Mechanism of Sodium Fluoride-Induced Volume Reduction in Rat Hippocampal Slices
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Abstract

Regulation of cell volume is an important aspect of cellular homeostasis during neural activity. This volume regulation is often to be mediated by activation of specific transporters, aquaporins, and volume-activated channels. In cultured astrocytes, swelling-induced MAP kinase activation is involved in the opening of volume-sensitive chloride channels, which are thought to be important in regulatory volume decrease and in the response to OIS to trauma and excitotoxicity. Moreover, NaF, a recognized G protein activator and protein phosphatase inhibitor, leads to significant MAP kinase activation in endothelial cells, but its role in volume regulation in OIS are unknown. We have investigated the mechanisms of NaF-induced volume reduction through MAP kinase pathways in rat hippocampal slices using optical intrinsic signal (OIS) recording with relative changes in intracellular and extracellular volume measured as changes in light transmittance. Decrease of light transmittance (phenomenon of volume) by NaF (1-5 mM) in slices was blocked by U0126 (50 µM), a specific inhibitor of MEK (MAPK/ERK) kinase, with 5-10 µM pre-incubation. Local field potentials evoked responses were reduced at the beginning, but gradually recovered to control level with co-application of NaF and U0126. This reduction induced by NaF did not depend on volume-sensitive anion channels, because increase of transmittance over control level (baseline volume) was observed when rutinum acid (NPA, 200 µM) and NPPB (200 µM), anion channel blocker, were treated after induction of volume reduction by NaF. In addition, decrease of light transmittance was observed when NaF was applied after induction of tissue swelling by anion channel blocker, which indicated volume reduction by NaF was not mediated via volume-sensitive anion channels. Amanitamide (0.01-0.1 µM, aquaporin 4 inhibitor), also failed to recover NaF-induced volume reduction. On the other hand, decreased light transmittance by NaF, was completely recovered to control level by KCC inhibitor, DIOA (10-20 µM). Therefore, tissue shrinkage induced by NaF was mediated by MAPK kinase activation followed by activation of potassium-chloride co-transporter instead of activation of volume-sensitive anion channels and aquaporins.

Material & Methods

How to get OIS in vivo

- Patch clamp recording
- Field potential recording

OIS = Difference images

Sliced reference images from others

Electrophysiology

- Patch Clamp recording

NaF-induced volume reduction by NaF

MAP Kinase

NaF-induced volume reduction by NaF

MAP Kinase

Aquaporin-water channel

NaF-induced volume reduction in hippocampal slices was mediated by OIS technique – which strongly implied the target cells of NaF in gial cells instead of Neurons

This volume reduction mediated via MAP kinase pathway by pre-incubation slices with MAPK inhibitor, U0126, completely blocked NaF-induced volume reduction in hippocampus slices

Aquaporin channel blockers, Niflumic Acid/NPPB, and aquaporin inhibitor, acetazolamide, failed to prevent NaF-induced volume reduction

NaF-induced volume reduction in hippocampal slices was reduced by OIS technique – which strongly implied the target cells of NaF in gial cells instead of Neurons

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