MECHANISMS THAT TURN-OFF INTRACELLULAR CALCIUM RELEASE CHANNELS

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1. ABSTRACT

Calcium release from intracellular stores is a common phenomenon in cells. Calcium release is mediated by two classes of Ca\(^{2+}\) release channels: the ryanodine receptors (RyRs) and the inositol trisphosphate receptors (IP\(_3\)Rs). There are three types of RyR and three types of IP\(_3\)R. Different cells have different complements of RyR and IP\(_3\)R. In most cases, it is clear what turns-on these channels. It is often unclear what turns them off. It appears that a composite of factors and/or processes may act in synergy to regulate these channels and terminate local intracellular Ca release events. This review details some of the potential negative control mechanisms that may govern individual RyR and IP\(_3\)R channel activity.

2. INTRODUCTION

Intracellular Ca\(^{2+}\) signaling is associated with a diverse array of cellular phenomena. The intracellular Ca\(^{2+}\) signals are generated by Ca\(^{2+}\) entry through the surface membrane and/or Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores. The endoplasmic and/or sarcoplasmic reticulum (ER or SR, respectively) are the primary intracellular Ca\(^{2+}\) storage/release repositories. Specialized Ca\(^{2+}\) release channels are present in the ER/SR membranes. There are two classes of Ca\(^{2+}\) release channels, ryanodine receptors (RyRs) and inositol trisphosphate receptors (IP\(_3\)Rs). The RyR channels bind the plant alkaloid ryanodine with nanomolar affinity and are the primary Ca\(^{2+}\) release effectors in the excitation-contraction coupling process in striated muscles. The IP\(_3\)R channels are activated by the ubiquitous second messenger inositol 1,4,5-trisphosphate and are involved in many other intracellular Ca\(^{2+}\) signaling events. The RyR and IP\(_3\)R channels are both large oligomeric structures formed by either four RyR or IP\(_3\)R subunits, respectively. The RyRs and IP\(_3\)R proteins share significant homology but have little homology with the more widely studied voltage-dependent Ca\(^{2+}\) channels found in the surface membrane (1, 2). The function of single RyR and IP\(_3\)R channels in striated muscle will be the focus of this review. Many of the concepts and principles discussed here, however, can be applied more generally.
RyR-mediated Ca\textsuperscript{2+} release events in heart cells called “Ca\textsuperscript{2+} sparks” (3). Analogous IP\textsubscript{3}-R-mediated Ca\textsuperscript{2+} release events have been identified in other types of cells (4, 5). Global Ca\textsuperscript{2+} release phenomena are thought to arise from the spatio-temporal summation of these local Ca\textsuperscript{2+} release events. Most studies of local Ca\textsuperscript{2+} release events have focused on RyR-mediated Ca\textsuperscript{2+} sparks in heart (e.g. 6).

The stereotypic RyR-mediated Ca\textsuperscript{2+} spark is thought to arise from the opening of multiple RyR channels arranged in discrete clusters of channels. The current estimates of the number of RyR channels involved in generating a Ca\textsuperscript{2+} spark range from 10 to 30 (6, 7, 8). The time course of the Ca\textsuperscript{2+} spark is thought to depend on the interplay of positive and negative control mechanism(s) that govern individual RyR channels in a stochastic cluster of multiple channels. The same is likely true for local IP\textsubscript{3}-R-mediated Ca\textsuperscript{2+} release events. Thus, it is likely that control of RyR and IP\textsubscript{3}-R channels likely depends on both the properties of the individual channels and the “group dynamics” between channels. My focus here is on single channel properties.

4. RyR-MEDIATED Ca\textsuperscript{2+} RELEASE

Surface membrane depolarization of mammalian cardiac myocytes is spread axially into the cell down surface membrane invaginations called transverse tubules (T-tubules). These T-tubules come into close association with the sarcoplasmic reticulum (SR). Depolarization of the T-tubule membrane activates voltage-dependent Ca\textsuperscript{2+} channels resulting in a small-localized Ca\textsuperscript{2+} influx (I\textsubscript{Ca}). This small local Ca\textsuperscript{2+} influx is the second messenger signal that activates the RyR channel. Opening of RyR channels is responsible for the large SR Ca\textsuperscript{2+} release signal that initiates muscle contraction. The process of Ca\textsuperscript{2+} activation of the RyR channel is called Ca-induced Ca\textsuperscript{2+} release (CICR).

The CICR process is inherently self-regenerating. The Ca\textsuperscript{2+} released by a RyR channel should intuitively feedback and promote further Ca\textsuperscript{2+} release from the same channel. Interestingly, the CICR process is finely graded by the amplitude of the initial Ca\textsuperscript{2+} trigger signal (i.e. no feedback). Small triggers produce small Ca\textsuperscript{2+} release events. Large triggers produce large Ca\textsuperscript{2+} release events. How can CICR be so stable and precisely controlled? This is the classical paradox of CICR in heart.

Many investigators using a variety of different methodologies have studied the control of intracellular Ca\textsuperscript{2+} release in heart. There must be some sort of RyR-based negative control mechanism(s) to counter the inherent positive feedback of the CICR process. The Nature of the mechanism(s) that turns-off RyR-mediated Ca\textsuperscript{2+} release in heart is frequently debated. Various candidate negative control mechanisms have been proposed. Some intriguing possibilities include Ca\textsuperscript{2+}-dependent inactivation, stochastic attrition, luminal Ca\textsuperscript{2+} inhibition and/or coupled gating of neighboring channels. Furthermore, the negative control mechanism(s) that control single RyR channels in heart may also be very different than those that control the much better defined voltage- and ligand-dependent ion channels found in the surface membrane. This would not be surprising considering the very different roles these different channels play. The conventional wisdom gleaned from other systems and other channels may thus not be directly applicable to the RyR channel. For example, it appears that incremental stimuli (e.g. I\textsubscript{ca}, caffeine or depolarization in skeletal muscle) induce transient and multiple SR Ca\textsuperscript{2+} release events (9, 10, 11). This phenomenon has been referred to as quantal or adaptive behavior. The existence of this type of phenomena suggests the underlying RyR control mechanisms may be quite unusual.

5. IP\textsubscript{3}-R-MEDIATED Ca\textsuperscript{2+} RELEASE

Activation of G-protein linked receptors generates inositol 1,4,5-trisphosphate (IP\textsubscript{3}), a ubiquitous soluble second messenger. The IP\textsubscript{3} is produced at the surface membrane by phospholipase hydrolysis of a phospholipid (i.e. phosphatidylinositol). The IP\textsubscript{3} diffuses through the cytosol and binds to the IP\textsubscript{3}R channel (12). Binding of IP\textsubscript{3} activates (opens) the IP\textsubscript{3}-R channel generating a rise in cytosolic Ca\textsuperscript{2+} levels (13). Such IP\textsubscript{3} mediated Ca\textsuperscript{2+} signals are important to several cellular phenomena including secretion, synaptic transmission, fertilization, nuclear pore regulation and transcription (14, 15). It would not be an overstatement to say that IP\textsubscript{3}-dependent intracellular Ca\textsuperscript{2+} signaling is an essential element in mammalian cell physiology (including the heart).

In heart, a small Ca\textsuperscript{2+} influx across the surface membrane activates the large RyR-mediated cytosolic Ca\textsuperscript{2+} elevations that govern contraction (see above). Like other mammalian cells, heart muscle cells contain IP\textsubscript{3}-R channels and IP\textsubscript{3}-dependent Ca\textsuperscript{2+} signaling cascades. Like RyR channels, IP\textsubscript{3}-R channels are activated by cytosolic Ca\textsuperscript{2+} elevations (16). Thus, there is a clear potential for cross talk between the RyR- and IP\textsubscript{3}-mediated Ca\textsuperscript{2+} signaling pathways in heart muscle. The extent and Nature of RyR-IP\textsubscript{3}-R cross talk will of course depend on the functional attributes of the individual IP\textsubscript{3}-R channels involved. Whether or not the IP\textsubscript{3}-R channels are governed by negative control mechanisms similar to those that regulate RyR channels remains an open question.

6. RyR: SINGLE CHANNEL PROPERTIES

The 3 different isoforms of the RyR protein (Figure 1) are encoded by 3 different genes (RyR1, RyR2 & RyR3) on different chromosomes (2, 17). At the amino acid level, the 3 RyR isoforms share about 70\% identity. Thus, the RyRs form a relatively small but well-conserved family of proteins. The different RyR isoforms are found in a variety of tissues. The RyR1 isoform is the most prominent type in skeletal muscle. The RyR2 isoform is the most abundant in cardiac muscle. The RyR3 isoform is found in a variety of smooth muscles, diaphragm, as well as several other tissues (including neurons). Typically, a particular tissue will contain more than one type of RyR protein. For example, aortic smooth muscle contains both the RyR1 and RyR3 forms (18). Cerebellum contains both
The Ryanodine Receptors

![Diagram showing the 3 isoforms of the RyR channel: Type-1, Type-2, Type-3, with Vm, Ca²⁺, Skeletal Muscle, Cardiac Muscle, and Ca²⁺ signs.]

**Figure 1.** Cartoon illustrating the 3 isoforms of the RyR channel. Most cells contain multiple types of RyR. Expression levels of any one particular isoform varies dramatically tissue to tissue. Tissues listed correspond to a primary source from which a particular channel isoform can be isolated for study. The type-1 RyR (from skeletal muscle) is primarily activated by transverse tubule membrane potential changes (Vm). All channels can be activated by calcium.

RyR1 and RyR2 (19). The importance of having multiple RyR isoforms in the same cell is not known. One might speculate that the morphological heterogeneity of channels present introduce functional heterogeneity that allows RyRs to participate in different Ca²⁺ signaling tasks. Recall that every cell must carry out a myriad of Ca²⁺ signaling tasks to survive. Just because skeletal muscle is specialized for contraction does not mean that it only has the Ca²⁺ signaling required for the cell to contract.

The RyR channels are modulated by Ca²⁺, ATP, Mg²⁺, phosphorylation, calmodulin, and several other ligands. Calcium and ATP are both potent activators of the RyR channel while Mg²⁺ is an inhibitor (particularly the RyR1 isoform). A classic property of the RyR channels is their bell-shaped steady-state cytosolic Ca²⁺ dependence. The RyR channels are activated by micromolar Ca²⁺ and inhibited by high Ca²⁺ concentrations. Inhibition at high Ca²⁺ is isoform specific. The RyR1 channel is almost entirely inhibited by 1 mM Ca²⁺ (20, 21). The RyR2 (and RyR3) channel are inhibited at Ca²⁺ concentrations in excess of 10 mM (22). It is not clear that such high cytoplasmic Ca²⁺ concentrations are ever reached in the cells. Thus, the physiological role of high Ca²⁺ inhibition of RyR2 channels is unknown.

The Mg²⁺ inhibition of the RyR channel is also somewhat isoform specific. For example, the RyR1 channel is more sensitive to Mg²⁺ than the RyR2 channel. It has also been suggested that phosphorylation of the RyR channel modulates its Mg²⁺ sensitivity (23). They suggest that dephosphorylated channels are inhibited by physiological Mg²⁺ concentrations while phosphorylated channels are not. However, this idea needs further experimental verification. The action of ATP on the RyR is isoform specific as well. The RyR2 channel is much less sensitive to ATP than the RyR1 channel. Nearly all RyR regulatory agents act on the cytoplasmic side of the channel. This is not surprising considering that about 90% of the RyR’s mass extend into the cytoplasm. Although only a small portion of the RyR is in the lumen of the SR, the possibility that this part of the RyR contains ligand regulatory sites has also been explored (24, 25).

The RyR channel may also be modulated by several closely associated regulatory proteins (e.g. dihydropyridine receptor, triadin, junctin, calsequestrin, FK-506 binding protein, sorcin, etc.). The dihydropyridine receptor (DHPR) protein is a voltage-dependent L-type Ca²⁺ channel found in the transverse tubules (T-tubules) of striated muscles. The DHPR is also found in the surface membranes of many other types of cells. In skeletal muscle, the DHPR is intimately involved in RyR regulation. It has been suggested that an integral SR protein called triadin may also be somehow involved in DHPR-RyR communication (26). The FK-506 binding protein (FKBP) is tightly bound to the RyR channel complex (27) but the impact of FKBP on RyR function is not yet clearly understood. Another potentially important protein that is closely associated with the RyR channel is calsequestrin. Calsequestrin is a low affinity, high capacity Ca²⁺ buffer that seems to be attached to the luminal surface of the RyR channel protein (28). The position of calsequestrin implies that it plays an important role in buffering Ca²⁺ near the mouth of the RyR channel.

In striated muscles, the RyR channels interact with the DHPR channels in the T-tubule membrane. Depolarization of the T-tubule membrane (i.e. excitation) induces conformational changes in DHPR that lead eventually to RyR channel activation. The process of DHPR-RyR communication is commonly referred to as excitation-contraction (E-C) coupling. Its role in striated muscle E-C coupling is probably the RyR channel’s most notable claim to fame. Defining RyR channel regulation during E-C coupling promises to generate important insights into how RyR channels are regulated in other cells.

**7. RyR: EXCITATION-CONTRACTION COUPLING**

The E-C coupling process is different in skeletal and cardiac muscle. In skeletal muscle, the DHPR communicates with the RyR1 channel through some sort of physical protein-protein link. Voltage-induced changes in DHPR conformation directly induce conformational changes in the RyR1 channel that trigger it to open. The voltage-induced conformational changes in the DHPR generate measurable non-linear capacitive currents called charge movements (29). Expressing mutant DHPRs in mouse myotubes that lacked endogenous DHPR provided convincing support for a physical DHPR-RyR1 communication (30). These studies revealed that a particular intracellular loop of the DHPR is involved in DHPR-RyR1 signaling. Signal transmission between the DHPR and RyR1 channel must be quite fast because skeletal E-C coupling process occurs during the very brief (~2 ms) skeletal muscle action potential. This action potential is apparently only long enough for the DHPR voltage sensor to move and induce RyR opening. It is not long enough to allow DHPR opening and significant Ca²⁺ entry through the DHPR Ca²⁺ channel. This is why skeletal...
muscle E-C coupling is independent of extracellular Ca²⁺ levels (31). It seems that skeletal muscle’s “need-for-speed” has transformed the DHPR from a Ca²⁺ channel to a specialized voltage-sensor.

The physiological role of the DHPR in cardiac muscle is quite different. During the long cardiac action potential (~100 ms), the DHPR Ca²⁺ channel has ample time to open and for a significant Ca²⁺ influx to occur. This Ca²⁺ influx is the signal that triggers RyR2 channel opening. Specifically, Ca²⁺ acts as a diffusible second messenger that binds to and then activates the RyR2 channel. This is the Ca²⁺-induced Ca²⁺ release process (32; CICR). Although the CICR process should be self-regenerating, it is not and this implies that some sort of programmed “fateful” inactivation (5) or low intra-SR Ca²⁺ levels (6). Coupled gating could also drive RyR1 channel closure (7).

8. RyR: NEGATIVE CONTROL MECHANISMS

The negative feedback that counters the inherent positive feedback of CICR may arise from a single mechanism. Alternatively, it may arise from a composite of factors/processes acting in synergy. I believe the latter possibility is more likely because no individual mechanism by itself seems sufficient. Potential negative control mechanisms are summarized in Figure 2 and discussed in detail below.

8.1. Stochastic Attrition

Stochastic attrition of single RyR channel activity may be responsible for terminating a local Ca²⁺ release event. Stern (34) suggested that a small cluster of SR Ca²⁺ release would “turn-off” automatically as a result of local Ca²⁺ reductions generated by the stochastic closing of single RyR channels in the cluster. Stochastic attrition of course would be very sensitive to the number of channels in the cluster. Stern (34) calculated that stochastic attrition could terminate local Ca²⁺ release only in clusters of less than 10 RyRs. In reality, it appears that RyR clusters in heart muscle are composed of 10-30 single RyRs (6, 8, 35, 36). Thus, it seems unlikely that local Ca²⁺ release is terminated solely due to stochastic attrition.

8.2. Calcium Dependent Inactivation

Ca²⁺-dependent inactivation was proposed by Fabiato (32) to be the negative feedback mechanism that terminates the SR Ca²⁺ release process. Specifically, studies in a skinned cardiac cell preparation suggested that inactivation was due to slow binding of Ca²⁺ to a high affinity site on the release channel. Later studies in intact cells reported contradictory results (37). Further, single RyR2 channel studies also presented no evidence for high affinity Ca²⁺ dependent inactivation (38). Thus, the existence of high affinity Ca²⁺ dependent inactivation is not clearly established yet.

There is evidence of low affinity Ca²⁺ dependent inactivation. In single RyR2 channel studies, spontaneous channel activity is inhibited at high steady state Ca²⁺ concentrations (22, 39, 40). This high Ca²⁺ inhibition is consistent with studies in SR vesicle preparations (38). The low affinity Ca²⁺ dependent inactivation here is evident at Ca²⁺ levels higher than ~500 µM. There are estimates that local Ca²⁺ levels during a Ca²⁺ spark may reach the 1 mM range (41). Thus, low affinity Ca²⁺ dependent inactivation may be a potential negative control mechanism that contributes to termination of the SR Ca²⁺ release process.

8.3. Adaptation

Until 1993, single RyR channels studies focused on defining RyR behavior under steady state conditions. Using laser flash photolysis of caged Ca²⁺, Gyorke and Fill (42) were the first to apply fast trigger Ca²⁺ signals to single RyR channels in artificial planar bilayers. They reported that single RyR2 channels activated rapidly reaching open probabilities well above that predicted in steady state studies. Channel activity then slowly decayed. A second fast trigger Ca²⁺ signal reactivated the apparently “inactivated” channels. These data suggested that a...
The Inositol Trisphosphate Receptors

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<th>Type-1</th>
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Figure 3. Cartoon illustrating the 3 isoforms of the IP_3R channel. Most cells contain multiple types of IP_3R. Expression levels of any one particular isoform varies dramatically from tissue to tissue. The type-1 receptor is found in high density in the cerebellum. This tissue has served as a primary source from which a particular channel isoform has been isolated for study. The IP_3R channels are all activated by IP_3 and calcium.

The adaptation phenomenon was controversial. It is observed only when the RyR2 channel is activated by a photolytically generated free Ca^{2+} waveform (42, 43, 44, 45, 46). This waveform in unique in that it has a very fast (< 1 ms) Ca^{2+} overshoot at its leading edge (47, 48, 49). Lamb et al. (50) proposed that the Ca^{2+} overshoot complicates data interpretation. They suggested that the adaptation phenomenon may simply be generated by slow Ca^{2+} deactivation following the overshoot. This possibility has been experimentally evaluated. So far, there is no published experimental evidence showing slow Ca^{2+} deactivation. Further, Ca^{2+} deactivation of individual RyR channels is far to fast for this to be true (45, 51, 52). Thus, it is not likely that the adaptation phenomenon is generated by slow Ca^{2+} deactivation. However, there is growing evidence that adaptation may be generated by a Ca- and time-dependent, transient shift in the modal gating of the RyR2 channel (52). It is clear that additional experimental evidence is required to definitively establish the origin of the adaptation phenomenon.

8.4. Activation Dependent or “Fateful” Inactivation

Pizarro et al. (11) reported that the Ca^{2+} release process in frog skeletal muscle was inhibited by a previous Ca^{2+} release. It appeared that previously activated RyR channels were not always available. They argued that RyR inactivation is strictly and “fatefully” linked to their activation (i.e. use-dependent). Again, additional experimental evidence is required to definitively establish the origin of this phenomenon.

8.5. Allosteric or Coupled RyR Gating

Stern et al. (53) reviewed published single RyR gating schemes and concluded that none of them satisfactorily accounted for local control of SR Ca^{2+} release. The lack of strong negative feedback and relatively low cooperativity in the RyR activation process were notable problems. Interestingly, Stern et al. (53) proposed that inter-RyR allosteric interactions could theoretically overcome these problems. Recently, it was suggested that neighboring RyR channels may be physically and functionally linked by the FK-506 binding protein (54). Is this evidence of the needed allosteric interactions? In the absence of more experimental evidence, there is no answer to this question. Intuitively, the thermodynamic considerations associated with microsecond functional synchrony of multiple large macromolecules like RyRs make it unlikely (53, 55). Nevertheless, coupled RyR gating is an interesting and provocative hypothesis that simply requires further verification.

9. RyR: MODAL GATING

Modal gating of single RyR channels has been reported (56, 57, 58). The RyR channel appears to open in discrete bursts. These bursts are temporally clustered into modes (high and low-Po modes). The channel also appears to occasionally move into a silent or “inactivated” mode (at high Ca^{2+} levels). At any set Ca^{2+} concentration, there appears to be a dynamic equilibrium between these different gating modes. Sudden Ca^{2+} concentration shifts upset this equilibrium forcing the system to re-equilibrate. This re-equilibration takes time and during this time channel activity will vary. Ca^{2+} dependent modal gating is not unique to the RyR channel. A similar process appears to govern the Ca^{2+} dependent gating of dihydropyridine receptor (DHPR) Ca^{2+} channels (59).

The modal gating of single RyR channels is interesting because it may account for certain aspects of RyR channel Ca^{2+} regulation. A model of RyR modal gating was first presented by Zahradníková and Zahradník (56). Theoretical simulations illustrated that modal gating can generate many of the known steady-state and non-stationary features of single RyR channel behavior. It may very well be that phenomena like steady-state Ca^{2+} dependence, adaptation and low affinity Ca^{2+} inactivation are three different manifestations of a common underlying mechanism (i.e. modal RyR gating).

10. IP_3R: SINGLE CHANNEL PROPERTIES

There are 3 homologous IP_3R proteins (sharing 60-70% homology) that are encoded by 3 different genes (Figure 3). These large proteins are highly conserved among different species but are differentially expressed in various tissues in any one species (14, 15). They tetramerize to form Ca^{2+} release channels that are activated by IP_3 and blocked by heparin. Each IP_3R protein is composed of three domains: a N-terminal IP_3 binding domain, a C-terminal channel domain, and a large interceding regulatory domain. The regulatory domain contains consensus phosphorylation, ATP-binding and Ca-binding sites (1). The IP_3R protein may also have sites that may interact with certain accessory proteins (e.g. calmodulin & FKBP). The type-1 IP_3R is found in high density in the mammalian cerebellum (12). The type-2 IP_3R...
Intracellular Ca\(^{2+}\) Release Channels

is found in high density in mammalian spinal cord, glial cells and cardiomyocytes (60). The type-3 IP\(_R\) is concentrated in the kidney, diaphragm, gastrointestinal tract and pancreatic islets (12). However, nearly all tissues will contain multiple IP\(_R\) isoforms.

The IP\(_R\) channels are regulated by several cytoplasmic ligands (12). The cytoplasmic Ca\(^{2+}\) sensitivity of the IP\(_R\) channel is isoform-specific (61). The Ca\(^{2+}\) sensitivity of the type-2 and type-3 receptors appears to be much broader than that of the type-1 receptor. Type-1 IP\(_R\) channel activity occurs over a relatively narrow range (~0.1 to ~1 \(\mu\)M) of free Ca\(^{2+}\) concentrations. Type-2 and type-3 IP\(_R\) channels can be active at much higher Ca\(^{2+}\) levels. The Ca\(^{2+}\) sensitivity of the IP\(_R\) channels is modulated by cytoplasmic IP\(_3\) levels (61). All 3 IP\(_R\) proteins bind IP\(_3\) but with different affinities (1, 12). The type-2 receptor has the highest affinity. The type-1 receptor has the lowest affinity. The IP\(_3\) binding affinities of the IP\(_R\) proteins are directly reflected in the EC\(_{50}\)’s of IP\(_R\) channel function (60). The activity of the IP\(_R\) channels is also governed by certain nucleotides (e.g. ATP, GTP, AMP). Among these, it appears that ATP has the highest efficacy. Low nucleotide concentrations seem to activate the channel while high nucleotide levels inhibit it (1, 61). This nucleotide action is dependent on cytoplasmic IP\(_3\) levels. The inhibitory action of ATP may in fact involve a competition between ATP and IP\(_3\) at a common site.

The permeation properties of the 3 different IP\(_R\) channel isoforms are similar (60). All 3 IP\(_R\)s form poorly selective Ca\(^{2+}\) channels (\(P_{Ca}/P_{K}\) ratio ~5) that have a unit Ca\(^{2+}\) conductance many times larger than that of voltage-dependent Ca\(^{2+}\) channels found in the surface membrane. The permeation properties of the IP\(_R\) channels are similar to those of the RyR channels. This likely permits both of these channels to accomplish their physiological role (i.e. mediate large local Ca\(^{2+}\) release events).

11. IP\(_R\): NEGATIVE CONTROL MECHANISMS

Like the RyR channels, the IP\(_R\) channels are Ca\(^{2+}\)-activated Ca\(^{2+}\) release channels. Like the RyR case, IP\(_R\)-mediated Ca\(^{2+}\) released should be regenerative. The Ca\(^{2+}\) released by an IP\(_3\) bound channel should feedback and activate the same channel further. In cells, IP\(_3\) mediated Ca\(^{2+}\) signaling is well controlled. Thus, there must be negative feedback mechanisms controlling these channels. Many of the negative feedback mechanisms discussed for the RyR channel may also apply to the IP\(_R\) channel. One clear difference is that IP\(_3\) generation and degradation ultimately define IP\(_R\) channel function. The IP\(_R\) channels turn-on when local IP\(_3\) levels rise and turn-off when they fall. The situation, however, is not so simple. The activity of an IP\(_R\) channel depends on the concerted actions of several ligands (e.g. IP\(_3\), Ca\(^{2+}\), ATP, calmodulin etc.). Consequently, the interaction of IP\(_R\) regulators is a current focus of investigation. Certain specific regulatory interactions are discussed below.

11.1. Calmodulin-IP\(_R\) Association

The type-1 IP\(_R\) channel has a bell-shaped Ca\(^{2+}\) sensitivity. Low Ca\(^{2+}\) levels activate while high Ca\(^{2+}\) concentrations (e.g. 1 \(\mu\)M) inhibit. This implies that Ca\(^{2+}\) release mediated by this channel may be self-limiting (released Ca\(^{2+}\) will feedback and turn-off the channel). In essence, this is analogous to the Ca\(^{2+}\) dependent inactivation proposed for single RyR channels (see above). Calcium dependent inactivation of the type-2 and type-3 IP\(_R\) channel occurs at substantially higher Ca\(^{2+}\) concentrations. The Nature of IP\(_R\) Ca\(^{2+}\) inactivation is controversial. Some IP\(_R\) channel studies argue that Ca\(^{2+}\) inactivation is absent when the channels are “purified” biochemically. One possibility is that “purification” removes calmodulin which may be a critical cofactor for Ca\(^{2+}\) inactivation. The implication here is that Ca\(^{2+}\) inactivation may not be the result of Ca\(^{2+}\) binding directly to the IP\(_R\) protein. It may be mediated by a Ca\(^{2+}\) dependent calmodulin-IP\(_R\) interaction. In other studies (60), however, the bell-shaped Ca\(^{2+}\) sensitivity was present in both purified and non-purified receptor IP\(_R\) channel. It is clear that the role of calmodulin in IP\(_R\) regulation is still poorly understood and requires more study.

11.2. Ca\(^{2+}\)-IP\(_3\) Interaction

The interaction of cytosolic Ca\(^{2+}\) and IP\(_3\) in the regulation of single IP\(_R\) channels is controversial. There is agreement that the type-1 receptor has a bell-shaped Ca\(^{2+}\) sensitivity at low IP\(_3\) concentrations and that this bell-shape dependency is lost at high IP\(_3\) concentrations. There is some disagreement as to how much IP\(_3\) is required to abolish the bell-shaped Ca\(^{2+}\) sensitivity of the channel (62, 63). There is also disagreement concerning the Nature of the Ca\(^{2+}\)-IP\(_3\) interaction involved. One group suggests that two IP\(_3\) binding sites with different affinities regulate the channel (62). Occupancy of the low affinity site is what alters the Ca\(^{2+}\) sensitivity of the channel. Another group suggests that a single high affinity IP\(_3\) binding site regulates the channel (63). Occupancy of this site “tunes” the Ca\(^{2+}\) sensitivity of the channel. Low occupancy results in a bell-shaped Ca\(^{2+}\) sensitivity. High occupancy abolishes the bell-shaped Ca\(^{2+}\) sensitivity. In any event, it is likely that Ca\(^{2+}\) and IP\(_3\) interact in interesting ways to regulate these channels.

12. RyR-IP\(_R\) CHANNEL CROSS TALK

Most cells contain both RyR and IP\(_R\) channels. In fact, most cells contain multiple types of each. The point is that cells can contain many types of intracellular Ca\(^{2+}\) release channels. The existence of multiple functionally distinct Ca\(^{2+}\) release channels implies that each may mediate different physiological processes in the cell. Interestingly, cytosolic Ca\(^{2+}\) is a modulator of all of these channels. This presents the possibility of inter-channel Ca\(^{2+}\) cross talk.

The situation in cardiac muscle is a good case in point (Figure 4). Moschella and Marks (64) suggested that the type-1 IP\(_R\) was the predominant IP\(_R\) in heart muscle cells. The single channel properties of the type-1 IP\(_R\) are well defined (Figure 4, solid line). At a constant activating IP\(_3\) level, channel activity is a bell-shaped function of cytosolic Ca\(^{2+}\) concentration. The channel is open at 200 nM Ca\(^{2+}\) and closed at Ca\(^{2+}\) levels greater than 2.5 \(\mu\)M. In heart
Intracellular Ca\(^{2+}\) Release Channels

![Graph showing Ca\(^{2+}\) changes in heart over time](image)

**Figure 4.** The Ca\(^{2+}\) sensitivity of single type-1 IP\(_{3}\)R channels in planar lipid bilayers are represented as solid lines. These plots are based on the work of Bezprozvanny et al. (1991). Dotted line represents the theoretical free Ca\(^{2+}\) changes that occur during the cardiac cycle.

Over the last decade, the roles of the RyR and IP\(_{3}\)R Ca\(^{2+}\) release channels in intracellular Ca\(^{2+}\) signaling have begun to be defined. Many basic properties of the different RyR and IP\(_{3}\)R Ca\(^{2+}\) release channels have been described. Additionally, small local elementary Ca\(^{2+}\) release events that are generated by these channels have been identified and the hierarchical *Nature* of Ca\(^{2+}\) signaling revealed. It appears that the Ca\(^{2+}\) mobilized by individual release channels generates local elemental Ca\(^{2+}\) release events. These elemental release events combine to produce the global Ca\(^{2+}\) signals that govern a host of cellular phenomena. It is apparent that the specific subcellular localization of particular types of intracellular Ca\(^{2+}\) release channels is an important factor in generating the complex spatiotemporal *Nature* of intracellular Ca\(^{2+}\) signaling. Although many key elements and general concepts are known, it is clear that there are many more that are either poorly understood or entirely unknown.

Over the next decade, I believe it will become increasingly evident that individual intracellular Ca\(^{2+}\) release channels are just one component in complex multi-protein Ca\(^{2+}\) signaling assemblies. These assemblies will likely have all the components (i.e. surface receptors, enzymes, regulatory proteins, release channels, structural elements, etc.) needed carry out specific and local Ca\(^{2+}\) signaling tasks. Thus, research emphasis will gradually shift away from defining the function of the individual Ca\(^{2+}\) signaling elements to defining the concerted operation of elements in local multi-protein Ca\(^{2+}\) signaling assemblies. In this context, defining mechanism will become even more important and challenging.

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