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Supplemental information

Cytosolic Ca^{2+} gradients and mitochondrial Ca^{2+} uptake in resting muscle fibers: A model analysis

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Supplementary text

Numerical data was obtained using an improved version of our model for calcium diffusion in skeletal muscle (1). The diffusion-reaction model of a cylindrical half sarcomere is divided in a mesh with 50 longitudinal and 20 radial compartments with an assumed total length L=1.25 μm and radius R=0.5 μm. Calcium diffusion coefficient is taken to be D=300 $\mu m^2/s$ (2). Indexing the radial compartments with the variable i and the longitudinal ones with the variable j the equation for the radial and longitudinal diffusion between compartments becomes (3, Chap. 8):

$$\frac{\partial[\mathrm{Ca}^{2+}]_{\mathrm{i,j}}}{\partial \mathrm{t}} = 4\mathrm{D}(\mathrm{n}/\mathrm{R})^2[[\mathrm{Ca}^{2+}]_{1,\mathrm{j}} - [\mathrm{Ca}^{2+}]_{0,\mathrm{j}}] + \mathrm{D}\left(\frac{\mathrm{m}}{\mathrm{L}}\right)^2 [[\mathrm{Ca}^{2+}]_{\mathrm{i,j+1}} - 2[\mathrm{Ca}^{2+}]_{\mathrm{i,j}} + [\mathrm{Ca}^{2+}]_{\mathrm{i,j-1}}] + \mathrm{F}\left([\mathrm{Ca}^{2+}]_{\mathrm{i,j}}, \mathrm{t}\right)$$

For the m inner radial compartments $(i = 0)$ and:

$$\frac{\partial[\mathrm{Ca}^{2+}]_{\mathrm{i,j}}}{\partial \mathrm{t}} = \frac{\mathrm{D}(\mathrm{n}/\mathrm{R})^2}{2i}\left[(2\mathrm{i}+1)[\mathrm{Ca}^{2+}]_{\mathrm{i+1,j}} - 4\mathrm{i}[\mathrm{Ca}^{2+}]_{\mathrm{i,j}} + (2\mathrm{i}-1)[\mathrm{Ca}^{2+}]_{\mathrm{i-1,j}}\right] + \mathrm{D}(\mathrm{m}/\mathrm{L})^2\left[[\mathrm{Ca}^{2+}]_{\mathrm{i,j+1}} - 2[\mathrm{Ca}^{2+}]_{\mathrm{i,j}} + [\mathrm{Ca}^{2+}]_{\mathrm{i,j-1}}\right] + \mathrm{F}\left([\mathrm{Ca}^{2+}]_{\mathrm{i,j}}, \mathrm{t}\right)$$

for all other compartments $(i \neq 0)$ up to $i = n - 1$. The radial index for the compartments representative of the sarcoplasmic reticulum being *n*.
$\mathrm{F}\left([\mathrm{Ca}^{2+}]_{\mathrm{i,j}}, \mathrm{t}\right)$ represents the sources and/or sinks factor for parvalbumin (PV), troponin C (TnC), calsequestrin (CASQ), mitochondrial buffers and extracellular buffers as well as the fluxes. All numerical values of the parameters are reported in the supplementary material Table 1. Buffers are modeled as

$$\frac{d[SCa^{2+}]}{dt} = k_{on}[Ca^{2+}]^n[S] - k_{off}[SCa^{2+}]$$

Where $[S]$ is the free buffer concentration, $[SCa^{2+}]$ the bound buffer concentration, with $[S^{Tot}] = [S] + [SCa^{2+}]$ and n simulates the cooperativity, equal to 1 if not present.

TnC, with two calcium binding sites (Tr_1 and Tr_2) with different affinities, is described by:

$$\frac{d[Tr_1Ca^{2+}]}{dt} = k_{Tr1on}[Ca^{2+}]^{n1}[Tr] - k_{Tr1off}[Tr_1Ca^{2+}] - k_{Tr2on}[Ca^{2+}]^{n2}[Tr_1Ca^{2+}] + k_{Tr2off}[Tr_2Ca^{2+}]$$

and:

$$\frac{d[Tr_2Ca^{2+}]}{dt} = k_{Tr2on}[Ca^{2+}]^{n2}[Tr_1Ca^{2+}] - k_{Tr2off}[Tr_2Ca^{2+}]$$

With the constraint $[Tr^{Tot}] = [\mathrm{Tr}] + [Tr_1Ca^{2+}] + [Tr_2Ca^{2+}]$.

The other components of $\mathrm{F}\left([\mathrm{Ca}^{2+}]_{\mathrm{I,j}}, \mathrm{t}\right)$ are calcium fluxes from and to the elements and compartments, through channels and pumps.
RyR channel has a constant leak computed as described in the text.
The reuptake of calcium in the SR is operated by the SERCA, here simulated in a saturable first-order kinetics (4):

$$\left.\frac{\mathrm{dCa}^{2+}}{\mathrm{dt}}\right|_{\mathrm{Up}} = \mathrm{V}_{\mathrm{max}} \frac{[\mathrm{Ca}^{2+}]_{\mathrm{x}}}{[\mathrm{Ca}^{2+}]_{\mathrm{x}} + \mathrm{K}_{\mathrm{m}}}$$

active in each compartment x which is in the i=n-1 radial position. A similar kinetics is imposed to the MCU flux or v_{MCU}

$$\mathrm{v}_{\mathrm{MCU}} = \left.\frac{d\mathrm{Ca}^{2+}}{\mathrm{dt}}\right|_{\mathrm{MCU}} = \mathrm{V}_{\mathrm{MCU}} \frac{[\mathrm{Ca}^{2+}]_{\mathrm{c}}{}^{\mathrm{h}}}{[\mathrm{Ca}^{2+}]_{\mathrm{c}}{}^{\mathrm{h}} + \mathrm{K_d}{}^{\mathrm{h}}}$$

Notably, the $[Ca^{2+}]_{cyto}$ sensed by the MCU is in the element i=n-2, j=m_{RyR}-2, being m_{RyR} the longitudinal index of the compartment which contains the RyR, roughly equating to the average distances inferred from the structural data (see text). The efflux of calcium from the mitochondria via the Sodium-Calcium-Lithium Exchanger (NCLX) follows the model proposed by Dash and Beard (5) with a 3:1 stoichiometry for Na^+ and Ca^{2+}

$$\left.\frac{dCa^{2+}}{dt}\right|_{NCLX} = V_{NCLX}\left(\frac{e^{+\frac{0.5\Delta\Psi_m F}{RT}}\frac{[Na^+]_c^3[Ca^{2+}]_m}{K_{Na}^3 K_{Ca}} - e^{-\frac{0.5\Delta\Psi_m F}{RT}}\frac{[Na^+]_m^3[Ca^{2+}]_c}{K_{Na}^3 K_{Ca}}}{1+\frac{[Na^+]_c^3}{K_{Na}^3}+\frac{[Ca^{2+}]_m}{K_{Ca}}+\frac{[Na^+]_c^3[Ca^{2+}]_m}{K_{Na}^3 K_{Ca}}+\frac{[Na^+]_m^3}{K_{Na}^3}+\frac{[Ca^{2+}]_c}{K_{Ca}}+\frac{[Na^+]_m^3[Ca^{2+}]_c}{K_{Na}^3 K_{Ca}}}\right)$$

In our simplified approach, the values of $[Na^+]_c$, $[Na^+]_m$, and $\Delta\Psi_m$, are assumed to be constant during the train of stimuli. A similar equation is used to simulate the Sodium-Calcium Exchanger (NCX) flux, as proposed in (6). MCU and NCLX have a constant flux of Ca^{2+} even in resting conditions. This would also create a local modification of the Ca^{2+} concentration, though smaller than the one generated by the RyR leakage. However, the size of the compartments is not small enough to appreciate this effect. Since the mitochondria occupy only one compartment, the two fluxes are simply balancing each other.

The influx from the extracellular compartment, operated by the SOCE, is meant to equilibrate the $[Ca^{2+}]_{SR}$ content to keep it at the quiescent level of 500 μM (7)

Supplementary Table 1: Parameters definitions, values adapted at 25°C, and reference (concentration values are express with reference to 1 Liter fiber volume, L_{fiber})

Parameter	Meaning	Value
L	length of half-sarcomere	1.25 µm
R	Radius of sarcomere	0.5 µm
V_{TC}	volume of Terminal Cisternae	3.5% V_{tot}
V_{SR}	volume of Sarcoplasmic Reticulum	5.5% V_{tot}
V_{mito}	Volume of mitochondrion	4.2%-5.6% V_{tot}
$V_{t\text{-}sys}$	Volume of T-tubule system	1% V_{tot}
D	free diffusion coefficient	300 $\mu m^2\ s^{-1}$
CSQ1	Calsequestrin 1 concentration	1200 µM
CSQ2	Calsequestrin 2 concentration	120 µM
k^{CS1}_{ON}	Calsequestrin 1 binding rate	3 10^{-3} µM s^{-1}
k^{CS1}_{OFF}	Calsequestrin 1 unbinding rate	3 s^{-1}
n^{CS1}	Calsequestrin 1 cooperativity	3
k^{CS2}_{ON}	Calsequestrin 2 binding rate	15 10^{-4} µM s^{-1}
k^{CS2}_{OFF}	Calsequestrin 2 unbinding rate	15 10^{-4} µM s^{-1}
n^{CS2}	Calsequestrin 2 cooperativity	1
PV	Parvalbumin concentration	1500 µM
k^{PaCa}_{ON}	PV-Ca binding rate	77.8 µM s^{-1}
k^{PaCa}_{OFF}	PV-Ca unbinding rate	1.7 s^{-1}
Tr	Troponin concentration	240 µM
k^{Tr1}_{ON}	Troponin 1 binding rate	707 µM s^{-1}
k^{Tr1}_{OFF}	Troponin 1 unbinding rate	2885 s^{-1}
k^{Tr2}_{ON}	Troponin 2 binding rate	162.6 10^{-4} µM s^{-1}
k^{Tr2}_{OFF}	Troponin 2 unbinding rate	31.1 s^{-1}
B_{tsys}	T-system buffer	400 µM
T	Absolute temperature	300 K
X_{NCE}	NCLX factor	see text
$\Delta\Psi$	Mitochondrial inner membrane potential	190 mV
K^{NCE}_{Ca}	Ca^{2+} binding constant of NCLX	2.1 µM
K^{NCE}_{Na}	Na^{+} binding constant of NCLX	8200 µM
V_{MCU}	MCU maximum flux rate	22.4 µM s^{-1}
h	Hill parameter for MCU	2
K_d	MCU binding constant for $[Ca^{2+}]$	20 µM
V_{max}	maximum pump rate for SERCA	$5.6{\cdot}10^3$ µM
K_m	SERCA binding constant for $[Ca^{2+}]$	0.5 µM

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