Central role of Ca\(^{2+}\)-dependent regulation of vascular tone in vivo

Arteriolar diameter is the primary regulator of peripheral vascular resistance, perfusion pressure, and regional blood flow. Ca\(^{2+}\) ions in smooth muscle cells (SMC) lining the vascular wall act as an important second messenger to regulate vascular tone. In these cells, a Ca\(^{2+}\)/calmodulin complex stimulates the activity of myosin light chain kinase (MLCK). MLCK phosphorylates the myosin regulatory light chain (RLC), triggering myosin-actin interaction, myocyte contraction, and ultimately, vasoconstriction. This process is reversed when the RLC is dephosphorylated by myosin light chain phosphatase (MLCP), resulting in SMC relaxation and vasodilation. Arterial diameter fluctuations are thus closely coupled with the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) of SMC and with the relative activity of MLCK and MLCP. Ca\(^{2+}\) influx via voltage-dependent Ca\(^{2+}\) channels after membrane depolarization is the primary signal for contraction in vascular SMC. In addition, Ca\(^{2+}\)-independent changes in the activity of MLCK and MLCP, generally referred to as Ca\(^{2+}\) sensitization and/or Ca\(^{2+}\) desensitization, also influence arterial diameter (6). The relative importance of [Ca\(^{2+}\)]\(_i\) vs. Ca\(^{2+}\) sensitization/desensitization in the control of vascular tone and regional blood flow is a subject of much discussion. At the heart of this issue is understanding how arteries are regulated in vivo, with each vascular bed presenting different needs based on the local environment. In this issue of the Journal of Applied Physiology, Brekke et al. (1) describe a method that permits the simultaneous measurement of SMC [Ca\(^{2+}\)]\(_i\) and diameter of arterioles in a living animal.

The hallmark of Ca\(^{2+}\) sensitization is vasoconstriction in the absence of a significant elevation of [Ca\(^{2+}\)]\(_i\), in response to a “sensitizing agonist” (6). Discrimination between alterations in vascular tone resulting from changes in [Ca\(^{2+}\)]\(_i\), or Ca\(^{2+}\) sensitization therefore requires precise measurement of SMC [Ca\(^{2+}\)]\(_i\), and arteriolar diameter in response to physiological stimuli. For example, elevation of intravascular pressure causes graded membrane potential depolarization, increases in [Ca\(^{2+}\)]\(_i\), and vasoconstriction in isolated cerebral arteries (5). SMC membrane depolarization, increases in [Ca\(^{2+}\)]\(_i\), and vasoconstriction also result when the extracellular potassium concentration is raised. Arterial diameter was found to be set by SMC [Ca\(^{2+}\)]\(_i\), independent of the constricting stimulus (pressure or potassium) (5). Intravascular pressure and nerve stimulation represent reasonable physiological stimuli when investigating the function of isolated arteries and arterioles. The contribution of Ca\(^{2+}\) sensitization to these stimuli is unclear without accurate simultaneous measurements of [Ca\(^{2+}\)]\(_i\). Furthermore, the responses of blood vessels to vasoconstrictor or vasodilator stimuli may be affected by the experimental conditions when studied in isolation. Arteries in vivo are composed of and influenced by other types of cells in addition to vascular SMC, including endothelial cells and perivascular nerves. Neuronal connections are disrupted when vascular beds are isolated and removed, and endothelial function may be disturbed or compromised during dissection and cannulation procedures, resulting in loss of neurotransmission or paracrine and autocrine factors produced by the endothelium. Furthermore, the influence of a number of in vivo factors that can influence Ca\(^{2+}\) dynamics and vascular function such as laminar shear stress, blood-borne endocrine factors, and arterial blood-gas tension are lost when vessels are studied in isolation.

In an elegant series of experiments, the Segal laboratory has extended the study of SMC [Ca\(^{2+}\)]\(_i\), in intact arterioles to the in vivo setting. To test the hypothesis that changes in SMC [Ca\(^{2+}\)]\(_i\), underlie arteriolar constriction and dilation in a vascular bed controlling regional blood flow, Brekke et al. (1) studied arterioles in the cheek pouches of anesthetized hamsters loaded in situ with the ratiometric Ca\(^{2+}\) indicator fura-PE3. Diameter and SMC Ca\(^{2+}\) changes were simultaneously recorded during a number of experimental protocols. These studies demonstrate that changes in Ca\(^{2+}\) precede changes in vessel diameter elicited by elevating superfusate O\(_2\) concentration from 0 to 21%. Significantly, constriction in response to this stimulus is typically not present when arteries are studied in isolation (3, 4), further demonstrating the value of the in vivo approach. Spontaneous vasomotion occurred during some of these experiments, with [Ca\(^{2+}\)]\(_i\) changes driving alterations in diameter, with a lag of ~2 s. Vasodilation induced by the endothelium-dependent vasodilator acetylcholine or constriction induced by the α\(_1\)-adrenergic receptor agonist phenylephrine was also preceded by appropriate changes in [Ca\(^{2+}\)]\(_i\). Changes in Ca\(^{2+}\) sensitivity were not observed in any of these experiments, suggesting that changes in [Ca\(^{2+}\)]\(_i\) dominate vascular control in this setting. Furthermore, a prior study found that elevated O\(_2\) tension caused SMC depolarization and that vasoconstriction induced by this stimulus is blocked by inhibitors of voltage-dependent Ca\(^{2+}\) channels (7). Thus the findings reported by Brekke et al. that arteriolar constriction in response to elevated O\(_2\) is associated with a rise in SMC [Ca\(^{2+}\)]\(_i\) are consistent with the hypothesis that electromechanical coupling and Ca\(^{2+}\) entry via voltage-gated Ca\(^{2+}\) channels is the primary signal for short-term control of arterial diameter and blood flow in vivo. Future experiments using this preparation could assess potential roles for Ca\(^{2+}\) sensitization in longer term vascular control or in pathophysiological situations.

The ability to study SMC Ca\(^{2+}\) dynamics in vivo will allow previously unresolved issues in microvascular physiology to be addressed. For example, in some vascular beds, vasomotor responses can be conducted to sites distant to the original stimulus, presumably via gap junctional communication (2). The techniques developed by the Segal laboratory will allow the role of SMC Ca\(^{2+}\) in conducted vascular responses to be to be investigated under physiological conditions during the control of tissue blood flow.

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