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Abstract and Introduction

Neuronal activity is often accompanied by intracellular acid loads which are cleared by acid-extruding proteins, particularly in glutamatergic terminals with synchronized multirelease burst such as photoreceptor synapses, auditory hair cell synapses, and the Drosophila melanogaster larval neuromuscular junction (NMJ).1 Mutations in human neuronal acid excluders are associated with hyperexcitability disorders and hereditary sensory loss.2 Growing evidence suggests rapid intracellular pH regulation is vital to neuronal function and we seek to uncover cellular mechanisms which integrate pH regulation with neuronal activity through live imaging and electrophysiological assessment of the glutamatergic Drosophila larval NMJ.

Materials and Methods

In Situ Larval NMJ Preparation

- The monitored cytosolic pH in Drosophila larval motor nerve (M) terminals in real-time through neuronal expression of calibrated genetically encoded pH indicators (GEpHs).
- Segmental nerves were dissected and drawn a loop intact nerve into a stimulating suction electrode.

Rationale and Design of a Novel Ratiometric GEPH

- Schematic of ratiometric GEPH (A), Schematic of GEPH in larval MN terminals (B).
- Figure 1 shows a ratiometric GEPH demonstrated pH response. C) Ratio of GEPH/FPP signals in B.

Results

Nerve Activity Generates Ca²⁺-Dependent Acid Loads in Situ and in Vivo

- Acid loads generated in MN terminals (A, B).
- A) Schematic of GEPH (where pH increases with changes in red/green ratio).
- C) Spontaneous bursts of nerve activity (indicated by increased cytosolic Ca²⁺ as detected by the membrane permeant Ca²⁺ indicator BCEA-SE) is removed and replaced with a calcium chelator (repeated measures ANOVA, p<0.05).

The Plasma Membrane Ca²⁺-ATPase (PMCA) is Responsible for Activity-Driven Acid Loads

- The PMCA is responsible for activity-induced acid loads (A).
- A) Molar acid influx during nerve stimulation correlates with the magnitude of cytosolic Ca²⁺ transients. B) Inhibition of the PMCA by high extracellular pH (pH extracellular pH (pH_e) reduces activity-induced acid influx and increases the magnitude of Ca²⁺ transients by impairing Ca²⁺ clearance (paired Student’s t test, p<0.05).

Acid Loads are Cleared by Vascular Acid Extruders Dependent on the Plasma Membrane pH Gradient

- Acid loads are cleared by vascular acid extruders in WT (p<0.05) and mutant flies expressing Tetanus toxin (TetxIMP), and TetxLC-expressing larva. C) Expression of tetanus toxin (inhibition of exocytosis) decreases resting pH and increases acidification of MN terminals (ANOVA, p<0.05). These results demonstrate loss of activity-driven acid clearance.

Conclusions and Future Directions

- The Plasma Membrane Ca²⁺-ATPase (PMCA) is responsible for activity-induced acid loads.
- Acid loads are cleared by vascular acid extruders in WT (p<0.05) and mutant flies expressing Tetanus toxin (TetxIMP), and TetxLC-expressing larva. C) Expression of tetanus toxin (inhibition of exocytosis) decreases resting pH and increases acidification of MN terminals (ANOVA, p<0.05). These results demonstrate loss of activity-driven acid clearance.

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References
