

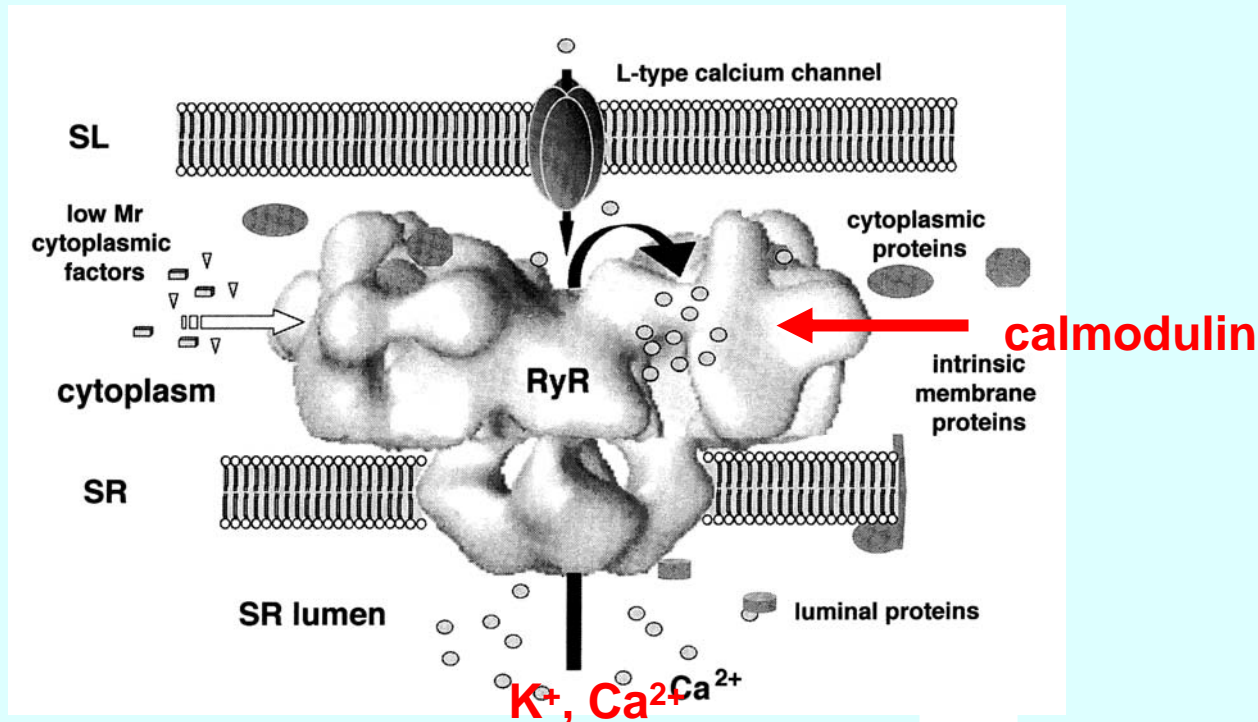
Cardiac ryanodine receptor and its regulation by calmodulin

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Ryanodine receptor ion channels (RyRs)

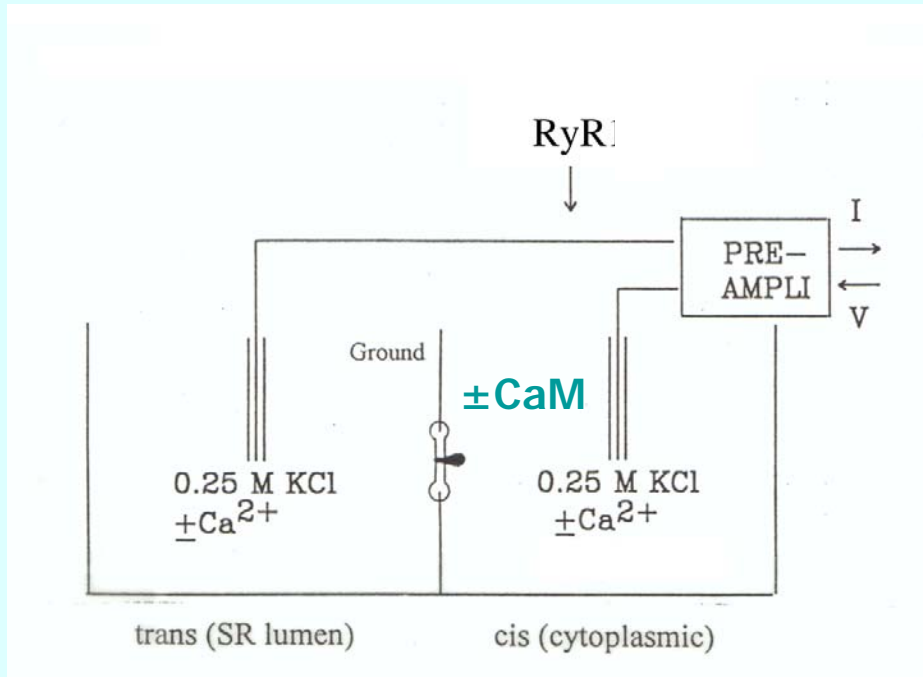
- Release in muscle Ca^{2+} from the sarcoplasmic reticulum, an intracellular Ca^{2+} -storing compartment.
- 4 subunits, each $\sim 585\text{kDa}$ and $\sim 12\text{kDa}$
- High conductance, ligand-gated Ca^{2+} channels
- Three isoforms: RyR1 or skeletal RyR, RyR2 or cardiac RyR
RyR3 or brain RyR



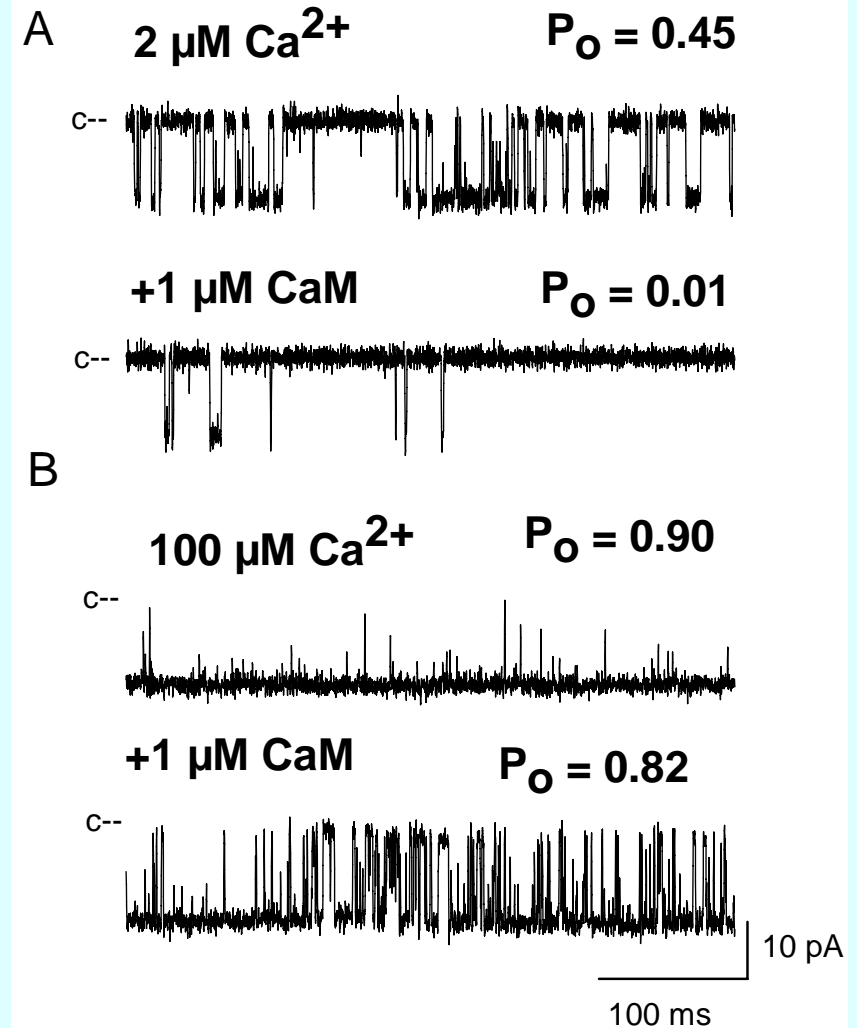
[³⁵S]CaM binding to Purified RyR2 indicates the presence of a single CaM binding site /RyR2 subunit

	CaM/RyR	K_D (nM)
0.1 μM Ca²⁺	3.8	54.0
100 μM Ca²⁺	3.9	5.0

CaM inhibition of single RyR2 ion channels

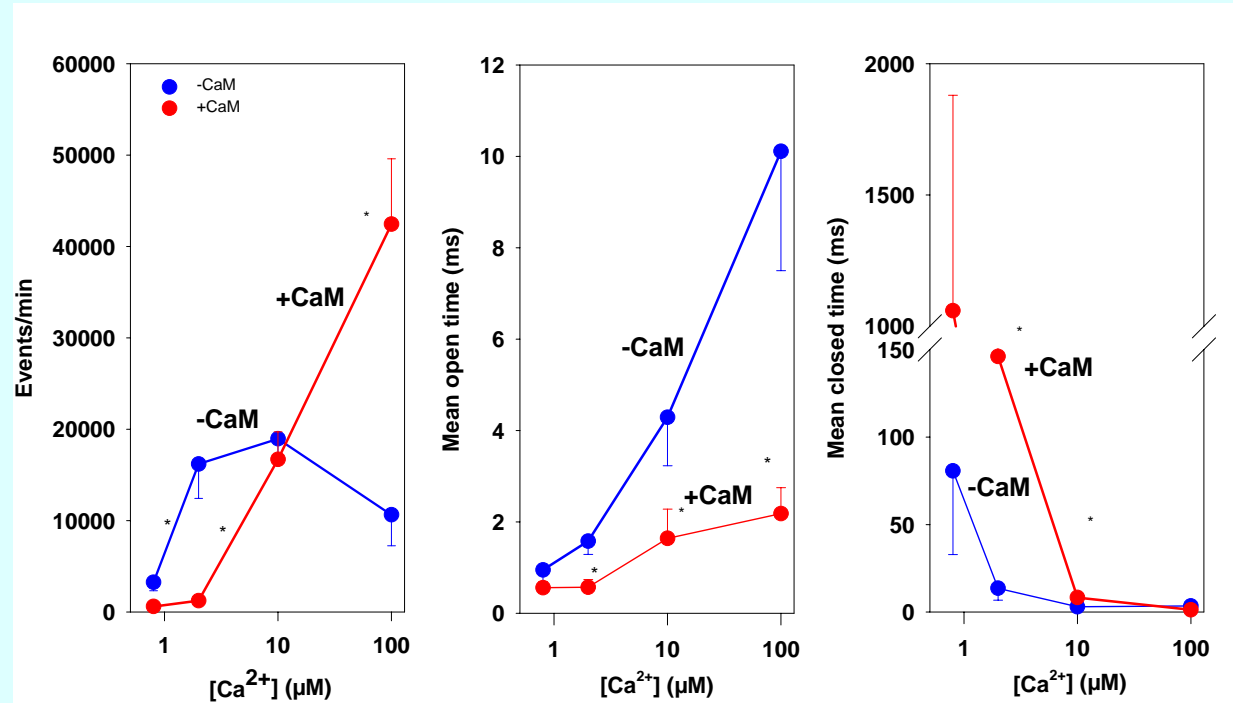
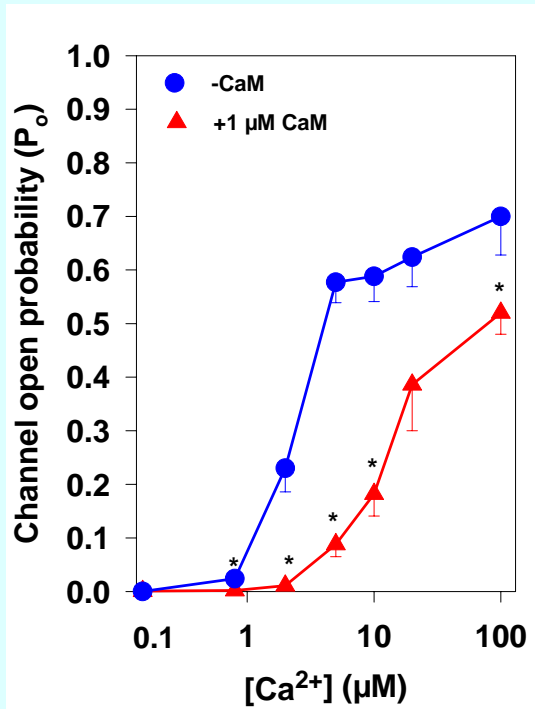


Measurement of single channel activities, using planar lipid bilayer

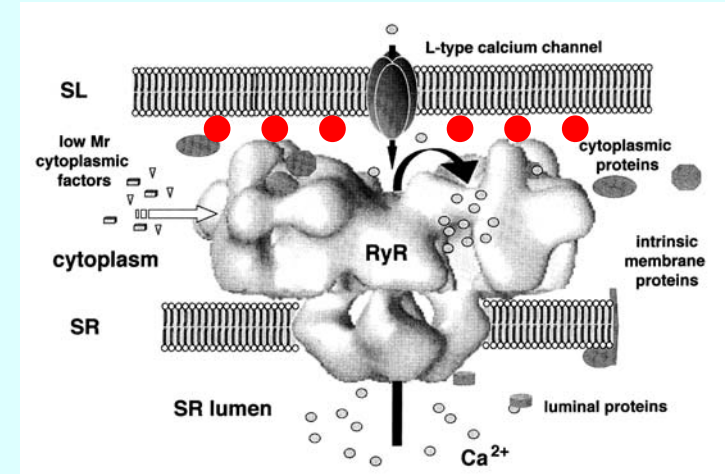
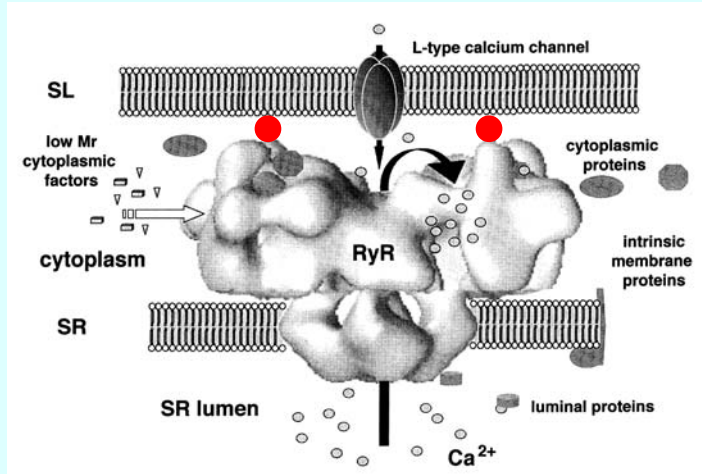


Current traces of RyR2

CaM inhibition of single cardiac Ca^{2+} release channel/RyR2 depends on cytosolic $[\text{Ca}^{2+}]$



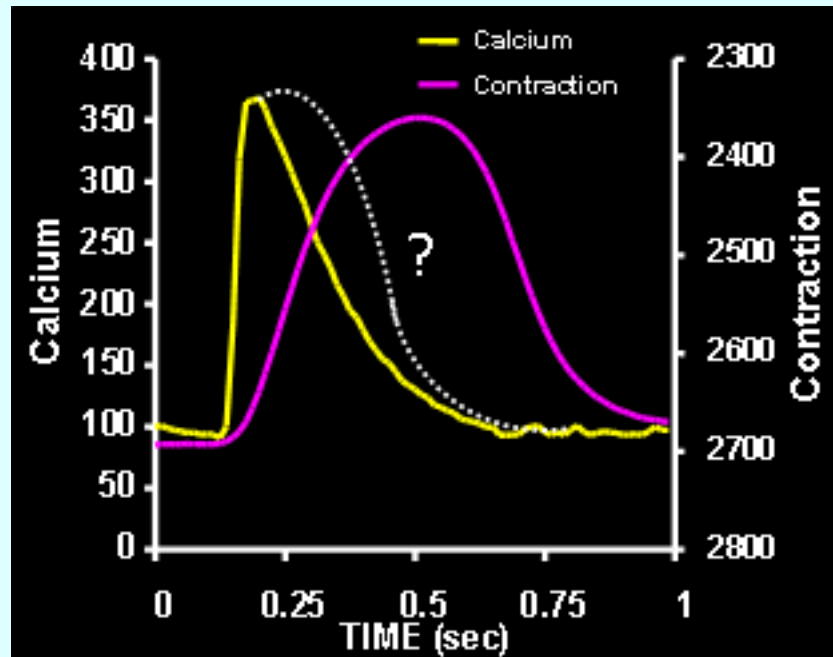
Proposed role of CaM in ending SR Ca^{2+} release



CaM facilitates termination of SR Ca^{2+} release by decreasing local activator [Ca^{2+}].

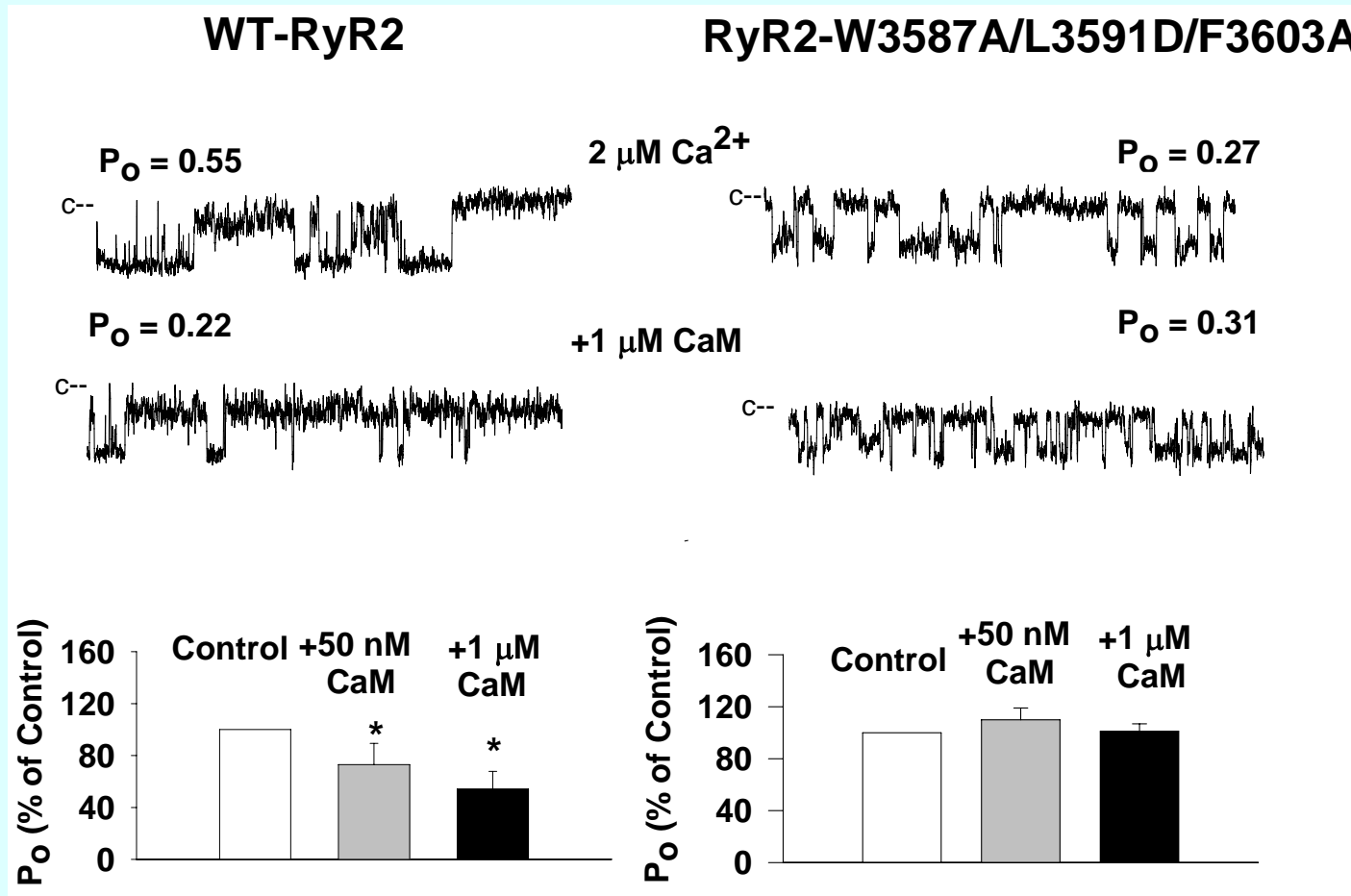
**No CaM inhibition:
Channels stay open longer
or can reopen.**

Role of CaM in *in vivo* SR Ca²⁺ release ?



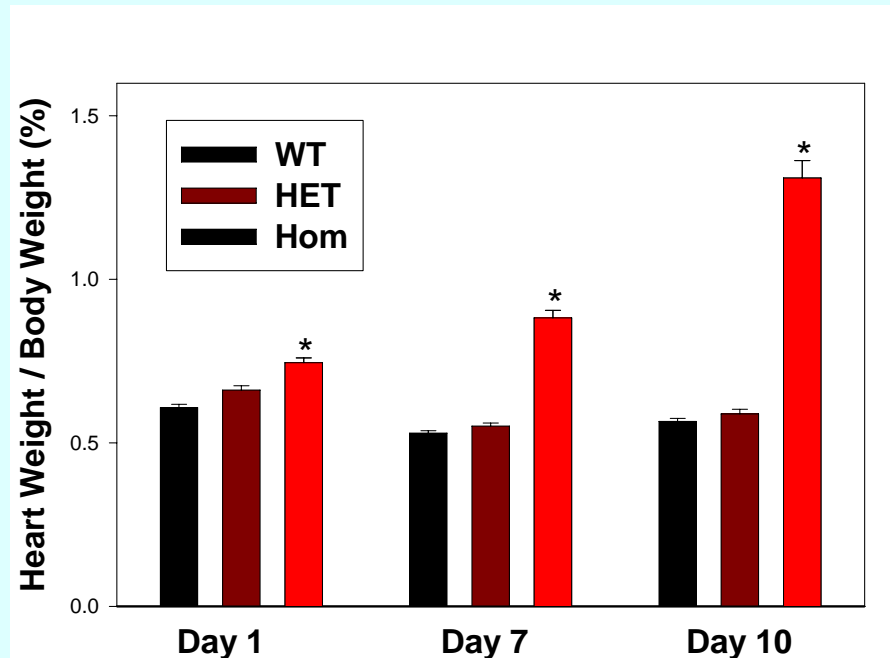
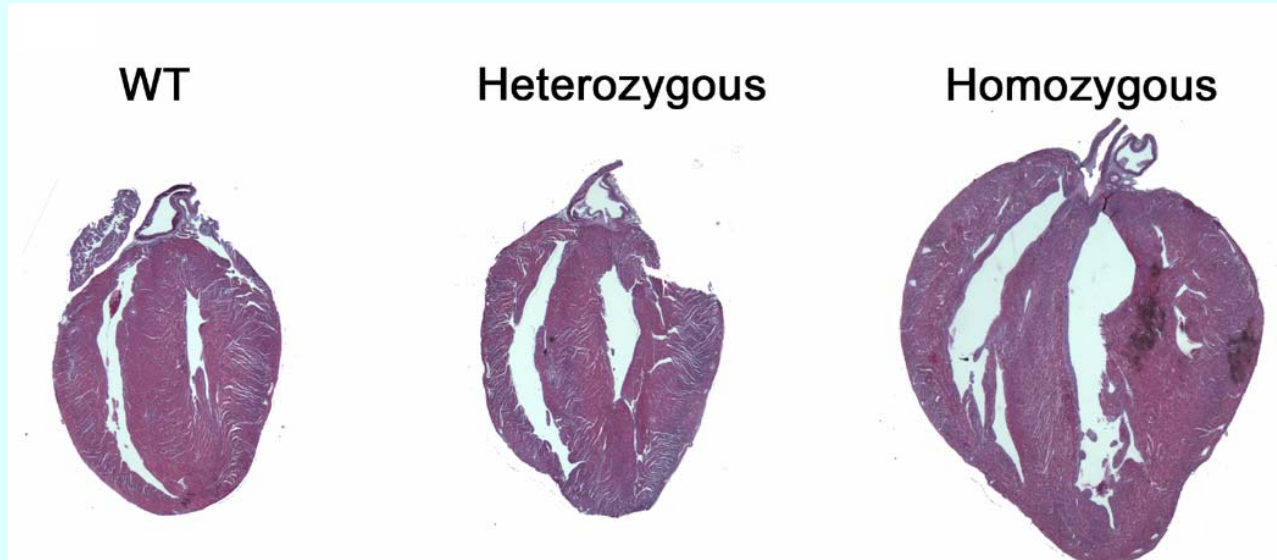
Create mouse model impaired in regulation of cardiac ryanodine receptor ion channel by calmodulin

Three amino acid substitutions in calmodulin binding domain of cardiac RyR2 ion channel eliminate regulation by calmodulin

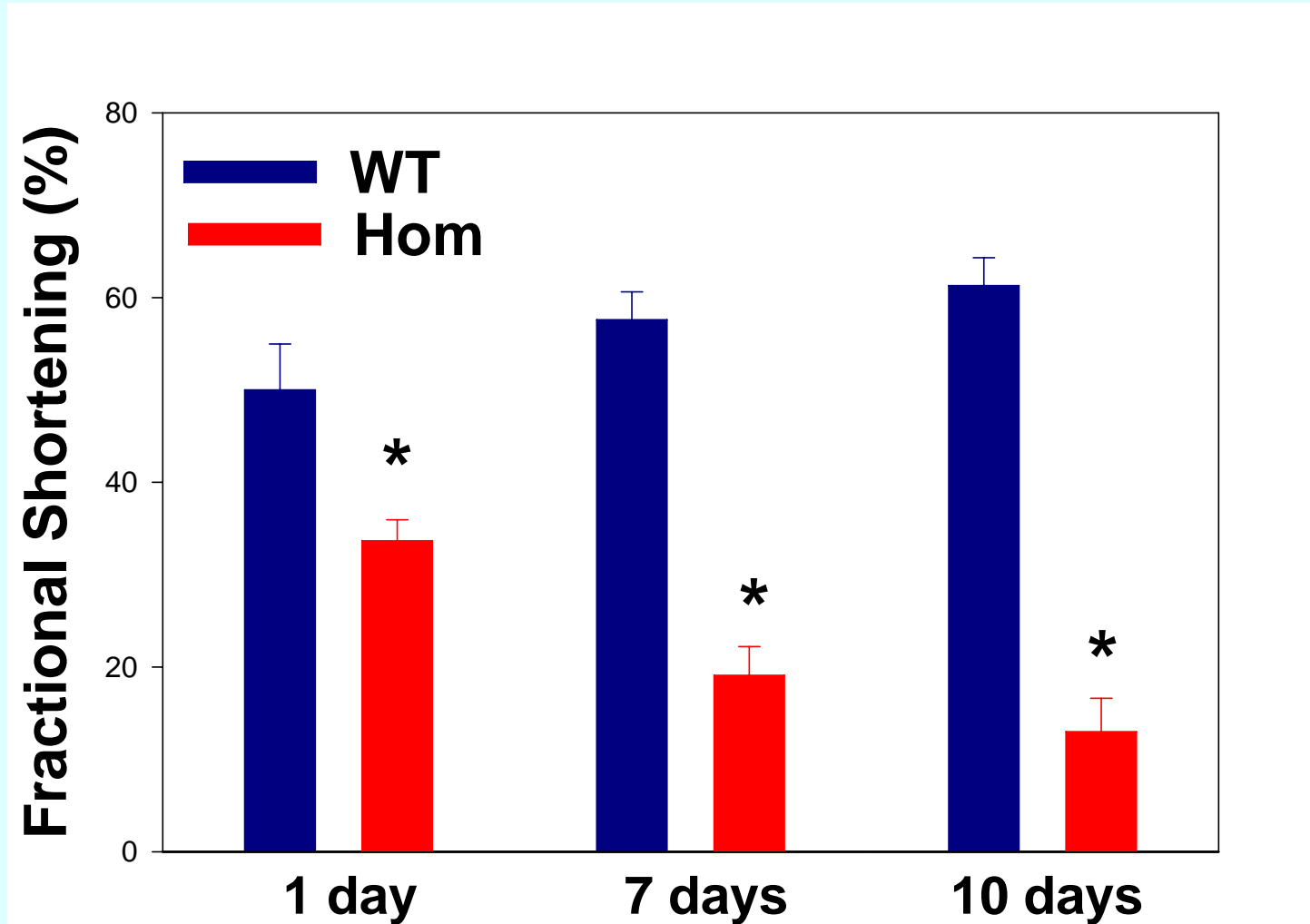


Mice with WLF mutation in CaM binding site of RyR2

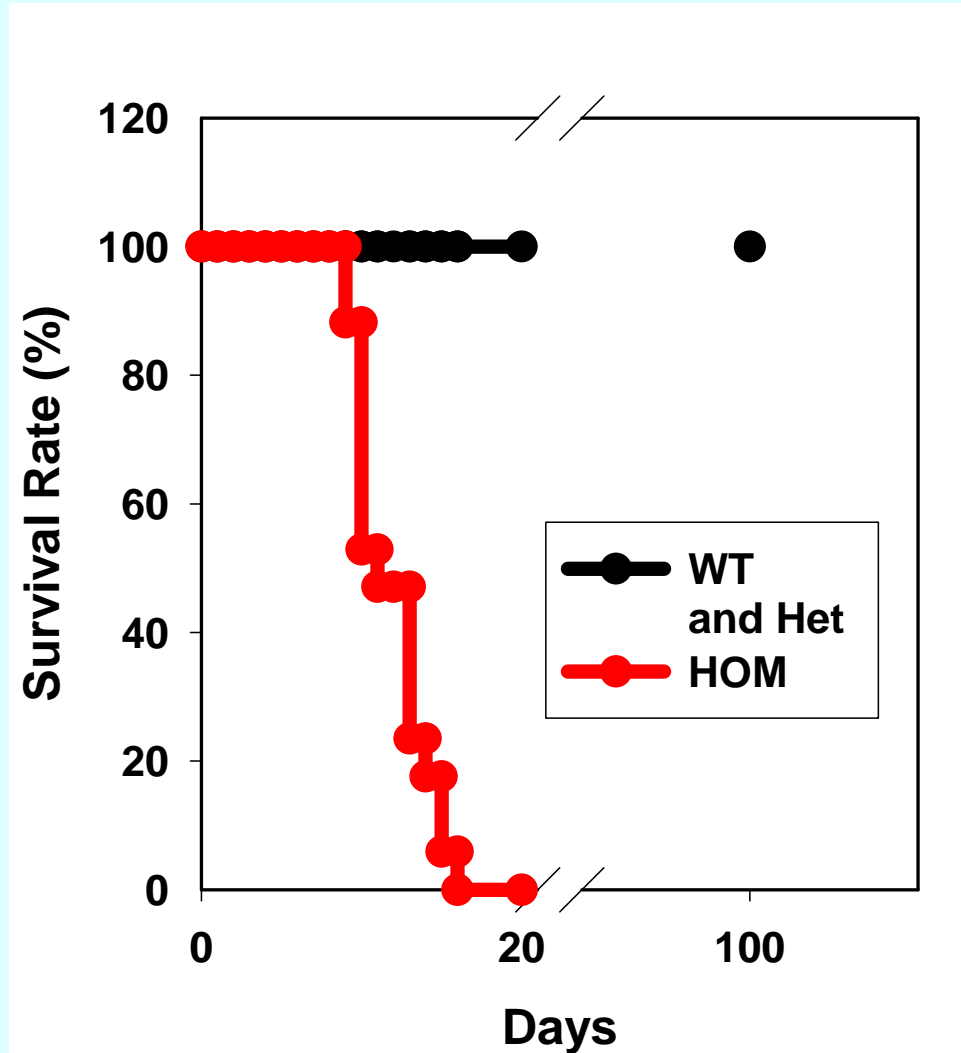
10-Day
hearts



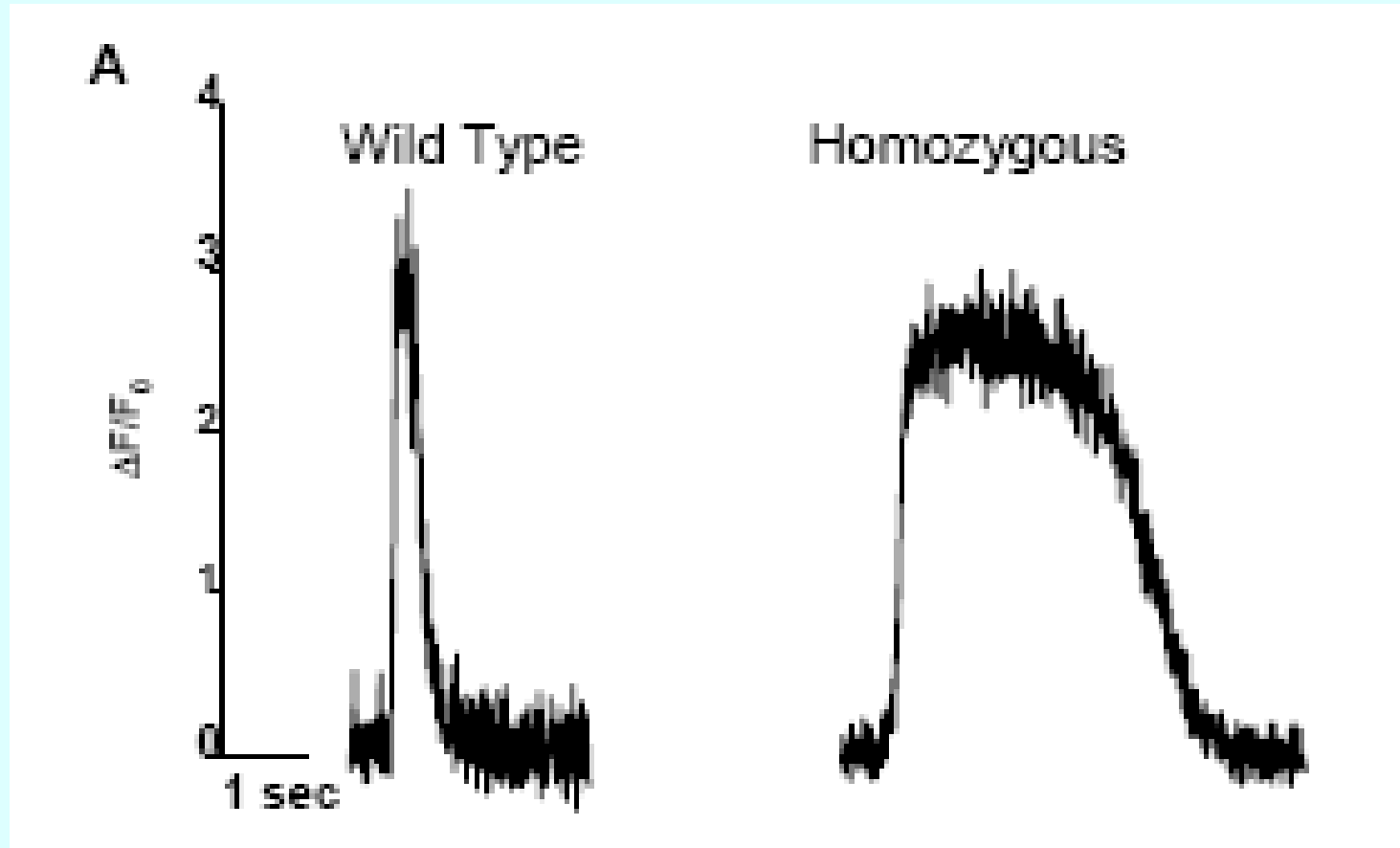
Cardiac hypertrophy is associated with left ventricular dysfunction of homozygous mutant mice



Homozygous mice with WLF mutation die early after birth



Ca²⁺ transients in spontaneously beating cardiomyocytes



Working Hypothesis

A defective SR Ca^{2+} release activates signaling mechanisms in the heart. Subsequent major changes in Ca^{2+} handling, cytoskeletal and extracellular molecules are associated with the rapid progression of cardiac hypertrophy in the mutant mice.

Conclusions

- RyRs are cation-selective ion channels that conduct monovalent and divalent cations, and are regulated by a large number of endogenous effectors that include calmodulin.
- Impaired calmodulin regulation of RyR2 leads to prolonged SR Ca^{2+} release, cardiac hypertrophy, and early death of homozygous mutant mice.
- Creation of mice impaired in calmodulin regulation of RyR2 provides a promising model to study the functional significance of Ca^{2+} , a key signaling molecule in normal cardiac function and hypertrophy.



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