Actions of Calcium on Smooth Muscle[a]

Historical Overview

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In 1883, Ringer alerted the biological world to the fact that calcium played an important role in muscle contraction.[1] In 1911, Cow described the extensive studies that he had carried out on isolated vascular smooth muscle.[2] He recorded the responses of this muscle to many pharmacological and physiological agents and studied the influence of calcium on these responses.

In the current review I will try to sum up what we have learned about the actions of calcium on smooth muscle since this early beginning. These actions of calcium will be reviewed from three aspects: (1) how calcium activates the contractile protein, (2) how the concentration of activator calcium is regulated, and (3) how calcium affects the plasma membrane.

CALCIUM AND THE CONTRACTILE PROTEINS

The central consideration given to the role played by calcium in muscle contraction is its activation of the chemomechanical transducing system that converts energy from the physiological fuel, ATP, to developed force. Calcium is the "spark plug" that puts the contractile proteins "in motion."

It is now well established that the role played by this activator calcium as the spark plug for contraction of vertebrate skeletal muscle is indirect. Ebashi *et al.* observed that when they combined purified actin (from which the troponin-tropomyosin system had been removed) with myosin, calcium was not necessary for super precipitation of the resultant, purified actomyosin.[3] In the intact, resting muscle and in extracted, native actomyosin, the ATPase activity of actomyosin is inhibited by a complex cooperative influence of troponin and tropomyosin. This inhibitory action of the troponin-tropomyosin system is eliminated when calcium combines with one of the troponins, TN-C. The binding of calcium to TN-C has the same calcium concentration dependence as does the activation of native actomyosin ATPase. The action of calcium is not a direct activation, but instead it is a disinhibition of the troponin-tropomyosin system. Because in the native state these inhibitory proteins are bound to actin in the thin filaments, this regulatory system is referred to as the actin or thin-filament regulatory system.

In 1965, we demonstrated that the calcium requirement for contraction of smooth muscle of the pig carotid artery was identical to that of the skeletal muscle of the rabbit psoas.[4] From these observations we concluded that the molecular mechanism by which calcium regulated the contraction of vascular smooth muscle was the same as that

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which Ebashi has characterized for striated muscle. Because of this conclusion, we credit ourselves with having delayed by a decade the correct understanding of the mechanism by which calcium activates the contractile process of smooth muscle.

It is now known that the calcium regulatory system of vertebrate smooth muscle is myosin linked as it is in molluscan muscle.[5] Bremel *et al.* observed that purified myosin from vertebrate smooth muscle could not be activated by pure actin from vertebrate skeletal muscle in the absence of calcium.[6] Calcium activated this hybrid actomyosin which had no TN-C.

In the smooth muscle cell, calcium activates a kinase that causes the phosphorylation of myosin light chain. Once phosphorylated, the actomyosin becomes an active ATPase and the myosin bridges cycle to generate active force.[7] The sensitivity of the activation of the myosin light chain kinase by calcium is increased tenfold by calmodulin.[8] In a recent study using aequorin to monitor intracellular calcium concentration in swine carotid media, Rembold and Murphy demonstrated convincingly the correlations between intracellular calcium concentration, myosin light chain phosphorylation, and cross bridge cycling rate.[9] They also observed that although these three variables were transient following stimulation of the muscle, the developed stress persisted in what they have called the "latch state." Based on these and other observations Hai and Murphy have described a kinetic model that hypothesized two types of cross bridge interactions: (1) cycling phosphorylated cross bridges and (2) noncycling dephosphorylated cross bridges ("latch bridges").[10] The only calcium-dependent regulatory mechanism in this model is myosin light chain kinase activation. They conclude that myosin phosphorylation is both necessary and sufficient for the development of the latch state.

REGULATION AND ACTIVATOR CALCIUM

These investigations have provided a comfortable understanding of the mechanism of contraction of vascular smooth muscle and have made it clear that this contraction is regulated by the concentration of calcium in the myoplasm. Parallel research has developed insight into the processes that regulate this calcium concentration. Broadly considered, these processes are involved either in the movement of calcium across the plasma membrane or in the sequestration and release of calcium from the sarcoplasmic reticulum.

In 1963 we found it possible to differentiate contractile responses to calcium from these two sources[11] when we observed that the response of the rabbit aorta was comprised of a fast and a slow component. We and others[12] established that calcium for the fast component had its origin from intracellular sequestered sites whereas that for the slow component had its origin from the extracellular calcium pool.

Calcium for the fast component of the contraction is released from the sarcoplasmic reticulum when a constrictor agonist activates its specific receptors in the plasma membrane. Somlyo and Somlyo have named this process pharmacomechanical coupling.[13] It can occur without a change in membrane potential. The role of an intracellular source of calcium in response to physiological agonist is demonstrated by the observation that vascular smooth muscle contracts phasically when stimulated with norepinephrine (NE) in a calcium-free medium.[12] Further evidence of this intracellular release of sequestered calcium in response to a receptor-agonist complex is found in the observation that ^{45}Ca efflux increases when ^{45}Ca-loaded vascular smooth muscle is stimulated with NE.[14] The signal for calcium release is transmitted to the sacroplasmic reticulum either by calcium-induced calcium release[15] or by inositol trisphosphate.[16]

In the absence of these signals the sarcoplasmic reticulum activity sequesters calcium, and it has been established that in vascular smooth muscle these microsomes hold sufficient calcium "to be both sink and source for the activator calcium in excitation-contraction coupling."[17] Van Breemen *et al.* have called our attention to the important role that the sarcoplasmic reticulum must play in buffering increments in intracellular calcium concentration.[18] They have presented evidence that NE blocks this buffering action and thereby causes a further increase in intracellular calcium concentration.

Extensive insight has also been developed into the roles played by the plasma membrane in the regulation of intracellular calcium concentration. Fundamental to this regulation is the fact that extracellular calcium concentration is approximately 10,000 times greater than that inside of the resting smooth muscle cell. For this reason the regulatory role of the plasma membrane is largely dependent on its permeability to calcium. This permeability is governed by the status of specific protein channels in the lipid bilayer of the membrane. Some channels open when the membrane is depolarized (potential-sensitive channels); others open when agonists react with their membrane receptors (receptor-operated channels).[19] Extensive evidence has established that these two classes of channels are different. For instance, they respond differently to calcium entry blockers[19] and to calcium agonists.[20]

The role of the membrane in regulating intracellular calcium concentration is influenced by the concentrations of potassium and sodium in its environment. Indirectly this influence reflects the roles that these monovalent cations have as determinants of membrane potential and hence on the opening of the potential-sensitive calcium channel. The concentration of these ions regulates membrane potential directly by their diffusion potentials and indirectly by their regulation of the activity of the electrogenic sodium pump. An increase in the activity of this pump causes membrane hyperpolarization, hence closure of the potential-sensitive channels and vascular smooth muscle relaxation.[21]

There is now being considered a more direct role for the sodium ion in regulating the transmembrane calcium movement in vascular smooth muscle. A specific sodium-calcium exchange mechanism was first critically characterized in the squid axon.[22] With the normal high transmembrane concentration gradient of sodium, the flow of this ion down its concentration gradient into the cell through this exchange system energizes the extrusion of calcium from the cell. When the sodium concentration gradient is reduced either by decreasing extracellular sodium or by increasing intracellular sodium, calcium concentration inside the cell increases. Briggs and Melvin observed that when rabbit aortic strips were exposed to a low sodium concentration, calcium influx increased by 225% and contracture developed.[23] Several investigators have attributed the contracture that develops when the sodium pump of vascular smooth muscle is inhibited, to the increase in intracellular sodium that dissipates the sodium gradient that is normally responsible for calcium extrusion via this sodium-calcium exchange system.[24-26] Although extensive arguments have been advanced supporting the physiological significance of this exchange system,[27] its importance is not universally accepted.[28]

Another substantive membrane system involved in the regulation of intracellular calcium concentration is the energy-requiring calcium-extrusion pump. When the smooth muscle cell is metabolically inhibited calcium accumulates intracellularly at the rate of its leak into the resting cell, demonstrating the essential homeostatic role of the calcium-extrusion pump.[29] The functional significance of this pump is suggested by the vasodilation by nitroglycerine that stimulates the pump,[30] and the vascular smooth muscle contraction that results from its inhibition with vanadate.[31]

CALCIUM AND THE PLASMA MEMBRANE

Not only is the plasma membrane equipped with many mechanisms that are important regulators of intracellular calcium concentration, but the function of the membrane is itself regulated by calcium. Intracellular functions are protected from excessive calcium concentration, since an increase in extracellular calcium concentration causes a decrease in cell membrane permeability to calcium. Increases in extracellular calcium concentration cause a decrease in ^{45}Ca influx in vascular smooth muscle.[32,33] This inactivation of calcium channels by increasing concentations of calcium can be demonstrated by the relaxation of vascular smooth muscle that occurs when extracellular calcium concentration is increased above physiological levels.[34] Increases in calcium concentration also cause a cessation of action potentials in the taenia coli.[35] The decrease in membrane permeability caused by the increase in calcium concentration is referred to as membrane stabilization. It is a generalized effect, as evidenced by the observations that it results in a decrease in potassium flux through the vascular smooth muscle membrane[36] and it produces identical permeability changes in the membrane of the lymphocyte.[37] The converse is also true in that a decrease in extracellular calcium concentration results in an increase in membrane excitability. Clinically this is recognized in hypocalcemic tetany, and we have observed that it causes an increase in membrane excitability in vascular smooth muscle.[11]

More recently we have used an alternate technique to evaluate the influence of membrane-bound calcium on its permeability.[38] In this technique the vascular smooth muscle is placed in a calcium-free solution for 5 minutes with the sodium pump shut down by a potassium-free solution. Calcium is then added back to the muscle bath causing a contraction, the time course of which is determined by the rate of calcium entry into the cell through the plasma membrane which, in turn, is regulated by the amount of calcium that had remained bound to the membrane during the 5 minutes exposure to the calcium-free solution. This regulatory role of calcium bound to the membrane is demonstrated by exposing the muscle to 1 mM EGTA in a calcium-free solution for 1 minute before the 5 minute period in a calcium-free solution without EGTA. The 1 minute exposure to EGTA removes calcium from the membrane. Now when the calcium is added back 5 minutes later it enters the cell easily giving rise to a much faster contractile response. Using this technique we have confirmed results of other studies[39,40] indicating that in experimental hypertension there is a deficiency of membrane binding sites on which calcium has this stabilizing action.

As characterized by Johnson *et al.* all aspects of the relationships of calcium to vascular smooth muscle functions are mediated through calcium-binding proteins.[26] The future of this field will depend on a further understanding of the role played by these proteins.

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