Release of calcium from IP$_3$-sensitive stores by synaptic activation of mGluRs paired with backpropagating action potentials

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Abstract

Increases in postsynaptic [Ca$^{2+}$]$_i$, can result from Ca$^{2+}$ entry through ligand-gated or voltage-gated Ca$^{2+}$ channels, or through release from intracellular stores. By imaging [Ca$^{2+}$]$_i$ in single neurons, we investigated the mechanism of synaptically activated Ca$^{2+}$ release and the role of backpropagating action potentials in the induction of Ca$^{2+}$ release. Repetitive synaptic activation of metabotropic glutamate receptors, paired with backpropagating action potentials, caused large, wave-like increases in [Ca$^{2+}$]$_i$, predominantly in the proximal apical dendrites (< 100µm) and soma of hippocampal CA1 pyramidal neurons. The wave initiated at branch points in the proximal apical dendrite. [Ca$^{2+}$]$_i$ changes of several micromolars could be reached by the synergistic effect of mGluR-generated IP$_3$ and spike-evoked Ca$^{2+}$ entry acting on the IP$_3$ receptor.

Introduction

Increases in postsynaptic [Ca$^{2+}$]$_i$, can result from entry through ligand-gated channels, entry through voltage-gated channels, or release from intracellular stores. Entry through NMDA receptor channels has received special attention since this entry is Hebbian, requiring both presynaptic activation and postsynaptic depolarization. Here we examine [Ca$^{2+}$]$_i$ increases mediated by metabotropic receptors. These receptors can be activated either by synaptic activity or by the modulatory action of bath applied t-ACPD or Carbachol. Of particular interest is the role of backpropagating action potentials in mediating Ca$^{2+}$ release since these spikes have been implicated in the induction of some forms of LTP and LTD.

Methods

Transverse hippocampal slices were prepared from 2-4 week old rats. Pyramidal neurons from the CA1 region were patched on the soma and loaded with Ca$^{2+}$ indicators (300 µM bis-fura-2 in most cases). Excitatory ionotropic responses were blocked with 10 µM CNQX and 100 µM AP-5. Synaptic stimulation was evoked with a tungsten electrode positioned close to the cell. Fluorescence changes, corresponding to [Ca$^{2+}$]$_i$,
changes, were detected with a high speed, cooled CCD camera.

**Results**

*Ca2+-wave in the main dendrite by repetitive synaptic activation (Fig. 1)*

In the presence of ionotropic glutamate receptor blockers, a train of synaptic stimuli at 100 Hz often caused a delayed increase in [Ca2+]i that spread as a wave over a restricted region of the pyramidal neuron. The electrical response of the cell was usually a small, slow depolarization. (A) Pseudocolor image of the [Ca2+]i change at the time of the arrow in C. (B) Bis-fura-2 filled pyramidal neuron with regions of interest and electrode positions indicated. (C) Time course of [Ca2+]i changes at the selected sites.

![Fig. 1 SYNAPTIC ACTIVATION CAUSES CALCIUM WAVES IN HIPPOCAMPAL PYRAMIDAL NEURONS](image)

*Ca2+ release induced by synaptic activation paired with backpropagating action potentials (Fig. 2)*

In many cells, weaker or less focused synaptic stimulation failed to evoke calcium waves. However, when synaptic stimulation was paired with backpropagating spikes, waves were much more reliably evoked. These waves were also restricted to the soma and proximal dendrites. Spikes alone evoked fast [Ca2+]i changes at all locations.
Ca\textsuperscript{2+} release induced by mGluR activation paired with backpropagating action potentials (Fig. 3)

Ca\textsuperscript{2+} release could also be evoked by spikes if 30 \mu M t-ACPD were added to the ACSF. The agonist acts through mGluR since release could be blocked by 1 mM MCPG.

**Fig. 2** Backpropagating action potentials enhance release of Ca\textsuperscript{2+} from internal stores when paired with synaptic stimulation

**Fig. 3** CALCIUM CAN BE RELEASED FROM INTERNAL STORES WHEN SPIKES ARE EVOKED IN ACSF CONTAINING t-ACPĐ
Conclusions

1. Localized regenerative increases in [Ca\(^{2+}\)]\(_i\) and Ca\(^{2+}\) waves, mediated by IP3-induced Ca\(^{2+}\) release and reaching \(\mu\)M level, were observed in dendrites of hippocampal CA1 neurons.

2. These could be induced by strong synaptic activation of metabotropic receptors coupled to IP3 production. Weaker synaptic activation was needed to induce the [Ca\(^{2+}\)]\(_i\) increase when combined with backpropagating action potentials.

3. Release could be also induced by metabotropic receptor agonists when combined with backpropagating action potentials.

4. The Ca\(^{2+}\)-waves initiated at branch points in the proximal apical dendrite.

5. Below is the possible mechanism of the synergistic activation of IP3 receptors by mGluR activation and backpropagating action potentials.

References

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