Barium Plateau Potentials of CA1 Pyramidal Neurons Elicit All-or-None Extracellular Alkaline Shifts Via the Plasma Membrane Calcium ATPase

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Makani S, Chesler M. Barium plateau potentials of CA1 pyramidal neurons elicit all-or-none extracellular alkaline shifts via the plasma membrane calcium ATPase. J Neurophysiol 104: 1438–1444, 2010. First published July 14, 2010; doi:10.1152/jn.00504.2010. In many brain regions, synchronous neural activity causes a rapid rise in extracellular pH. In the CA1 region of hippocampus, this population alkaline transient (PAT) enhances responses from postsynaptic, pH-sensitive N-methyl-D-aspartate (NMDA) receptors. Recently, we showed that the plasma membrane Ca2+-ATPase (PMCA), a ubiquitous transporter that exchanges internal Ca2+ for external H+, is largely responsible for the PAT. It has also been shown that a PAT can be generated after replacing extracellular Ca2+ with Ba2+. The cause of this PAT is unknown, however, because the ability of the mammalian PMCA to transport Ba2+ is unclear. If the PMCA did not carry Ba2+, a different alkalinizing source would have to be postulated. Here, we address this issue in mouse hippocampal slices, using carboxyeosin and therefore could be entirely attributed to proton transport by the PMCA. These results suggest that the PMCA activities of the pyramidal neurons that likely undergo a prolonged cycle of Ba2+ entry and extrusion during the long plateau potential. Thus the exaggerated, sustained electrical response from rat hippocampus (Grichtchenko and Chesler 1996; Smith and Chesler 1999) and lateral geniculate nucleus (Tong and Chesler 1999), robust PATs were noted when Ba2+ was substituted for Ca2+. If Ba2+ were ineffectively carried by the PMCA, the mechanism for the generation of these Ba2+-dependent PATs would have to differ in some marked fashion. For example, the responses might arise from a distinct transport mechanism or they could be due to significant alterations in the electrophysiology of the cell. The latter possibility warrants particular attention, because Ba2+ blocks most K+ channels and can have pronounced effects on the regenerative behavior of excitable cells, often leading to the occurrence of prolonged plateau depolarizations (Blitzer et al. 1991; Eckert and Chad 1984), sustained depolarization might enable sufficient entry of this ion to activate the PMCA.

We recently described the generation of surface pH changes on single CA1 pyramidal neurons studied in normal saline (Makani and Chesler 2010). Here, we used the same approach to study the surface alkaline transient (SAT) in Ba2+ media. We show that, in Ba2+ saline, an antidiromic stimulus to the alveus elicited an all-or-none plateau potential in these neurons that could last many seconds. The plateau potentials were accompanied by a large, all-or-none, extracellular PAT in stratum pyramidale. Using a prerecorded plateau response as the voltage-clamp command, SATs could be elicited on single pyramidal neurons that were approximately one seventh the amplitude of the PAT. The SATs were blocked by intracellular carboxyeosin and therefore could be entirely attributed to proton transport by the PMCA. These results suggest that the large PAT in Ba2+ media is caused by the sustained summated PMCA activities of the pyramidal neurons that likely undergo a prolonged cycle of Ba2+ entry and extrusion during the long plateau potential.

Because many VGCCs are highly permeable to Ba2+ (Hille 2001), this ion can readily enter and accumulate in neurons in response to membrane depolarization. However, the degree to which the PMCA can subsequently extrude this ion has been debated. Recently, Thomas (2009) reported that the PMCA of snail neurons could indeed transport Ba2+, albeit five times less effectively than Ca2+. Earlier studies suggested that the transporter uses Ba2+ very poorly (Duncan 1976; Pfleger and Wolf 1975; Yamaguchi et al. 1989) or not at all (Zhang et al. 1992; Zhao and Dhalla 1988).

In view of these studies, it is unclear whether the PMCA of mammalian neurons could extrude Ba2+ in exchange for H+ and thereby generate a PAT in brain slices. However, in slices from rat hippocampus (Grichtchenko and Chesler 1996; Smith and Chesler 1999) and lateral geniculate nucleus (Tong and Chesler 1999), robust PATs were noted when Ba2+ was substituted for Ca2+. If Ba2+ were ineffectively carried by the PMCA, the mechanism for the generation of these Ba2+-dependent PATs would have to differ in some marked fashion. For example, the responses might arise from a distinct transport mechanism or they could be due to significant alterations in the electrophysiology of the cell. The latter possibility warrants particular attention, because Ba2+ blocks most K+ channels and can have pronounced effects on the regenerative behavior of excitable cells, often leading to the occurrence of prolonged plateau depolarizations (Blitzer et al. 1991; Eckert and Chad 1984), sustained depolarization might enable sufficient entry of this ion to activate the PMCA.

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INTRODUCTION

Calcium enters neurons through a variety of pathways, including voltage-gated Ca2+ channels (VGCCs) and N-methyl-D-aspartate (NMDA) receptors. A consequence of Ca2+ influx is the activation of the plasma membrane Ca2+-ATPase (PMCA), a ubiquitous transporter that extrudes cytosolic Ca2+ in exchange for extracellular protons (Carafoli 1991; Carafoli and Stauffer 1994). This cation exchange can cause a measurable increase in the surface pH of a single neuron (Kreitzer et al. 2007; Makani and Chesler 2010; Molina et al. 2004; Schwiening et al. 1993). Moreover, when many neurons are activated synchronously, the PMCA of the cell population can give rise to a large, widespread rise in extracellular pH (Makani and Chesler 2010). This population alkaline transient (PAT) can in turn augment additional Ca2+ entry by increasing the current through pH-sensitive NMDA receptors (Makani and Chesler 2007).

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responses that are characteristic in Ba\(^{2+}\) media are likely to be accompanied by similarly aberrant changes in the transmembrane pH gradient.

**METHODS**

**Preparation and solutions**

**BRAIN SLICE PREPARATION.** Transverse hippocampal slices were prepared from P6–P14 mice of either sex. All procedures were performed with the approval of the New York University School of Medicine Institutional Animal Care and Use Committee. The brain was blocked in ice-cold artificial cerebrospinal fluid (ACSF) and cut into 250 \(\mu\)m slices using a Vibratome. The slices were incubated in standard ACSF at room temperature for \(\approx 1\) h before use. Experiments were conducted in a submersion-style incubation chamber at 32°C. ACSF was equilibrated with 95% O\(_2\)-5% CO\(_2\) (pH 7.4), as described in Table 1. The poorly permeant carbonic anhydrase inhibitor benzolamide (10 \(\mu\)M) was included in all solutions to decrease the effective extracellular buffering capacity, thereby keeping all extracellular pH changes relatively large and independent of possible differences in extracellular carbonic anhydrase activity (Chen and Chesler 1992a). To prevent HCO\(_3\)\(^-\) efflux through GABA\(_A\) anion channels, which could cause a confounding extracellular alkalosis (Chen and Chesler 1992b; Kaïa and Voipio 1987), picrotoxin was included in all experiments.

**DRUGS.** Drugs were added to the external ACSF or to the intracellular pipette solution, as noted, in the following concentrations (in \(\mu\)M): 50 D-2-amino-5-phosphonovalerate (APV), 10 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX), 10 benzolamide, 100 picrotoxin, and 5 carboxyeosin. Benzolamide was a gift from Dr. Erik Swenson (University of Washington, Seattle, WA). All other agents were obtained from Sigma-Aldrich (St. Louis, MO).

**Concentric pH-sensitive microelectrodes**

\(pH\)-sensitive microelectrodes with response times of a few milliseconds were fabricated as described previously (Fedirko et al. 2006). In brief, a thin-walled borosilicate glass capillary with outer diameter (OD) of 2.0 mm and inner diameter (ID) of 1.5 mm (6185, A-M Systems, Carlsborg, WA) was pulled to a tip size of 2–4 \(\mu\)m and silanized by injection of pure N,N-dimethyltrimethylsilylamine (Fluka 41720), followed by heating with a hot air gun. A pH-selective mixture (Fluka 95291) was introduced into the tip by suction. The inner micropipette was pulled from thin-walled glass (OD, 1.2 mm; ID, 0.9 mm; #6160; A-M Systems) to a tip diameter of \(\sim 0.5\) \(\mu\)m. This pipette was filled with phosphate-buffered 3 M KCl (pH 7.4) and inserted within the outer, ion-selective barrel and into the ion exchange column. With its tip 4–6 \(\mu\)m from the end of the outer pipette, the inner pipette was secured in place with dental wax. All pH electrodes were fitted with Ag–AgCl junctions that fed a high-input impedance (>10\(^{13}\) \(\Omega\)) head stage and were calibrated in 50 mM K\(^+\)/Na\(^+\) phosphate buffers of pH 6.87 and 7.42. The slope response for the concentric electrodes was 57–59 mV per decade change in H\(^+\) activity. The mean response time constant of similar concentric pH microelectrodes constructed in this laboratory was reported to be 15 ms (Fedirko et al. 2006).

**Whole cell recording**

The somata of CA1 pyramidal neurons were visualized under infrared differential interference contrast microscopy using a Zeiss Axioscope 2 Plus, fixed-stage microscope, fitted with a 40\(\times\), water immersion objective (0.75 numerical aperture), and an Olympus Optical 150 video camera. Patch pipettes were pulled from 1.5 mm OD \(\times 1.12\) mm ID borosilicate tubing (World Precision Instruments, Sarasota, FL) using a Narishige PP-830 two-stage puller. The intracellular filling solution contained the following (in mM): 120 K-glucuronate, 20 KCl, 2.0 MgCl\(_2\), 25 Na-HEPES, and 2 Mg\(^{2+}\)-ATP. After adjusting the pH to 7.3 with KOH, the final osmolarity was 280–290 mOsm. Pipettes had resistances of 3–5 M\(\Omega\). In current-clamp experiments, current was first injected to bring the resting membrane potential to nominal –80 mV. Data were acquired using an Axopatch 1D amplifier and Digidata board 1320A, controlled by Clampex 8.2, and analyzed using ClampFit (Molecular Devices, Union City, CA). Data were accepted if cells had a series resistance of <25 M\(\Omega\) that did not change by >20% during the experiment.

**Antidromic stimulation of the CA1 neuronal population**

Constant current pulses of 300 \(\mu\)s duration were delivered to the alveus by a pair of 50 \(\mu\)m diameter, Teflon-insulated platinum-iridium wires in saline containing 10 \(\mu\)M DNQX, 50 \(\mu\)M APV, and 100 \(\mu\)M picrotoxin. These agents insured that activation did not occur via synaptic transmission. Pure antidromic invasion was confirmed by monitoring the short-latency, extracellular, population spike.

**Recording of population alkaline transients**

The concentric pH microelectrode and a separate reference micropipette were mounted on a dual micromanipulator, with a tip separation of 5–10 \(\mu\)m, as described by Fedirko et al. (2006). The array was advanced into the CA1 cell body layer until the antidromic population spike was maximal, which typically occurred at a depth of 100–150 \(\mu\)m. Capacitance neutralization was used to match the time constant of the reference and pH electrodes, as judged by the response to a 1 ms, 1 mV calibration pulse in the common ground circuit. Slow DC potentials recorded on the reference barrel were continuously subtracted from the pH recording to yield the H\(^+\) signal. Stimuli were delivered every 2 min to obtain raw traces.

**Recording of surface alkaline transients**

Surface alkaline transients were recorded as described by Makani and Chesler (2010). In brief, a gigohm seal was obtained on the targeted soma with a standard patch pipette before placement of the surface pH microelectrode. The concentric pH microelectrode was subsequently advanced until its tip made contact with the same cell body. Brief suction was applied to the patch pipette to break through

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**TABLE 1. Composition of the ACSF solutions**

<table>
<thead>
<tr>
<th>Solution</th>
<th>NaCl</th>
<th>KCl</th>
<th>CaCl(_2)</th>
<th>BaCl(_2)</th>
<th>MgCl(_2)</th>
<th>NaHCO(_3)</th>
<th>NaH(_2)PO(_4)</th>
<th>Glucose</th>
<th>EGTA</th>
<th>HEPES</th>
</tr>
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<tbody>
<tr>
<td>Ca(^{2+})</td>
<td>124</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1.5</td>
<td>26</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0 Ca(^{2+})</td>
<td>123</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>27</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ba(^{2+})</td>
<td>122</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1.5</td>
<td>27</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ba(^{2+})-HEPES</td>
<td>120</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1.5</td>
<td>29</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

All values are added concentrations in millimolar. The free Ba\(^{2+}\) concentration in the presence of 1 mM EGTA was calculated using the data of Martell and Smith (1982) to generate an apparent association constant of 4.48 (expressed as log base 10), assuming an extracellular pH of 7.2 (Harrison and Bers 1987). The effect of Mg\(^{2+}\) on estimated free Ba\(^{2+}\) was negligible.
into the whole cell configuration. The depth of targeted cells ranged from the slice surface to ~100 μm and did not correlate with the size of surface pH responses. Surface responses contained a minor, contaminating, DC component. These were determined to be an artifact arising from the current path from bath to ground, and records were corrected accordingly (Makani and Chesler 2010).

Surface responses were generated by simulated antidromic invasion. First, we recorded the whole cell current-clamp response to a single antidromic stimulus in Ba²⁺-containing, Ca²⁺-free ACSF. This resulted in a prolonged plateau potential (see Results). A representative plateau response was used as the voltage command in subsequent experiments to evoke surface alkalinations. Surface pH electrode records were elicited at 30 s intervals and filtered at 2 kHz.

**Data analysis and presentation**

Statistics were presented as means with SE. Values of n refer to the number of neurons or slices studied, as indicated. Dual comparisons between mean values were made with a two-tailed, Student’s paired t-test. Multiple comparisons were made using paired ANOVA with a Student-Newman-Keuls post hoc test. Three to five raw traces of all responses were averaged before data analysis. In all records, alkaline shifts were presented as upward deflections.

**RESULTS**

**Population alkaline transients supported by Ca²⁺ versus Ba²⁺**

In initial studies, PATs were elicited by stimulating the alveus with a 20-shock, 50 Hz antidromic stimulus train (n = 6 slices). With Ca²⁺ as the divalent charge carrier, the PAT measured 0.053 ± 0.010 unit pH in peak amplitude, had a time-to-peak of 2.7 ± 0.8 s, and had a half-time of decay of 4.7 ± 0.7 s. On removal of Ca²⁺ from the ACSF, the pH change was virtually abolished, measuring 0.001 ± 0.001 unit pH. After subsequent addition of Ba²⁺ to the ACSF, all parameters of the resulting PAT were augmented. The peak amplitude, time-to-peak, and half-time of decay were increased to 0.162 ± 0.018 unit pH (P < 0.01), 8.9 ± 0.5 s (P < 0.001), and 14.6 ± 2.4 s (P < 0.05), respectively. Examples of PATs in Ca²⁺ and Ba²⁺-containing media are shown in Fig. 1.

The Ba²⁺ saline had no added Ca²⁺, but it was unclear whether intracellular Ca²⁺ stores were depleted in this medium. In principle, these could serve as a source of cytosolic Ca²⁺ and thereby enable Ca²⁺-H⁺ exchange via the PMCA. Therefore to insure depletion, we used caffeine (5 mM) to empty endoplasmic reticulum (ER) Ca²⁺ stores (Rousseau and Meissner 1989) and cyclopiazonic acid (CPA, 100 μM) to block the reuptake of Ca²⁺ by the ER Ca²⁺ ATPase (Seidler et al. 1989). In five slices, a control PAT in Ba²⁺ media was first evoked by a 20-shock (50 Hz) stimulus train. This was followed by 30 min of superfusion with CPA and a second 30 min superfusion with CPA plus caffeine. During wash-in of these agents, the stimulus train was repeated at 5 min intervals. The PATs obtained in control Ba²⁺ media, after addition of CPA and after subsequent addition of caffeine, were not statistically different (0.162 ± 0.018, 0.181 ± 0.021, and 0.129 ± 0.023 unit pH, respectively; P > 0.05, repeated-measures ANOVA; data not shown). These results suggested that internal Ca²⁺ reservoirs did not contribute substrate for the PMCA when Ba²⁺ was substituted for external Ca²⁺.

Previous studies in turtle cerebellum showed that the Ca²⁺-dependent PAT undergoes temporal summation as the number of stimuli increase at a given frequency, whereas a single stimulus does not produce an evident pH change (Chesler and Chan 1988). Indeed, in normal Ca²⁺ saline, a single antidromic stimulus did not elicit a detectable PAT, whereas a subsequent 20 shock, 50 Hz stimulus train evoked a sizable response (peak amplitude of 0.050 ± 0.011 unit pH, time-to-peak of 1.8 ± 0.3 s, and a half-time of decay of 3.1 ± 0.5 s, n = 5 slices; Fig. 2A). Separate current-clamp recordings indicated that each antidromic stimulus gave rise to a single corresponding somatic action potential (Fig. 2B).

In stark contrast, a single antidromic stimulus in Ba²⁺ saline elicited a PAT with peak amplitude of 0.166 ± 0.064 unit pH, time-to-peak of 12.4 ± 1.5 s, and decay half-time of 18.5 ± 1.6 s (Fig. 3A, left trace). With a 20 shock, 50 Hz antidromic stimulus, the PAT was not significantly different (P > 0.5) in any of these parameters (peak amplitude of 0.162 ± 0.065 unit pH, time-to-peak of 12.8 ± 1.8 s, and half-time of decay of 18.1 ± 1.9 s, n = 7 slices; Fig. 3A, right trace).

The whole cell current-clamp response to antidromic invasion in Ba²⁺ ACSF was similarly all-or-none in nature. A
single antidromic stimulus elicited a fast, overshooting depolarization. The plateau phase began during the repolarization of this initial spike, at a mean absolute membrane potential of $-3.4 \pm 3.2$ mV. The membrane depolarized further to a peak of $+12.6 \pm 3.3$ mV, slowly decayed, and started its sudden repolarization at $-12.3 \pm 3.6$ mV. Plateaus were often followed by a brief hyperpolarization. In paired comparisons made while recording from individual neurons, the plateau potential evoked by a single shock (Fig. 3B, left trace) had a mean half-width of $14.7 \pm 2.8$ s, which was not different from that of the subsequent single plateau potential elicited when giving a train of 20 antidromic stimuli at 50 Hz ($17.7 \pm 4.8$ s, $P > 0.5, n = 6$ cells; Fig. 3B, right trace). Thus the first shock of the antidromic stimulus train had initiated a long plateau potential that precluded further antidromic invasion and therefore rendered the subsequent stimuli ineffectual. After pooling the data from six additional neurons, the mean half-width of plateau potentials elicited by a single shock was $41.0 \pm 11.8$ s ($n = 12$ cells; range 7–138 s). Although the plateau potentials varied little in a single neuron, there was considerable variation in the shape and duration of the response among different cells, as shown in Fig. 4.

**Surface alkaline transients supported by Ba$^{2+}$**

To test whether the all-or-none voltage and PAT responses were related to PMCA activity, we studied SATs on individual CA1 neurons (Makani and Chesler 2010). To standardize the initiating stimulus, we used a plateau potential of $\sim 10$ s duration (previously recorded from a different cell) as the command potential in whole cell voltage clamp.

![Diagram of PAT and whole cell voltage response in 1 mM extracellular Ba$^{2+}$.](image)

**FIG. 3.** Relationship between PAT and whole cell voltage response in 1 mM extracellular Ba$^{2+}$. A: both 1 and 20 antidromic shocks (50 Hz) elicited PATs of similar magnitudes. B: both 1 and 20 shocks elicited whole cell plateau potentials of similar duration.

As shown in Fig. 5A (right), this voltage command initiated an SAT that was sustained throughout the duration of the plateau potential, with a mean amplitude of $0.023 \pm 0.006$ unit pH ($n = 5$ cells). On repolarization, the surface pH shift began to decline immediately. To insure that these surface responses were genuine alkaline changes that depended on replacement of external Ca$^{2+}$ with Ba$^{2+}$, we augmented the extracellular buffering capacity by the addition of 5 mM HEPES to the bicarbonate-buffered media. After the addition of HEPES, the amplitude of the SAT was reduced by $94.7 \pm 1.9\%$ ($n = 5; P < 0.05$; Fig. 5B). This reduction could not be attributed to rundown. In six cells, the SAT measured $0.021 \pm 0.003$ unit pH after 1 min and $0.020 \pm 0.003$ unit pH after 5 min, an interval consistent with the wash-in time of HEPES ($P > 0.5$; Fig. 5C). Because the SAT is abolished in the absence of a divalent charge carrier (Makani and Chesler 2010), these data indicated that the surface responses were genuine alkaline changes that depended on replacement of external Ca$^{2+}$ with Ba$^{2+}$.

We recently reported a Ca$^{2+}$-dependent SAT on CA1 neurons, elicited by simulated antidromic firing at 100 Hz for 2 s. These responses had measured $0.0012 \pm 0.0005$ unit pH at 200 ms and $0.017 \pm 0.0004$ unit pH at 2 s (Makani and Chesler 2010). Despite being elicited by a longer duration stimulus (a 10 s plateau potential), the Ba$^{2+}$-dependent SAT was not significantly different in magnitude from the former Ca$^{2+}$-dependent SAT.
response, measuring 0.0007 ± 0.0002 unit pH at 200 ms ($P = 0.34$) and 0.012 ± 0.002 unit pH at 2 s ($P = 0.24$).

Previously, we showed that the SAT in normal Ca\textsuperscript{2+} ACSF could be blocked by inclusion of the PMCA inhibitor carboxyeosin in the patch pipette (Makani and Chesler 2010). If the Ba\textsuperscript{2+}-dependent SAT was mediated by the PMCA, it should similarly be inhibited by intracellular carboxyeosin. We tested this hypothesis by again using a 10 s plateau potential as the voltage command. With 5 μM carboxyeosin in the pipette ($K_0 = 20$ nM), the SAT measured 0.015 ± 0.005 unit pH at 1 min after break-in to whole cell recording mode. At 5-min after break-in, this response was reduced by 95.7% (n = 5 cells; $P < 0.05$; Fig. 6A). There was no change in the membrane resistance or holding current in recordings obtained at 1 and 5 min, indicating that the reduction of the SAT was not because of loss of cell viability. Thus the SAT observed in Ba\textsuperscript{2+} media could be attributed to H\textsuperscript{+} transport mediated by the PMCA.

To test whether the effect of carboxyeosin on the SAT might be caused by a block of Ba\textsuperscript{2+} entry through VGCCs (Choi and Eisner 1999), Ba\textsuperscript{2+} plateau potentials were evoked by brief (120 ms) current injection at 1 and 5 min after breakthrough into whole cell mode, with 5 μM carboxyeosin in the pipette. The plateau was unaffected by carboxyeosin, as the half-width measured 33.2 ± 10.1 s at 1 min and 36.9 ± 11.6 s at 5 min after break-in ($n = 5$ cells; $P > 0.5$; Fig. 6B). Because carboxyeosin did not diminish the Ba\textsuperscript{2+} plateau potentials, it is unlikely that it had a significant effect on the entry of Ba\textsuperscript{2+} into these cells.

**DISCUSSION**

Synchronous neural activity causes a PAT in the hippocampus and several other brain regions (Chesler 2003). It was suggested that these phenomena might arise via Ca\textsuperscript{2+}-H\textsuperscript{+} exchange mediated by the neuronal PMCA (Schwiening et al. 1993). We recently provided direct evidence that this is indeed the case by recording alkaline transients on the surface of hippocampal CA1 pyramidal neurons in situ. The SATs on individual cells amounted to >10% of the PAT, were dependent on external Ca\textsuperscript{2+}, and were fully inhibited by intracellular carboxyeosin (Makani and Chesler 2010). Although these data in concert made a strong case for the PMCA hypothesis, the ability of Ba\textsuperscript{2+} to support the PAT remained a source of uncertainty (Grichtchenko and Chesler 1996; Smith and Chesler 1999; Tong and Chesler 1999), because there were no data regarding the ability of the PMCA to use this ion in intact mammalian neurons. If Ba\textsuperscript{2+} was not a substrate for this transporter, an alternative mechanism would have to be postulated to account for the associated PAT. Here we provide an array of evidence that indicates Ba\textsuperscript{2+} transport via the PMCA gives rise to both the SAT and PAT responses.

In stark contrast to results in Ca\textsuperscript{2+} ACSF, it was shown that, in Ba\textsuperscript{2+} media, both the PAT and the prolonged whole cell current-clamp response of individual neurons were all-or-none and could be triggered by a single antidromic stimulus. This suggested that the Ba\textsuperscript{2+}-supported PAT was the result of many neurons undergoing individual plateau potentials. Because the initial plateau depolarization precluded further antidromic invasion, the remaining shocks of the stimulus train had no effect.

To test whether the Ba\textsuperscript{2+}-dependent depolarization triggered a PMCA-dependent extracellular alkalosis, we recorded SATs on single pyramidal neurons elicited by a prerecorded plateau potential used as the voltage command. In Ba\textsuperscript{2+} ACSF, this protocol evoked a rapid SAT that was virtually abolished when the pipette contained the PMCA inhibitor carboxyeosin. Although carboxyeosin also inhibits organellar Ca\textsuperscript{2+}-H\textsuperscript{+} exchange (Kosterin et al. 1996; O’Neal et al. 1979; Watson et al. 1992; Zhao and Dhalla 1988). Because those studies used preparations from rat synaptosomes and heart, it is plausible that the discrepancies with these results are caused by a difference in species or tissue. Another possibility is that only some forms of the transporter can carry Ba\textsuperscript{2+}, with numerous splice variants (Strehler and Zacharias 2001). PMCA 2 is the predominant isoform in stratum pyramidale, where our measurements were performed, whereas PMCA 3 is more prevalent in the dendritic layer (Kip et al. 2006). In the heart, the main isoforms are PMCA 1 and 4 (Strehler and Zacharias 2001). PMCA 2 is optimally transported by the pyramidal neuron PMCA.

The ability of the PMCA to transport Ba\textsuperscript{2+} has been the source of some controversy. For example, several early reports, which measured enzymatic activity from membrane fractions, suggested that the PMCA carries Ba\textsuperscript{2+} ineffectively (Duncan 1976; Pfleger and Wolf 1975; Yamaguchi et al. 1989; Zhang et al. 1992; Zhao and Dhalla 1988). Because those studies used preparations from rat synaptosomes and heart, it is plausible that the discrepancies with these results are caused by a difference in species or tissue. Another possibility is that only some forms of the transporter can carry Ba\textsuperscript{2+}, with numerous splice variants (Strehler and Zacharias 2001). PMCA 2 is the predominant isoform in stratum pyramidale, where our measurements were performed, whereas PMCA 3 is more prevalent in the dendritic layer (Kip et al. 2006). In the heart, the main isoforms are PMCA 1 and 4 (Strehler and Zacharias 2001).

The use of intact cells to assay PMCA activity in external Ba\textsuperscript{2+} ACSF might also account for these results. Indeed, our data are in substantial agreement with a recent report of surface pH on giant snail neurons, which similarly showed a Ba\textsuperscript{2+}-supported surface alkalosis generated by the PMCA (Thomas 2009). It is possible that one or more cytosolic constituents were required for Ba\textsuperscript{2+} to be optimally transported by the PMCA, allowing for the detection of the surface alkalinations in that study, as well as this one.

**FIG. 6.** Ba\textsuperscript{2+} SAT was caused by the PMCA. A: internal voltage-clamp solution contained carboxyeosin (5 μM), and SATs were measured at 1 and 5 min after breakthrough into whole cell mode. B: in separate experiments, the addition of carboxyeosin to the internal solution had no effect on the Ba\textsuperscript{2+} plateau potentials.
It can be argued that an SAT and PAT would occur even if the affinity of the PMCA for Ba\textsuperscript{2+} was far less than that for Ca\textsuperscript{2+}. The long plateau potential, coupled with the greater conductance of VGCCs with Ba\textsuperscript{2+} as the charge carrier, would allow for a large intracellular accumulation of this ion. Moreover, cytosolic buffering of Ba\textsuperscript{2+} is relatively poor (Ahmed and Connor 1979; Connor and Ahmed 1984), and it has been reported that Sarco-endoplasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA) pumps do not transport this ion (Méme and Léoty 2001). Thus, compared with Ca\textsuperscript{2+}, the cytosolic concentration of free Ba\textsuperscript{2+} may attain a substantially higher level. Accordingly, even if the $K_a$ for Ba\textsuperscript{2+} were greater than that for Ca\textsuperscript{2+}, the PMCA might still be able to support the same rate of H\textsuperscript{+} transport.

Consistent with this notion, the magnitude of the Ba\textsuperscript{2+}-dependent SAT generated by a 10 s plateau potential was similar in size to the previously reported Ca\textsuperscript{2+}-dependent SAT elicited by a simulated, 100 Hz antidromic train of action potentials of just 2 s duration (Makani and Chesler 2010). Although these observations do not directly address the relative affinity of the PMCA for these ions, they do indicate that similar transport rates can be achieved whether Ca\textsuperscript{2+} or Ba\textsuperscript{2+} is the divalent charge carrier.

The contention that the SAT is the underlying basis of the PAT is based in part on its large relative amplitude. In Ba\textsuperscript{2+} media, the mean peak amplitude of the SAT generated by one cell was nearly one seventh that of the PAT, elicited by the activation of thousands of neurons. As discussed previously (Makani and Chesler 2010), the PAT would be expected to arise from the summated contributions of single cells, because the H\textsuperscript{+} uptake by each cell would effectively act as a source of extracellular base. With an increased number of cells activated, the total concentration of base at an adjacent extracellular recording site would rise accordingly, leading to the generation of a PAT.

The long duration plateau potentials most likely resulted in a prolonged cycle of Ba\textsuperscript{2+} entry and extrusion in exchange for H\textsuperscript{+}. Thus the net contribution of base from individual cells could be expected to undergo temporal summation, leading to a large and long-lasting PAT. Indeed, the SAT of single neurons began a return to baseline only when the plateau command was terminated. With antidromic activation of the neuronal population, the plateau response would start at approximately the same instant; however, the durations of the plateau would most likely have varied among cells (Fig. 4). This might account for both the long time to peak and the slow decay of the Ba\textsuperscript{2+}-supported PAT.

Prolonged Ba\textsuperscript{2+} plateau potentials have been described previously and were attributed to a large sustained influx of Ba\textsuperscript{2+} through VGCCs (Blitzer et al. 1991; Eckert and Lux 1976; Hagiwara and Naka 1964; Houhsgaard and Nicholson 1983; Lilás and Sugimori 1980). Factors that are likely to contribute to this response include the direct block of K\textsuperscript{+} channels by Ba\textsuperscript{2+} (Armstrong et al. 1982; Rudy 1988), the failure to recruit Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductances (Gorman and Hermann 1979; Hagiwara et al. 1975), and the inability of intracellular Ba\textsuperscript{2+} to inactivate VGCCs (Choi and Eisner 1999; Eckert and Chad 1984). In the current setting, the PAT itself may have been an additional contributing factor, because both high- and low-voltage activated Ca\textsuperscript{2+} channels are partially inhibited by extracellular H\textsuperscript{+} in the physiological pH range (Chen et al. 1996; Tombaugh and Somjen 1996, 1998; Tytgat et al. 1990). Indeed, we previously reported that, in standard Ca\textsuperscript{2+} saline, the PAT generated by synaptic activation of CA1 neurons acts to enhance the postsynaptic afterdepolarization. This enhancement was largely attributable to the relief of the proton block of NMDA receptors; however, the effect was diminished with n-600 in the patch pipette (Makani and Chesler 2007), suggesting that the Ca\textsuperscript{2+}-dependent PAT was also boosting current through L-type Ca\textsuperscript{2+} channels. Given the large size of the PAT in Ba\textsuperscript{2+} saline, similar enhancement of Ba\textsuperscript{2+} currents might have occurred. Accordingly, the VGCCs that triggered the PAT could have been the target of positive feedback mediated by this rise in extracellular pH.

In summary, these results indicate that the PMCA of CA1 pyramidal neurons can transport Ba\textsuperscript{2+} in exchange for extracellular H\textsuperscript{+} and can thereby generate stimulus-evoked extracellular alkaline shifts. Because of the long duration of Ba\textsuperscript{2+}-dependent plateau potentials, these extracellular pH responses can be especially large and prolonged. Given the influx of H\textsuperscript{+} via the PMCA, a long-lasting intracellular acidification could also be anticipated. Thus, use of Ba\textsuperscript{2+} as the divalent charge carrier may result in significant pH changes on both sides of the plasma membrane. In view of the pH sensitivity of numerous channels and enzymes, awareness of the possible effects of these pH shifts may be warranted in studies where Ba\textsuperscript{2+} is used as the principal divalent charge carrier.

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DISCLOSURES

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