PRINCIPLES OF PHYSIOLOGY G6001

MUSCLE: EXCITATION-CONTRACTION COUPLING

LECTURE 10-9-2003

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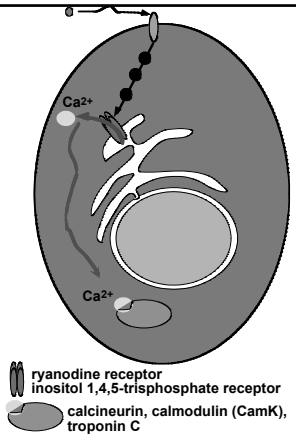
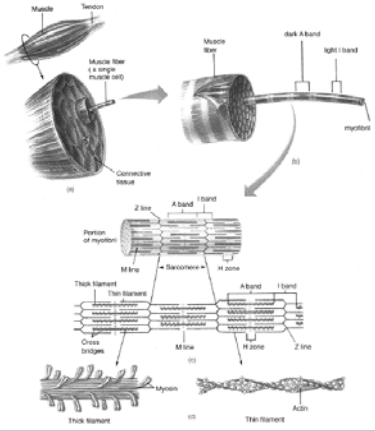
Assigned text: Physiology, 4rth Edition by R.M.Berne and M.N.Levy
Chapters 17 & 18

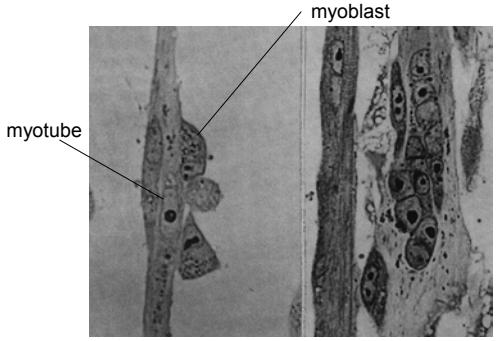
Topics to be covered:

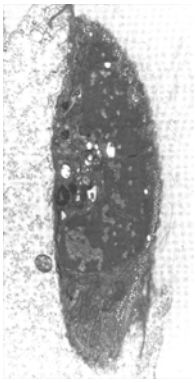
1) Excitation-contraction coupling
2) Cardiac muscle vs skeletal muscle
3) Diseases of ECC

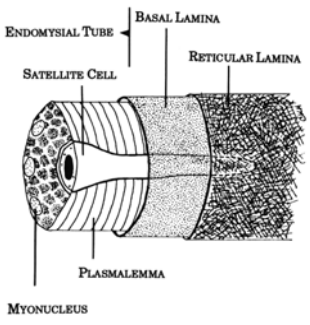




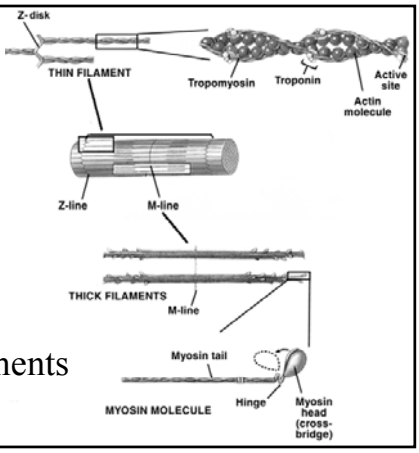
Myotubes in culture

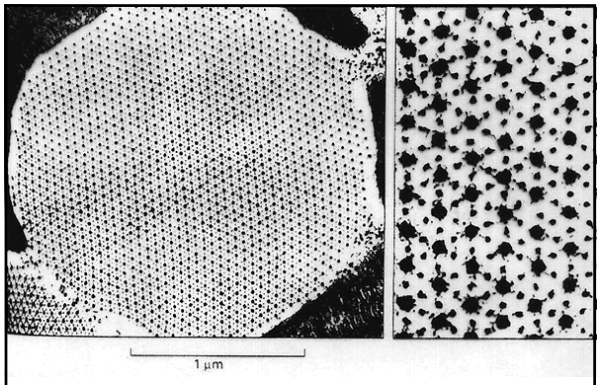






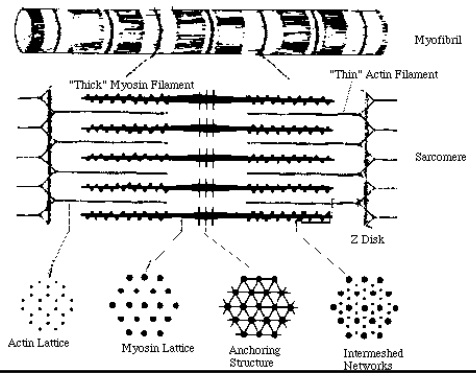
Myofilaments



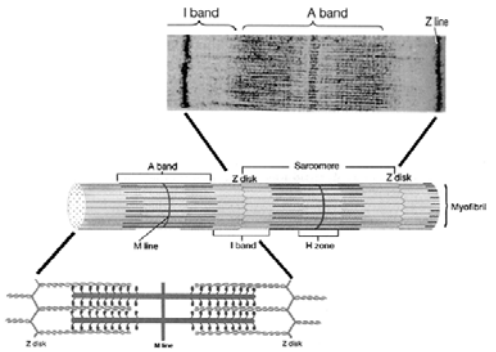


Skeletal muscle cross section EM

cross section,

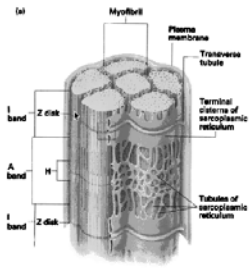


Sarcomeres



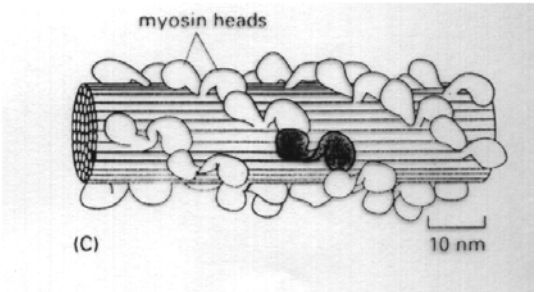
SR and T tubules in skeletal muscle fiber

Skeletal muscle fiber membranes

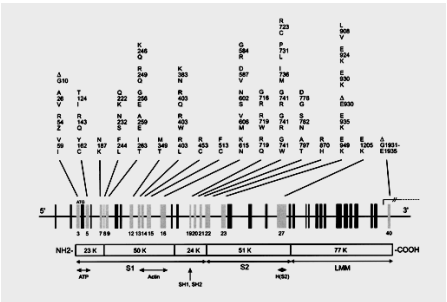


Thick filament II

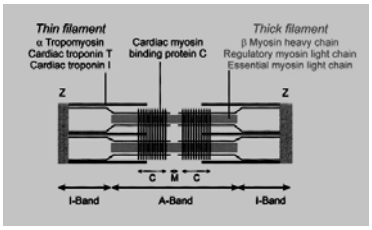
Thick filament structure



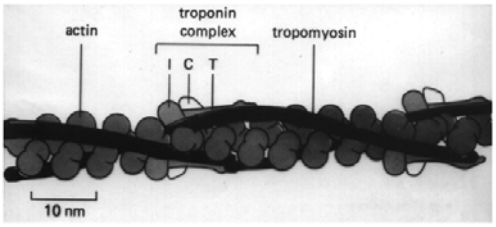
Myosin heavy chain mutations in FHC



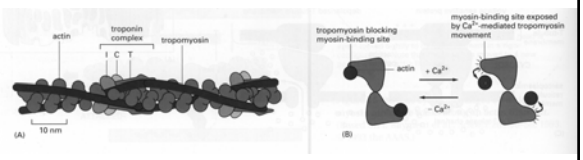
Elements of hypertrophic cardiomyopathy



Structure of Thin Filament

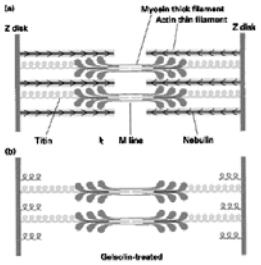


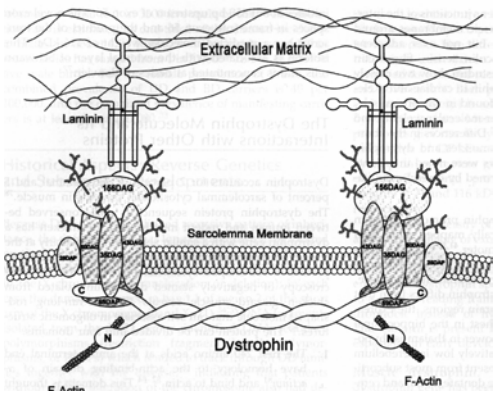
Tropomyosin and Troponin



Nebulin and Titin

Intermediate filaments in the sarcomere: nebulin and titin



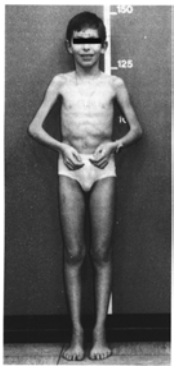


Emery-Dreifuss Muscular dystrophy

Cardiac pacemaker @18 y/o

Mutations in genes encoding nuclear membrane/lamina proetins emerin and lamin A/C

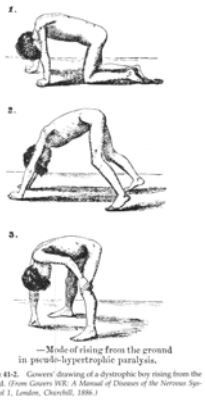
X-linked (emerin mut.) Autosomal dom.



Myotonic stiffness

Becker's Muscular Dystrophy

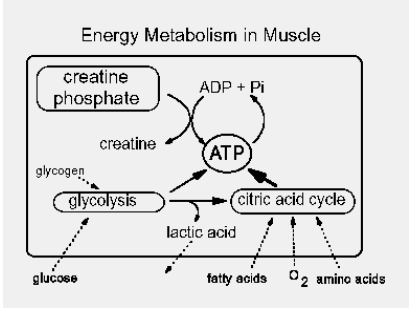
Rest, tight fist 3 sec, relax (>10 sec)



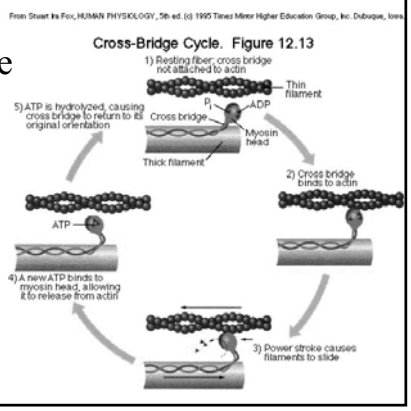
—Mode of rising from the ground in pseudo-hypertrophic paralysis.

FIGURE 43-2. Gowers' drawing of a dystrophic boy rising from the ground. (*From Gowers WR: A Manual of Diseases of the Nervous System, vol 1, London, Churchill, 1886.*)

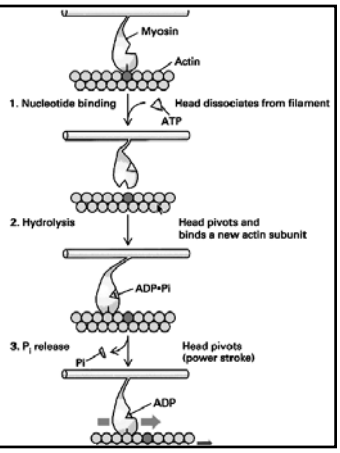
Energy required for contraction comes from three main sources:



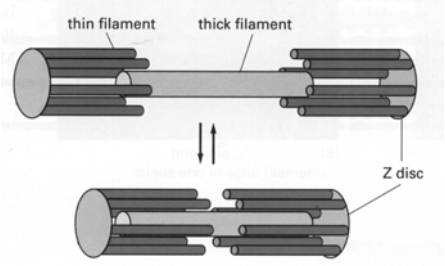
myosin crossbridge



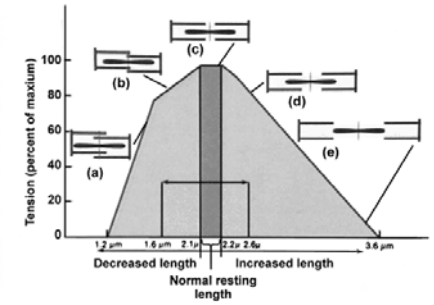
X-bridge: ATP hydrolysis



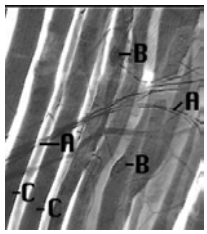
Sliding filament model of muscle contraction: actin and myosin filaments slide paste one another

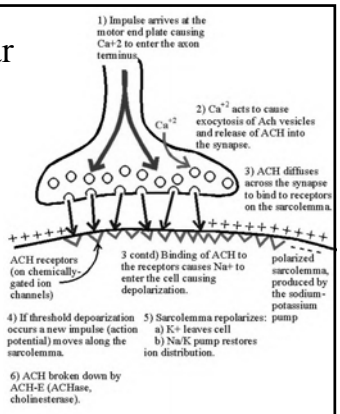


Filament Overlap Hypothesis

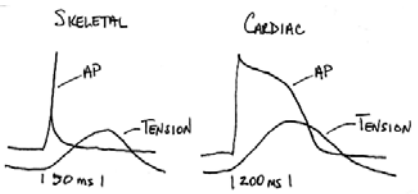


Neuromuscular Junction

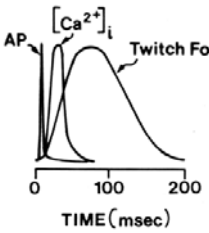




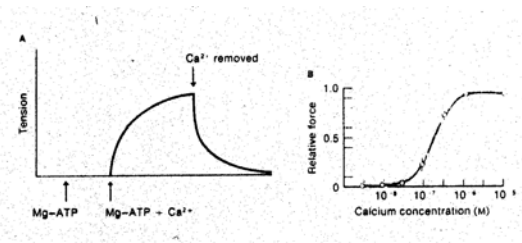
Action Potential



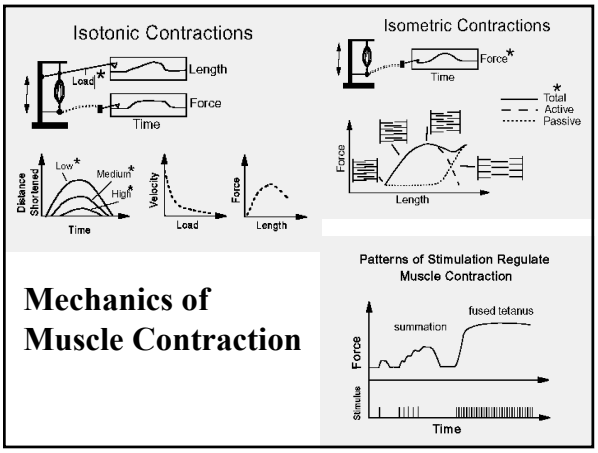
Twitch



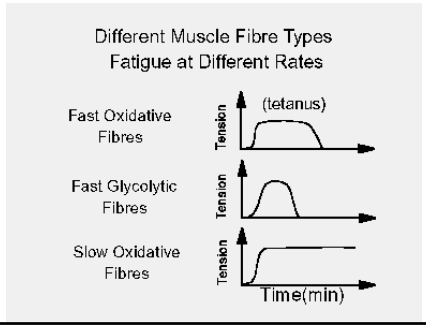
Role of Ca++ in contraction



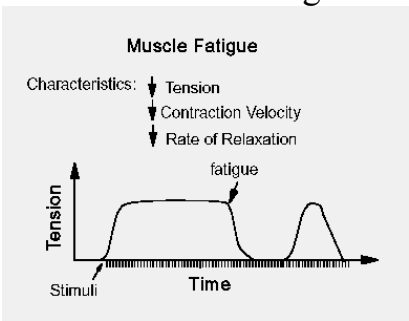
Mechanics of Muscle Contraction



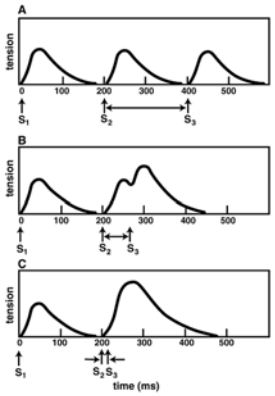
SPECIALIZED MUSCLE FIBRES



What causes muscle fatigue?



Summation



Tetanus

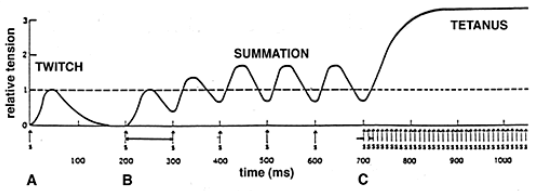


TABLE I Duration of Key Steps in the Activation of a Fast-Twitch Skeletal Muscle

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.

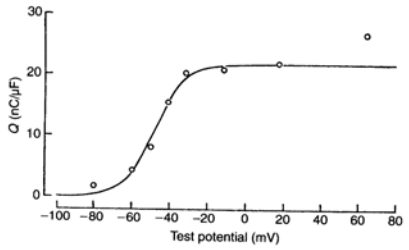
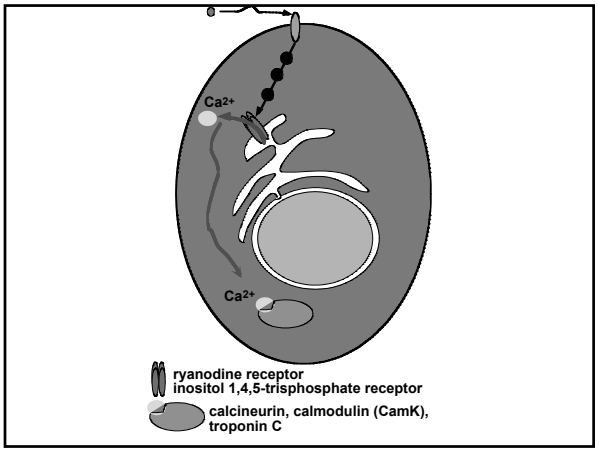
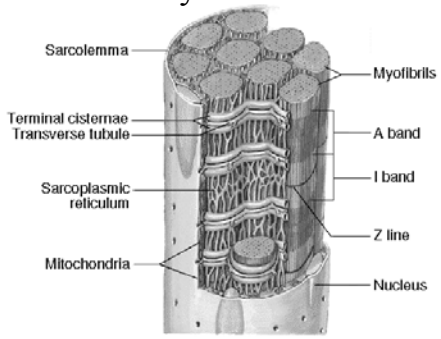
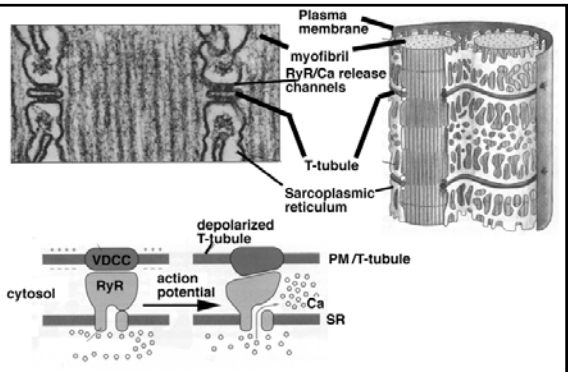


FIGURE 12. Relationship between charge movement (nC/μF) and membrane potential (mV). Charge moved at each potential was obtained by integrating the voltage-dependent capacity current, normalized to the fiber linear capacitance near the resting potential. Symbols represent the mean values of charge moved 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.)

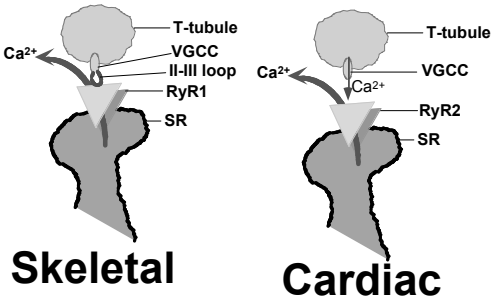


Sarcoplasmic Reticulum (SR) / T Tubule System



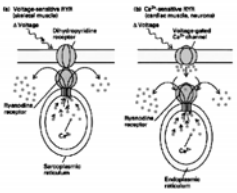


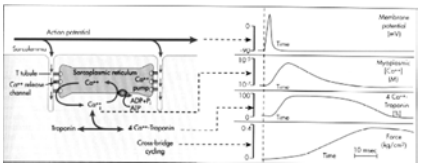
ECC membranes

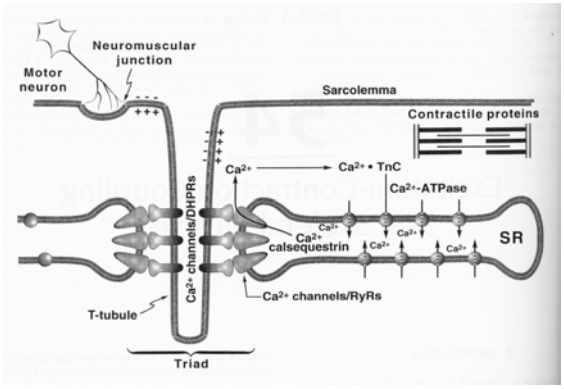


Skeletal vs Cardiac ECC

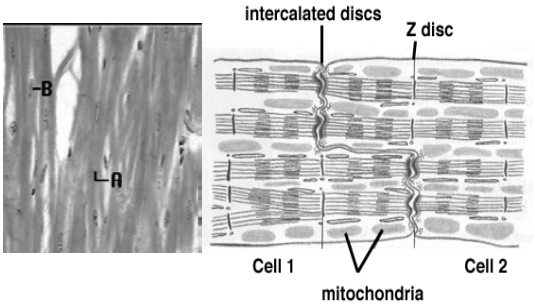
T-tubule channel and RyR on SR: skeletal vs cardiac muscle







Heart Muscle Structure



Ion fluxing membranes in muscle

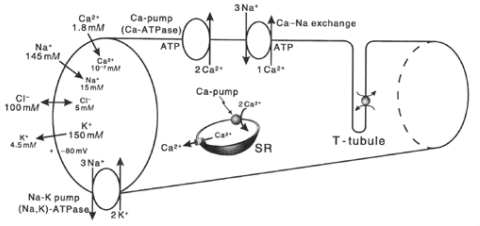
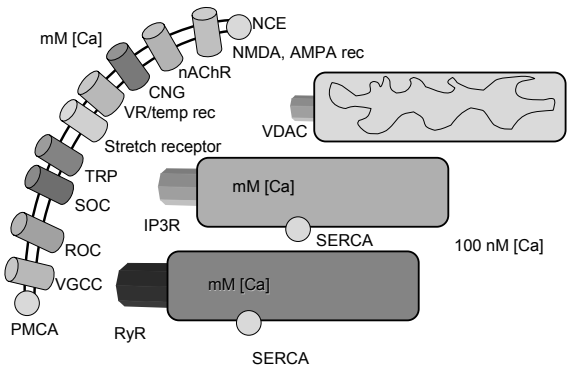
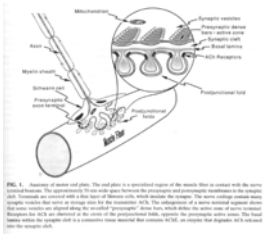
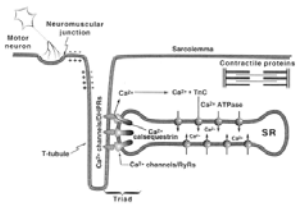


FIG. 1. Intracellular and extracellular ion distributions in vertebrate skeletal muscle fibers. Also shown are the polarity and magnitude of the resting potential. Arrows indicate direction of the net electrochemical gradient. The Na^+–K^+ pump and Ca^{2+}–Na^+ exchange carrier are located in the cell surface membrane. A Ca-ATPase and Ca^{2+} pump, similar to that in the sarcoplasmic reticulum (SR), is located in the cell membrane.



vertebrate skeletal muscle contraction

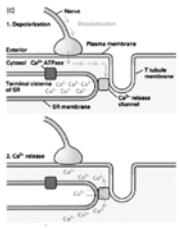




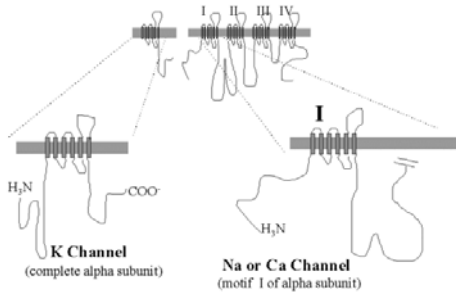
- Resting $[Ca]_i$ ~0.1 μM
- Neuromuscular transmission
- Action potential propagation along sarcolemma and into T-tubules
- Signal transduction from voltage-dependent Ca^{2+} channels to Ca^{2+} release channels at triad junctions
- Ca^{2+} release from SR
- Cytosolic $[Ca]_i$ reaches 1–10 μM
- Diffusion 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

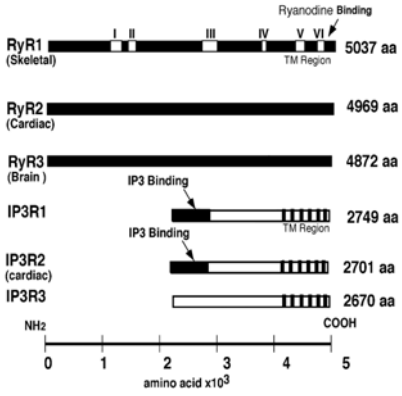
ECC

T tubule, SR interaction in excitation-contraction



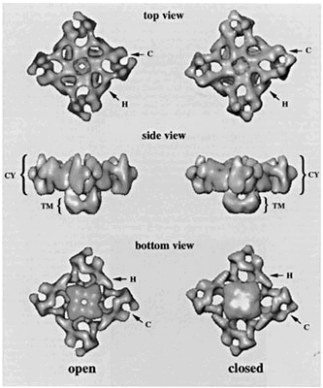
Voltage-gated ion channels

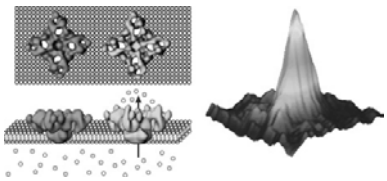


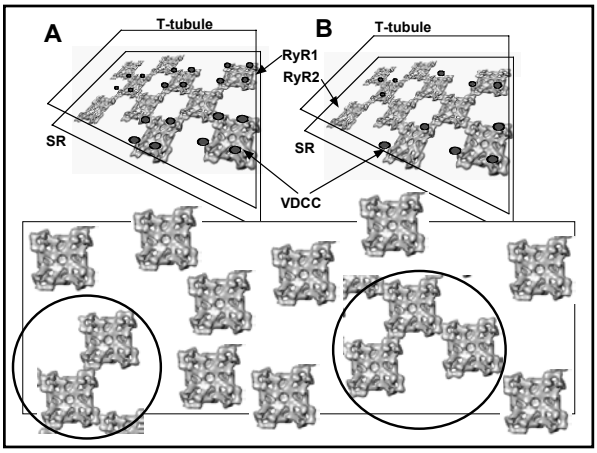


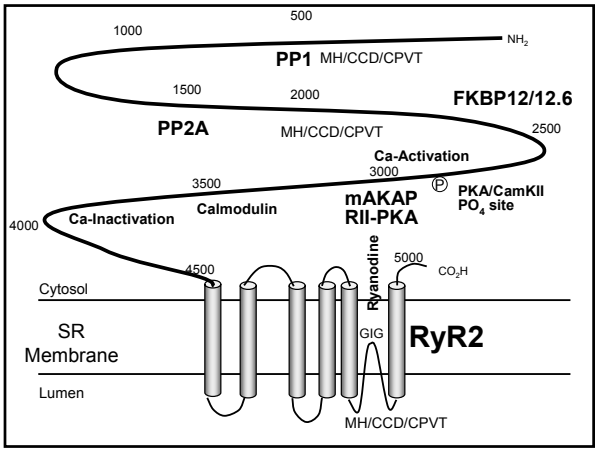
Property	RyR1	RyR2	RyR3
Sed coef.	30s	30s	30s
Ryan K_D	<10 nM	<10 nM	<10 nM
Single ch. cond. (γ_{max}) Ca	~120 pS	~120 pS	~110 pS
Act by Ca^{2+}	mM	mM	mM
Act by caffeine & ATP	Yes	Yes	Yes
Inhibited by mM Ca^{2+} & Mg^{2+}	Yes	Yes	Yes
Mod by ryanodine	Yes	Yes	Yes

RyR

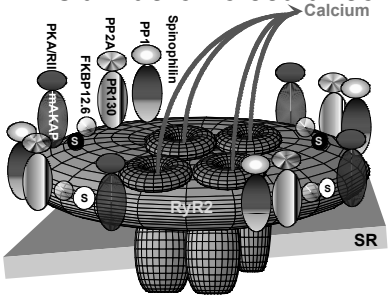






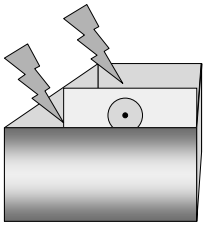


RyR2 is a macromolecular complex

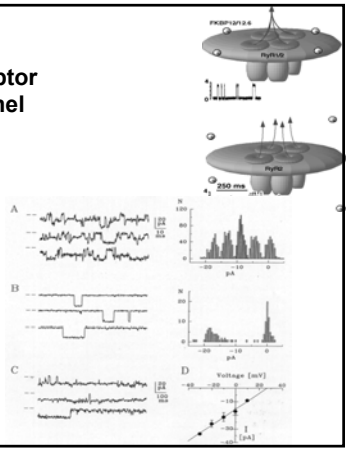


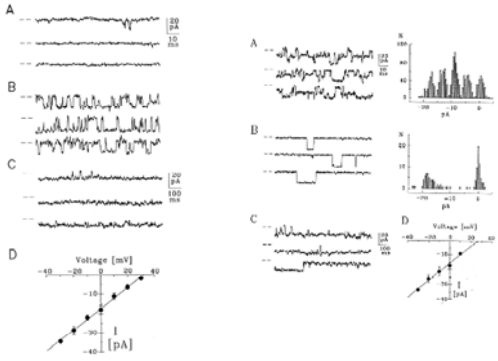
RyR2 PKA phosphorylation *dissociates* FKBP12.6 & increases Ca^{2+}-induced activation of channel. RyR2 PKA hyperphosphorylation (3/4-4/4 sites) dissociates most FKBP12.6 & channels are "leaky"

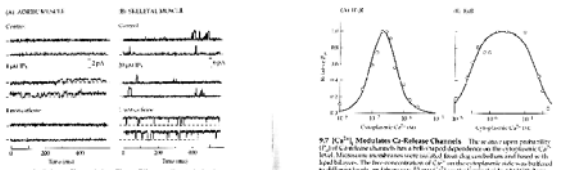
FKBP12/12.6 required for normal function of the ryanodine receptor calcium release channel

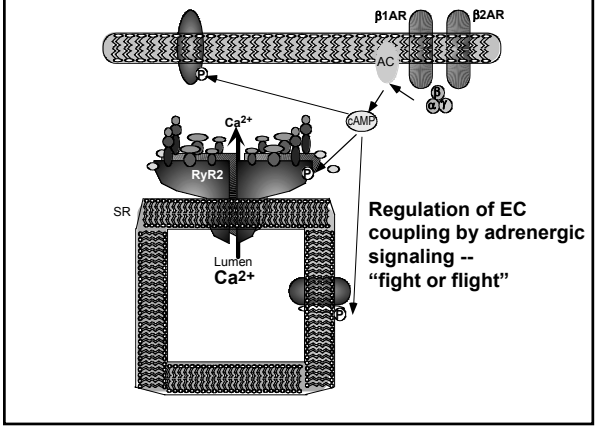


Planar lipid bilayer chamber



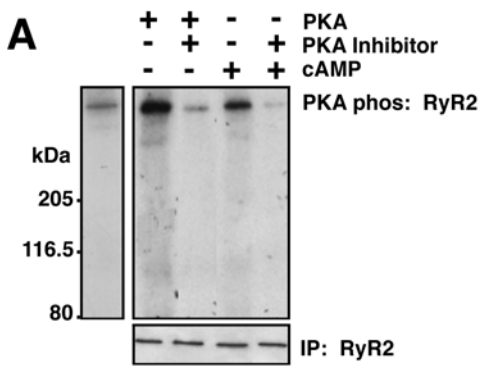




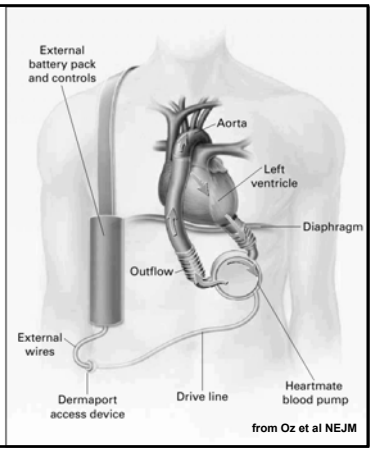


Regulation of EC coupling by adrenergic signaling -- "fight or flight"

RyR2/calcium release channel phosphorylated by addition of cAMP alone

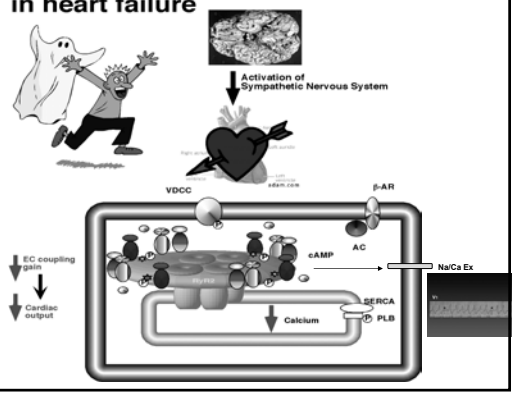


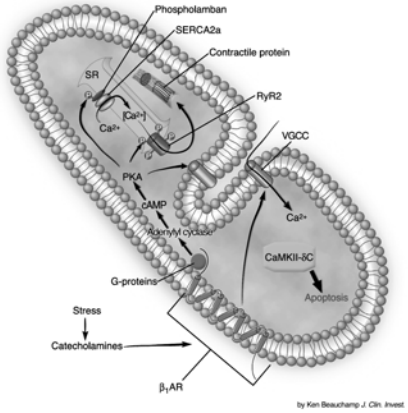
Left Ventricular Assist Device

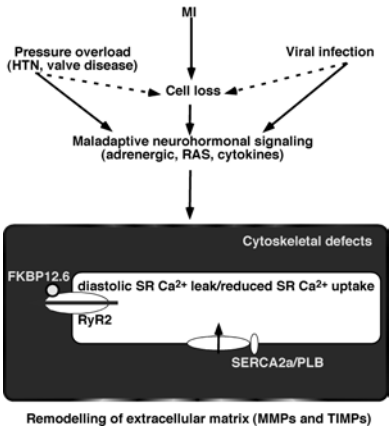


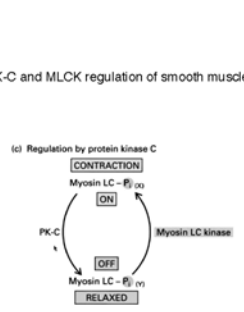
from Oz et al NEJM

Maladaptation of the fight or flight response in heart failure

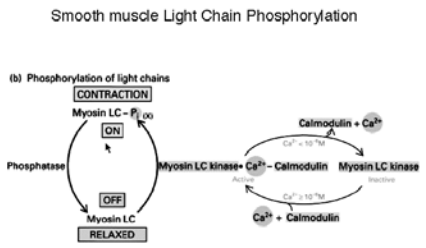






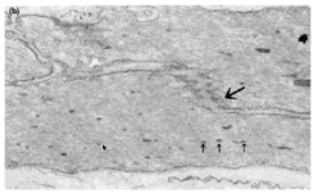


Smooth muscle contraction

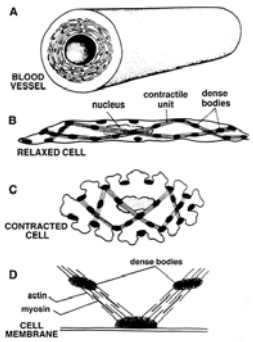


SM-LC phosphorylation

Smooth muscle structure - EM

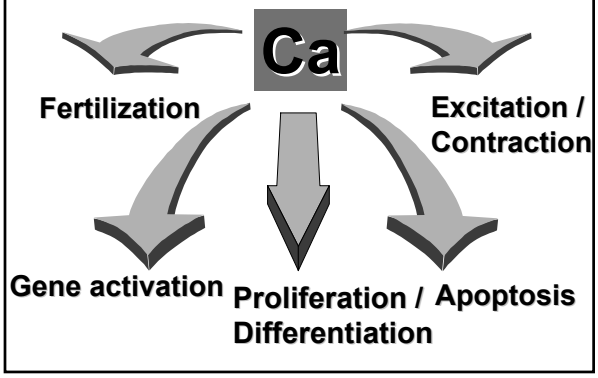


Smooth muscle

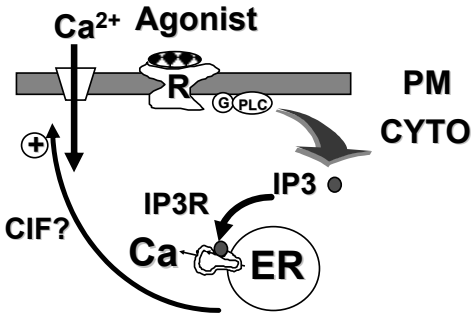


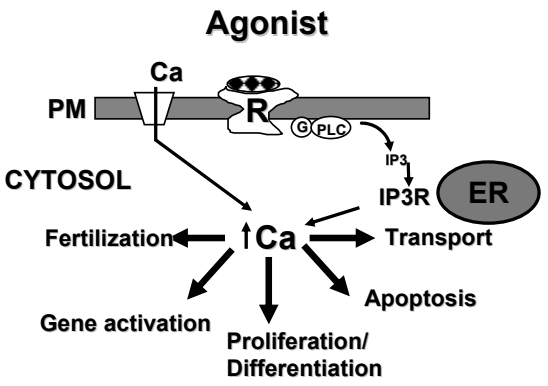
	IP3R	RyR
Skel muscle	+	+++
Smooth m.	+++	+
Neurons	+++	+++
IP3	Activates	None
Ryanodine	None	Locks open/closes
Caffeine (5 mM)	Inhibits	Activates
Ca^{2+}	IP3R1 - biphasic IP3R2/3 - opens	biphasic
RR	None	inhibits
Heparin	inhibits	activates

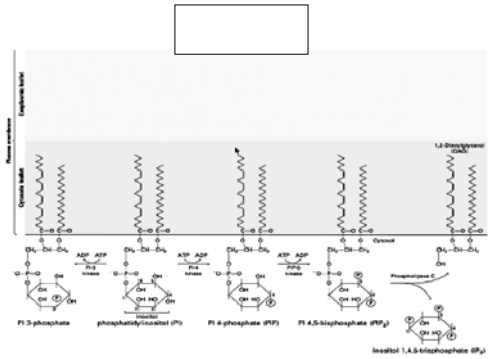
Ca signaling



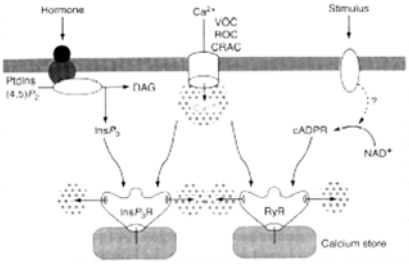
Ca^{2+} elevation: 2 pathways

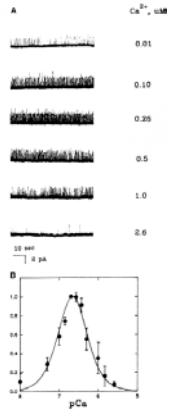


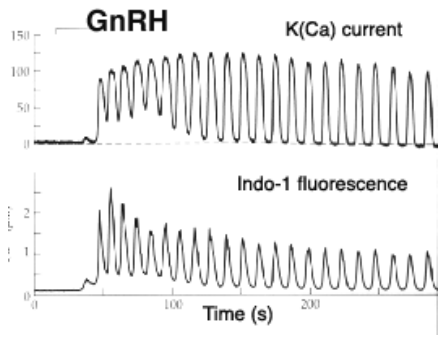

Agonist
Ca
PM
R
G
PLC
IP3
CYTOSOL
IP3R
ER
Fertilization
↑Ca
Transport
Apoptosis
Gene activation
Proliferation/
Differentiation

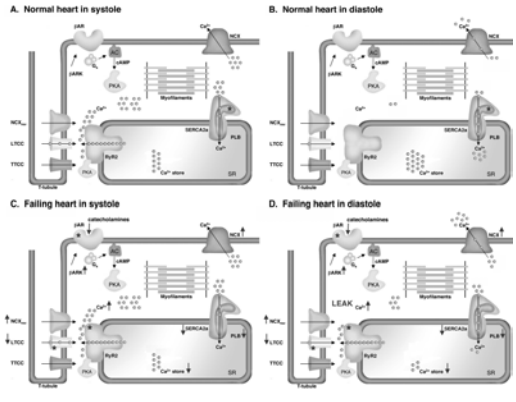


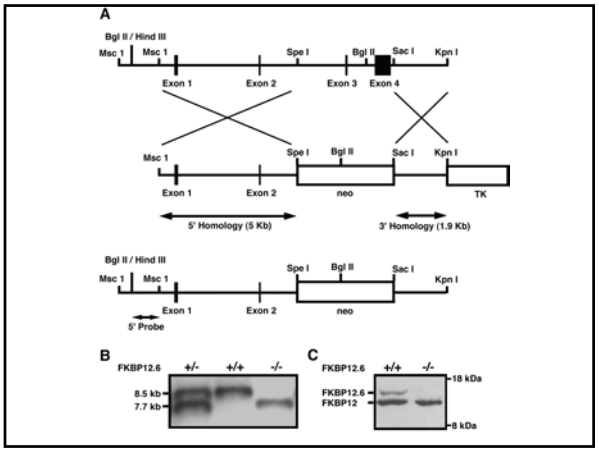
IP3 pathway

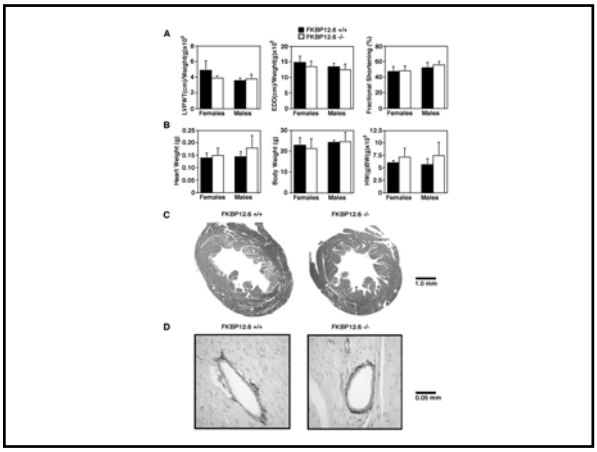

Hormone
Ca2+
VOC
ROC
CRAC
Stimulus
PtdIns
(4,5)P2
DAG
InsP3
cADPR
NAD+
InsP3R
RyR
Calcium store

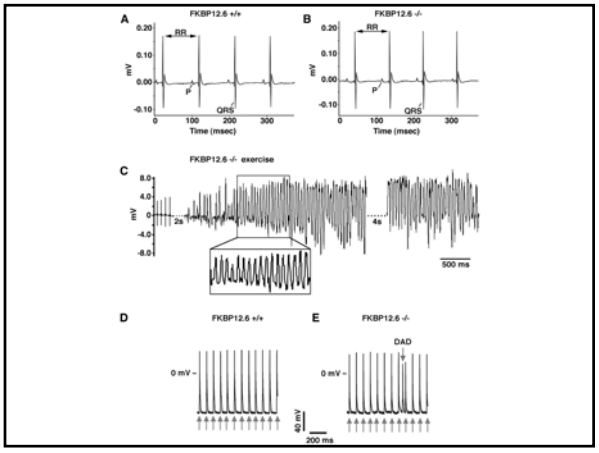

A
Ca2+, uM
0.01
0.10
0.25
0.5
1.0
2.6
10 sec
B
pCa

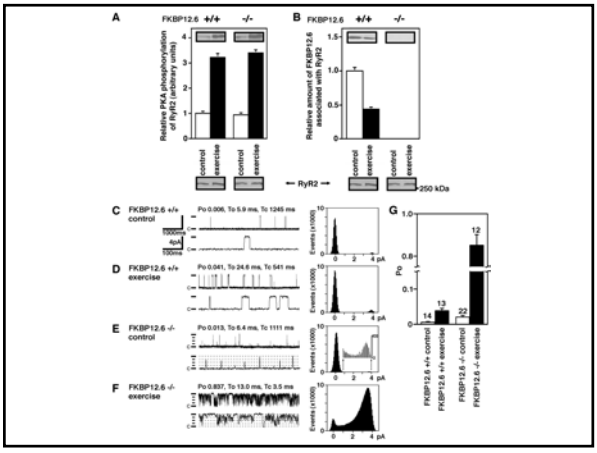

GnRH
K(Ca) current
Indo-1 fluorescence
Time (s)

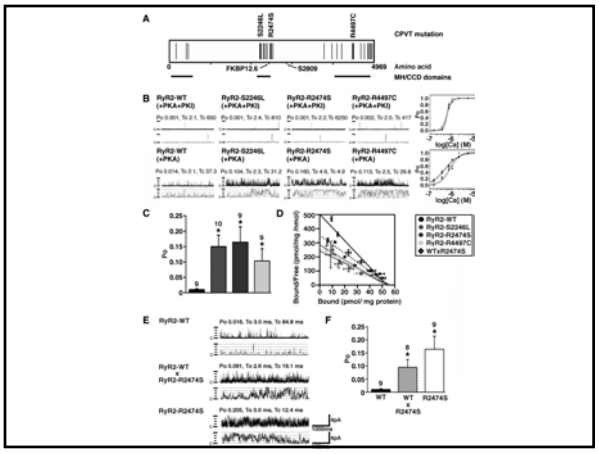

A. Normal heart in systole
B. Normal heart in diastole
C. Failing heart in systole
D. Failing heart in diastole
LEAK

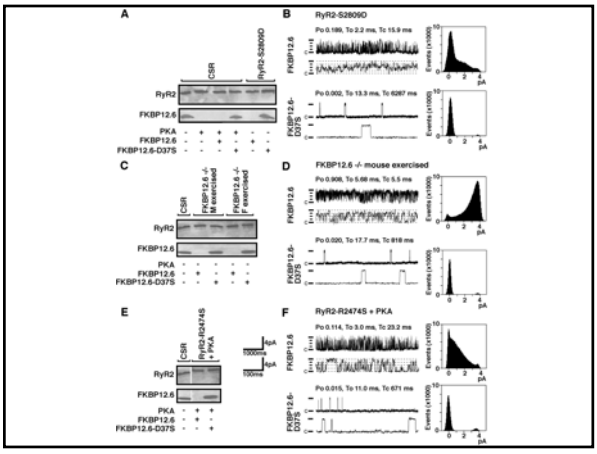

A
Bgl II / Hind III
Msc 1
Msc 1
Spe I
Bgl II
Sac I
Kpn I
Exon 1
Exon 2
Exon 3
Exon 4
neo
TK
5' Homology (5 Kb)
3' Homology (1.9 Kb)
5' Probe
B
FKBP12.6
+/-
+/+
-/-
8.5 kb
7.7 kb
C
18 kDa
FKBP12.6
FKBP12
8 kDa

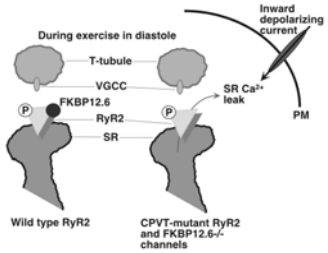

FKBP12.6 +/+
FKBP12.6 -/-
Females
Males
Fractional Shortening (%)
Heart Weight (g)
Body Weight (g)
1.0 mm
0.05 mm

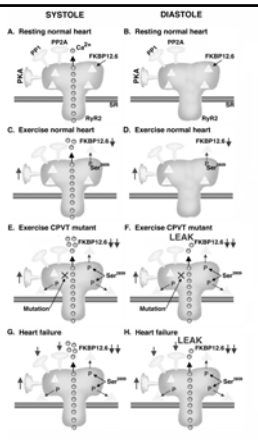

FKBP12.6 +/+
FKBP12.6 -/-
RR
QRS
Time (msec)
FKBP12.6 -/- exercise
2s
4s
500 ms
DAD
0 mV
40 mV
200 ms


FKBP12.6 +/+
-/-
Relative PKA phosphorylation of RyR2 (arbitrary units)
Relative amount of FKBP12.6 associated with RyR2
control
exercise
RyR2
250 kDa
FKBP12.6 +/+ control
FKBP12.6 +/+ exercise
FKBP12.6 -/- control
FKBP12.6 -/- exercise


CPVT mutation
Amino acid
MH/CCD domains
FKBP12.6
S2809
RyR2-WT (+PKA+PKI)
RyR2-S2246L (+PKA+PKI)
RyR2-R2474S (+PKA+PKI)
RyR2-R4497C (+PKA+PKI)
RyR2-WT (+PKA)
RyR2-S2246L (+PKA)
RyR2-R2474S (+PKA)
RyR2-R4497C (+PKA)
log[Ca] (M)
Bound/Free (pmol/mg /nmol)
Bound (pmol/ mg protein)
RyR2-WT
RyR2-S2246L
RyR2-R2474S
RyR2-R4497C
WT+R2474S
Po
WT
R2474S


CSR
RyR2-S2809D
FKBP12.6
FKBP12.6-D37S
PKA
FKBP12.6 -/- exercised
RyR2-R2474S + PKA
1000ms
100ms
1nA


During exercise in diastole
Inward depolarizing current
T-tubule
VGCC
SR Ca2+ leak
FKBP12.6
RyR2
SR
PM
P
P
Wild type RyR2
CPVT-mutant RyR2 and FKBP12.6-/- channels


SYSTOLE
DIASTOLE
A. Resting normal heart
B. Resting normal heart
PP2A
PP1
Ca2+
FKBP12.6
PKA
SR
RyR2
C. Exercise normal heart
D. Exercise normal heart
E. Exercise CPVT mutant
F. Exercise CPVT mutant
LEAK
Mutation
G. Heart failure
H. Heart failure
LEAK