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Scientific Update™

Molecular Biology of Calcium Channels in the Cardiovascular System

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Calcium is an intracellular mediator of critical importance in the cardiovascular system. In order to mediate or regulate its actions, a complex system of families of proteins has evolved. This system contains many redundancies, underscoring its great physiological relevance. There are two calcium cycles: an extracellular calcium cycle, which is small in quantity but greatly important in signalling, and an intracellular cycle, which is quantitatively larger and is responsible for activating muscle contraction. In a simplified overview, the system responsible for maintaining the integrity of calcium homeostasis and signalling in the cardiovascular system can be divided into: 1) proteins that regulate calcium entry into the cytosol, 2) proteins that recognize calcium in the cytosol and lead to the activation of the process that leads to muscle contraction, and 3) proteins that remove calcium from the cytosol and facilitate muscle relaxation, e.g., those in the intracellular cycle, or prevent cellular calcium overload, e.g., cell membrane calcium pumps from the extracellular calcium cycle.

From a clinical and pharmacological standpoint, the most interesting and important of these proteins are the cell membrane calcium channels that regulate the extracellular cycle. There are at least two important calcium channels in the cardiovascular system: L-type channels and T-type channels. The former bind the familiar calcium channel blockers, such as diltiazem, verapamil, and the dihydropyridines. The latter were more recently discovered and have unique pharmacological properties, such as a possible role in the maladaptive response of cardiac hypertrophy and dilatation seen in heart failure. Of note, the first agent to act on the T-type calcium channels, mibefradil, has been shown to be equivalent to an ACE inhibitor in improving survival in an animal model of heart failure.

Introduction

The function of calcium as an intracellular messenger and the complex system of families of proteins that mediate or regulate its actions are of critical physiological importance. The diversity of those systems includes three different processes active in calcium signalling: (1) proteins that regulate calcium entry into the cytosol, (2) proteins that recognize calcium in the cytosol, and (3) proteins that remove calcium from the cytosol.

Striated cardiac and skeletal muscle cells are very large cells that require systems to effect very rapid contraction. In order to provide calcium signalling effectively

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and in sufficiently high quantities, a dual activator system has evolved: calcium enters from outside the cell (the extracellular calcium cycle) and calcium is also released from stores within the cell (the intracellular calcium cycle). Calcium enters the cells by plasma membrane calcium channels and leaves the cells in exchange for sodium through the sodium/calcium exchanges. In the adult myocardium, the extracellular cycle is rather small in quantity but very important in signalling. Far more important quantitatively and in activating cardiac muscle is the intracellular calcium cycle, in which calcium is released from stores in the sarcoplasmic reticulum, thereby binding to proteins like troponin and leading to contraction, then is pumped back into the sarcoplasmic reticulum where it moves again from the uptake to the release cycle.

The two cycles are connected. The extracellular calcium cycle is responsible for triggering the opening of the large intracellular calcium channels, which are in turn responsible for the activation of the contractile proteins.

Proteins that Recognize Calcium in the Cytosol

The intracellular calcium binding proteins are called E-F Hand proteins. They are members of a family of proteins related in evolution which received that name because of a specific amino acid sequence in the calcium binding area. These amino acid domains take the structure of two protruding fingers in a hand, in which oxygen-containing groups from the amino acids get together to form a site at which calcium binds with very high affinity. This motif is seen throughout biology for all of the intracellular calcium binding proteins, and these proteins transduce or mediate a variety of intracellular calcium signals. In skeletal and cardiac muscle, proteins named troponins have four E-F Hand units. Calcium binds to troponin in the thin filament and rearranges it so that actin can interact with myosin, resulting in contraction. In smooth muscle, the calcium binding E-F Hand protein is calmodulin, a soluble calcium receptor.

Proteins that Remove Calcium from the Cytosol

Removal of calcium from the cytosol is accomplished by energy-dependent calcium pumps. They constitute an entirely different kind of protein with a complicated structure, including 10 membrane-spanning subunits formed by alpha helices. There are distinct calcium pumps for the sarcoplasmic reticulum (intracellular cycle) and the plasma membrane (extracellular calcium cycle). These two calcium pumps are related to each other but are regulated differently. The calcium pump of the sarcoplasmic reticulum is formed by 10 membrane-spanning alpha helices and phospholamban, which is a regulatory protein that transduces the signal produced by epinephrine and causes the activation of the calcium pump, one of the factors responsible for accelerating relaxation of the muscle, which is important, for instance, during exercise. In the case of the plasma membrane calcium pump, the regulation is by calcium itself. This protein is activated by calcium overload and protects the cells against "drowning" in calcium. In this case, the regulation is by a calcium-calmodulin complex that binds to a specific amino acid sequence in the C-terminal end of the protein. The sodium/calcium exchangers that move calcium out of the cell derive energy from the sodium gradient rather than from ATP.

Proteins that Regulate Calcium Entry to the Cytosol

A. Intracellular Calcium Cycle

These are the proteins that begin calcium signalling and they function as intracellular calcium release channels. These proteins are involved in the very large intracellular calcium cycle and are most important in the release of calcium for excitation-contraction coupling. In cardiac muscle, this protein is called the ryanodine receptor and it is opened by calcium entry into the cell by way of the L-type calcium channels present in the cell membrane. Calcium entry by the L-type calcium channels sprays on the ryanodine receptor and opens up the intracellular calcium channel that delivers calcium to the contractile apparatus through the E-F Hand proteins.

There is another large family of intracellular calcium release channels that respond to a signal transducer called inositol triphosphate (IP_3). The IP_3 receptors are related to the ryanodine receptors and play a role in the pharmacomechanical coupling in smooth muscle, which is one of the ways in which calcium gets released in the smooth muscle cells, where they may also have growth regulatory

effects. The role of the IP_3 receptors in the cardiac muscle is not well established.

B. Extracellular Calcium Cycle

Of all of the factors that regulate calcium signalling, the ones that are clinically most interesting and important are the plasma membrane calcium channels. These proteins regulate the extracellular calcium cycle and are members of a large family of ion channels, such as the sodium channels, the potassium channels, and the chloride channels. In working, contracting cardiac myocytes, these channels play an important role in excitation-contraction coupling: in the SA node, they play a role in pacemaker activity and, in the AV node, they play a role in conduction. Calcium channels are formed by four covalently linked domains, which each have six membrane-spanning alpha helices. There are at least two important types of calcium channels in the cardiovascular system: (1) L-type: this is a channel that binds the familiar types of calcium channel blockers, such as diltiazem, verapamil and the dihydropyridine calcium antagonists. They are present in working cardiac myocytes, where they mediate the excitation-contraction coupling and are responsible in part for the long plateau of the cardiac action potential. (2) T-type: these calcium channels were discovered approximately 10 years ago and are quite different in most of their properties from the L-type calcium channels. They are probably not present in significant numbers in adult cells, as they are predominantly expressed in fetal cells. In general, they are found in relatively high levels in growing cells. Their expression decreases markedly when the cells stop growing, suggesting that they may be involved in growth regulation. Because of their particular properties, they are unlikely to participate either in controlling the membrane potential of the heart or in the excitation-contraction coupling. However, they exist in the SA node with a clear role in pacemaker activity, but it is not clear whether they exist in the AV node. In the smooth muscle cells, in contrast, they play a role in excitation-contraction coupling similar to the L-type channels and therefore participate in vasoconstriction.

There are exciting possibilities that may be opened by new agents that target specifically the latter type of calcium channel as illustrated by a recently published study.[1]

In this study, the investigators utilized the coronary ligation model of myocardial infarction/heart failure in rats that was used originally by Pfeffer to demonstrate the effectiveness of ACE inhibitors. A new T-type calcium channel blocker, mibefradil, was compared to the ACE inhibitor cilazapril. At 270 days, approximately half of the rats in the control group, not receiving either agent, were dead. In contrast, the new calcium channel blocker mibefradil matched the ability of the ACE inhibitor to prolong survival. This model has correlated well with increased survival in patients treated with other agents, such as ACE inhibitors. Given that in heart failure, there is an abnormal maladaptive growth response in the myocardiocyte, the possibility exists that the effect of mibefradil is not due only to vasodilator properties but potentially to an effect on cellular growth. Thus, it would be tantalizing to speculate that the growth and hypertrophy inhibitory properties of the T-type calcium channel blockers may have the same, or similar, effects as ACE inhibitors. As mentioned above, there are very few T-type calcium channels in the adult myocardium, but this does not hold true in heart failure. In response to the overload, the heart reverts back to a re-expression of fetal gene patterns with changes in the isoforms of the contractile proteins and re-expression of atrial natriuretic peptide, for instance. There appears, as well, to be a re-expression of T-type calcium channels, which play a deleterious role in the progressive maladaptive response of hypertrophy and dilatation of the overloaded heart.

Conclusion

The exploration of the unique and novel properties of the T-type specific calcium channel blockers is one of the most exciting developments to occur in this field in many years. These new agents could become a valuable addition to our armamentarium of agents to use in heart failure.

This presentation by Dr. Katz reporting the basic science of calcium signalling and, in particular, the role of the T-type calcium channels in the cardiovascular system, was followed by a presentation by Dr. Barry M. Massie, who reviewed the clinical research carried out, to date, with mifebramil. A discussion of Dr. Massie's presentation will appear in Part II of this Update.

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Related Reading

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