Calcium and its role in muscle relaxation

Calcium is the most abundant mineral in the human body. The body stores 99% of its calcium in bones and teeth. The other 1% is stored in the plasma, muscle, or other fluids and cells. Though only 1% of calcium is used outside the bones, it is a crucial nutrient for proper physiological functioning. Calcium is needed for muscle contraction and plays a large role in the cardiovascular system. If calcium levels are insufficient in the cells, the body will pull calcium from the bones.

When a nerve impulse is sent to contract a muscle fiber, calcium is released into the muscle fiber from the sarcoplasmic reticulum (SR). The calcium binds with the protein troponin, changing its shape and thus revealing the myosin binding site. The muscle is now ready to contract.

Calcium is incredibly important in contracting muscle tissue. but it can also be used to relax muscles. After contraction, calcium must be actively transported from the cytosol and back into the SR. Relaxed muscles have a much higher concentration of calcium in the SR than the sarcoplasm. When another nerve impulse reaches the muscles, calcium gates open and calcium migrates to the sarcoplasm.

Calcium lactate is a chelated (combined with an organic acid) form of calcium. In this case, calcium is bound to lactic acid. Chelated compounds tend to be more easily absorbed. Additionally, calcium lactate is one of the more acidic forms of calcium. Many integrative practitioners believe that the acidic form is more useful in addressing the needs of the body for calcium *outside* the bones, i.e., tissue calcium. Additional minerals that play a role in muscle relaxation are magnesium and zinc.

Relaxation of Arterial Smooth Muscle by Calcium Sparks. Local increases in intracellular calcium ion concentration ([Ca2+]i) resulting from activation of the ryanodine-sensitive calcium-release channel in the sarcoplasmic reticulum (SR) of smooth muscle cause arterial dilation. Ryanodine-sensitive, spontaneous local increases in [Ca2+]i (Ca2+ sparks) from the SR were observed just under the surface membrane of single smooth muscle cells from myogenic cerebra arteries. Ryanodine and thapsigargin inhibited Ca2+ sparks and Ca2+-dependent potassium (KCa) currents, suggesting that Ca2+ activate KCa channels. Furthermore, KCa channels activated by Ca2+ sparks appeared to hyperpolarize and dilate pressurized myogenic arteries because ryanodine and thapsigargin depolarized and constricted these arteries to an extent similar to that produced by blockers of KCa channels. Ca2+ sparks indirectly cause vasodilation through activation of KCa channels, but have little direct effect on spatially averaged [Ca2+]i, which regulates contraction. [Nelson MT, Cheng H, Rubart M, et al. Relaxation of Arterial Smooth Muscle by Calcium Sparks. *Science.* 1995 Oct;270:633-637.]

Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. The role of calcium in the relaxations evoked by methacholine and A23187 in intact rabbit aortic rings was investigated. Methacholine (10(-8) to 10(-6) M) and the calcium ionophore A23187

(10(-8) to 10(-6) M) produced dose-dependent relaxations of rings which had been contracted with the alpha-adrenergic agonist phenylephrine. The ability of a ring to relax in this manner was correlated with the presence of endothelium as judged by transmission and scanning electron microscopy. Purposely disrupting the endothelium led to a loss of the relaxation response. In these rings methacholine caused dose-dependent contractions at concentrations greater than 10(-7) M. Deletion of Ca++ from the incubation medium inhibited maximum methacholine-induced relaxations by 67% and A23187-induced relaxations by 92%. The Ca++-channel blockers verapamil (10 microM) and nifedipine (0.5 microM) inhibited maximum methacholine-induced relaxations by 39% and 45%, respectively. The blockers had no effect on the methacholine ED50 (2.5 x 10(-7) M) for relaxation. Verapamil and nifedipine also inhibited maximum A23187-induced relaxations by 43% and 47% with no effect on the ED50 (6 x 19(-8) M) for relaxation. A structurally dissimilar vasodilator, sodium nitroprusside (10(-7) M), had no effect on the A23187-induced relaxation. These data are consistent with a role of Ca++ in regulating either the production or release of endothelial-derived relaxing factor(s). [Singer, HA, Peach MJ. Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. *Hypertension.* 1982;4:19-25.]

Mechanisms of calcium relaxation of vascular smooth muscle. We examined mechanisms of relaxation in intact and endothelium-denuded thoracic aortic rings of rat in response to various concentrations of calcium (from 1.6 to 10.1 mM) after contraction induced by 30 mM KCl. The relaxation to calcium was concentration dependent in intact and denuded rings. This effect was greater in intact preparations than in denuded preparations or in intact preparations treated with methylene blue (10(-5) M). This indicates that the relaxation by calcium is partly mediated by releasing endothelium-derived relaxing factors (EDRFs). In the presence of A23187, the relaxation to calcium was attenuated in intact rings but not in denuded rings, whereas calcium relaxation was not significantly altered by sodium nitroprusside. These observations suggest that the calcium-induced relaxation is reduced if EDRF has already been released by A23187. We conclude that, paradoxically, the same increase in extracellular Ca2+ concentration that causes an increase in endothelial intracellular calcium concentration ([Ca2+]i) to produce EDRF also results in membrane stabilization of the vascular smooth muscle cell to decrease [Ca2+]i and cause relaxation. These two mechanisms are additive in producing calcium relaxation in the intact artery. [Wu CC, Bohr DF. Mechanisms of calcium relaxation of vascular smooth muscle. *Am J Physiol*. 1991 Nov;261(5 Pt 2):H1411-H1416.]

Force decline during muscle relaxation promotes calcium release to the cytosol. During relaxation of skeletal muscle, an initial rapid decline of intracellular Ca2+ concentration ([Ca2+]i) (phase 1), is followed by a brief phase in which the decline of [Ca2+]i is markedly slowed or even reversed (phase 2). Phase 2 appears as a prominent "bump" on records of the time course of declining [Ca2+]i during relaxation. The goal of this study was to test the hypothesis that phase 2 represents a release of Ca2+ to the cytosol that occurs with net cross-bridge detachment during relaxation. The experimental approach was to measure [Ca2+]i with

indo 1 in stimulated bullfrog semitendinosus muscles and to determine if phase 2 was diminished during relaxation of contractions in which cross-bridge interactions had been reduced by two different methods: 1) stretching muscles to reduce the overlap between actin and myosin filaments or 2) decreasing stimulus duration. The results showed that, when either method was used to reduce cross-bridge interactions during contraction, then the size of phase 2 during relaxation was also decreased. Phase 2 was eliminated during relaxation of contractions in which cross-bridge interactions had been reduced to a lower contraction force approximately 30% of maximum. These findings are consistent with the hypothesis that the phase 2 of [Ca2+]i decline represents a release of Ca2+ to the cytosol that occurs with net cross-bridge detachment during relaxation. This conclusion is consistent with previous studies that suggest that cross-bridge detachment lowers the affinity of troponin for Ca2+. [Baker AJ, Weiner MW. Force decline during muscle relaxation promotes calcium release to the cytosol. *Am J Physiol.* 1997 Jul;273(1 Pt 1):C85-C91.]

The cellular basis of contraction and relaxation in cardiac and vascular smooth muscle. Changes in cytoplasmic levels of free-ionized calcium regulate the contraction and relaxation of cardiac and vascular smooth muscle. Aside from changing the intrinsic rate of energy use within the cell, an intervention that alters the strength of contraction of cardiac or vascular smooth muscle must in general alter either the intracellular calcium level, change the calcium requirements of the contractile apparatus, or exert both effects. Intracellular calcium handling is modulated by at least three other important second messengers: cyclic adenosine monophosphate, cyclic guanosine monophosphate, and inositol 1,4,5 triphosphate. These substances modulate intracellular calcium handling at multiple steps in the excitation-contraction coupling scheme. In vascular smooth muscle, diacylglycerol appears to play an important role in the regulation of myofilament calcium requirements. Intracellular calcium homeostasis is maintained through the action of sarcolemmal mechanisms that extrude calcium into the extracellular space; these mechanisms include a sodium-calcium exchange mechanism and an energy-dependent calcium pump. Abnormalities in electrophysiologic properties, sarcoplasmic reticulum function, energy use and supply, and contractile element interaction have been identified in experimental studies of animals and patients with various cardiovascular diseases. These abnormalities may be differentially affected by therapeutic agents that act on the heart or vasculature; therefore it is important to understand the underlying cellular abnormalities and subcellular actions of inotropic, vasoconstrictor, and vasodilatory drugs to apply rational therapeutics in the clinical setting. [Morgan JP, Perreault CL, Morgan KG. The cellular asis of contraction and relaxation in cardiac and vascular smooth muscle. *Am Heart J.* 1991 Mar;121(3 Pt 1):961-968.]

Regulation of vascular smooth muscle tone. Vascular smooth muscle tone is regulated primarily by the sarcoplasmic free Ca2+ concentration, which determines the level of myosin phosphorylation. Stimulation of the muscle results in an increase in free [Ca2+], whereupon Ca2+ binds to calmodulin, inducing a conformational change enabling calmodulin to interact

with and activate myosin light chain kinase. The active Ca2+.calmodulin.myosin light chain kinase complex catalyses the phosphorylation of serine-19 of the two 20-kDa light chains of myosin; this triggers cross-bridge cycling and the development of force. Relaxation follows restoration of free [Ca2+] to the resting level, whereupon calmodulin dissociates from myosin light chain kinase, which is thereby inactivated, and myosin is dephosphorylated by myosin light chain phosphatase and remains detached from actin. Overwhelming evidence now exists in favour of the central role of myosin phosphorylation-dephosphorylation in smooth muscle contraction-relaxation. However, considerable evidence supports the existence of additional, secondary mechanisms that can modulate the contractile state of smooth muscle either by altering the Ca2+ sensitivity of the contractile response or otherwise modulating one of the molecular events occurring downstream of the Ca2+ signal, e.g., the interaction of phosphorylated myosin heads with actin. The interplay of several regulatory elements confers on the contractile response of vascular smooth muscle the high degree of flexibility and adaptability required for the effective regulation of blood pressure. [Walsh MP. Regulation of vascular smooth muscle tone. *Can J Physiol Pharmacol*. 1994 Aug;72(8):919-936.]

Calcium Ion in Skeletal Muscle: Its Crucial Role for Muscle Function, Plasticity, and Disease. Mammalian skeletal muscle shows an enormous variability in its functional features such as rate of force production, resistance to fatigue, and energy metabolism, with a wide spectrum from slow aerobic to fast anaerobic physiology. In addition, skeletal muscle exhibits high plasticity that is based on the potential of the muscle fibers to undergo changes of their cytoarchitecture and composition of specific muscle protein isoforms. Adaptive changes of the muscle fibers occur in response to a variety of stimuli such as, e.g., growth and differentition factors, hormones, nerve signals, or exercise. Additionally, the muscle fibers are arranged in compartments that often function as largely independent muscular subunits. All muscle fibers use Ca2+ as their main regulatory and signaling molecule. Therefore, contractile properties of muscle fibers are dependent on the variable expression of proteins involved in Ca2+ signaling and handling. Molecular diversity of the main proteins in the Ca2+ signaling apparatus (the calcium cycle) largely determines the contraction and relaxation properties of a muscle fiber. The Ca2+ signaling apparatus includes1) the ryanodine receptor that is the sarcoplasmic reticulum Ca2+ release channel, 2) the troponin protein complex that mediates the Ca2+ effect to the myofibrillar structures leading to contraction, 3) the Ca2+pump responsible for Ca2+ reuptake into the sarcoplasmic reticulum, and 4) calsequestrin, the Ca2+storage protein in the sarcoplasmic reticulum. In addition, a multitude of Ca2+-binding proteins is present in muscle tissue including parvalbumin, calmodulin, S100 proteins, annexins, sorcin, myosin light chains, β-actinin, calcineurin, and calpain. These Ca2+-binding proteins may either exert an important role in Ca2+-triggered muscle contraction under certain conditions or modulate other muscle activities such as protein metabolism, differentiation, and growth. Recently, several Ca2+signaling and handling molecules have been shown to be altered in muscle diseases. Functional alterations of Ca2+handling seem to be responsible for the pathophysiological conditions seen in dystrophinopathies, Brody's disease, and malignant hyperthermia. These also

underline the importance of the affected molecules for correct muscle performance. [Berchtold, MW, Brinkmeier H, Müntener M. Calcium Ion in Skeletal Muscle: Its Crucial Role for Muscle Function, Plasticity, and Disease. *American Physiological Society.* 2000 Jul:80(3):1215-1265.]