Capacitative (Store-Operated) Calcium Entry
Dr. James W. Putney, Jr.

Cellular Ca\(^{2+}\) Signalling
- Contraction
- Secretion
- Metabolic effects
- Growth
- Differentiation
- Death etc.

Two Phases of Calcium Signalling
In a Single Lacrimal Acinar Cell

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Ca\textsuperscript{2+} Signaling Is Comprised of Ca\textsuperscript{2+} from Two Sources

Could IP\textsubscript{3} Be the Signal for Ca\textsuperscript{2+} Entry?
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1. The Regulation of the Cellular Ca\(^{2+}\) Pool

2. Regulation of Ca\(^{2+}\) Release by Carbachol and Atropine

3. Refilling of Ca\(^{2+}\) Pool

Putney, J.W., Muscarinic, α1adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland, J. Physiol., (Lond.) 268: 139-149, 1977


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Capacitative Calcium Entry

Ca$^{2+}$

PLC

Gp

Agonist

Putney, J.W., A model for receptor-regulated calcium entry, Cell Calcium 7:1-12, 1986

SERCA Inhibitors

Cyclopiazonic acid

2,5-di-[tert-butyl]-14-benzohydroquinone (DBHQ)

Thapsigargin

The Effect of Thapsigargin:

a SERCA Inhibitor

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Summary: Ca\(^{2+}\) Exchange

The Effect of TG on Ca\(^{2+}\) Stores and Exchange

I\(_{\text{CRAC}}\)
Calcium Release-Activated Calcium current


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Varying Ionic Selectivity of CCE Channels

<table>
<thead>
<tr>
<th>Highly selective</th>
<th>Moderately selective</th>
<th>Not selective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic cells</td>
<td>Endothelial cells</td>
<td>Smooth muscle</td>
</tr>
<tr>
<td>$I_{\text{calc}}$</td>
<td>A431 cells</td>
<td>Pancreatic acinar cells (mouse)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta cells</td>
</tr>
</tbody>
</table>

Capacitative Calcium Entry (CCE)

- Also called store-operated calcium entry
- Ubiquitous in non-excitable cells, occurs in many excitable cells
- Maintenance of ER/SR calcium homeostasis: a requirement for life
- Involved in calcium signaling, either directly (thymocytes) or in a supporting role (pancreas)
- CCE specifically implicated in inflammation, Alzheimer’s disease, immune deficiencies, oncogenesis, actions of PCBs, heavy metals

The Pharmacology of CCE

Channel blockers: ZAPB, SKF900665, Divalent cations

"Mechanism" inhibitors: Wortmannin, Vas. trafficking inhibitors, Ionophores

SERCA inhibitors: Thapsigargin, Tributyltin, PAHs
Capacitative Calcium Entry:
Key Questions

1. The signal
2. The channel molecule

The Signal for Capacitative Ca\(^{2+}\) Entry Channel

"CIF" Exocytosis

Conformational coupling

STIM

DT40 B-Lymphocytes

Control IP\(_3\)-R-KO

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Partial Purification of CIF

- Human platelets
- Controls: Thapsigargin-activated
- Acid extraction, barium precipitation (removes inositol phosphates)
- Activity detected by injection into oocytes
- Biogel P-2 chromatography
- Activity detected by injection into oocytes
- Anion exchange HPLC
- Heat stable, 600 daltons, phosphate (?)

Csutora, et al., Calcium influx factor is synthesized by yeast and mammalian cells depleted of organellar calcium stores, Proc. Nat. Acad. Sci. USA, 96: 121126, 1999

I\textsubscript{CRAC} Activation in Cell-Lines

I\textsubscript{CRAC} in RBL Cells Activated by IP\textsubscript{3}
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Conclusion

Thapsigargin-stimulated platelets produce a soluble activity that activates the calcium-selective, store-operated current, $I_{\text{CRAC}}$, without discharging intracellular calcium stores.

The Results of RNA Interference Knockdown of STIM1 in HEK293 Cells

Liou et al., Current Biology 15: 1235-1241, 2005

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STIM1 and SOC Activation

EYFP-STIM1

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HEK293 Cells Co-Transfected with YFP-STIM1 and M5-Receptor All Cells Initially in Media Lacking Ca\(^{2+}\) With or Without 5 µM Gd\(^{3+}\)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>11</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 µM CCh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 µM Atrp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1.8 mM Ca(^{2+})</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

TIRF Imaging: EYFP-STIM1

Control  TG, t = 5min

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The Effect of STIM1 Mutant on Ca\(^{2+}\) Signaling

Thapsigargin-activated calcium entry

Constitutive calcium entry

Superimposition of constitutive entry
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The TRP (Transient Receptor Potential) Superfamily

Common Structural Features of Mammalian TRPCs and Drosophila TRP

The Structure of a Functional Channel
Capacitative (Store-Operated) Calcium Entry

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The Regulation of TRPC Channels by Store Depletion

TRPC3 is capacitative

Birnboumer, CHO

Schultz, HEK293

Muallem, HEK293

Putney, HEK293

Putney, DT40

Gill, DT40

HEK293 Cells

DT40 B Lymphocytes

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DT40 B Cells

WT-DT40 Cells, 100µg TRPC3 cDNA-CMV

TRPC3 in DT40 B-Cells

<table>
<thead>
<tr>
<th>Low expression</th>
<th>High expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitative</td>
<td>Non-capacitative</td>
</tr>
<tr>
<td>(probably through DAG)</td>
<td></td>
</tr>
<tr>
<td>Gd&lt;sup&gt;3+&lt;/sup&gt; sensitive (1 µM)</td>
<td>Not Gd&lt;sup&gt;3+&lt;/sup&gt; sensitive (1-10 µM)</td>
</tr>
</tbody>
</table>
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Signalizing Pathways for HTRPC3-Mediated Calcium Entry

- **ATP** → **IP3** → **Ca2+**
- **Ca2+** activates **PKC**
- **PKC** activates **TRPC3**

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Assembly of Functional Store-Operated Channels

- **Ca2+** triggers channel assembly

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Incomplete and Non-Functional Store-Operated Channels

- **Ca2+** fails to trigger channel assembly

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Addendum

After completion and submission of this talk, a series of papers appeared establishing newly discovered proteins, designated as Orai1, 2 and 3, as likely subunits of the CRAC channels. Key references are given below:


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