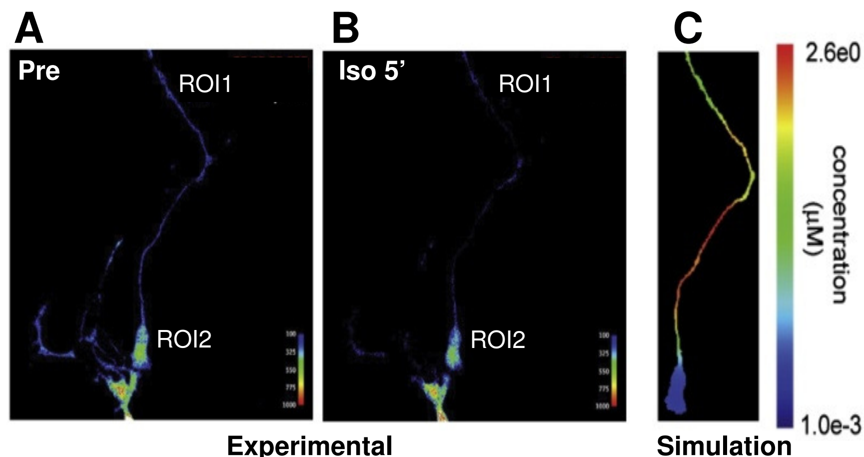
Visualizing the dynamics of cAMP formation in neurons using FRET



Dynamics of cAMP formation in live primary hippocampal neurons (DIV 4) using the cAMP FRET sensor, EPAC1. cAMP FRET was performed on ROI1 (dendrite) and ROI2 (cell body). The images show the calculated nF prior to addition of isoproterenol (panel A) and 5 min post addition (10 mM, panel B). This experimental cAMP FRET was then compared with the simulation results (panel C) in the corresponding locations.

Cellular signal transduction processes often involve the local production of small molecule second messengers, such as cAMP, and local activation of signaling components. This local activation gives rise to spatial segregation of signal flow and leads to the formation of microdomains of activated signaling components. Understanding the origins and dynamics of these microdomains is important for unraveling cellular complexity.

In this study, researchers developed a computational model of signal flow based on the β-adrenergic receptor-cAMP/PKA-MAPK signaling network in neurons. Using this model, they performed numerical simulations to analyze the effect of various factors on microdomain dynamics, before experimentally verifying the results.

As part of this study, the dose response for cAMP formation in response to the receptor agonist isoproterenol was examined. They placed the β-adrenergic receptor and adenylyl cyclase (which synthesizes cAMP) at the cell membrane with an even concentration distribution and started the simulations by activating the receptors with isoproterenol. They showed that although the cAMP production system was activated globally, distinct cAMP microdomains were formed in the distal dendrites with little to no increase in the cell body.

To experimentally verify this, primary hippocampal neurons were transfected with the cAMP FRET probe, EPAC1, and the stimulation of cAMP by isoproterenol was visualized in live neurons as loss of FRET (see figure). FRET occurs between fluorescent proteins fused to the cAMP-binding domain of Epac1. The Epac1-based fluorescent cAMP sensor changes its conformation upon cAMP binding, which results in the decrease in FRET.

FRET was calculated using the net FRET (nF) method in Volocity® 3D Image Analysis Software. The Volocity FRET analysis allows accurate FRET measurements and generation of net FRET (or "corrected" FRET) and normalised FRET images for publication.

The experimental results agreed with the computational simulations with respect to the preferential accumulation of cAMP at the dendrites, providing evidence that the computational model reasonably recapitulates the biochemical dynamics of the signaling network. The researchers went on to suggest mechanisms for the control of spatial information transfer and microdomain characteristics within cells.

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