EC Coupling in Heart

Calcium Cycle in Cardiac Muscle

General scheme of Ca cycle in a cardiac myocyte. Ca can enter via Ca channels and Na/Ca exchange. Ca current may also control the SR Ca release by the SR Ca release channel/ryanodine receptor/foot protein. Ca is removed from the myofilaments (MF) and cytoplasm by the SR Ca-ATPase pump and the sarcolemmal Ca-ATPase pump and Na/Ca exchange.
Calcium Transients.
Sidney Ringer clearly recognized that extra cellular Ca is required for contraction in heart.

In heart cells contraction can be graded rather than all or none.

To produce graded contractions EC coupling in heart must possess mechanisms that eliminate positive feedback.

Mechanisms and theories of EC coupling must accommodate these facts.
Ca$^{2+}$ sparks and the Ca$^{2+}$ transient

From Fawcett and Mc Nutt, 1969
Ca Induced Ca Release: Trigger Hypothesis

Terminal Cisternae

L type Ca Channel

Ca

Sr Release Channel (RyR)

3Na
Regeneration

Cell Membrane

SR

RyRs

Ca

Ca

PMT

Light Source

Laser

Objective

Specimen

Focus

Beam Splitter

Source Aperture

Detector Aperture
Cell loaded with Fluo 3

T- Tubule
Emission Line 1

Local Ca transient
Emission Line 2

x
Stacked Lines
t

Line Scan Plot of Evoked Mouse Sparks

58 ms
92 µm

255
0
Preliminary Ideas on Couplons

Sarcolemma

LCC

Ca\(^{2+}\) trigger

SR

Ca\(^{2+}\) RyR

Ca\(^{2+}\) trigger

SR

Ca\(^{2+}\) RyR
Small Junction: Ca can rise to 30 μM and gate release channels and produce local Ca release events.

Numerous junctions spaced to ensure adjacent junctions not activated by Ca release.

Is a Single RyR Responsible for Producing a Spark

- It is important to understand two aspects of the structure of junctional regions.
- The first is the number of RyRs responsible for generating a spark.
- The second is the number of L-type Ca channels required to trigger a spark.
Single Channel Sparks

- Open time frequency histogram exhibits an exponential distribution
- Spark amplitude \( \propto \int \text{Ca flux}_{\text{yr}} \cdot \text{dt} \)
- Therefore we expect spark amplitudes to distribute exponentially if they are produced by a single channel
The Structure of the Junction

Although it appears that a cluster of RyRs contribute to the formation of a spark, it is not clear how many L-type Ca\(^{2+}\) channels are required to ensure that a spark is triggered. We investigated this by examining both the properties of Ca\(^{2+}\) sparks evoked by action potentials and single channel activity in rabbit ventricular myocytes.
Sparks and T-tubule locations

Sulforhodamine B
occupied T-tubules

10 µm

T-tubule locations

50 ms

5 µm

Average T-tubular spacing = 1.7 µm.

Sparks were evoked during action potentials. They were abolished after treatment with thapsigargin.

Average of 23 F/F₀ images

Sparks appeared coinciding with T-tubules.

Sparks produced by sequential stimuli

50 series of action potentials and F/F₀ images

Sparks observed at a fixed location and confocal plane appeared at the beginning of nearly every action potential. The amplitude of the sparks was variable among sites, but approximately constant at a given site.
Properties of signal-averaged spark

Averaged single spark

Spark size = 1.8 µm (at half maximum width)

Spark profiles and action potential

10% of the peak of every spark occurred with latency of 2 - 6 ms from the peak of every action potential.

Probability of null sweeps

♦ We detected all open events in 1000 sweeps during the period 2 - 6 ms after the clamp pulses.
♦ Open events are detected at half-amplitude threshold.
♦ From this, we calculated the probability of null sweeps at +10 mV and +50 mV.

\[ P_{\text{null}+10\text{mV}} = 0.58 \]

\[ P_{\text{null}+50\text{mV}} = 0.17 \]
Summary - Ca\textsuperscript{2+} sparks

• The properties of sparks in rabbit ventricular myocytes are similar to those in other species.

• Sparks at fixed sites during a series of action potentials occurred at the beginning of nearly every action potential, i.e., they appeared with a probability ~ 100%. Close examination of spark probability revealed a small proportion of spark failures, i.e., 0.3%.

• Sparks occur in a limited interval 2 - 6 ms after the action potential peak.