Intracellular pH Buffering Shapes Activity-Dependent Ca$^{2+}$ Dynamics in Dendrites of CA1 Interneurons

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In Dendrites of CA1 Interneurons at high but not low firing rates. These data suggest that activity-threshold Ca channels are the principal route of neuronal-dependent pH i shifts can blunt voltage-gated Ca$^{2+}$ entry during strong depolarization, and cells must restrict this Ca$^{2+}$ signal both temporally and spatially to ensure its specificity. Because Ca currents are most sensitive to H$^-$ inhibition near resting pH (Dixon et al. 1993), Ca channels may be part of a negative feedback circuit in which Ca$^{2+}$ influx during intense periods of activity triggers a local acid shift that then restricts Ca channel conductance. Such negative feedback could prevent a potentially dangerous rise in [Ca$^{2+}$], while allowing Ca$^{2+}$ to fulfill its varied role as an intracellular messenger. In this scenario, intracellular acidosis would effectively act as a low-pass filter for a coincident Ca$^{2+}$ signal.

The goal of this study was to test the possibility of such a feedback circuit by examining the impact of internal pH buffering on dendritic Ca$^{2+}$ transients triggered by brief spike trains. The working hypothesis predicts that submembrane acidosis during cell firing should be less pronounced in strongly pH buffered cells, resulting in reduced Ca channel blockade and facilitating a greater rise in cytosolic Ca$^{2+}$. Because the size of activity-driven H$^-$ transients has been previously correlated with firing rate, hippocampal interneurons were chosen for study as they exhibit high firing rates with little spike frequency adaptation. Overall, the results support a physiological role for pH shifts in mammalian neurons and sharpen the growing view that H$^+$ ions act as important regulatory signals in excitable cells.
METHODS

Slice preparation

Transverse hippocampal slices (300 μm) were prepared from male Sprague-Dawley rats (16–24 days old, 20±40 g) with a vibrating tissue slicer (4 male Sprague-Dawley rats (16 ± 24 days old, 20± 40 g) with a vibrating tissue slicer (4 male Sprague-Dawley rats (16 ± 24 days old, 20± 40 g) with a vibrating tissue slicer (4 male Sprague-Dawley rats (16 ± 24 days old, 20± 40 g) with a vibrating tissue slicer (4 male Sprague-Dawley rats (16 ± 24 days old, 20± 40 g) with a vibrating tissue slicer) and pK were derived with PClamp 6.0 software. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP.

Intracellular recordings

Interneurons located in the s. radiatum region of CA1 were viewed through a Zeiss 40× WI objective (0.75 NA) with transmitted light. No rigorous cell selection criteria were applied, except that large pyramidal shaped cells recently described as “radiation giant cells” were avoided (Maccarelli and McBain 1996). High-resistance seals (~2 GΩ) and subsequent whole cell current-clamp recordings were made with patch pipettes (3–4 mΩ) filled with K-glucuronate-based solutions containing either a nominally “high” or “low” concentration of pH buffers (β). The “high β” solution contained (in mM) 100 K-glucuronate, 40 K-N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 5 3-(N-morpholino)propanesulfonic acid (MOPS), 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP. The “low β” solution contained (in mM) 135 K-gluconate, 5 K-HEPES, 2 MgCl₂, 0.5 K₄-bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid (BAPTA), 2 Na₂ATP, and 0.01 Na-GTP.

FIG. 1. OGB-1 fluorescence is not affected by H⁺ ions in a physiological pH range. The Hill plot depicts background-corrected OGB-1 fluorescence at various pH values in free solution normalized to that recorded at pH 7.2 (F/F₇.₂). In vitro measurements were performed in a slide-mounted cuvette filled with the same high β internal solution (K-glucuronate) used for intracellular recording. This solution also contained 0.5 mM bis-(o-aminophenol)-N,N',N'',N'''-tetraacetic acid and 200 nM free Ca²⁺. Solution pH was adjusted to 7.2 with gluconic acid. In some cases, the pH of the low β solution was adjusted to 6.9; in other experiments, K-methylsulfate (ICN Biomedicals) was used in place of K-glucuronate. For Ca current recording, Cs-gluconate, Cs₂-BAPTA, and the free-acid of HEPES were substituted for their respective potassium salts, and 5 mM TEA-Cl was added (high β: 95 mM Cs-glue; low β: 130 Cs-glue); pH was adjusted to 7.2 with CsOH. Junction potentials for both K- and Cs-based solutions were determined to be ~10 mV (Neher 1992). The concentration of BAPTA in the pipette solutions was estimated to yield a free [Ca²⁺] of ~15–20 nM (Tsien and Pozzan 1989).

The normal internal pH buffering capacity (β) of hippocampal interneurons is not known. However, β can be estimated during whole cell recording if one makes the following two assumptions. First, the rate of buffer exchange from the pipette is slow relative to the kinetics of internal pH transients; second, rapid pH buffering depends primarily on intrinsic buffers rather than membrane transporters, which operate on a second-to-minute timescale. Under these assumptions, the cell can be viewed as a closed system from a pH buffering standpoint. In the presence of 5% CO₂, which is about twice the PCO₂ in vivo, and assuming a normal pH, of 7.2,
Intracellular HCO$_3^-$ is ~18–20 mM and corresponds to a $\beta$ value of ~45 mM (Chesler et al. 1994). Intrinsic pH buffering, provided primarily by large dialysis-resistant proteins and organelles, is ~10 mM (Chesler 1990). Additional buffering provided by the monobasic pH buffers in the pipette solution are 23 and 3 mM for the high and low $\beta$ solutions, respectively. Thus both solutions yield a higher nominal internal $\beta$ (high, 78; low, 58 mM) than thought to exist in vivo (~40 mM), owing largely to the higher concentrations of CO$_2$ present. HEPES and MOPS are rapid diffusable buffers that act to collapse H$^+$ gradients, an effect analogous to that of mobile Ca$^{2+}$ buffers (e.g., BAPTA, Fura-2) on cytosolic Ca$^{2+}$ gradients. The relative importance of HEPES/MOPS buffering becomes more striking when one considers that the speed of HCO$_3^-$ buffering will be reduced as soluble carbonic anhydrase is washed out by internal dialysis.

All cells were initially held in voltage clamp ($V_h = -60$ mV) to measure input resistance ($R_{in}$), series resistance ($R_s$), and cell capacitance. After these measurements, the amplifier (Axopatch 200A) was switched to current-clamp mode, and injected current was adjusted to keep the pipette potential near ~60 mV. Actual $V_m$ was estimated to be near ~70 mV caused by the junction potential offset, although no posthoc correction for this offset was performed. Depolarizing current pulses (400 ms) of varying amplitude (20–250 pA) were applied at 30-s intervals to evoke trains of action potentials (APs). In experiments designed to record Ca currents, cells were held continuously in voltage clamp ($V_h = -60$ mV); Ca currents were then evoked by a series of depolarizing voltage steps (50 ms) between ~50 mV and +20 mV in 10-mV increments at 20-s intervals. For determination of Ca current rundown, repeated voltage steps (50 ms, ~10 mV) were applied every 20 s for 20 min. Capacitive artifacts were canceled electronically and series resistance (6–15 m$\Omega$) was compensated 60–85%. Current and voltage records were sampled at 5–10 kHz, filtered at 5 kHz, and stored on a PC.
Fluorescence imaging

For optical recording of Ca\(^{2+}\) transients, patch pipette tips were filled with internal solution and then backfilled with the same solution containing 100 \(\mu\)M of the Ca\(^{2+}\) indicator Oregon Green 488 BAPTA-1 (OGB-1; Molecular Probes, Eugene, OR). Reducing the dye concentration to 50 \(\mu\)M or less noticeably reduced signal/noise, whereas omitting BAPTA resulted in elevated baseline fluorescence signals that limited detection of small activity-driven transients. The pH sensitivity of OGB-1 was determined in vitro by recording its fluorescence in high \(\beta\) internal solutions containing 200 nM free Ca\(^{2+}\) plus 10 \(\mu\)M of the Ca\(^{2+}\) indicator. The pH of these solutions was adjusted between 4 and 8 by the addition of concentrated HCl/NaOH.

Cells were allowed to “fill” with the indicator for 5–10 min before laser scanning. Cells were illuminated with 488 nm light from a krypton-argon laser; the emitted light was reflected by a dichroic mirror (560LP) and collected by a photomultiplier tube. Fluorescence data were collected by “time-course” software through a 520 / 16 nm band-pass filter. Rectangular regions of interest (ROIs) were positioned along a proximal dendritic segment 10–50 \(\mu\)m from the cell soma and over an adjacent area (for recording background fluorescence). ROI selection in this region was arbitrary, except that dendritic branch points or varicosities were deliberately avoided. In many cases, the dendritic ROI was limited by dendrite orientation or the extent of dye diffusion. Dendrites generally lacked spines, but scattered spines could be clearly resolved along both primary and secondary dendrites in several well-filled cells. Occasionally, ROIs were also placed over sections of the cell soma well separated from the nucleus. Average fluorescence intensities were collected by “time-course” software (TCSM, BioRad) at 3 Hz for short periods (3–10 s) to minimize bleaching and phototoxicity.

Data analysis

AP height, firing frequency, and 50% repolarization time (RT) were measured as described elsewhere (Zhang and McBain 1995). For a given spike train, changes in the RT of the last spike were expressed as a percent of the RT of the first spike. Peak aAHP amplitude (occurring \(\leq 100\) ms after the current pulse) was measured relative to membrane voltage before the current pulse.

Input resistance (\(R_m\)) was determined by applying small negative voltage steps (20 mV) or hyperpolarizing current pulses (10–50 pA). Series resistance (\(R_s\)) and cell capacitance were read directly from the amplifier controls. Cells in which \(R_s\) exceeded 20 m\(\Omega\) or which displayed abrupt shifts in \(R_m\), \(V_m\), or holding current during the course of an experiment were rejected. For voltage-clamp records of Ca currents, steady-state leak currents were subtracted from laser scans. Cells were illuminated with 488 nm light Raw fluorescence values were corrected for background fluorescence before laser scanning. Cells were illuminated with 488 nm light from a krypton-argon laser; the emitted light was reflected by a dichroic mirror (560LP) and collected by a photomultiplier tube. Raw fluorescence values were corrected for background fluorescence and expressed as a percent change in fluorescence relative to membrane voltage before the current pulse. Analysis of the Hill plot yielded a pK of 10.2 ± 0.3. For between-group comparisons, \(\Delta F\) values were normalized by grouping them according to the number of associated APs. The decay of Ca\(^{2+}\) transients was fit with a single exponential and used as an index of Ca\(^{2+}\) clearance (Markram et al. 1995). All analyses of voltage and current records, including curve fitting, were performed with PClamp software (v. 6.0).

Unpaired statistical comparisons were made with a two-tailed Student’s \(t\)-test (Statview 4.0). All data are presented as means ± SE.

RESULTS

Effects of pH, buffering on membrane and dye properties

Because the emission spectra of fluorescein-based dyes tend to be highly pH sensitive, it was important to determine the pH sensitivity of OGB-1, a fluorescent indicator composed of F\(_2\)-fluorescein (Oregon Green; Molecular Probes) conjugated to the Ca\(^{2+}\) chelator BAPTA. Fluorescence data obtained in vitro in the presence of 200 nM free Ca\(^{2+}\) were used to construct a Hill plot in which average fluorescence at a given pH was normalized to that measured at pH 7.2. pH adjustments within the range expected to occur in living cells (pH 6.5–7.5) caused no detectable change in dye fluorescence (Fig. 1). Analysis of the Hill plot yielded a pK of 6.5 ± 0.5.

FIG. 4. Dendritic Ca\(^{2+}\) transients are larger and decay more rapidly than somatic transients. A: fluorescence image of a hippocampal interneuron (s. radiatum) dialyzed with 100 \(\mu\)M OGB-1 (high \(\beta\) solution). Rectangular regions of interest are positioned over the cell soma (S) and proximal dendrite (D). B: transient changes in the normalized fluorescence signal (background corrected) from these 2 regions evoked by a 400-ms current pulse (250 pA). Peak OGB-1 fluorescence in the soma was scaled to that in the dendrite; the fluorescence decay in each region was fit with a single exponential. Tau values represent the time constant of decay.
Differences in the nominal pH buffering ring rates (6 ± 10 mAPs). In the presence of cadmium (200 μM), spike broadening still occurred in both groups, but the difference in RT between groups did not occur at lower firing rates (6–10 APs: high β 87 ± 1 mV, low β 87 ± 6 mV; >25 APs: high β 76 ± 3 mV, low β 78 ± 1 mV, n = 10).

To assess whether differences in β of the internal solutions directly affected Ca channel function, whole cell Ca currents were monitored 10 min after membrane rupture with Cs-based pipette solutions containing the identical type and concentration of pH buffers used in the current-clamp experiments (see METHODS). In both high and low β groups, Ca currents activated near −40 mV, peaked near −10 mV, and exhibited a small degree of rundown as reported previously (Lambert and Wilson 1996). No differences in either peak Ca current amplitude, voltage dependence, or rundown rate (0.7%/min) were detected between the two groups (Fig. 3). In contrast, deliberate acidification of the internal solution (high β) from pH 7.2 to 6.9 depressed Ca currents by ~20% (Fig. 3B).

**Effects of phi buffering on Ca²⁺ transients**

Depolarizing current pulses large enough to trigger a train of APs also evoked Ca²⁺ transients in both the cell body and proximal dendrites. The size of these transients were well correlated to the number of spikes, although dendritic transients were consistently larger and decayed more rapidly than those in the soma (Fig. 4). Ca²⁺ transients in the soma decayed with a time constant of 2.8 ± 0.2 s (n = 5, high β, 250-pA pulse), which did not vary significantly with the current pulse or spike number. Unstable fluorescence signals in the cell soma sometimes precluded reliable optical recordings, a problem rarely encountered in dendrites. Therefore, rigorous unpaired comparisons of somatic Ca²⁺ changes between high and low β cells were not made.

When dendritic Ca transients were normalized for the number of APs, high β cells exhibited larger transients than those recorded in low β cells (Fig. 5), a difference that became more pronounced at higher firing rates (i.e., during larger current pulses). In contrast, Ca transients decayed with a time constant that was not significantly affected by the difference in pH buffering and that did not vary significantly with spike number (Fig. 5). Whole cell dialysis with gluconate-based solutions was reported to depress I_Ca in CA1 pyramidal neurons (Zhang et al. 1994); however, substitution of K-glucuronate with K-methlysulfate (n = 7, low β) did not significantly alter the amplitude of Ca transients (6–15APs: Kgluc 42 ± 4%, KMeSO₄ 42 ± 4%; >25 APs: Kgluc 71 ± 6%, KMeSO₄ 69 ± 5%).

APs in a train tended to increase in duration and decrease slightly in amplitude (Fig. 6A), a feature observed in both high β and low β cells. The initial AP repolarized with a half-time (RT) of 1.5 ± 0.1 ms in both high β and low β groups. In high β cells spike broadening became more pronounced at higher firing rates (26–35 Aps, Fig. 6B). This difference in RT between groups did not occur at lower firing rates (6–10mAPs). In the presence of cadmium (200 μM) spike broadening still occurred in both groups, but the effect of elevated pH buffering on AP repolarization was abolished.

4.9, very close to the pK value of 4.7 reported by the supplier (Molecular Probes) for the unconjugated fluorophore (Oregon Green).

To validate comparisons between high β and low β-dialyzed cells, intrinsic membrane properties were measured in the two groups. Differences in the nominal pH buffering capacity had no detectable effect on $R_m$ (high β: 260 ± 9 mΩ; low β: 267 ± 15 mΩ), $R_i$ (high β: 11 ± 1 mΩ; low β: 10 ± 1 mΩ), or cell capacitance (high β: 14 ± 1 pF; low β: 15 ± 1 pF, n = 34). Similarly, AP number or frequency did not differ significantly between the two experimental groups (Fig. 2). AP height was nearly identical regardless of firing rate (6–10 APs: high β 87 ± 1 mV, low β 87 ± 6 mV; >25 APs: high β 76 ± 3 mV, low β 78 ± 1 mV, n = 10).

**FIG. 5.** Dendritic Ca²⁺ transients are amplified in high β cells. A: examples of Ca transients evoked by 400-ms current pulses (0–250 pA) in a high β and low β cell. B: peak amplitude of these transients were pooled and normalized for the number of action potentials (AP) evoked during the current pulse; *P < 0.05, **P < 0.01 (n = 6, high β; n = 9, low β). C: Ca transient decay (τ) was plotted against the number of APs evoked by the current pulse (derived from data in B).
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FIG. 6. Spike repolarization occurs more slowly in high β cells. A: examples of individual APs from spike trains evoked by small (50 pA) or large (250 pA) current injection (high β). The 1st and last APs are superimposed to illustrate the general feature of spike broadening during a current pulse. B: change in spike repolarization time (RT) of the last spike is expressed as a percent of the RT of the 1st spike. This index of spike repolarization was determined for both high β and low β cells at 2 levels of spike frequency. Note that cadmium (200 μM) abolished the difference in RT between groups by reducing ΔRT in high β cells. * P < 0.05; n = 10 (control), n = 5 (cadmium).

Effects of phi buffering on the sAHP

Trains of APs were usually followed by a slow afterhyperpolarization (sAHP) that lasted for several seconds and could be blocked by cadmium (not shown). The size of this component was determined by measuring its peak amplitude within 100 ms after the current pulse. After “strong” cell depolarization (current pulse ≥150 pA), peak sAHP amplitude was moderately enhanced in high β cells (∼15%, Fig. 7) but was unaffected by substitution of KMeSO\(_4\) for K-glucurate (250 pA: Kgluc -6.7 ± 1 mV; KMeSO\(_4\) -6.8 ± 1 mV, n = 12). No significant difference in sAHP amplitude between low and high β cells was seen with smaller depolarizing pulses. sAHP decay could usually be described by two exponentials. The fast AHP component (tau1) decayed more rapidly in high β cells with increasing cell depolarization, and the decay of the slower AHP component (tau2) was not affected (Fig. 8).

In a few cells, two distinct components of the sAHP could be seen, a rapidly decaying potential followed by one that activated and decayed more slowly (Fig. 9A). In the majority of cells, however, this visual separation was less clear. In such cases, the SK-channel antagonist apamin (50 nM) selectively blocked the early component of the sAHP, which peaked within 50–100 ms after the current pulse. This apamin-sensitive component, after isolation by subtraction (Fig. 9B), could be fit with a single exponential and decayed with a time constant of 150–200 ms (174 ± 12 ms, n = 5).

DISCUSSION

The key findings are that dendritic Ca\(^{2+}\) transients, sAHP amplitude, and sAHP decay in hippocampal interneurons are sensitive to pH\(_i\) buffering. Each of these effects emerged with increased cell activity and could not be ascribed to baseline differences in Ca channel function. These data suggest that during rapid cell firing H\(^+\) transients occur with a magnitude and in a time domain capable of modulating intracellular Ca\(^{2+}\) dynamics very close to dendritic membranes.

During cell activity H\(^+\) may rise gradually in response to continued Ca\(^{2+}\) influx (Ahmed and Conner 1980; Ballanyi et al. 1994; Rose and Deitmer 1995; Trapp et al. 1996b), and this rise in H\(^+\) may limit Ca\(^{2+}\) accumulation by blunting Ca channel activity. According to this model, H\(^+\) inhibition of Ca\(^{2+}\) influx would be expected to be most pronounced near the end of the spike train. Although Ca currents could not be measured directly during current-clamp recordings, a modest increase in AP RT was detected at the end of a
spike train in high β cells during rapid firing rates. This is consistent with an enhanced high-threshold Ca current that can broaden the repolarization of the AP (Llinas and Yarom 1981). That progressive spike broadening occurred in the presence of Cd in both β groups clearly indicates that this phenomenon is not dependent on Ca influx. However, the increase in RT caused by elevated internal β was abolished in the presence of cadmium (which blocks Ca influx during each spike) and was not observed at slow firing rates, when H⁺ shifts were presumably small. This suggests that internal β influenced Ca channels via a mechanism that was sensitive to the level of cell activity.

The most likely explanation for these data is a H⁺-mediated depression of Ca channel conductance, as changes in pH do not alter the kinetics of macroscopic Ca currents (Mironov and Lux 1991). However, one cannot rule out inhibitory H⁺ effects on other channel types. In general, H⁺ modulation of Na and K channels in mammalian neurons is small within a physiological pH range (pH 6.5–8.0) (Tombaugh and Somjen 1998). As an exception, rapid H⁺ inhibition of large-conductance BK channels can occur at physiological pH and could in principle increase spike width (Habartova et al. 1994; Zhang and McBain 1995). If such inhibition occurred during cell firing, one would expect that it would be more pronounced in low β cells, promoting a greater increase in spike width. However, in this study the opposite was observed, arguing against K channel blockade as a dominant mechanism. In the presence of any inhibitory effect of H⁺ on K channels, measurements of AP repolarization may have actually underestimated the degree of H⁺-mediated Ca channel blockade.

**Submembrane H⁺ and Ca²⁺ dynamics**

Ca²⁺ transients in the proximal dendrites were consistently larger and decayed more rapidly than those evoked in
that were detected fluorometrically. One early component of the sAHP also decayed more rapidly in high β cells, suggesting that, in addition to limiting Ca influx, H+ accumulation interfered with Ca2+ sequestration or buffering near the cell membrane. As possible mechanisms, proton accumulation may have impaired H+–Ca2+ exchange (Trapp et al. 1996a) or competed with Ca2+ ions for nonselective binding sites.

In contrast to its effect on sAHP decay, pH buffering had no apparent effect on the decay of OGB-1 fluorescence after a spike train. This discrepancy may reflect the action of high-affinity mobile Ca2+ buffers (e.g., OGB-1, BAPTA). These compounds enhance Ca2+ diffusion away from the membrane (Zhou and Neher 1993), where Ca2+ ions activate KCa channels, and into the bulk cytosol, where the fluorescent Ca2+ signal was actually measured. Such an increase in Ca2+ mobility may have obscured fluorescent detection of a more subtle effect of H+ on Ca2+ clearance near the membrane. Thus fluorescent Ca2+ measurements averaged across even relatively confined regions of a cell may be inadequate for drawing conclusions about the behavior of Ca2+ signals close to their source.

The presence of mobile Ca2+ buffers presumably contributes to the generally slow decay of Ca2+ transients reported here and in other studies (Jaffe et al. 1994). Prolonged Ca2+ transients may also reflect the delayed contribution of Ca2+-induced Ca2+ release (CICR) from internal stores. CICR was proposed to explain the slowly decaying sAHP component seen in rat CA1 pyramidal cells and vagal motoneurons (Lancaster and Zucker 1994; Lasser-Ross et al. 1997; Sah 1992; Sah and McLachlan 1991). Whether CICR occurs in hippocampal interneurons is not known.

The KCa channels that were presumably affected by a H+-mediated reduction in Ca2+ influx are likely to include SK channels. This conclusion is based on the kinetic identity between an early, rapidly decaying component of the sAHP and that component that was blocked by apamin. This component is similar if not identical to GKCa previously described in sympathetic ganglion neurons (Cassell and McLachlan 1987). In addition, SK channels appear to be functionally coupled to N-type Ca channels (Davies et al. 1996), which are more sensitive to internal H+ than the structurally related L-type Ca channel (Tombaugh and Somjen 1997). These findings strengthen the idea that H+ can depress the sAHP indirectly by reducing Ca2+ influx (Church 1992), but they need not preclude other mechanisms. For example, the depression of sAHP in low β cells could reflect a direct inhibitory action of H+ on SK channels themselves.

**Possible sources of error**

In this study, Ca fluorimetry yielded some important insights into the physiological effects of internal pH buffering, but the conclusions regarding H+ dynamics per se remain indirect. Attempts to measure dendritic pH shifts directly with the fluorescent dye SNARF-1 were empirically difficult. In retrospect this was not surprising given the results of some pilot experiments with cultured hippocampal neurons in which SNARF’s pK exhibited an apparent alkaline shift and its isosbestic point shifted to longer wavelengths when...
introduced into cells (G. Tombaugh, unpublished observations). Such changes have been described elsewhere (Owen 1992) and not only limit one’s ability to detect small acid transients but, with a fixed wavelength detection system, also prevent one from making reliable ratiometric measurements.

In considering the results of this study, three additional points should be made. First, fluoroceilin-based Ca\(^{2+}\) indicators can be highly pH sensitive, which raises a valid question about the ion specificity of OGB-1 fluorescence. However, in this study intracellular H\(^+\) shifts were presumably too small (<1.0 pH unit) to affect OGB-1 fluorescence directly. The Ca\(^{2+}\) affinity of BAPTA-based indicators such as OGB-1 are essentially unaffected by protons between pH 6 and 8 (Tsien 1980), and OGB-1 fluorescence is insensitive to H\(^+\) ions within a wide physiological pH range (Fig. 1). Nevertheless, it should be stated that the pK of OGB-1 within cells may differ from that recorded in vitro.

Second, intracellular dialysis with HEPES-buffered solutions may have impaired normal pH regulation via washout of carbonic anhydrase. Moreover, one could argue that, in the face of ongoing metabolic activity, low \(\beta\) cells had a lower resting pH, than high \(\beta\) cells, which could have in turn reduced (1) baseline Ca channel activity or (2) the transmembrane driving force by elevating resting \([Ca^{2+}]_i\). (Ouyang et al. 1994). Neither of these possibilities appear likely given that Ca currents evoked in low and high \(\beta\) cells exhibited nearly identical peak amplitudes, I-V profiles, and rundown rates.

Third, pH\(_{b}\) buffering may have somehow affected the ability of Na\(^+\) APs to invade the proximal dendrites, a process that dictates the size and range of Ca\(^{2+}\) signals in the dendrites of CA1 pyramidal neurons (Jaffe at al. 1992, 1994). A rise in internal H\(^+\) capable of blocking Ca channels would, however, have little direct effect on Na\(^+\) channels, whose unitary conductance, activation, and inactivation properties are relatively insensitive to internal H\(^+\) in a physiological acid pH range (Carbone et al. 1981; Daumas and Anderson 1993). This is important in light of recent reports that back-propagating APs exhibit an activity-dependent decrease in amplitude arising from prolonged Na channel inactivation (Colbert et al. 1997; Jung et al. 1997).

The above argument applies if dendritic APs propagated actively but not in the extreme case in which dendritic Na channels were absent and APs propagated passively. Passive AP propagation into dendrites can be limited by large (2- to 3-fold) differences in internal resistivity (Jaffe et al. 1994), although it is difficult to imagine that such a large difference existed between cells dialyzed with the low and high \(\beta\) internal solutions used in this study. The time course of an AP also influences the extent of its attenuation, with sharper spikes traveling shorter distances (Spruston et al. 1994). However, no significant difference in spike amplitude or rise time (data not shown) was detected between high and low \(\beta\) cells.

**Possible physiological significance**

Active dendritic Ca\(^{2+}\) conductances, specifically those in the proximal dendrites, have been implicated in the integration and amplification of coincident synaptic potentials (Markram et al. 1995; Seamans et al. 1997). By limiting \(G_{Ca}\), a rise in dendritic H\(^+\) may reduce excitatory postsynaptic potential amplification and thus the efficiency with which dendrites conduct electric signals to the cell soma. In addition, when APs invade dendritic membranes, they generate sizable submembrane Ca\(^{2+}\) signals that appear necessary for at least one form of associative synaptic plasticity (Magee and Johnston 1995). Such signal processing may not be limited to parent dendrites but might also occur in dendritic spines and presynaptic terminals, which experience large voltage-gated Ca\(^{2+}\) transients during synaptic activity (Mi-yakawa et al. 1992). If synthetically evoked H\(^+\) transients arise in these spatially restricted regions, the resulting reduction of Ca\(^{2+}\) influx may limit transmitter release or postsynaptic receptor modification in an activity-dependent manner.

Are the size of activity-driven pH\(_{i}\) transients measured previously consistently with the depression of \(G_{Ca}\) in the present study? Internal acid shifts are generally correlated with cell activity but are relatively small (≈0.1 pH), probably reflecting the slow firing rates (≈10 Hz) of the cell types studied (Ahmed and Conner 1980; Ballanyi et al. 1994; Rose and Deitmer 1995; Trapp et al. 1996b). However, as these authors point out, even the most reliable measurements of rapid H\(^+\) transients may represent spatially and temporally filtered reflections of true local changes in pH\(_{i}\), especially in the narrow confines of the submembrane space (Chesler 1990). Thus localized H\(^+\) transients, much like brief Ca\(^{2+}\) transients that can occur in restricted dendritic regions (Ellers et al. 1995), may greatly exceed estimates based on lumped tissue averages or even fluorescent pH\(_{i}\) measurements recorded from cell bodies. In low \(\beta\) cells dendritic pH may have fallen transiently by ≈0.5 pH units, an estimate based on the degree to which Ca\(^{2+}\) transients were influenced by \(G_{Ca}\), the observed 20% depression of \(I_{Ca}\), by internal acidification of 0.3pH units, and earlier findings in isolated neurons (Dixon et al. 1993; Tombaugh and Somjen 1997).

In sum, activity-dependent decreases in pH\(_{i}\) appear to modulate intracellular Ca dynamics in part by limiting Ca\(^{2+}\) influx through voltage-gated Ca channels. Given the correlation between firing rate and acidification, this feedback mechanism may represent an important strategy for inhibitory and perhaps other fast-spiking neurons in which the expression of both Ca\(^{2+}\) permeable AMPA receptors and Ca\(^{2+}\)-binding proteins implies an especially strong need for tight Ca\(^{2+}\) regulation (Gulyas and Freund 1996; Isa et al. 1996). To what extent the present findings apply to other cell types and to adult neurons in situ remains to be tested. On a broader level, however, these results expand the concept of pH regulation in the nervous system and present H\(^+\) ions in a new light, specifically as a conditioner of Ca\(^{2+}\) signals in excitable cells.

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