A basic model of calcium homeostasis in non-excitable cells

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Supporting Information S6 Program

Determination of first-order rate constant and Michaelis-Menten as well as hysteretic parameters from the experimental results by Luik et al. [1] and Camello et al. [2], respectively.

Overview

The two folders `'Camello et al'` and `'Luik et al'` contain the extracted data together with the programs estimating the rate parameters for the Ca^{2+} leakages out of the ER. For both the Camello et al. data and the Luik et al. data the Mac program `GraphClick`, (Softonic.com) was used to extract numerical data from the published graphs.

`'Luik et al'` data

Fig S1 gives an overview of the extracted data using Jurkat E6-1 cells. The red dots show the point-wise extracted Ca^{2+} concentration time data made from the authors' published plot. The extracted data are found in the file `Luik_leakage_data.txt` in the subfolder `gnuplot`.

In the subfolder `gnuplot` the Perl script `graph_Ca_leak.pl` contains the gnuplot commands to plot the extracted data and to fit these data to the exponential function

$$A = A_0 \cdot \mathrm{e}^{-\mathrm{k_{exp}} \cdot \mathrm{t}} \tag{S1}$$

where A represents the concentration of Ca^{2+} (in μM) inside the ER at time t (in s) and A_0 is the concentration of Ca^{2+} at t=0.

The file `graph_Ca_leak.pl` is run by pointing the Mac Terminal or the Windows Command Prompt (cmd) to the folder `gnuplot` and writing:

```
perl graph_Ca_leak.pl
```

This will create the files `graph_Ca_leak_Luik.pdf` and `fit.log`. The file `graph_Ca_leak_Luik.pdf` contains the graph of the extracted data (red dots) together with the fitted function Eq S1 (green solid line). The file `fit.log` contains the final set of parameters A_0 and k_{exp} from Eq S1 together with their asymptotic standard errors (Fig S1).

From these data the estimated initial velocity v_0 of Ca^{2+} entering the cytosol can be calculated as:

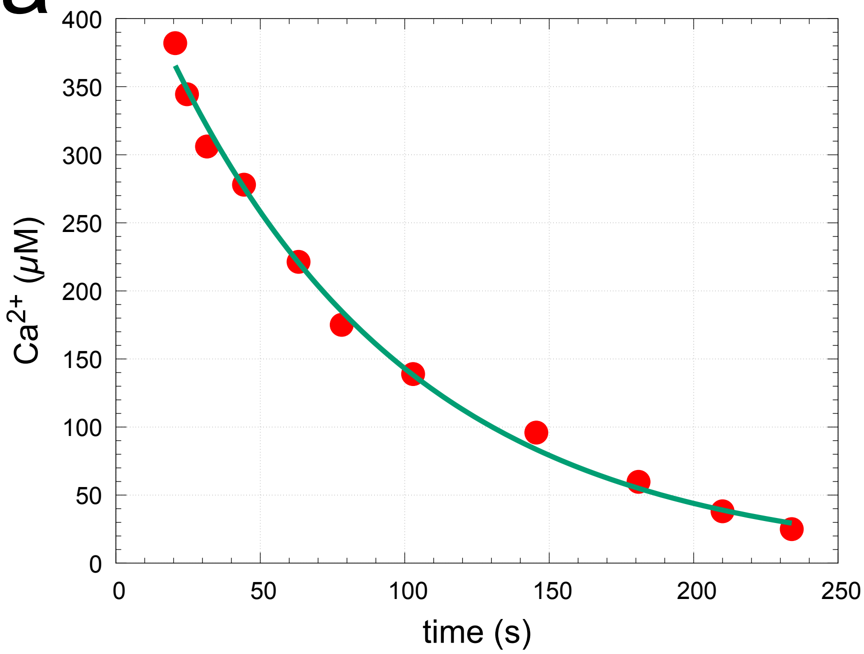
$$v_0 = A_0 \cdot k_{exp} = 465.947 \times 0.01181 \mu M/s = 5.5 \mu M/s \tag{S2}$$

The estimated rate at t=230s is:

$$v = 5.5 \cdot e^{-0.01181 \times 230} \mu M/s = 0.364 \mu M/s \tag{S3}$$

Taken together, the data by Luik et al. clearly suggest that the leak of Ca^{2+} ions from the ER into the cytosol is a first-order process with a rate constant of $0.01181 s^{-1}$.

a



b

```
Final set of parameters            Asymptotic Standard Error
=======================            ==========================
kexp            = 0.0118101        +/- 0.0004463     (3.779%)
A0              = 465.947          +/- 10.31         (2.213%)
```

Figure S1. Panel a: Extracted Ca^{2+} ER concentrations as a function of time (red solid points) from Luik et al. [1] (Fig 1a, lower panel). Panel b: The numerical values of the fitted parameters k_{exp}, and A_0 together with their asymptotic standard errors.

`'Camello et al'` **data**

Camello et al. used pancreatic acinar cells to investigate the calcium leakage from the ER. We have analyzed their data when the SERCA inhibitor thapsigargin (Tg) is applied (Fig 1B, upper panel in [2]). Fig S2a shows, while the ER leaks Ca^{2+}, a graph of the extracted and decreasing luminal Ca^{2+} concentration as red dots. The green solid line shows the calculated luminal Ca^{2+} concentration as a function of time when leakage rate is described as a Michaelis-Menten process where $V_{max}(t)$ is allowed to change (increase hysteretically) with time, i.e.

$$v_{leak} = \frac{V_{max}(t) \cdot Ca^{2+}_{lum}}{K_M + Ca^{2+}_{lum}} \tag{S4}$$

with

$$V_{max}(t) = V_{max}^{final}\left(1 - e^{-\gamma \cdot t}\right) \tag{S5}$$

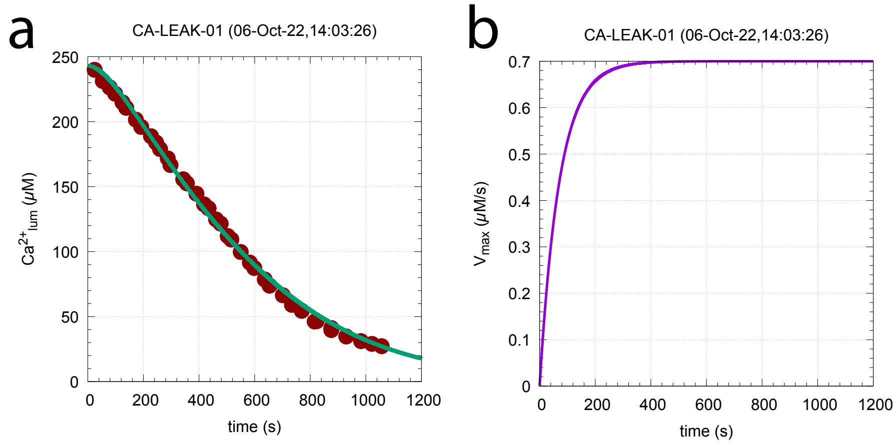


Figure S2. Analysis of Ca leakage data by Camello et al. Panel a: Red dots are the extracted data from [2] (Fig 1B, upper panel). The extracted data are found in file `camello_leak_data_fig1b.txt` located in the `fortran` subfolder. The green solid line is an eye-balled fit by calculating v_{leak} from Eq S4 together with Eq S5. K_M, V_{max}^{final}, and γ were used as adjustable parameters. Panel b: Calculated V_{max} as a function of time using γ=0.014s^{-1} and V_{max}^{final}=0.7μM/s.

Fig S2b shows the change of V_{max} as a function of time when the following parameter values are used: γ=0.014s^{-1}, V_{max}^{final}=0.7μM/s, and K_M=215μM. The initial concentration of Ca_{lum}^{2+} is 243μM.

Running the Michaelis-Menten model (Fig S2)

In the subfolder `fortran` the source file `CA_LEAK.f` is compiled as follows:

Mac OSX: `f77 -o ca_leak -m64 -O2 CA_LEAK.f libV77.a`
Windows: `f77 -o ca_leak.exe -m64 -O2 CA_LEAK.f vms.lib`

which creates the binary files `ca_leak` (Mac) or `ca_leak.exe` (Windows). If both Perl and gnuplot are installed run on Mac in the Terminal the shell script `ca_leak.sh` by the command:

```
./ca_leak.sh
```

Note that the file `ca_leak.sh` should be given execution rights. On Windows computers, open the Command Prompt (Cmd) and write:

```
ca_leak.cmd
```

In both cases (Win or Mac) the two graphs of Fig S2 will appear.

In case gnuplot is not installed execute only the binary files `ca_leak` or `ca_leak.exe` and use other plotting software with respect to the following (comma-delimited) column information:

File `CA-LEAK-01.txt`:
column 1: time (s)
column 2: Ca^{2+}_{lum} (μM)
column 3: not used column 4: $\mathrm{V}_{\mathrm{max}}$ (μM)

File `camello_leak_data_fig1b.txt`:
column 1: extracted time (s) from Ref. [2] (Fig 1B, upper panel)
column 2: extracted Ca^{2+}_{lum} (μM) from Ref. [2] (Fig 1B, upper panel)

Parameter values can be changed through the input file `CA_LEAK.INP`

References

1. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. Nature. 2008;454(7203):538–542.

2. Camello C, Lomax R, Petersen OH, Tepikin A. Calcium leak from intracellular stores—the enigma of calcium signalling. Cell Calcium. 2002;32(5-6):355–361.