Calcium signalling in health and disease

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Calcium in biology

Information transfer by intracellular Ca$^{2+}$
Bio-functions of calcium
“on the path from plasma membrane to store”
Ca\textsuperscript{2+} - the main “secondary messenger”
Cell signaling by calcium (neurona & glia)
Calcium binding proteins
Secondary messengers and $\text{Ca}^{2+}$-oscillations / *cross-talk* of the plasma membrane and $\text{Ca}^{2+}$ stores.
Calcium & neurodegeneration
Calcium fluorescent probe

Measurement of intracellular Ca^{2+}
From Ca$^{2+}$ bufer to Ca$^{2+}$ indicator (sintesis of fluo-3)
Family of Fluo-indicators

![Fluo-indicators molecule](image)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>$K_d$(Ca$^{2+}$)</th>
<th>$R^2$</th>
<th>$R^7$</th>
<th>$R^5$</th>
<th>$R^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluo-3</td>
<td>0.39 µM</td>
<td>Cl</td>
<td>Cl</td>
<td>CH$_3$</td>
<td>H</td>
</tr>
<tr>
<td>Fluo-4</td>
<td>0.35 µM</td>
<td>F</td>
<td>F</td>
<td>CH$_3$</td>
<td>H</td>
</tr>
<tr>
<td>Fluo-5F</td>
<td>2.3 µM</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>H</td>
</tr>
<tr>
<td>Fluo-5N</td>
<td>90 µM</td>
<td>F</td>
<td>F</td>
<td>NO$_2$</td>
<td>H</td>
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<tr>
<td>Fluo-4FF</td>
<td>9.7 µM</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
</tbody>
</table>

**Emission spectra of fluo-3**

- $39.8$ µM
- $1.35$
- $0.60$
- $0.23$
- $0.10$
- $0.017$

Ex = 488 nm
Intensity of fluo-3 fluorescence vs. \( \text{Ca}^{2+} \) concentration

Fluorescencia vs. \([\text{Ca}] \) (\( \mu \text{M} \))

\( K_d = 390 \text{ nM} \)
Raciometric dyes

- BAPTA
- EGTA
- Fura-2
- Calcium Green-1

**Indo-1**

Ex = 338 nm

**Fura-2**

39.8 μM free Ca$^{2+}$

Em = 510 nm
In vivo calibration of the Ca$^{2+}$ signal

\[
\left[Ca\right]_{in}(t) = K_d \cdot \frac{F_0^{380}}{F_{\text{max}}^{380}} \cdot \frac{R(t) - R_{\text{min}}}{R_{\text{max}} - R(t)}
\]
Space & time dynamics of Ca\textsuperscript{2+} in a multicellular system
Probe entrance into the cell (1)

(Roger Y. Tsien)
Probe entrance into the cell (2)

Ext

In
Ca\textsuperscript{2+}-sensitive proteins
Aequorin
Videomicroscopy *in vivo*
Experimental study cases
Amyotrophic lateral sclerosis (ALS)

- Late-onset neuromuscular disorder
- Death of large motor neurons in spinal cord and brainstem
- sALS - sporadic and fALS - familial (5-10%) forms.
- 15-20% familial ALS -> mutant form of Cu$^{+2}$/Zn$^{+2}$ SOD (SOD1)
- Transgenic animal models – mice and rat with mutant hSOD1
Effect of ALS IgGs on astrocytes in culture

CASE 1
Milena Milošević, Centar za lasersku mikroskopiju

Robert Zorec, Matjaž Stenovec, Institut za Patofiziologiju, Laboratorija za Neuroendokrinologiju-Molekularnu ćelijsku fiziologiju, Ljubljana, Slovenija

Zorica Stević, Institut za Neurologiju, Klinički centar Srbije

Vladimir Petrušić, Ljiljana Dimitrijević, Institut za viruslogiju, vakcine i serume - “Torlak”, Srbija
Transfer to mouse induces degeneration of motor neurons, increase in calcium containing organelles (Pullen et al. 2004)

Apoptotic cell death in hybrid motor neuron cell line (Alexianu et al. 1994) and human neuroblastoma cells (Yi et al. 2000)

Activates caspase-3 pathway and induces selective apoptosis of neurons in rat mixed primary spinal cord cultures whereas astrocytes are less susceptible (Demestre et al. 2005)

Increased P/Q type Ca\(^{2+}\) currents in Purkinje cells (Llinas et al., 1993), or decreased Ca\(^{2+}\) currents of cultured granule cells (Zhainazarov et al., 1994).

Cultured hippocampal neurons: increased frequency, but not amplitude of the spontaneous and miniature glutamatergic currents; partly independent of external Ca\(^{2+}\) (Andjus et al. 1997)

Modulate calcium transients (Andjus et al., 1996)

Enhance mobility of acidic vesicles in cultured astrocytes (Stenovec et al., 2011) by affecting calcium homeostasis
Primary cortical astrocytic culture from Wistar P2

Laser scanning confocal microscopy of astrocytes loaded with fluo-calcium indicators Fluo-3 AM and Fluo-4 AM

Relative change in intracellular calcium concentration calculated from the absolute change in the indicator’s intensity normalized by the basal fluorescence:

\[
I_t = \frac{\Delta F}{F_0} = \frac{F_t - F_0}{F_0}
\]

Signal parameters: peak amplitude, time to peak, and time integral

\[
SIgG = \sum_{i=s}^{e} (I_i \times \Delta t)
\]
ALS IgGs evoke calcium transients in cultured rat astrocytes
Various types of transients

(i) single transients
(ii) high frequency bursts
(iii) repetitive calcium transients with variable peak amplitude
Extracellular Ca\textsuperscript{2+} shapes calcium transients

\begin{itemize}
\item 2 mM Ca\textsuperscript{2+}
\item 0 Ca\textsuperscript{2+}
\end{itemize}

\begin{itemize}
\item 10 A.U.
\item 100 s
\item ALS IgG
\item 1 mM ATP
\end{itemize}
Calcium transients are abolished by selective blockers of **IP$_3$-sensitive** but not of **Ryanodine-sensitive** receptors on the endoplasmic reticulum membrane.
Enzymes and/or plasma membrane structures involved in ALS IgG-evoked calcium transients

@PLipazaC  @IP3Kinase

A

<table>
<thead>
<tr>
<th>Cells responding (%)</th>
<th>100</th>
<th>80</th>
<th>60</th>
<th>40</th>
<th>20</th>
<th>0</th>
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<tbody>
<tr>
<td></td>
<td>17</td>
<td>19</td>
<td>43</td>
<td>17</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>515</td>
<td>262</td>
<td></td>
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<td></td>
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</table>

B

<table>
<thead>
<tr>
<th>S IgG / cell (norm. F/F0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
</tr>
</tbody>
</table>

CCE 0 Ca^{2+}

La^{3+}, Gd^{3+}: + + + + + + +
W: + + + - - - -
2-APB: + - - - - - -
U73122: - - - - + - -
Ca^{2+} (mM): 2 2 2 2 2 2 0

@SOCE-memb. ch.
CONCLUSION 1

- IgG from sALS patients, but not from non-ALS controls, evoked complex calcium transients in ~50% of treated astrocytes.
- The probability to evoke calcium transients by ALS IgG did not depend on extracellular calcium.
- ~60% of calcium involved in these responses originates from intracellular organelles, while the remaining ~40% of calcium originates from the extracellular space.
- ALS IgG-evoked Ca^{2+} transients depend on IP₃R, while RyR is not involved.
- The influx of extracellular calcium through SOCE channels prolongs the responses.
- Inhibition of PLC diminishes, while the inhibition of PI3K completely prevents ALS IgG evoked calcium response.

ALS IgG affect calcium homeostatic system in astrocytes by IP₃ mediated calcium release from the endoplasmic reticulum and entry of extracellular calcium through SOCE channels, with the activation of PI3K upstream of PLC.
Effects exogenous mutant SOD1

CASE 2
Aleksandar Bajić, Milena Milošević, Danijela Bataveljić Centar za lasersku mikroskopiju

Ljilja Nikolić Institut za biološka istraživanja “Siniša Stanković”

Jean Pierre Julien Department of Psychiatry and Neuroscience of Laval University, Quebec
Mutant SOD1

Wild-type SOD1

- Aberrant oxidative chemistry from wrong substrate

Mutant SOD1

- Aggregates
- Loss of protein function through co-aggregation

- Aggregates
- Reduced chaperone activity

- Aggregates
- Reduced proteasome activity

Nature Reviews | Neuroscience
Exogenous mSOD forms tetrameric structures that are incorporated in lipid bilayers and from pores

*Allen et al. 2011*
M&M

- Astrocyte cultures from postnatal 2d Wistar rats
- Laser scanning confocal microscopy with Fluo-4 AM
- Application of metalised (Cu, Zn) recombinant mSOD1 i wtSOD1 (Jean Pierre Julien, Université Laval, Quebec)
- Measured parameters: percent if cells with change in intracellular calcium and time integral of transients
mSOD vs wtSOD – Ca^{2+} oscillations
Acute Ca$^{2+}$ response
Data summary

![Graph showing data summary]

- **Integral (ΔF/F₀) over 5 minutes**
  - Spontaneous activity
  - SOD

- **% responding cells**
  - wt SOD (n=15)
  - mSOD (n=26)
Electrophysiological exploration of SOD1 effect on astrocytes
Change in membrane resistance, $R_m$

![Graph showing change in membrane resistance for wtSOD and mSOD](image)

**wtSOD**

- Control (kонтрола)
- 3'
- 5'
- 8'
- 13'

**mSOD**

- Control (kонтрола)
- 3'
- 5'
- 8'
- 13'

Percentages of change in membrane resistance:

- wtSOD: 26%, 40%, 37%, 27%
- mSOD: 35%, 56%, 64%, 55%
Change in current density at -150 mV

wtSOD

mSOD
Thanx!

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- NENS – FENS