S-2 [10:30-11:00]

Diversity of atrial local Ca^{2+} signaling

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Atrial myocytes, lacking t-tubules, have two functionally separate groups of ryanodine receptors (RyRs): those at the periphery colocalized with dihydropyridine receptors (DHPRs), and those at the cell interior not associated with DHPRs. We have previously shown that the Ca^{2+} current (I_{Ca})-gated central Ca^{2+} release has a fast component that is followed by a slower and delayed rising phase. The mechanisms that regulate the central Ca^{2+} releases remain poorly understood. The fast central release component is highly resistant to dialyzed Ca^{2+} buffers, while the slower, delayed component is completely suppressed by such exogenous buffers. Here we used dialysis of immobile Ca^{2+} buffers (EGTA) into voltage-clamped rat atrial myocytes to isolate the fast component of central Ca^{2+} release and examine its properties using rapid (240 Hz) two-dimensional confocal Ca^{2+} imaging. We found two populations of rat atrial myocytes with respect to the ratio of central to peripheral Ca^{2+} release ($R_{c/p}$). In one population ("group 1", ~60% of cells), $R_{c/p}$ converged on 0.2, while in another population ("group 2", ~40%), $R_{c/p}$ had a Gaussian distribution with a mean value of 0.625. The fast central release component of group 2 cells appeared to result from in-focus Ca^{2+} sparks on activation of I_{Ca}. None of the group 1 cells showed t-tubule while most of group 2 cells showed rudimentary t-tubule-like structures in the cell interior. The central release sites specifically did not correspond to the faint membrane staining. Peripheral sparks, immediately activated by depolarizations, are larger in the group 1 cells compared to the group 2 cells. In contrast, both fast and slow central sparks in the group 2 cells are larger than those in the group 1 cells. Quantification of total Ca^{2+} content of sarcoplasmic reticulum (SR) using brief exposure to 10 mM caffeine consistently showed larger central Ca^{2+} stores in group 2 cells. On the other hand the caffeine-releasable peripheral Ca^{2+} stores were larger in group 1 cells. The ratio of central to peripheral Ca^{2+} release was larger at more positive and negative voltages in the group 1 cells. In contrast, in the group 2 cells, the $R_{c/p}$ was constant at all voltages. Nevertheless, the voltage-dependence of the fast central release component was bell-shaped and similar to that of I_{Ca} in both cell groups. Removal of extracellular Ca^{2+} or application of Ni^{2+} (5 mM) suppressed equally I_{Ca} and Ca^{2+} release

from the central release sites triggered by I_{Ca} at +60 mV. Depolarization to +100 mV, where I_{Ca} is absent and Na^+–Ca^{2+} exchanger acts in reverse mode, did not trigger the fast central Ca^{2+} releases in either group, but brief reduction of $[Na^+]_o$ to levels equivalent to $[Na^+]_i$ facilitated fast central Ca^{2+} releases in group 2 myocytes, but not in the group 1 myocytes. In the group 2, long lasting (>1 min) exposures to caffeine (10 mM) or ryanodine (20 μM) significantly suppressed I_{Ca}-triggered central and peripheral Ca^{2+} releases. Our data suggest significant diversity of local Ca^{2+} signaling in rat atrial myocytes. In one group I_{Ca}-triggered peripheral Ca^{2+} release propagates into the interior triggering central Ca^{2+} release with significant delay, and in another group of cells I_{Ca} triggers a significant number of central sites as rapidly and effectively as the peripheral sites, thereby producing more synchronized Ca^{2+} releases throughout the myocytes. We suggest that the two populations of rat atrial myocytes maybe related to the differential development of rudimentary t-tubules but that the fast activation of central release sites during I_{Ca} may require larger SR Ca^{2+} content in the cell interior, thereby generating larger central sparks in the latter group.

Diversity of atrial local Ca^{2+} signaling
:evidence from 2-D confocal imaging in rat atrial myocytes

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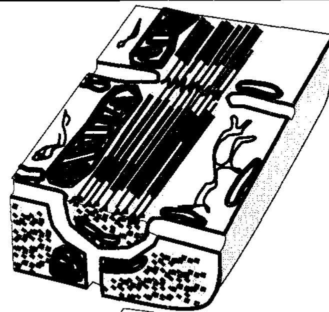
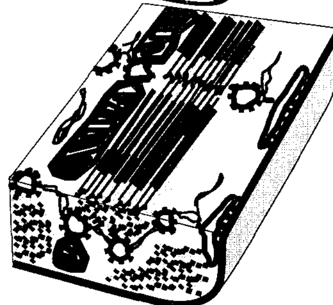
Ventricle

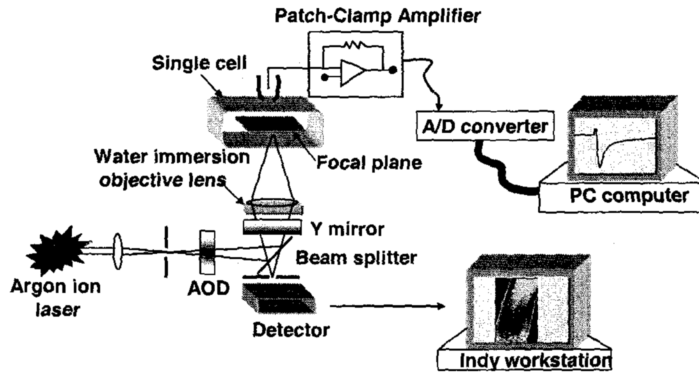


Atrium

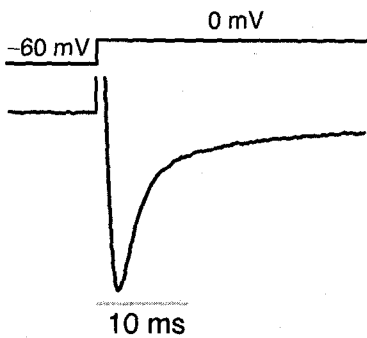


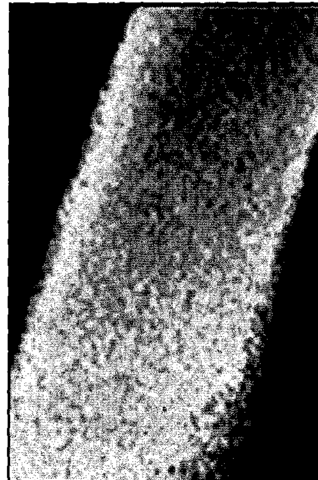
EMs from Carl, Felix, Caswell, Brandt, Ball, Vaghy, Meissner & Ferguson, 1995

Real Time 2D Confocal Imaging Setup (4 ms/image)

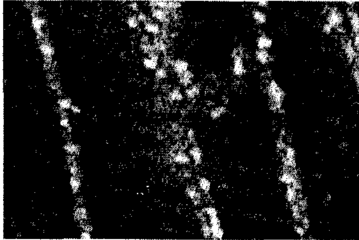


Ventricular myocyte

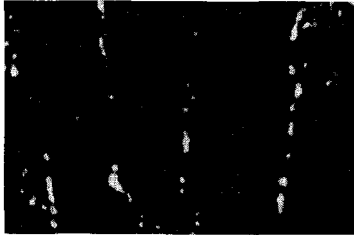




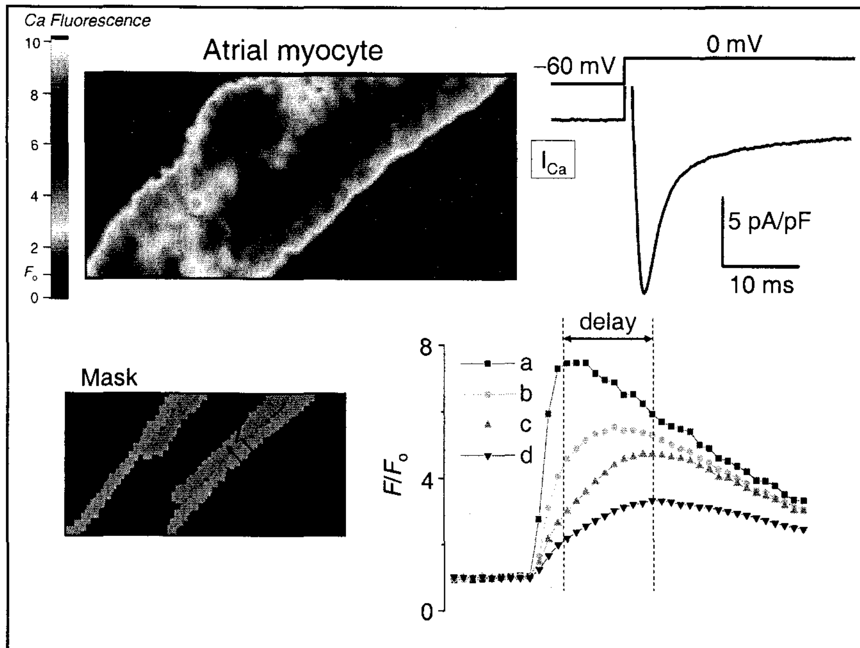
Atrium RyR+DHP
RyR



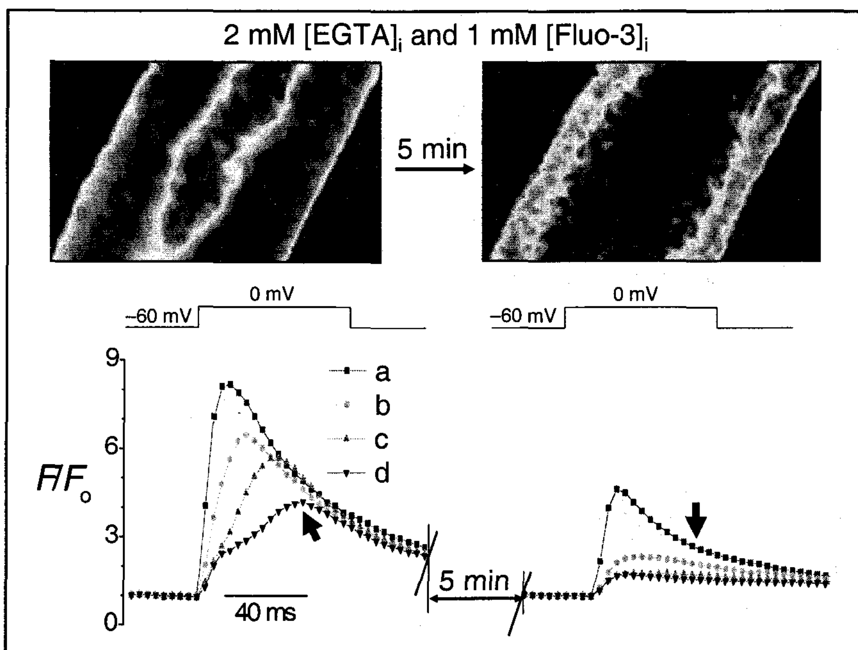
Atrium DHP



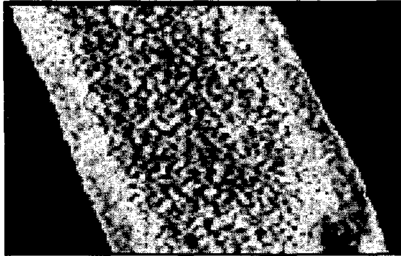
Carl, Felix, Caswell, Brandt, Ball, Vaghy, Meissner & Ferguson, J. Cell. Biol. 1985

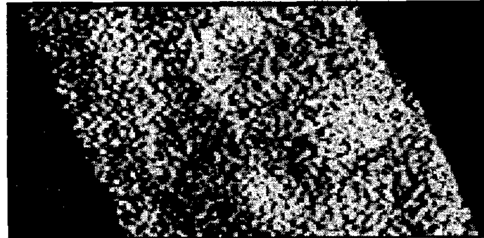


To resolve distinct sparks we introduced high concentrations of Ca^{2+} dye and EGTA into the atrial myocytes.



Two types of local Ca^{2+} signaling in Ca^{2+} buffered atrial myocytes

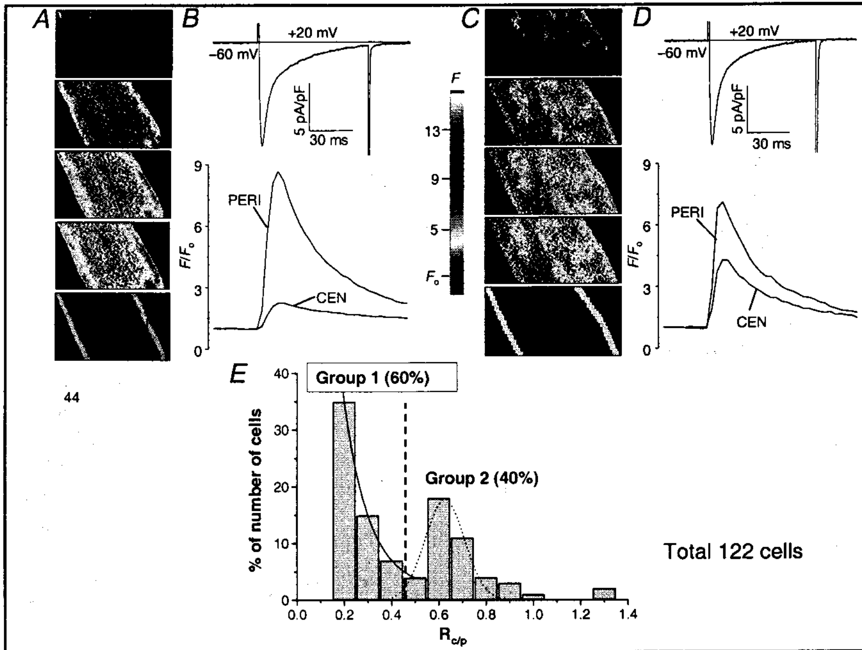


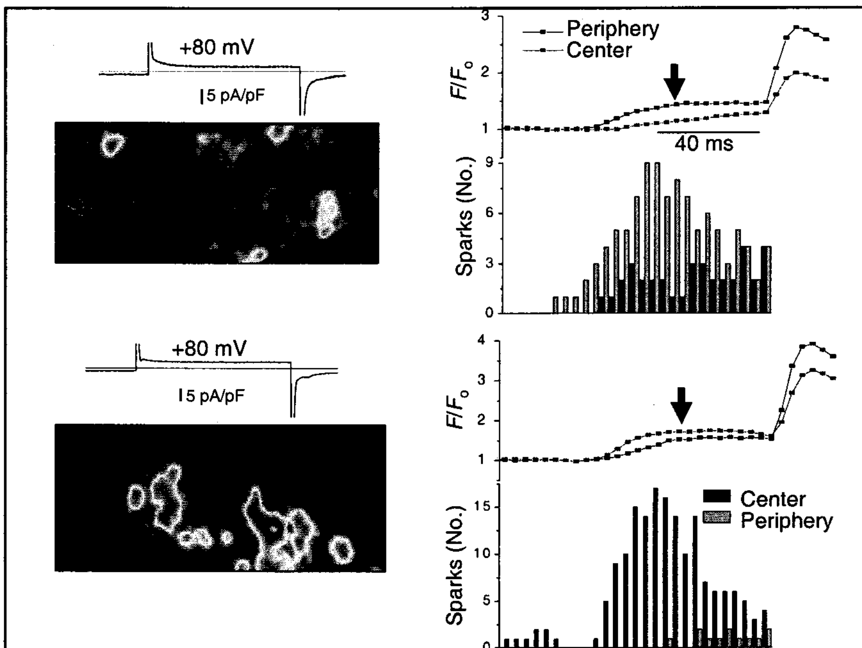


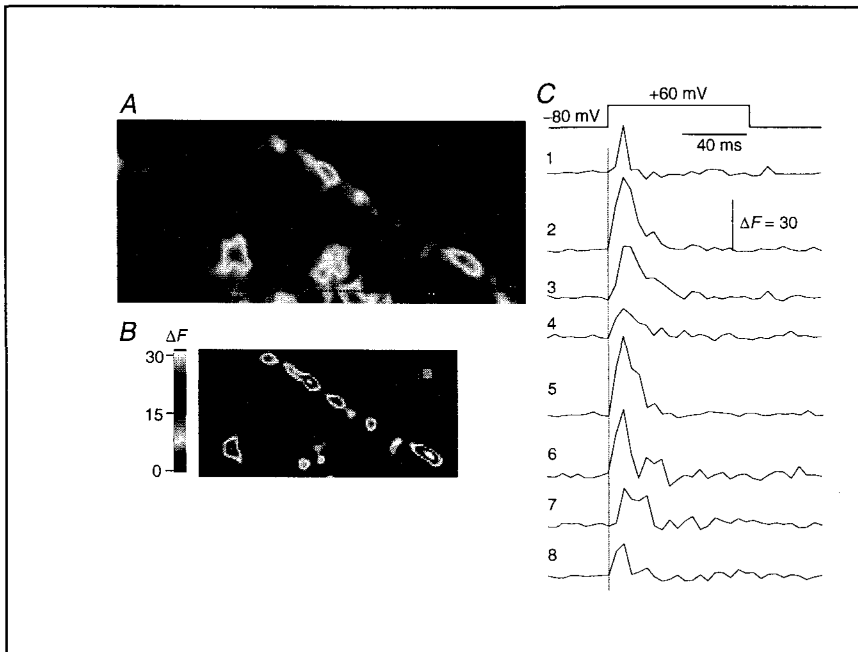
Rat atrial myocytes

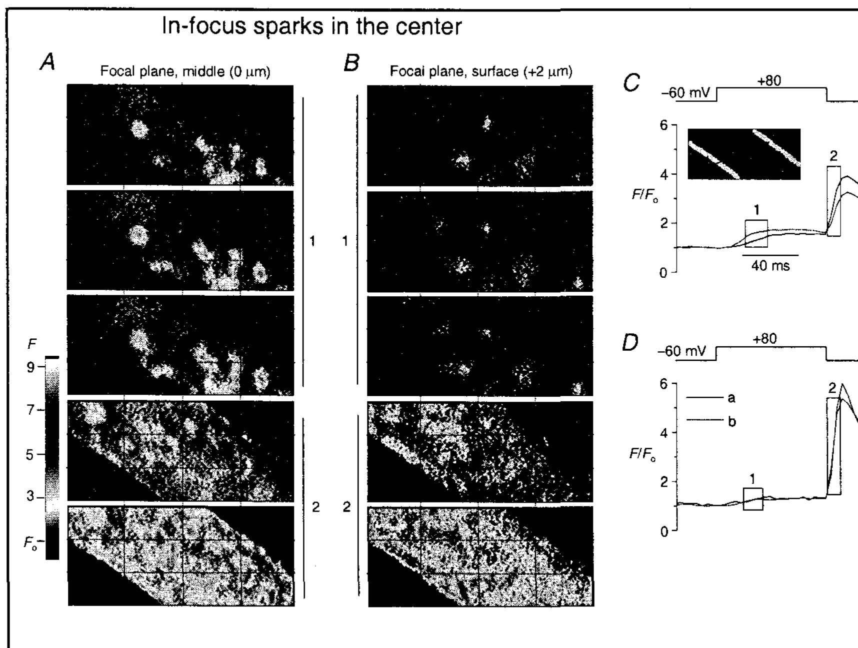
- Primary t-tubules, longitudinal tubules, narrower than those in ventricles
- Invaginated from the surface sarcolemma

Ayettey & Navaratnam, 1978, *J Anat* **127**, 125


A
B
+20 mV
-60 mV
5 pA/pF
30 ms
PERI
CEN
F/Fo
C
F
13
9
5
Fo
D
+20 mV
-60 mV
5 pA/pF
30 ms
PERI
CEN
F/Fo
44
E
Group 1 (60%)
Group 2 (40%)
% of number of cells
Total 122 cells
Rc/p

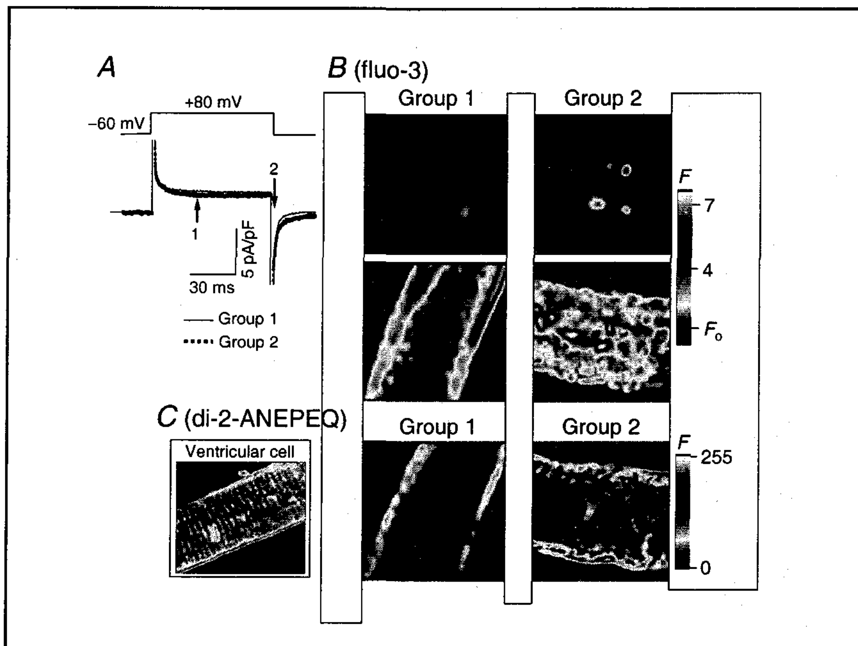

+80 mV
5 pA/pF
Periphery
Center
F/Fo
40 ms
Sparks (No.)
+80 mV
5 pA/pF
F/Fo
Center
Periphery
Sparks (No.)

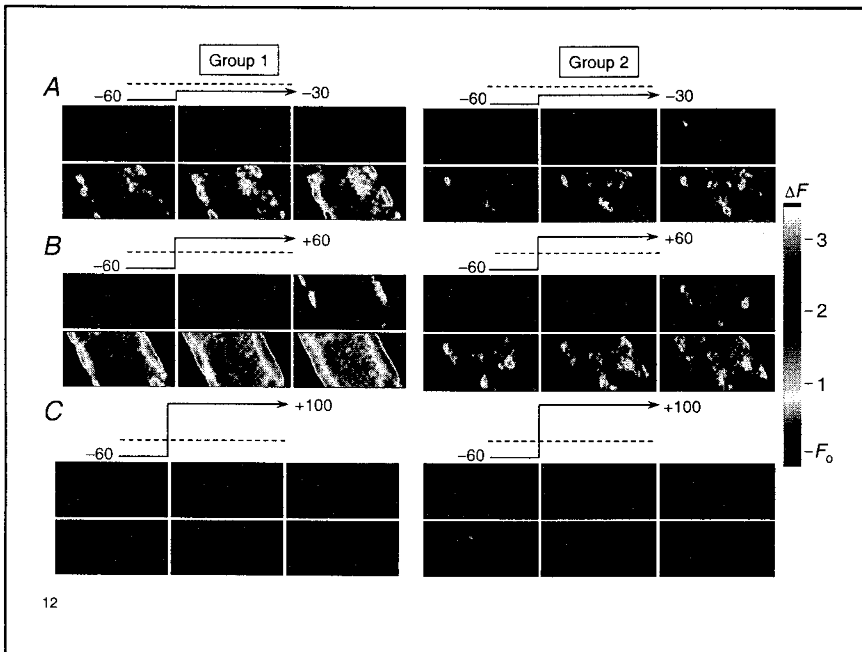

A
B
ΔF
30
15
0
C
+60 mV
-80 mV
40 ms
1
2
ΔF = 30
3
4
5
6
7
8


In-focus sparks in the center
A
Focal plane, middle (0 μm)
B
Focal plane, surface (+2 μm)
F
9
7
5
3
Fo
1
2
C
-60 mV
+80
F/Fo
40 ms
D
a
b

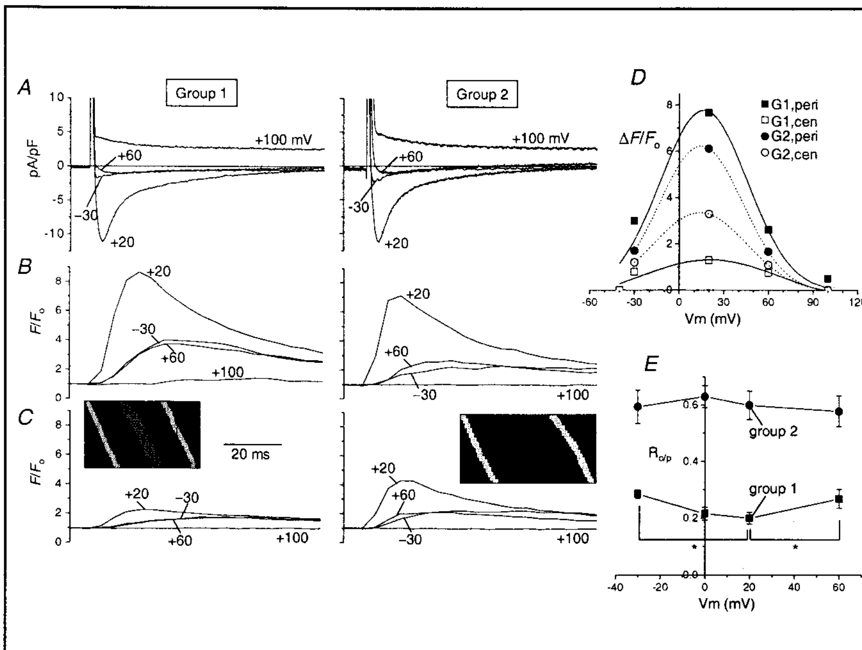
Comparison of the unitary properties of Ca^{2+} sparks from surface and middle of the atrial myocytes

	Surface (+2 μm) (n = 12)	Middle (0 μm) (n = 12)
Amplitude (F_1/F_o)	1.14 ± 0.32	1.77 ± 0.22*
FWHA (μm)	2.28 ± 0.46	1.87 ± 0.35
Size ($μm^2$)	6.71 ± 1.34	8.03 ± 1.71*
Release time (msec)	14.3 ± 1.9	16.6 ± 0.97

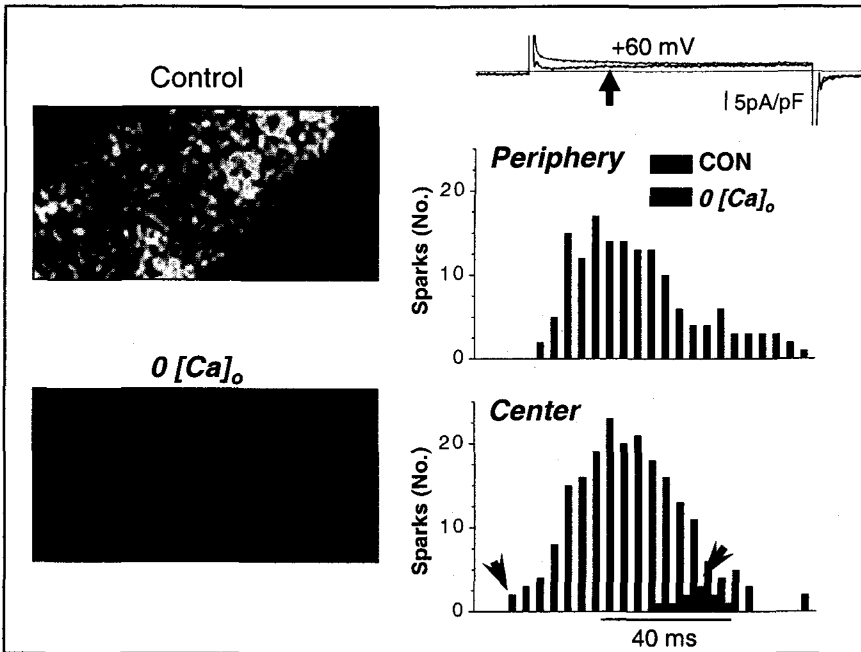


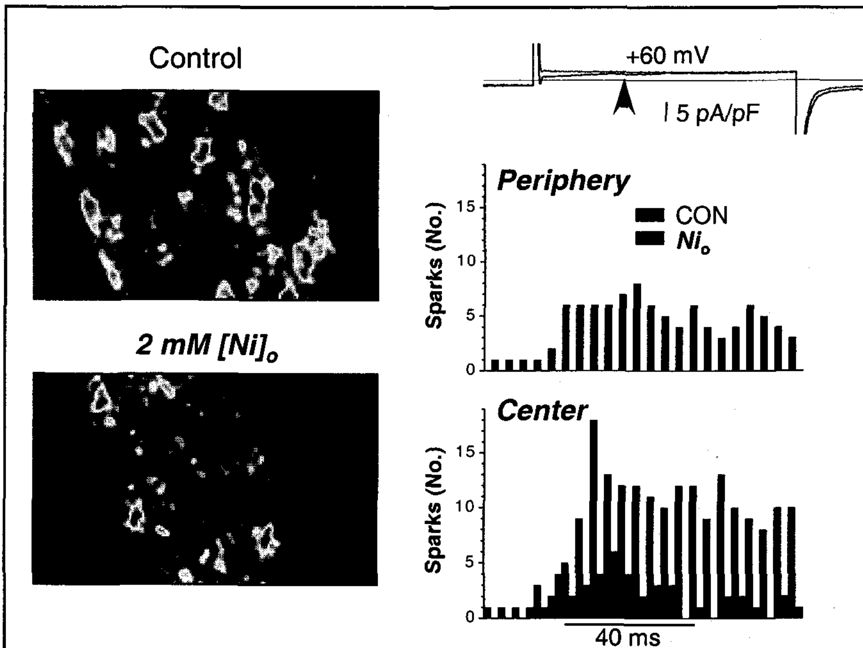


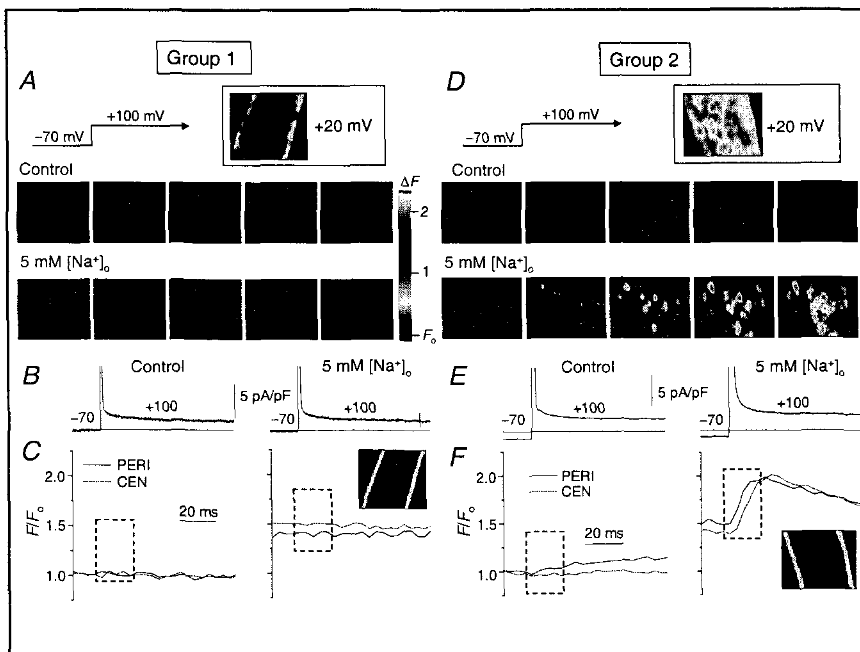
Group 1
Group 2
A
−60
−30
B
+60
C
+100
ΔF
3
2
1
F_o
12



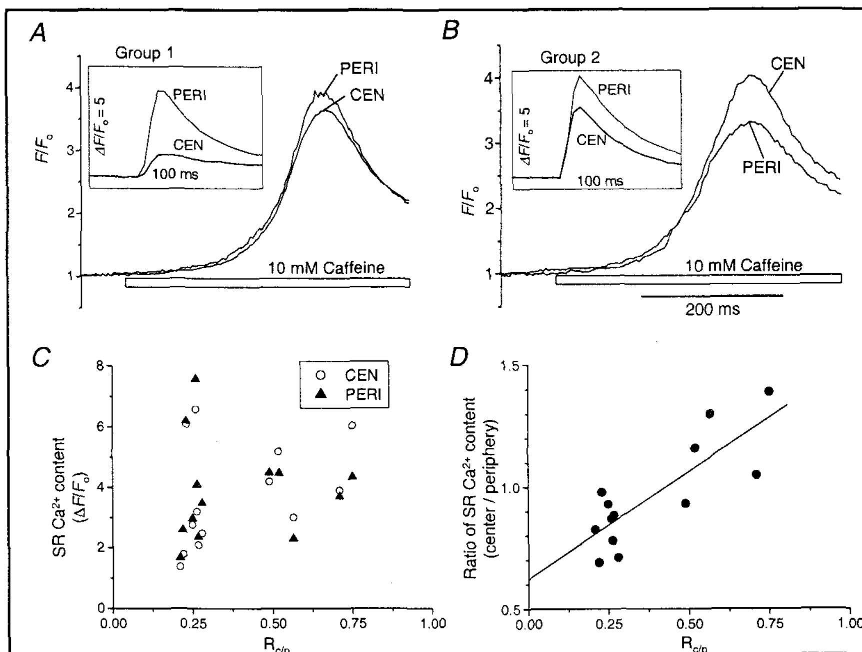
A
Group 1
Group 2
pA/pF
+100 mV
+60
-30
+20
B
F/F_o
+20
-30
+60
+100
C
20 ms
D
ΔF/F_o
G1,peri
G1,cen
G2,peri
G2,cen
Vm (mV)
E
R_o/p
group 2
group 1
Vm (mV)


Control
0 [Ca]o
+60 mV
5pA/pF
Periphery
CON
0 [Ca]o
Sparks (No.)
Center
40 ms

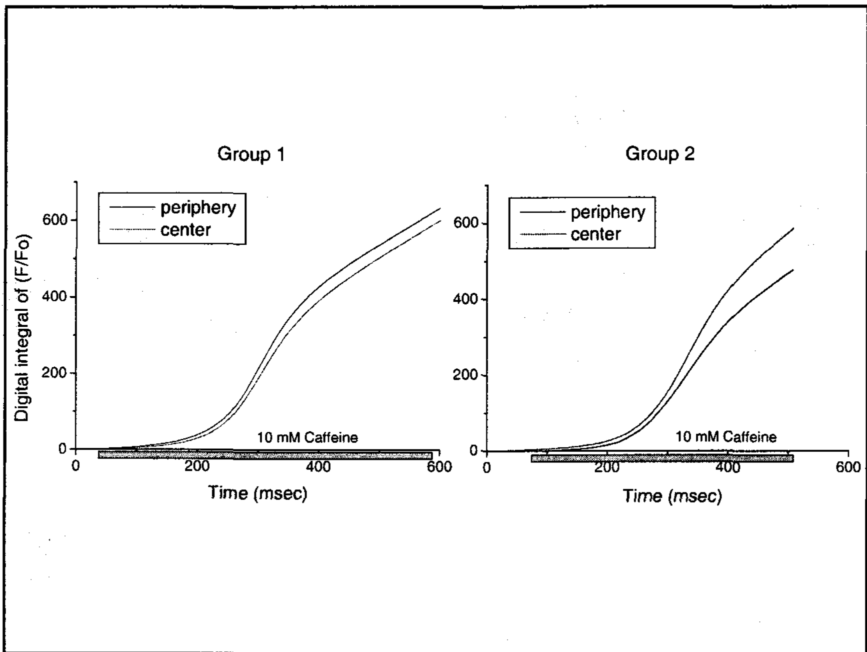

Control
2 mM [Ni]o
+60 mV
5 pA/pF
Periphery
CON
Nio
Sparks (No.)
Center
40 ms

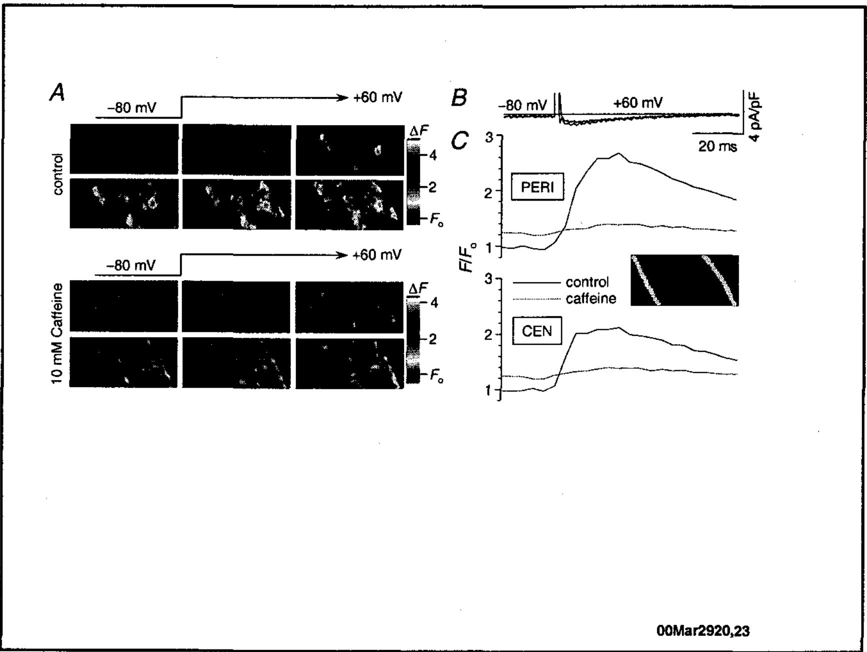


Group 1
Group 2
A
D
+100 mV
-70 mV
+20 mV
Control
ΔF
2
1
F₀
5 mM [Na⁺]ₒ
B
Control
5 mM [Na⁺]ₒ
5 pA/pF
+100
-70
E
C
F
PERI
CEN
20 ms
2.0
1.5
1.0
F/F₀



A
Group 1
PERI
CEN
ΔF/F₀ = 5
100 ms
F/F₀
10 mM Caffeine
B
Group 2
200 ms
C
SR Ca²⁺ content (ΔF/F₀)
CEN
PERI
R c/p
D
Ratio of SR Ca²⁺ content (center / periphery)


Group 1
Group 2
periphery
center
Digital integral of (F/Fo)
10 mM Caffeine
Time (msec)


A
B
C
-80 mV
+60 mV
control
10 mM Caffeine
PERI
CEN
control
caffeine
20 ms
4 pA/pF
00Mar2920,23

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Thank you!