**SUPPLEMENTAL MATERIALS AND METHODS**

**H⁺ Buffering by the Cytosol**

The purpose of this section is to compare two assumptions regarding H⁺ buffering by the muscle cytosol. The assumptions start from two possible definitions of buffering power. The usual way is to define it in terms of pH, as

\[
\text{pH}_{\text{bound}} = k \frac{[\text{H}^+]}{[\text{EGTA}]}.
\]

By contrast, in the present work, we have chosen to define it linearly, as increase in bound H⁺ per unit [H⁺] (Eq. 10). Within the framework of each description, it is convenient to assume that buffering power remains constant within the relevant range of [H⁺]. To evaluate the adequacy of these assumptions we start from the differential equation of reaction between Ca²⁺ and EGTA

\[
d[\text{CaEGTA}] / dt = k_1 [\text{Ca}^2+][\text{EGTA}] - k_{-1}[\text{CaEGTA}].
\]  

Hence,

\[
[\text{Ca}^2+] = K_{\text{Dapp}} \frac{[\text{CaEGTA}]}{[\text{EGTA}]} + \frac{d[\text{CaEGTA}]/dt}{k_1[\text{EGTA}]}.
\]  

Here \( K_{\text{Dapp}} = k_{-1}/k_1 \) is an “apparent” dissociation constant because the reaction between Ca²⁺ and EGTA is influenced by H⁺ in some ranges of pH.

[Ca²⁺] is therefore the sum of two terms, the concentration in equilibrium with the existing forms of EGTA and a term (roughly) proportional to the rate of change of [CaEGTA]. Therefore the first term in S2 is roughly proportional to the total calcium released, while the second term is roughly proportional to the rate of release or release flux. These are approximate because [EGTA] is not strictly constant. Additionally, the change in [CaEGTA] is not exclusively due to release, there is some removal, largely by the pump, which also changes [CaEGTA].

The corresponding set of equations for [H⁺] are written under two alternatives: one is that the change in H⁺ bound to cell buffers, \( d[\text{HL}] \), is proportional to the change in pH. Then

\[
d[\text{HL}] / d(pH) = -\beta = \Delta[\text{HL}] / \Delta(pH)
\]  

(the last equality is true if \( \beta \) is constant).

The change in bound H⁺ is equal to the amount of H⁺ released by EGTA, which is two times the change in [CaEGTA], hence

\[
d[\text{CaEGTA}] / d(pH) = -\beta/2 = \Delta[\text{CaEGTA}] / \Delta(pH).
\]  

This implies that [Ca²⁺] at times when there is no release (so that it is equal to the first term in Eq. S2) should have a linear relationship with pH. This assumption (constant \( \beta \)) therefore predicts proportionality between \( \Delta[\text{H}^+] \) and \( \Delta[\text{Ca}^2+] \). The proportionality constant can be derived if \( \Delta[\text{Ca}^2+] \) is represented by the first term of Eq. S2, then substituted in Eq. S4:

\[
\frac{\Delta[\text{Ca}^2+]}{\Delta(pH)} = K_{\text{Dapp}} \frac{\Delta[\text{CaEGTA}]}{[\text{EGTA}]} = -\beta/2 K_{\text{Dapp}}.
\]  

In the alternative assumption:

\[
d[\text{HL}] / d[\text{H}^+] = \alpha = \Delta[\text{HL}] / \Delta[\text{H}^+].
\]  

From this and the equality between \( \Delta[\text{HL}] \) and \( 2\Delta[\text{CaEGTA}] \), an equation corresponding to Eq. S4 is obtained, which is linear in concentration rather than pH:

\[
d[\text{CaEGTA}] / d[\text{H}^+] = \alpha/2 = \Delta[\text{CaEGTA}] / \Delta[\text{H}^+].
\]
And then, a linear relationship between \([Ca^{2+}]\) and \([H^+]\) replaces Eq. S5. Using Eqs. S2 and S7:

\[
\frac{\Delta[Ca^{2+}]}{\Delta[H^+]} = \frac{K_{\text{dissp}}[Ca\text{EGTA}]}{[EGTA]} = \frac{\alpha}{2} \frac{K_{\text{dissp}}}{[EGTA]}. \tag{S8}
\]

These assumptions are compared in Fig. S1. \(\Delta[Ca^{2+}]\) in the OFF portions of a complete set of data (including reference, conditioning to \(+60\) mV of various durations between 10 and 700 ms, and tests) is plotted versus \(\Delta(pH)\) measured simultaneously. The plot should be linear according to Eq. S5. In B, the same \(\Delta[Ca^{2+}]\) are plotted versus \(\Delta[H^+]\). These should be linear according to Eq. S8. Each set of data was fitted with a linear function (best fit in black trace) or the sum of a line plus an exponential (red). While both linear approximations are good, plot B is closer to linearity. This is seen both in a slightly higher correlation coefficient and lower curvature of the exponential term (figure legend). The difference, however, is small. Either assumption leads to satisfactory results, which is partly a consequence of the limited change of pH in these experiments.

A second way to test the alternative definitions of buffering power is through text Eq. 12, which implies that constancy of \(\alpha\) requires that \(d[H^+] / dt\) and \(\dot{R}(t)\) be linearly related (a relationship that could be modified by a usually negligible pump term). Fig. S2 illustrates the quantitative comparison of \(\dot{R}(t)\) and \(d[H^+] / dt\) in the same experiment. For every test pulse, the \(\dot{R}(t)\) records were plotted against the derivative of simultaneously recorded \([H^+]\) \((t)\). The reference records and the tests after 1 s depletion are plotted versus time for direct comparison in insets. The relationship is linear, except in the regions of rapid change (where the second time derivative of \([H^+]\) or the first of \(\dot{R}(t)\) are high). The traces fall near the same regression line in every test (black, dashed trace), implying constancy of \(\alpha\) over the range of SR content spanned by the records (which in this experiment reached from 100 to 10% of CaSR(0)).

The slope of the line (traced for all data in the figure) was 0.0955, corresponding to \(\alpha = 2.09 \times 10^4\). This number should be compared with the value obtained by Pape et al. (1995) for \(\beta = 22\) mM bound \(H^+\) per log unit of \([H^+]\). The corresponding value of \(\alpha\) can be calculated as \(\beta \Delta \log[H^+] / \Delta[H^+]\). When the test records were obtained, the average \([H^+]\) was 0.55 \(\mu M\), thus \(\alpha\) would be \(4.0 \times 10^4\) according to the estimates of Pape et al. (1955). Again, rough consistency is found between the two approaches.

It is worth examining the small differences between \(\dot{R}(t)\) and \(d[H^+] / dt\) revealed by the comparison in Fig. S2. The arrow represents the direction of time in the parametric plots. During the periods of rapid change, the deviations from linearity are systematic. Before

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**Figure S1.** Comparing two definitions of buffering power. (A) \(\Delta[Ca^{2+}]\) (from ApIII absorbance changes) vs. pH determined simultaneously during application of a complete set of pulse patterns (reference-conditioning test). Only OFF regions are plotted, different colors correspond to applications with different conditioning durations in the range 10–700 ms. Black: linear regression, of slope = −0.200 (\(\mu M/pH\) unit), intercept = 0.141 \(\mu M\), and \(r^2 = 0.957\). Red, dashed: best fit with function \(A + B \exp (k \cdot pH)\), with \(A = −0.014\) \(\mu M\), \(B = 0.099\) \(\mu M\), and \(k = −2.85\). (B) \(\Delta[Ca^{2+}]\) vs. \(\Delta[H^+]\) for the same set of records. 1st order regression slope = 0.223, intercept = −0.008 \(\mu M\), \(r^2 = 0.969\). Exponential fit: \(A = −0.16\) \(\mu M\), \(B = 0.290\) \(\mu M\), \(k = 1.02\) \(\mu M^{-1}\). Identifier 1727.
each peak, the curves tend to be below the regression line, and the opposite happens after the peak. In other words, the H\(^+\) displacement signal lags behind the release flux derived from ApIII signals. This mismatch, a consequence of the slow reaction rate constants of EGTA, is analogous to a redistribution of Ca\(^{2+}\), from fura-2 to EGTA, observed by Pape et al. (1995) upon Ca\(^{2+}\) release by an action potential, and used in that report to evaluate \(\beta\). It is initially surprising that an apparently good agreement between the EGTA/phenol red and the “removal” evaluations of release flux is still found, for both kinetic phases, in spite of the kinetic lag manifest in this plot. The answer lies in the fact that the differences become relatively less important at the peak of release. This fact is illustrated in Fig. S2 by the pink dashed trace, which is the first order regression line through the points that plot maxima of \(d[H^+]_0/\partial t\) versus maxima of \(\check{R}(t)\) (the black circle near the top right corner marks one point in that plot). The regression line through the maxima is nearly indistinguishable from that through all the points (black, dashed).

**Figure S2.** A measure of H\(^+\) buffering power. Rate of change of [H\(^+\)] vs. \(\check{R}(t)\) in a complete experiment. Records were plotted for reference (black) and tests following conditioning pulses of 10 to 700 ms to +60 mV. The arrow marks the direction of time in the parametric plots. Insets plot \(d[H^+]_0/\partial t\) and \(\check{R}(t)\) vs. \(t\) for reference and test after 400 ms depletion. \(d[H^+]_0/\partial t\) is the noisier record in both pairs. Regression line (black, dashed) has intercept \(-0.04\), slope 0.10, and correlation coefficient \(r^2 0.88\). A value of buffering power \(\alpha\) is derived from this slope (see text). The black circle near the top right corner has the maximum of \(\check{R}(t)\) in reference as abscissa, and the corresponding maximum of \(d[H^+]_0/\partial t\) as ordinate. The linear regression through similarly plotted maxima in all records is the pink dashed line, of intercept \(0.16\), slope 0.10, and \(r^2 0.97\). Identifier 1727. Removal parameters had the same values as for ID 1726 (listed in legend of Fig. 6) but for the following, which were obtained from fits to the \([Ca^{2+}](t)\) records. [Parvalbumin] = 2 mM, \(k_{on, Ca \text{EGTA}} = 9.05 \mu M^{-1} s^{-1}\), \(k_{off, Ca \text{EGTA}} = 5.23 s^{-1}\), maximum pump rate = 3.50 mM/s.

![Graph showing the relationship between the rate of change of H\(^+\) and \(\check{R}(t)\).](image-url)