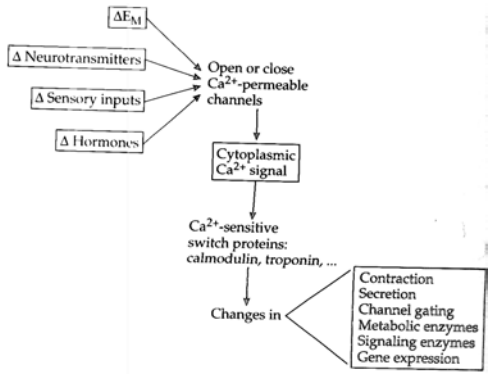


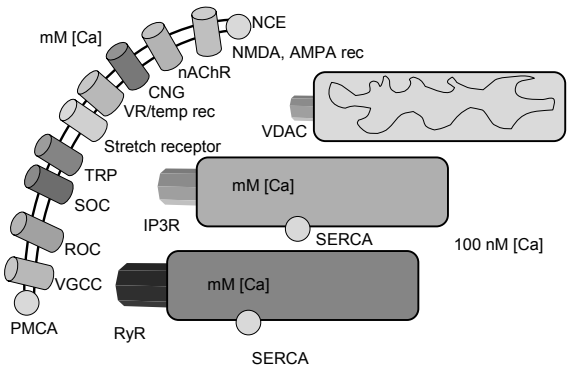
Why Calcium?

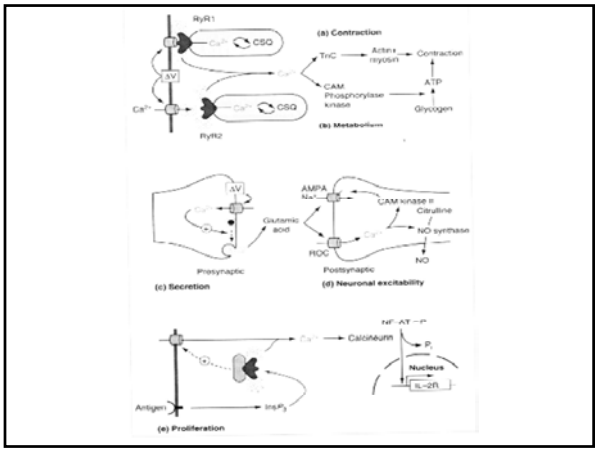
Double positive charge provides increased affinity for negatively charged proteins but lower affinity than larger divalent cations such as Cu, Zn, or Mn. The coordination chemistry of Ca is higher and more flexible than for Mg.

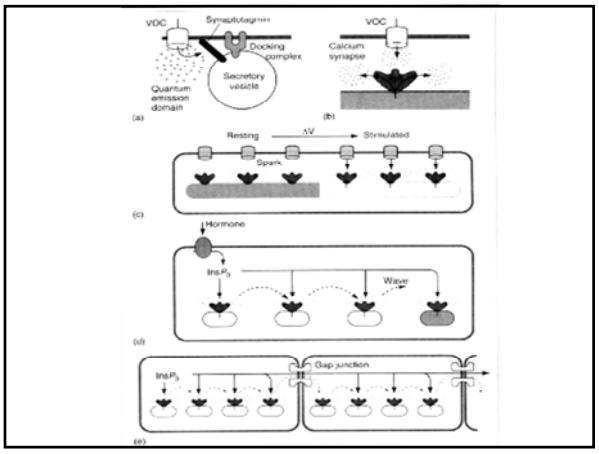
The fact that Ca complexes with inorganic compounds and to proteins suggests that the maintenance of low [Ca] intracellularly would require less energy than for other cations. The maintenance of a large transmembrane gradient is critical for a second messanger ion.

This large transmembrane gradient provides the signal-to-noise ratio required for efficient signal transduction.

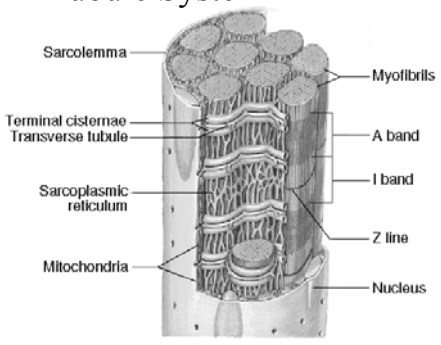
Resting intracellular [Ca] is ~100 nM versus mM extracellular [Ca] or a concentration gradient of ~10,000.

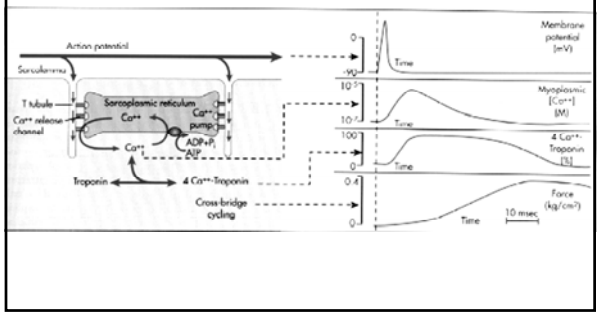






Sarcoplasmic Reticulum (SR) / T Tubule System





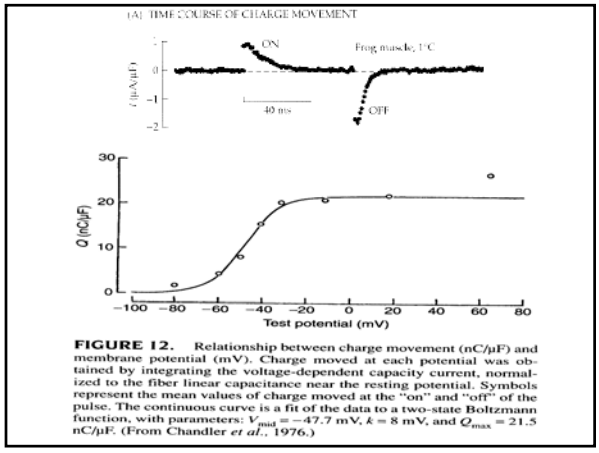
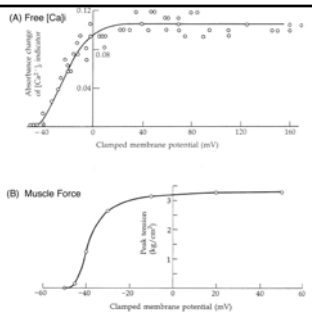


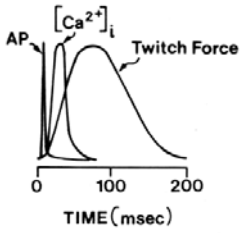
FIGURE 12. Relationship between charge movement (nC/μF) and membrane potential (mV). Charge moved at each potential was obtained by integrating the voltage-dependent capacity current, normalized to the fiber linear capacitance near the resting potential. Symbols represent the mean values of charge moved at the "on" and "off" of the pulse. The continuous curve is a fit of the data to a two-state Boltzmann function, with parameters: $V_{mid} = -47.7$ mV, $k = 8$ mV, and $Q_{max} = 21.5$ nC/μF. (From Chandler *et al.*, 1976.)



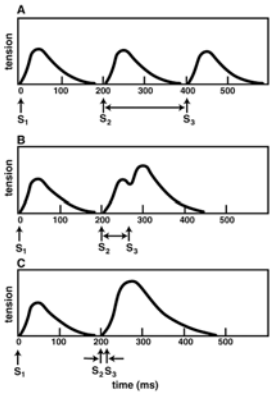
14 VOLTAGE-DEPENDENT Ca RELEASE IN MUSCLE

Two manifestations of the excitation-contraction coupling mechanism in frog skeletal muscle. (A) Optical measurement of intracellular free Ca^{2+} during applied membrane depolarizations. The membrane is depolarized in a voltage-clamp step while $[Ca^{2+}]_i$ is monitored from the absorbance changes of arsenazo III injected into a single muscle fiber. The applied depolarizations of the muscle surface membrane release Ca^{2+} from the intracellular sarcoplasmic reticulum. Note that unlike Ca^{2+} entry from the outside, this Ca^{2+} release does not diminish as E_m approaches E_{Ca}. [From Miledi et al., 1977.] (B) Peak tension elicited in a single frog muscle fiber depolarized for 100 ms by a two-microelectrode voltage clamp to various potentials. $T = 14°C$. [From Caputo et al., 1984.]

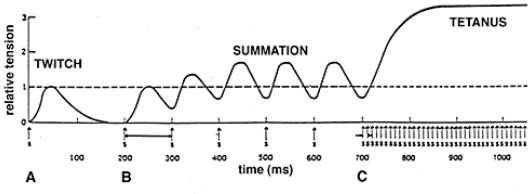
Twitch



Summation



Tetanus



Role of Ca++ in contraction

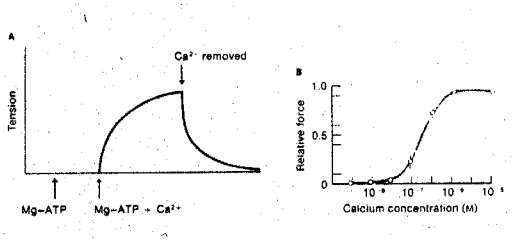


TABLE I Duration of Key Steps in the Activation of a Fast-Twitch Skeletal Muscle

EC coupling steps	Duration[a]
Action potential propagation along sarcolemma	5–10 ms
Action potential propagation to center of fiber along T-tubules	~ 0.7 ms
Signal transduction at triad junction, from T-tubule depolarization to activation of RyR on SR	~ 0.5 ms
Peak rate of Ca^{2+} release to peak Ca^{2+} binding to TnC (start of tension)	2–3 ms
Peak myoplasmic Ca^{2+} change to peak tension	15–25 ms

[a]Durations were calculated for a hypothetical frog fiber of 50 μm diameter and 5 cm length, having a central end plate. Literature values for conduction velocity and duration of intermediate steps (Gonzales-Serratos, 1971; Vergara and Delay, 1986; Jong *et al*., 1996) were adjusted to 18 °C.

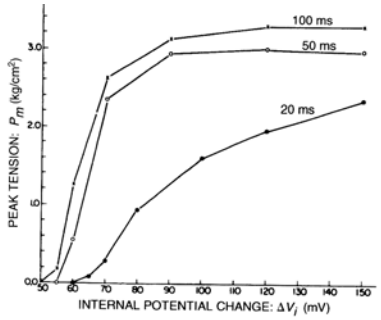
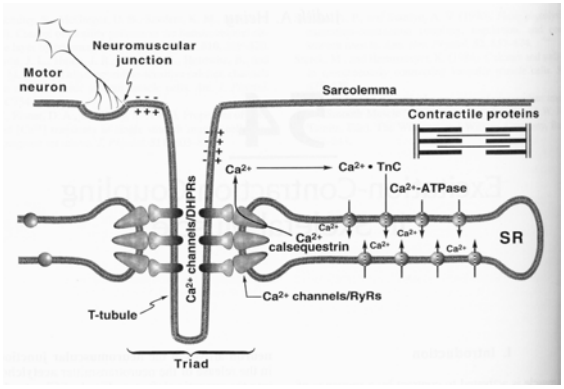
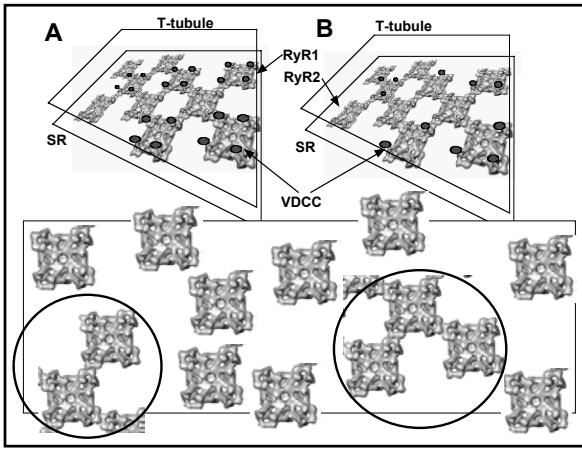
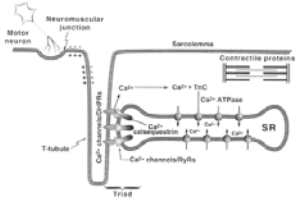


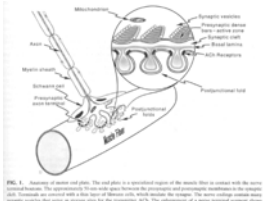
FIGURE 11. Relationship between peak tension and membrane potential in the absence of an action potential. The fibers were depolarized by holding the membrane potential constant for 50 ms with a voltage-clamp. Tension was measured simultaneously with a transducer attached to one tendon. (Reproduced from *The Journal of General Physiology*, 1984, **84**, 133–154, by copyright permission of The Rockefeller University Press.)





vertebrate skeletal muscle contraction



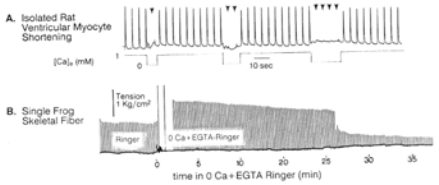


- Resting $[Ca]_i$ ~0.1 μM
- Neuromuscular transmission
- Action potential propagation along sarcolemma and into T-tubules
- Signal transduction from voltage-dependent Ca^{2+} channels to Ca^{2+} release channels at triad junctions
- Ca^{2+} release from SR
- Cytosolic $[Ca]_i$ reaches 1–10 μM
- Diffusion and binding of Ca^{2+} ions to TnC
- Removal of troponin inhibition of the contractile proteins
- Contractile proteins shorten to generate force
- $[Ca]_i$ returns to resting levels via:

  Reuptake of Ca^{2+} into SR by Ca^{2+}-ATPase

  Inactivation of Ca^{2+} release channels

  Binding of Ca^{2+} to calsequestrin



Ca_o-free solution abolishes contractions immediately (< 1 sec) in an isolated cardiac myocyte (**A.**), but does not decrease contraction in a single skeletal muscle fiber (**B.**) for >25 min. **A.** $[Ca]_o$ was changed by a rapid solution switching device between stimuli. The cell was stimulated at a continuous frequency of 0.2 Hz and arrowheads indicate stimulations in Ca_o-free solution (from Rich *et al.*, 1988, with permission). **B.** A single frog skeletal fiber stimulated at 0.1 Hz except during the switch to a Ca_o-free solution containing 1 mM EGTA. The eventual decline in force after ~26 min was attributed to gradual membrane depolarization (from Armstrong *et al.*, 1972, with permission).

Excitation-contraction coupling

Ryanodine receptors

Properties of Calcium Release Channels

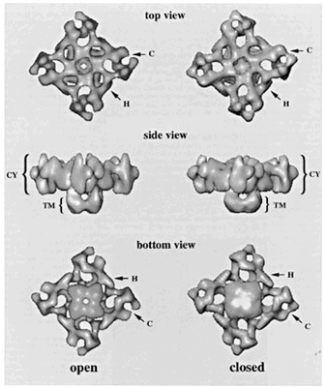
Ryanodine Receptors

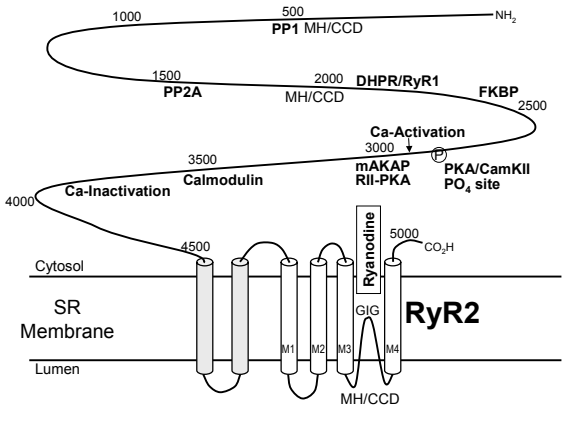
	RyR1	RyR2	RyR3
Size (amino acids) of monomer	5,037	4,970	4,870
Size (daltons) of monomer	~565, 000	~560, 000	~560, 000
Sedimentation coefficient of tetramer	30 S	30 S	30 S
Stoichiometry of FKBP/RyR	4	4	4
Single channel conductance in Ca 50mM	~120 pS	~120 pS	~100 pS
Single channel conductance in Cs 250mM	~540 pS	~540 pS	?
Endogenous modulators			
μM Ca activates	yes	yes	yes
mM Ca inhibits	yes	yes	yes
mM Mg inhibits	yes	yes	yes
kinases	yes	yes	?
phosphatases	yes	yes	?
DHPR interaction	yes	?	?
calmodulin	yes	yes	yes
adenine nucleotides	yes	yes	yes
MgATP	yes	yes	yes
NO	yes	yes	?

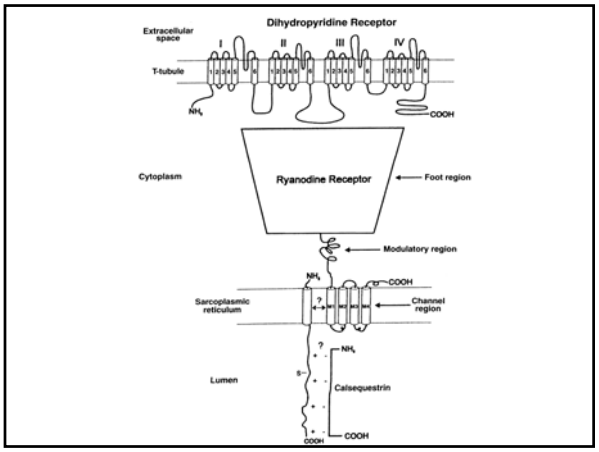
Pharmacology of Ryanodine Receptors

	RyR1	RyR2	RyR3	Site of action
Xanthines (caffeine)	activates	activates	activates	Ca activation sites
Ryanodine /ryanoids	subconductance state	subconductance state	subconductanc e state	carboxy terminus
ruthenium red	inhibits	inhibits	inhibits	Ca binding site(s)/channel pore
Antraquinones (doxorubicin)	activates	activates	activates	?
FK506 & rapamycin	activates	activates	activates	FKBP12/12.6
Purinergic agonists/ antagonists (adenosine)	activates	activates	activates	ATP binding sites
Calmodulin antagonist	inhibits	inhibits	inhibits	calmodulin binding site
Local anesthetics (tetracaine)	inhibits	inhibits	inhibits	?
Dantrolene	inhibits	inhibits	?	?
Phenol derivatives (4-chloro-m-cresol)	activates	activates	?	?
NO generating compounds	Activates/inhibits	Activates/inhibits	?	?

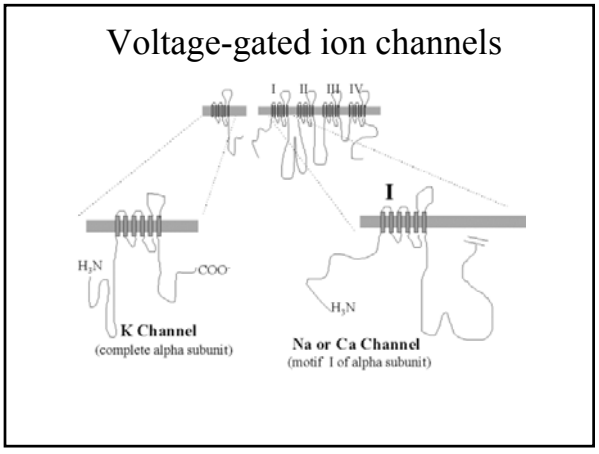
RyR

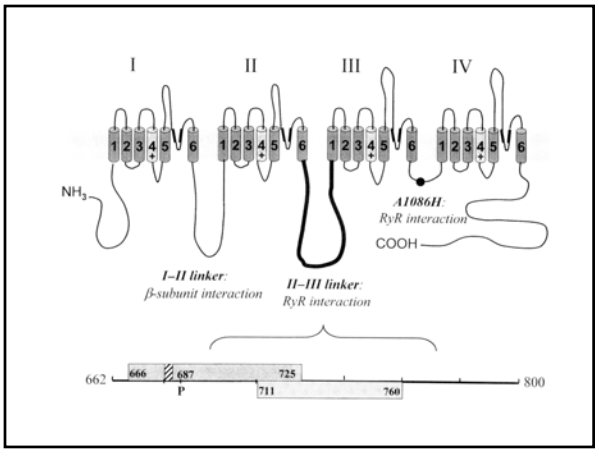


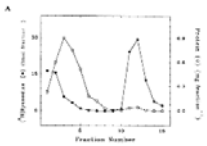


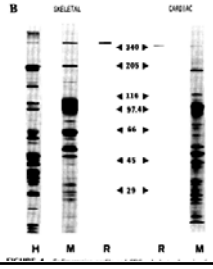


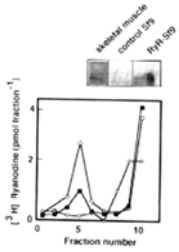
Voltage-gated ion channels

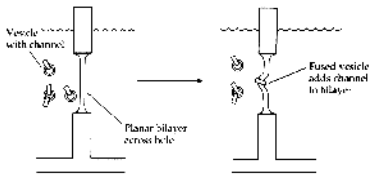


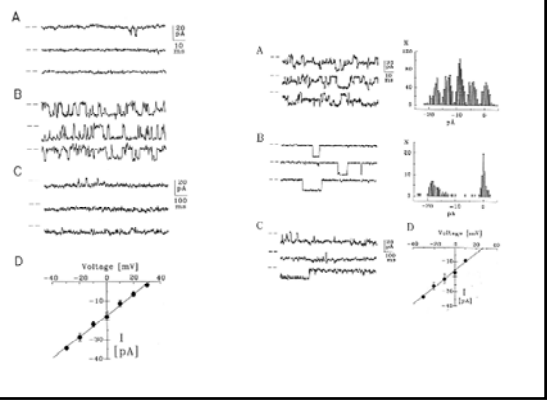


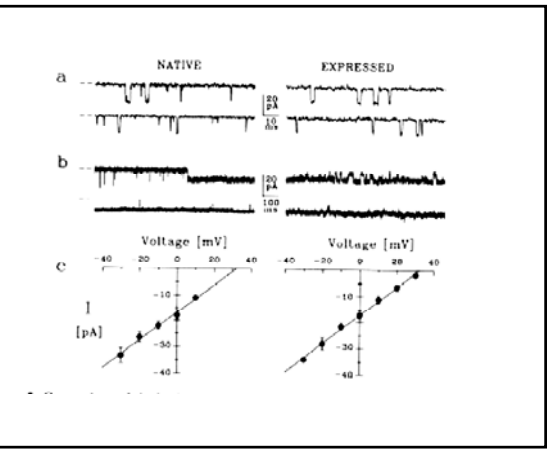


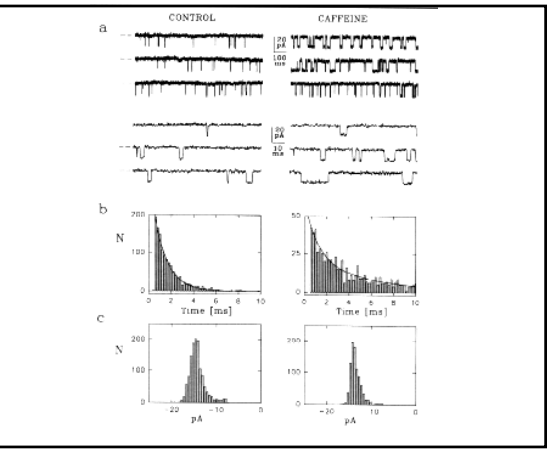

B
SKELETAL
CARDIAC
240
205
116
97.4
66
45
29
H
M
R
R
M

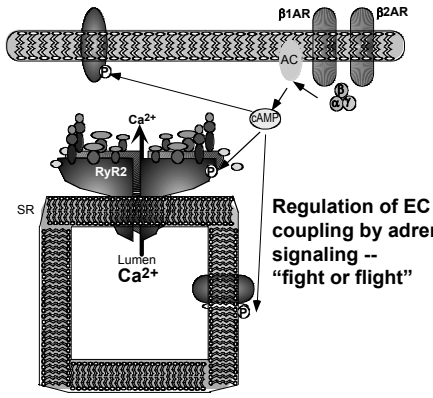

skeletal muscle
control 5I9
Ryr1 5I9
[3H] Ryanodine (pmol fraction-1)
4
2
0
0
5
10
Fraction number


Vesicle with channel
Planar bilayer across hole
Fused vesicle adds channel to bilayer


A
B
C
D
Voltage [mV]
I
[pA]

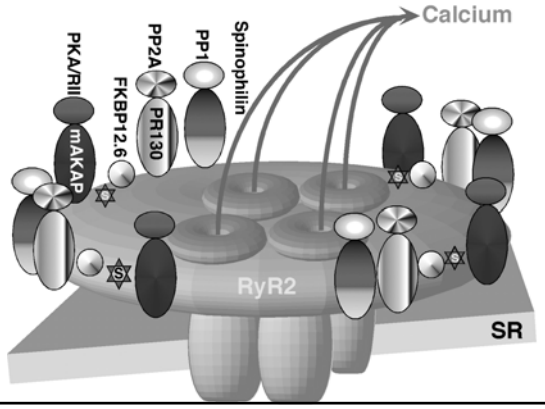

NATIVE
EXPRESSED
a
b
c
Voltage [mV]
Voltage [mV]
I
[pA]


CONTROL
CAFFEINE
a
b
c
N
Time [ms]
Time [ms]
pA
pA

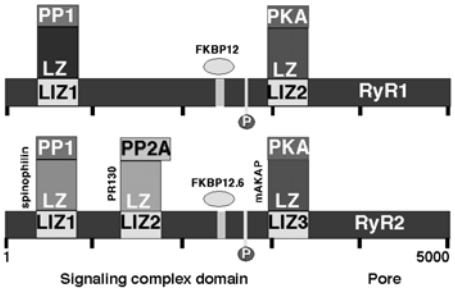


Regulation of EC coupling by adrenergic signaling -- "fight or flight"

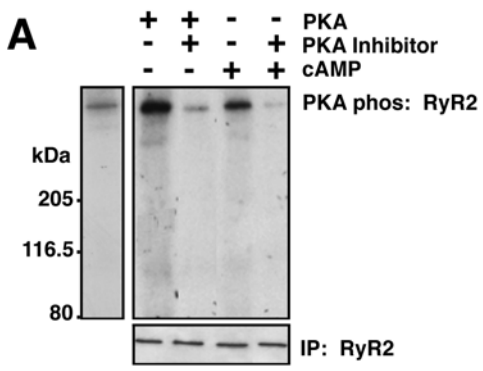
RyR2 macromolecular complex

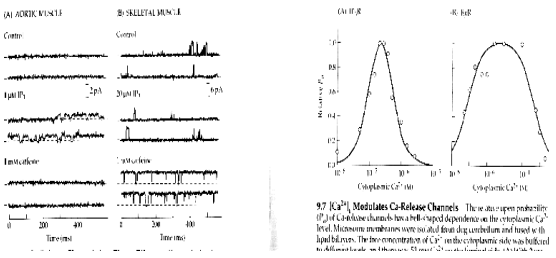


RyR macromolecular complexes are held together by leucine/isoleucine zippers

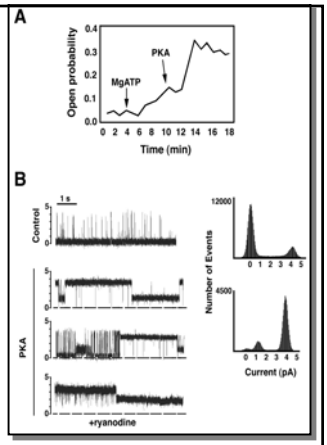


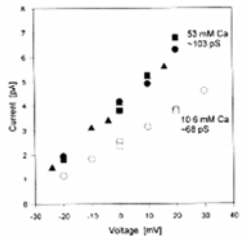
RyR2/calcium release channel phosphorylated by addition of cAMP alone

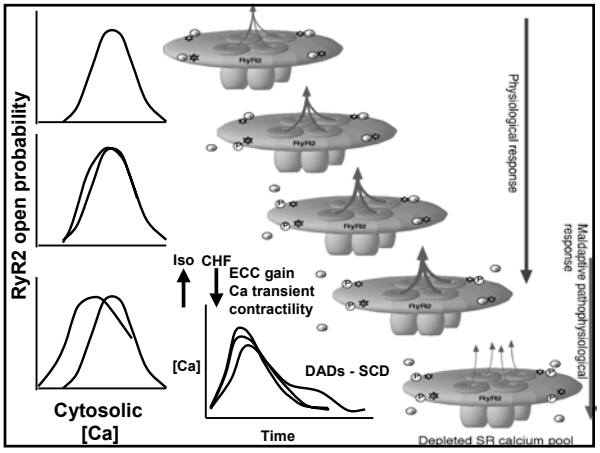


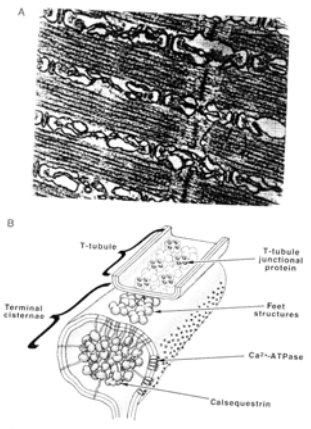


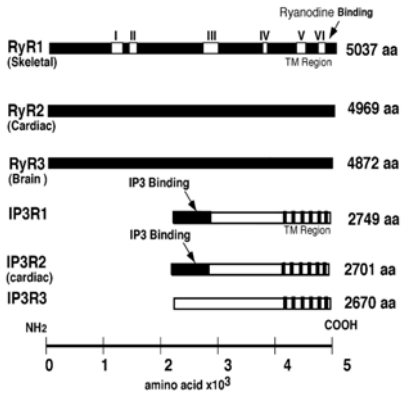
PKA phosphorylation activates RyR2 and induces subconductance states




53 mM Ca
~103 pS
10·6 mM Ca
~68 pS
Current [pA]
Voltage [mV]


RyR2 open probability
Cytosolic [Ca]
Iso
CHF
ECC gain
Ca transient
contractility
[Ca]
Time
DADs - SCD
RyR2
Physiological response
Maladaptive pathophysiological response
Depleted SR calcium pool


A
B
T-tubule
T-tubule junctional protein
Terminal cisternae
Feet structures
Ca2+-ATPase
Calsequestrin



	IP3R	RyR
Skel muscle	+	+++
Smooth m.	+++	+
Neurons	+++	+++
IP3	Activates	None
Ryanodine	None	Locks open/closes
Caffeine (5 mM)	Inhibits	Activates
Ca^{2+}	IP3R1 - biphasic IP3R2/3 - opens	biphasic
RR	None	inhibits
Heparin	inhibits	activates

Excitation-secretion coupling

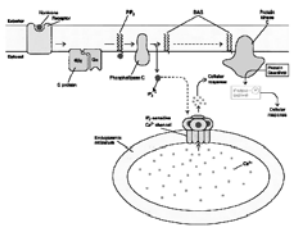
Inositol 1,4,5-trisphosphate receptors

IP3 Receptors

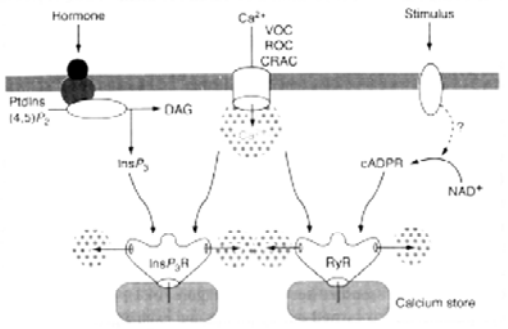
	IP3R1	IP3R2	IP3R3
Size (amino acids) of monomer	2,749	2,691	2,685
Size (daltons) of monomer	313,000	~300,000	~300,000
Stoichiometry of FKBP/RyR	4	4	?
Endogenous modulators			
IP3	activates	activates	activates
nM Ca activates	yes	yes	yes
μM Ca inhibits	yes	yes	no
ATP <2 mM	activates	activates	activates
ATP >2 mM	inhibits	inhibits	inhibits
tyrosine kinases	activates	?	?
phosphatases	?	?	?

Pharmacology of IP3 Receptors

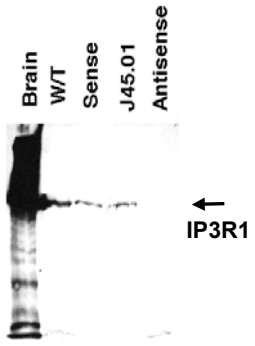
	IP3R1	IP3R2	IP3R3	Site of action
(caffeine)	inhibits	inhibits	inhibits	Ca activation sites
heparin	inhibits	inhibits	inhibits	?
2-APB	inhibits	inhibits	inhibits	?
xestospongin	inhibits	inhibits	inhibits	?

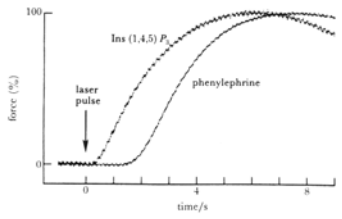


IP3 signaling

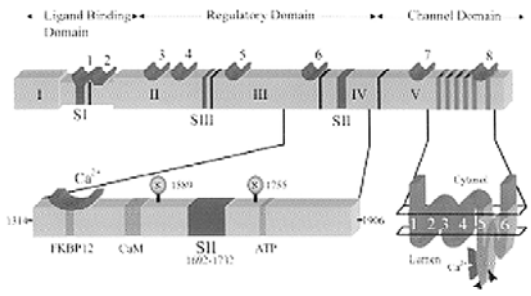


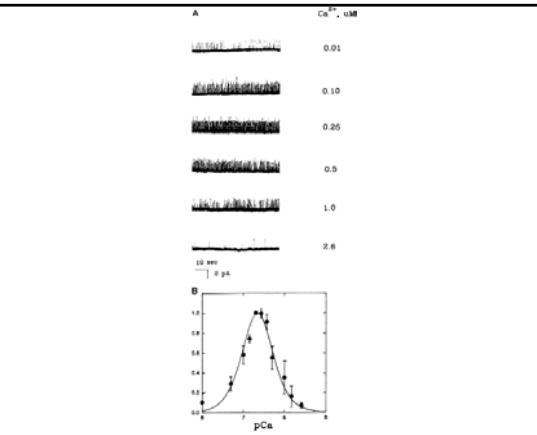
Stable transfectant Jurkat T cells do not express IP3R1



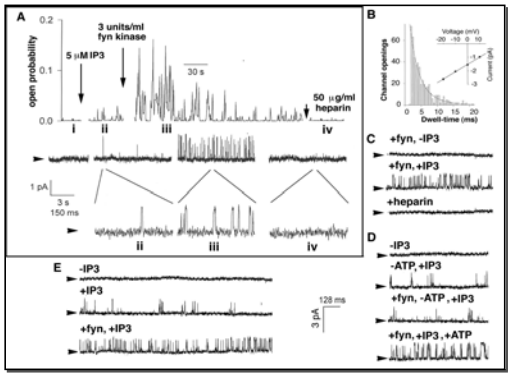


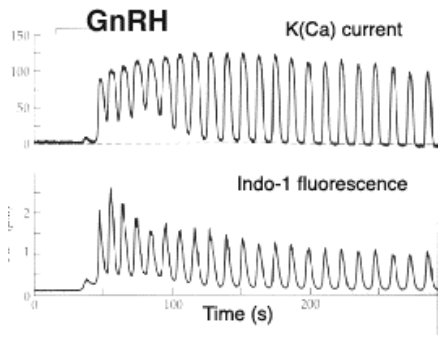
Activation of contraction in smooth muscle strips by photolysis of "ca
$Ins(1,4,5)P_3$ or "caged" phenylephrine (from Somlyo *et al.*, 1988, with permission).
guinea-pig portal vein strip had been depolarized by 143 mM KCl for the phenyleph
experiment and permeabilized by 50 mg/ml saponin for the $Ins(1,4,5)P_3$ experim
About 10% of the 50 μM phenylephrine and 10 μM $Ins(1,4,5)P_3$ were released by th
nsec laser pulse.





Activation of IP3-gated Ca^{2+} channel by *fyn*





Calcium signaling during apoptosis