

InsP₃/Ryanodine Receptors

Key References

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Overview

The low calcium concentration that characterizes the cytosol of all eukaryotic cells is maintained by active calcium transport into the extracellular space and the intracellular stores, largely the endoplasmic and sarcoplasmic reticulum. The resulting steep electrochemical gradients for calcium allow opening of calcium channels in these membranes to generate rapid increases in cytosolic calcium concentration in response to appropriate extracellular stimuli.

Two related families of calcium channels, inositol 1,4,5-trisphosphate (InsP₃) and ryanodine receptors, are largely responsible for mediating calcium release from intracellular stores. Additional, less extensively characterized channels are likely to mediate the calcium mobilization evoked by such intracellular signals as β-NAADP (nicotinic acid adenine dinucleotide phosphate), sphingosine-1-phosphate, and possibly other signals evoked by receptors for which the links with calcium mobilization have not been defined. Most of these intracellular calcium channels are regulated by several intracellular messengers; there are also important, though still rather poorly understood, interactions between the different calcium channels. These features undoubtedly contribute to the complexity of the calcium signals observed in intact cells. Because calcium diffuses only slowly in cytoplasm, the free calcium concentration near open calcium channels can far exceed that of the rest of the cytoplasm. Such spatially restricted increases in calcium concentration allow local communication between calcium channels and intracellular targets; mitochondria, for example. These "calcium synapses" increase enormously the versatility of calcium as an intracellular messenger.

Ryanodine and InsP₃ receptors share both structural and functional characteristics. Both receptor families are made up of subunits that are among the largest of all known proteins and for each the functional channel comprises a tetramer in which six membrane-spanning helices, near the carboxy terminus of each subunit, form a calcium channel. Whereas InsP₃ receptors exist as both homotetramers and heterotetramers, only homotetrameric ryanodine receptors have so far been reported. The enormous cytosolic domains of these receptors provide sites through which diverse intracellular signals modulate their behavior. Such signals include proteins that bind directly to the receptors (e.g., calmodulin, FKBP12), protein kinases that phosphorylate them, and many small second messengers. The N-terminal region of these receptors may also contact the calcium channels in the plasma membrane that mediate capacitative calcium entry. Such "conformational coupling" also occurs in skeletal muscle where L-type calcium channels are coupled to activation of type 1 ryanodine receptors. Perhaps the most important of the small messengers that regulate intracellular calcium channels is calcium itself, which regulates both families of receptors, and thereby allows each to mediate propagation of intracellular calcium signals by means of calcium-induced calcium release.

The ability of calcium channels in the plasma membrane to selectively allow the passage of calcium into the cell requires that they very effectively discriminate between calcium and monovalent cations. The need for such discrimination is less acute for the intracellular calcium channels, because calcium pumps concentrate calcium within the intracellular stores,

and monovalent cation channels within the membrane of the endoplasmic reticulum ensure that there is no steep gradient for monovalent cations. In effect, responsibility for the selective passage of calcium through intracellular calcium channels has been delegated to the calcium pumps of the stores. It is not surprising therefore that both InsP₃ and ryanodine receptors are far less calcium-selective than are plasma membrane calcium channels.

While intracellular and plasma membrane calcium channels provide access to different sources of calcium, there are important interactions between them. Calcium entering the cell across the plasma membrane can directly trigger release of calcium from intracellular stores by activating ryanodine receptors; the best example is provided by cardiac myocytes. Conversely, depletion of intracellular calcium stores in most non-excitabile cells generates an unknown signal that leads to activation of "capacitative" calcium entry across the plasma membrane. The important point is that there are dynamic interactions between intracellular and extracellular sources of calcium and the calcium channels that provide regulated access to them.

InsP₃/Ryanodine Receptors

	InsP ₃ receptors		Ryanodine receptors	
STRUCTURES	Tetramer (4 x ~313 kDa)		Tetramer (4 x ~560 kDa)	
SUBTYPES ^e	InsP ₃ R1-3 ^{a,f}	RyR1 ^f	RyR2 ^f	RyR3
ALTERNATE NAMES	—	skeletal, (α)	cardiac	(β)
STRUCTURAL INFORMATION	InsP ₃ R1: 2695 aa (human) InsP ₃ R2: 2701 aa (human) InsP ₃ R3: 2671 aa (human)	—	—	—
DISTRIBUTION	Most cells express several isoforms	Skeletal muscle and elsewhere	Heart, brain, smooth muscle and elsewhere	Brain, smooth muscle and elsewhere
CALCIUM CONDUCTANCE	~50 pS mean open time ~4 ms	100-150 pS mean open time ~20 ms	100-150 pS mean open time ~20 ms	100-150 pS mean open time ~20 ms
PERMEATION	Ba ²⁺ > Sr ²⁺ > Ca ²⁺ > Mg ²⁺ > Mn ²⁺ P _{Ba} /P _K = 6.3	Ba ²⁺ > Sr ²⁺ > Ca ²⁺ > Mg ²⁺ P _{Ba} /P _K = 4-8	Ba ²⁺ > Sr ²⁺ > Ca ²⁺ > Mg ²⁺ P _{Ba} /P _K = 4-8	Ba ²⁺ > Sr ²⁺ > Ca ²⁺ > Mg ²⁺ P _{Ba} /P _K = 4-8
PHARMACOLOGY AGONISTS	(Ins(1,4,5)P ₃ nM) (I 7012 , I 9766) and related compounds (e.g., Ins(2,4,5)P ₃ (I 0146), Ins(4,5)P ₂ (I 3264), Ins(1,4,5)P ₃) Adenophostins (A 5094)	Ryanodine (nM-μM) (R 6017) Caffeine (mM) (C 0750) Heparin (μg/ml) (H 3393 (p), H 0777 (b))	Ryanodine (nM-μM) (R 6017) Caffeine (mM) (C 0750) Heparin (μg/ml) (H 3393 (p), H 0777 (b))	Ryanodine (nM-μM) (R 6017) Caffeine (mM) (C 0750) Heparin (μg/ml) (H 3393 (p), H 0777 (b))
PHARMACOLOGY ANTAGONISTS	Heparin (μg/ml) (H 3393 (p), H 0777 (b)) Decavanadate (μM) (Caffeine, mM) (C 0750) Xestospongins (X 2628) (<μM) ^h 2-Aminoethoxydiphenyl borate (>μM) ^h	Ruthenium red (μM) (R 2751) Ryanodine (>10 μM) (R 6017) Imperatoxin (nM) (I-148)	Ruthenium red (μM) (R 2751) Ryanodine (>10 μM) (R 6017) 8-Amino cyclic ADP ribose (A 6705) ^d	Ruthenium red (μM) (R 2751) Ryanodine (>10 μM) (R 6017)
PHYSIOLOGY STIMULATION	Cytosolic Ca ²⁺ (<μM) ATP (μM) (A 2383)	Cytosolic Ca ²⁺ (μM) ATP (mM) (A 2383) Calmodulin (P 2277) ^c Cyclic ADP-ribose (C 7344) ^b	Cytosolic Ca ²⁺ (μM) ATP (mM) (A 2383) Calmodulin (P 2277) ^c Cyclic ADP-ribose (C 7344) ^b	Cytosolic Ca ²⁺ (μM) ATP (mM) (A 2383) Calmodulin (P 2277) ^c Cyclic ADP-ribose (C 7344) ^b
PHYSIOLOGY INHIBITION	Cytosolic Ca ²⁺ (>μM) Calmodulin (InsP ₃ R1) (P 2277 , P 1431) ^g	Cytosolic Ca ²⁺ (mM) Calmodulin (μM) (P 2277 , P 1431) ^c	Cytosolic Ca ²⁺ (mM) Calmodulin (μM) (P 2277 , P 1431) ^c	Cytosolic Ca ²⁺ (mM) Calmodulin (μM) (P 2277 , P 1431) ^c
RADIOLIGANDS OF CHOICE	[³ H]-Inositol 1,4,5-trisphosphate	[³ H]-Ryanodine [¹²⁵ I]-Calmodulin	[³ H]-Ryanodine [³ H]-Cyclic ADP-ribose [¹²⁵ I]-Calmodulin	[³ H]-Ryanodine [¹²⁵ I]-Calmodulin

ABBREVIATIONS

Ins(1,4,5)P₃: Inositol 1,4,5-trisphosphate

Ins(2,4,5)P₃: Inositol 2,4,5-trisphosphate

Ins(1,4,5)P₃S: Inositol 1,4,5-trisphosphorothioate

Ins(4,5)P₂: Inositol 4,5-bisphosphate

2-APB: 2-Aminoethoxydiphenyl borate

b: bovine

p: porcine

Additional messengers capable of mobilizing intracellular calcium stores, which include sphingosine-1-phosphate and β-NAADP, are omitted from the chart because the calcium channels they regulate have not yet been identified.

a Roman and Arabic numerals are presently used interchangeably to distinguish between InsP₃ receptor subtypes, the latter are preferable. Although partial sequences for putative types 4 and 5 InsP₃ receptors were reported, they are now thought to represent species differences between the type 2 InsP₃ receptor subtype.

b The site to which cyclic ADP-ribose binds has yet to be identified.

c Calmodulin, both with and without bound calcium, can bind to multiple sites to cause either inhibition or stimulation of ryanodine receptor function.

d 8-Amino cyclic ADP ribose is a competitive antagonist of the cyclic ADP-ribose-binding site. 8-Bromo-cyclic-ADP ribose is an antagonist with lower affinity, but it is more membrane permeant.

e While InsP₃ receptors are known to exist as both heterotetramers and homotetramers, only homotetrameric assemblies of ryanodine receptor subunits have so far been demonstrated.

f InsP₃R1, InsP₃R2, RyR1 and RyR2 are also expressed as alternative splice variants.

g Calmodulin-mediated inhibition of InsP₃ binding to InsP₃R1 is calcium-independent.

h The specificity of these antagonists, neither of which acts at the InsP₃-binding site, has been challenged by work suggesting that among many additional effects they also inhibit the endoplasmic reticulum calcium pump. In addition, 2-APB appears to directly block capacitative calcium entry without affecting other calcium entry pathways.

FOOTNOTES