely mediated by 'calcium sensitization') pathways^{27,28}. Cold-induced cutaneous dilation, which may restrain this non-adrenergic response in the mouse model, was observed only during the initial response to cold and was not evident during prolonged cooling in these human volunteers^{27,28}. A component of the late non-adrenergic cutaneous vasoconstriction to local cooling in humans may be mediated by a cold-induced decrease in NO dilator signaling²⁹. Impaired activity of NO activity could result in part from the cold-induced generation of ROS, which can inactivate NO, or to cold-induced activation of Rho/ROCK, which can inhibit NO signaling at multiple sites^{30,31}.

As described above, cold-induced cutaneous constriction is restrained by simultaneous cold-induced cutaneous vasodilation, which can be observed *in vitro* and *in vivo*^{6,7,28}. In addition

to stimulating constriction through the Rho/ROCK pathway, increased generation of mitochondrial ROS in VSMs can also initiate vasodilation. Mitochondrial ROS can increase the frequency of calcium sparks, which are highly-localized intracellular calcium $[Ca^{2+}]_i$ transients generated in response to the opening of Ca^{2+} release channels on the sarcoplasmic reticulum (SR), in VSMs³². Although Ca^{2+} sparks elevate $[Ca^{2+}]_i$ to micromolar concentrations in the local vicinity of the release site, they do not contribute directly to global $[Ca^{2+}]_i$ because of their transient and localized nature³². However, calcium sparks activate large-conductance calcium-activated potassium channels (K_{Ca}) inducing membrane hyperpolarization and relaxation³². It is tempting to speculate that this mechanism may contribute to cold-induced vasodilation in cutaneous and other blood vessels, and that the same thermosensor, i.e. VSM mitochondria, initiates both cold-induced vasoconstriction and cold-induced vasodilation. Indeed, the ability of mitochondrial ROS to couple to these divergent pathways may determine whether the response to cold is vasodilation, as observed in deep blood vessels, or a combination of vasodilation and vasoconstriction, as observed in cutaneous blood vessels.

Stress Signaling and Raynaud's Phenomenon

Raynaud's phenomenon can be of idiopathic origin or can occur in response to mechanical (Hand-Arm Vibration Syndrome or vibration white finger), immunological (autoimmune diseases) or chemical stress (antineoplastic chemotherapeutic agents including bleomycin) in cutaneous blood vessels¹³. It is intriguing that these pathological stressors act to amplify what is a normal physiological stress response to cold exposure.

Indeed, α_2C -ARs may function as stress receptors within cutaneous arteries^{10,33}. Stimuli that mimic arterial injury or inflammation cause a dramatic and selective increase in α_2C -AR expression in cutaneous VSMs, which is associated with partial mobilization of α_2C -ARs to the cell surface^{10,33}. Therefore, although these receptors appear to be carefully regulated in VSMs resulting in their intracellular retention under normal conditions, in response to physiological or pathophysiological stress they can be upregulated and/or relocated to the cell surface. α_2C -ARs may have signaling qualities that are desirable under conditions of vascular stress but are less beneficial under normal circumstances. For example, unlike α_2A or other cell-surface receptors, α_2C -ARs are resistant to agonist-induced desensitization³⁴⁻³⁶. α_2C -ARs may therefore have a functional advantage for responses that require prolonged activation (e.g. vascular remodeling, prejunctional regulation) but may not be beneficial for normal vascular function. While such unusual regulation may not be required for agonists that are present only during stress, norepinephrine is always present in the blood vessel wall. This remarkable regulation may provide a unique ability for norepinephrine to engage in certain vascular responses. However, inappropriate regulation of α_2C -ARs may precipitate pathological changes in the blood vessel wall, including enhanced cold-induced vasoconstriction and Raynaud's phenomenon. Interestingly, although the female sex hormone estrogen increases expression of α_2C -ARs in cutaneous VSMs, it increases α_2 -AR vasoconstriction only during cold exposure³⁷. Therefore, increased expression of α_2C -ARs may act to increase a mobile pool of intracellular α_2C -ARs and thereby selectively increase cold-induced vasoconstriction.

The other signaling components involved in cold-induced vasoconstriction (ROS, Rho/ROCK) are also known to have increased activity during pathological stress and the development of vascular diseases^{30,31}. It is therefore tempting to consider that Raynaud's phenomenon may arise from altered activity of components of the signal transduction pathway involved in the thermosensor or thermoeffector (including ROS, Rho, ROCK, α_2C -ARs) function of cutaneous arteries.

A Mechanism-Based Therapeutic Approach to Raynaud's Phenomenon

Although multiple mechanisms may contribute to the amplified cold-induced vasoconstriction of Raynaud's phenomenon, it is unlikely that the cutaneous arteries have developed a distinct thermosensor compared to control arteries. Therefore, therapies targeting the signal transduction pathway responsible for cold-induced constriction of cutaneous arteries (ROS/Rho/ROCK) should provide an effective mechanism-based therapeutic intervention for this condition. Given the complexities of ROS and REDOX signaling, the most promising candidate may be ROCK, especially considering that several pharmaceutical companies are developing inhibitors for these enzymes^{30,31}. Because of the role of ROCK in regulating numerous pathological processes including vasoconstriction, vascular remodeling and fibrosis, ROCK inhibitors may be especially beneficial in treating scleroderma. Many of the cholesterol-independent or 'pleiotropic' vascular protective effects of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors (statins) are thought to result from their ability to inhibit Rho/ROCK signaling in the blood vessel wall^{30,31}. Therefore, the promising preliminary results obtained with statins in Raynaud's phenomenon and scleroderma^{38–41} may herald an even more impressive outcome for ROCK inhibitors.

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