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Novel approach to analyzing steady-state intracellular pH and the recovery from NH₄⁺-induced acidosis in rat hippocampal neurons and astrocytes

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Abstract

Optimal function in the brain, especially in hippocampus—an area involved in learning and memory—requires tight regulation of intracellular pH (pHi) within neurons and neuroglial. The Na-H exchangers (NHEs) are the major family of acid/base proteins involved in regulating pHi in the absence of CO₂/HCO₃. In the present study, we used the pH-sensitive dye BCECF to examine the regulation of steady-state pHi and the recovery of pHi from NH₄⁺-induced intracellular acid loads in HC neurons and astrocytes, co-cultured from embryonic (E18-20) Sprague Dawley rats, and studied in CO₂/HCO₃ --free HEPES buffered (“HEPES”) solutions. After at least 14-days in a CO₂/HCO₃ – incubator, cells were removed, loaded with BCECF, and placed in a recording chamber with flowing HEPES. At the beginning of each experiment, we measured pHi (checkpoint A) after allowing pHi to stabilize for 5 minutes (checkpoint C), and reported mean “initial pHi”/SEM for neurons as 7.351/0.0597; N=37 (astrocytes: 7.189/0.0118, N=25) the value at checkpoint C = (pHi)_C. After using the twin paired NH₄⁺-pulse protocol to acid load cells, we find that—after the pHi recovery from the first acid load—the average neuronal steady-state pHi (now at checkpoint E; (pHi)_E) is 6.953/0.0601 (astrocytes: 7.037/0.0081). After the second NH₄⁺ pulse the neuronal steady-state pHi (now at checkpoint F; (pHi)_F) in neurons is 6.937/0.010 (astrocytes: 7.020/0.0062). The recovery from acidosis is fit with a double exponential (DExp) which we replot as dpHi/dt vs pHi. With this traditional approach, dpHi/dt, the fit as it approaches the

asymptotic pH_i , becomes slightly non-linear. To exploit the mainly linearity portion of the dpH_i/dt vs. pH_i plot (from the DExp fit) of the double exponential, we fit these dpH_i/dt vs. pH_i points with a DExp with a quasi- single exponential (SExp) to produce a quasi-single-exponential rate constant (k_{qSExp}) measured as dpH/dt . 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. The mean $k_{\text{qSExp}1}$ in neurons is 0.0054/ 0.0008 (astrocytes: 0.0107/0.0002) whereas the mean $k_{\text{qSExp}2}$ in neurons is 0.0055/0.0008 (astrocytes: 0.0010/0.0003). We summarize the twin pH_i recoveries from individual experiments in which we display as thumbnails the quasi-single-exponential dpH_i/dt line segments that represent the pH_i recoveries from the first and second $\text{NH}_3/\text{NH}_4^+$ pulses. These new analytical approaches may ultimately provide mechanistic insight into cell-to-cell heterogeneity of pH_i regulation in the nervous system.

This is the full abstract presented at the Experimental Biology meeting and is only available in HTML format. There are no additional versions or additional content available for this abstract.



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