The FASEB Journal / Volume 34, Issue S1

Physiology

A new approach for analyzing pH_i recoveries from NH_4^+-induced acid loads, applied to rat hippocampal neurons and astrocytes

Vernon Ruffin, Walter Boron

First published:18 April 2020
https://doi.org/10.1096/fasebj.2020.34.s1.05675

Abstract

Regulation of intracellular pH (pH_i) in brain cells (neurons and neuroglia) is essential for optimal cell function and primarily maintained by acid/base transporters in the SLC9 and SLC4 families. Large deviations in pH_i can lead to disrupted cellular function or even cell death, whereas small changes have been proposed as a signaling method due to the pH-sensitive residues within protein structure. In the hippocampus (HC), tight regulation of pH_i underlies the cascading reactions and impacts the structure of proteins necessary for learning and memory formation. The present work provides a detailed examination of the methods used in the analysis of steady-state pH_i and the recovery from NH_4/NH_3 induced acidosis by the SLC9s in the absence of CO_2/HCO_3^-, when SLC4 (HCO_3^--sensitive) acid/base transporters are largely inactive. In the present study, we use the pH-sensitive dye BCECF to examine HC neurons and astrocytes, co-cultured from embryonic (E18–20) Sprague Dawley rats. In the protocol, two sequential NH_4/NH_3 pulses are delivered and the steady-state pH_i values is identified before (checkpoint C) and after (E for pulse 1 and F for pulse 2) the NH_4/NH_3 pulse. Typically, steady-state pH_i values at C were more alkaline than E and F, most likely due to the inactivity of HCO_3^--dependent acid loaders in the SLC4 family. The recovery from acidosis is fit with a double exponential (DExp) which we replot as dpH_i/dt vs pH_i. With this traditional approach, dpH_i/dt, as it approaches the asymptotic pH_i, becomes slightly non-linear. To exploit the mainly linear portion of the

dpH_i/dt vs. pH_i plot (from the DExp fit), we fit these dpH_i/dt vs. pH_i points with a single exponential (SExp) to produce a quasi–single-exponential rate constant. This analysis—when transformed to the pH_i vs. time domain—generally produces a very good fit to the original pH_i vs. time data. We summarize the twin pH_i recoveries from individual experiments in thumbnails in which we display the quasi–single-exponential dpH_i/dt line segments that represent the pH_i recoveries from the first and second NH_3/NH_4^+ pulses. For each line segment in the thumbnail, the slope represents the quasi–single-exponential rate constant, and the projected pH_i-axis asymptote represents the predicted new steady-state pH_i. The thumbnails permit a quick assessment of the pH_i recoveries from acidosis in many cells, including a sense of the variability between the first and second pulses, or the variability among cells. The advantage to this approach is that our method of analysis allows an entire population of cells to be examined individually but quickly.

Support or Funding Information

This work was supported by the NIH National Heart, Lung, and Blood Institute. Grant # R01 NS018400