Relationships Between Intracellular Calcium and Afterhyperpolarizations in Neocortical Pyramidal Neurons

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Abel, H. J., J.C.F. Lee, J. C. Callaway, and R. C. Foehring. Relationships between intracellular calcium and afterhyperpolarizations in neocortical pyramidal neurons. J Neurophysiol 91: 324–335, 2004. First published August 13, 2003; 10.1152/jn.00583.2003. We examined the effects of recent discharge activity on [Ca\(^{2+}\)]\(_i\) in neocortical pyramidal cells. Our data confirm and extend the observation that there is a linear relationship between plateau [Ca\(^{2+}\)]\(_i\) and firing frequency in soma and proximal apical dendrites. The rise in [Ca\(^{2+}\)]\(_i\) activates K\(^+\) channels underlying the afterhyperpolarization (AHP), which consists of 2 Ca\(^{2+}\)-dependent components: the medium AHP (mAHP) and the slow AHP (sAHP). The mAHP is blocked by apamin (Al 1999; Yuste et al. 1994). Until buffering mechanisms restorerestoring resting Ca\(^{2+}\) levels (Gibney et al. 2002; Kannurpatti et al. 2000; Markram et al. 1995; Miller et al. 1991; White and Reynolds 1995), cytoplasmic free Ca\(^{2+}\) regulates critical cellular functions, including neurotransmitter release, gene transcription, and channel modulation. Cytoplasmic [Ca\(^{2+}\)] may also provide the cell with an index of recent spiking activity (Helmcchen et al. 1996).

Ca\(^{2+}\) entry during repetitive firing serves as a feedback regulator of firing rate (Wang 1998), activating Ca\(^{2+}\)-dependent K\(^+\) channels that produce afterhyperpolarizations (AHPs) and spike frequency adaptation (Lancaster and Nicoll 1987; Lorenzon and Foehring 1995; Schwindt et al. 1988b). This feedback increase dynamics firing rate (Engel et al. 1999) and modifies information content of spike output, based on recent firing behavior (Fuhrmann et al. 2002; Wang 1998).

In neocortical pyramidal neurons, 3 AHP components follow an action potential train (Lorenzon and Foehring 1992; Schwindt et al. 1988a). The fast AHP (fAHP) immediately follows spike repolarization. Several different ionic conductances underlie the fAHP (reversal potential about −65 mV; Lorenzon and Foehring 1992; Schwindt et al. 1988a). The mAHP is elicited by a single spike, blocked by apamin, and decays with a \(\tau\) of about 150 ms (Lorenzon and Foehring 1992; Schwindt et al. 1988b). The sAHP is observed only after several spikes (\(\tau\) decay about 1–2 s; Lorenzon and Foehring 1992; Schwindt et al. 1988b), insensitive to apamin, and modulated by several neurotransmitters (Foehring and Lorenzon 1999; Nicoll 1988). Apamin-sensitive small-conductance Ca\(^{2+}\)-dependent K\(^+\) (SK) channels are generally accepted as responsible for the mAHP (Alger et al. 1994; Hirschberg et al. 1998; Vergara et al. 1998). More elusive, however, are the channels underlying the sAHP.

Three SK subunits have been cloned: SK1, SK2, and SK3 (Hirschberg et al. 1998). SK1 and SK2 channels are expressed in the rat neocortex (Köhler et al. 1996; Stocker and Pedarzani 2000). The apamin insensitivity of SK1 channels in Xenopus oocytes (Bond et al. 1999) suggested that the pyramidal cell sAHP might be attributed to SK1 channels, or perhaps a heteromer of SK subunits (Sah and Faber 2002). In mammalian cell lines, however, the apamin sensitivity of SK1 approaches that of SK3 (Shah and Haylett 2000; Strobaek et al. 2000). Furthermore, although the sAHP decays much more slowly than the apamin-sensitive mAHP, currents through cloned SK1 and SK2 channels in expression systems exhibit similar kinetics and calcium affinities (Shah and Haylett 2000; Vergara et al. 1998).

Channels with biophysical properties consistent with the sAHP have been observed in CA1 pyramidal neurons (Hirschberg et al. 1999; Lancaster et al. 1991; Sah and Isaacson 1995; Selyanko et al. 1998; Valiante et al. 1997). Attempts to pinpoint the channels responsible for the sAHP have proven inconclusive, however. Bekkers (2000) was unable to locate sAHP channels in cell-attached or nucleated patches from the soma or dendrites of pyramidal cells. Furthermore, amputation of the apical dendrite or the axon resulted in only a small decrease in sAHP current.

Because of the importance of Ca\(^{2+}\) in pyramidal cell function, we examined the effects of recent spiking history on changes in [Ca\(^{2+}\)]\(_i\). To gain insight into the many unanswered questions concerning the elusive sAHP channels, we examined the relationships between the number and frequency of spikes, AHPs, and [Ca\(^{2+}\)]\(_i\) in the soma and proximal dendrites of pyramidal neurons. In particular, we examined whether the mAHP and sAHP channels respond in a similar way to changes in [Ca\(^{2+}\)]\(_i\).
METHODS

The brain was removed from metofane-anesthetized Sprague-Dawley rats (P13-19) and then sliced into 300-μm-thick coronal sections using a vibrating tissue slicer (WPI). The tissue was sliced in an ice-cold, high-sucrose solution (pH = 7.3–7.4, 300 mMosi) containing (in mM): 250 sucrose, 2.5 KCl, 1 Na3PO4, 11 glucose, 4 MgSO4, 0.1 CaCl2, 15 HEPEs. The primary somatosensory and primary motor cortices (sensomotor cortex) were dissected from the slices and then transferred to a mesh surface in a chamber containing artificial cerebrospinal fluid (aCSF) at room temperature. The aCSF contained (in mM): 125 NaCl, 3 KCl, 2 CaCl2, 5 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 20 glucose (pH = 7.4, 310 mMosi) and was bubbled with a 95% O2–5% CO2 (carbogen) mixture. For recording, slices were placed in a recording chamber on the stage of an Olympus BX50WI upright microscope. Slices were bathed in carbogenated aCSF pumped at 2 ml/min and heated with an in-line heater (Warner) to 31–32°C. All pharmacological agents (except Cd2+) were prepared as concentrated stocks in H2O (apamin, isoprotenerol, ZD 7288) or ethanol (linopirdine) and then thawed and added to the aCSF just before recording. In the linopirdine-containing aCSF, the concentration of ethanol was <0.05%, which we previously showed to have no effect on the AHPs (Pineda et al. 1998). The Cd2+-containing aCSF was made up in advance and contained (in mM): 125 NaCl, 3 KCl, 2 CaCl2, 5 MgCl2, 26 NaHCO3, 0.4 CdCl2, and 20 glucose (NaH2PO4 was omitted to avoid precipitation).

Pyramidal neurons in layers II and III were visualized with infrared/differential interference contrast (IR/DIC) video-microscopy (Dodd and Ziegglansberger 1990; Stuart et al. 1993) using a 40× (0.8 NA) water immersion objective. Simultaneous whole cell patch clamp and Ca2+ fluorescence imaging records were acquired using an Axocam 2A (Axon Instruments; current clamp) or an Axopatch 200B (Axon Instruments; voltage clamp) amplifier in combination with a cooled CCD camera (Sensicam: PCO, Kellheim, Germany). Recordings were taken using borosilicate electrodes (4–8 MΩ resistance) produced with a horizontal electrode puller (Sutter Instruments) and filled with a solution containing (in mM): 130.5 KMeSO4, 10 KCl, 7.5 NaCl, 4 MgCl2, 10 HEPES, 2 adenosine 5′-triphosphate (ATP), and 0.2 guanosine 5′-triphosphate. Unless otherwise specified, 100 μM fura-2 (Molecular Probes; pentapotassium salt) was added to the intracellular solution. Data were collected only from cells forming a 1-GΩ or tighter seal

Optical data were obtained by exciting the dye (usually fura-2) at a wavelength of 380 ± 10 nm and measuring fluorescence changes at an emission wavelength of 520 ± 40 nm (filters from Chroma Technology, Brattleboro, VT). Electrical and optical data were synchronously acquired on a single Windows platform PC running software written by Dr. J. C. Callaway, based on software developed by Lasser-Ross et al. (1991). Electrical records were digitized with 16-bit resolution at 10 kHz, and corrected for the liquid junction potential (10 mV).

The relative change in fura-2 fluorescence (ΔF/F) is closely proportional to the calcium concentration for changes less than about 50% ΔF/F (Lef-Lam et al. 1992). We used a calcium calibration buffer kit (Molecular Probes) to prepare solutions of known ratios of Ca2+ to EGTA in the internal recording solution, for which we could calculate [Ca2+]free. This allowed us to determine the K0 for fura-2 in vitro to be 222 nM. We acquired pairs (at excitation wavelengths of 340 ± 10 and 380 ± 10 nm) of fluorescence intensities from solutions containing [Ca2+]free ranging from 0 to 400 nM. The resulting calibration curve was used to estimate resting calcium in our cells (from ratiometric measurements taken at a holding potential of −70 ± 5 mV).

In our experiments, fluorescence values (at 380 nm) were converted to Ca2+ concentrations using a modification of the method described by Lev Ram et al. (1992). These were converted to calcium concentration using the equation

\[
[Ca^{2+}]_i = \frac{\Delta F}{F} \cdot \frac{K_0 + [Ca^{2+}]_i}{K_0} \cdot \left( \frac{\Delta F - 1}{Sb_{380}} \right) \left( \frac{F}{Sb_{380}} + 1 \right) \quad (1)
\]

(where [Ca2+]i is the resting Ca2+ level) to estimate [Ca2+]i, from %ΔF/F. This formula was derived by Wilson and Callaway (2000) and used here because it did not require a measurement of the maximal possible fluorescence change, which requires loading the cell with calcium. Sb380/Sb580 is the ratio of bound to free fura-2 fluorescence (see Grynkiewicz et al. 1985), which we determined in our calibration to be 10. Corrections for photobleaching were made by subtracting the Ca2+ signal from an equal-length control sweep containing no stimulus. Tissue autofluorescence was accounted for by subtracting the fluorescence of a non-fura-loaded area of tissue near the cell.

Unless otherwise stated, data are presented as means ± SE. Further analysis was conducted using Igor Pro (Wavemetrics, Lake Oswego, OR) and Kaleidagraph (Synergy Software). Curve fits used the Lev-Enberg–Marquardt algorithm to determine the best fit by minimizing x2 values. Additional components were reported for curve fits if the additional component constituted ≥10% of the amplitude.

RESULTS

Recordings were obtained from cells from rats between postnatal days 13–19. Cells were visually identified under IR/DIC and fluorescence imaging as pyramidal cells in layer II or layer III. These cells fired repetitively in a regular-spiking (RS) pattern (McCormick et al. 1985). From a practical standpoint, using immature animals facilitated imaging because there was less myelin-induced light scattering. Some quantitative aspects of our findings will differ from the adult neocortex. For instance, spike frequency adaptation and the sAHP are more prominent in immature pyramidal cells than in their mature counterparts (Lorenzon and Foehring 1993, 1995; McCormick and Prince 1987). In current-clamp recordings, cells with a resting potential negative to −60 mV, action potentials (APs) that overshoot 0 mV and fired repetitively in response to a 500-ms depolarizing current injection (e.g., “RF cells”: Lorenzon and Foehring 1993) were deemed healthy and included in this study. Table 1 summarizes membrane and firing properties of the cells studied.

<table>
<thead>
<tr>
<th>Number of Cells</th>
<th>Vrest (mV)</th>
<th>Vthreshold (mV)</th>
<th>AP Height (mV)</th>
<th>Half-width (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>−72.9 ± 0.8</td>
<td>−45.4 ± 0.5</td>
<td>83.2 ± 1.1</td>
<td>1.7 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. AP height is defined as the difference between the action potential peak and the resting potential. Half-width is defined as the spike width, halfway between the resting potential and the peak of the action potential.

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average intracellular resting calcium concentration to be 116 ± 16 nM (n = 16). In most cases, data will be presented in terms of percentage change in fluorescence intensity, which can be translated into calcium concentration by means of Eq. 1 (METHODS). All imaging data were corrected for tissue autofluorescence and photobleaching (METHODS).

\[ [Ca^{2+}]_i \] versus number and frequency of spikes

In current-clamp mode, cells were held using DC current injection at a potential between −60 and −70 mV. Suprathreshold current injections (500 ms) elicited repetitive firing. Although changes in \([Ca^{2+}]_i\) were not typically observed in response to subthreshold current injections, a \(Ca^{2+}\) transient occurred after a single spike, and more spikes resulted in greater calcium entry (Fig. 1). A mAHP followed a single spike (the fAHP was minimal because the cells were held near its reversal potential). Multiple spikes elicited a larger mAHP and also elicited a sAHP (see following text).

To quantify the influence of spike frequency and number on AHPs and \([Ca^{2+}]_i\), trains of action potentials were induced with a series of 5-ms suprathreshold current steps of constant frequency. Just enough current was injected to elicit one action potential per step. At a given frequency, the peak \([Ca^{2+}]_i\) increased with the number of spikes (Fig. 2). Trains of just subthreshold stimuli did not elicit \(Ca^{2+}\) transients (data not shown). During a prolonged train of action potentials, proximal dendritic \([Ca^{2+}]_i\) (measured in the apical dendrite, 20–50 μm from the soma) eventually reached a steady-state plateau level, indicating a balance between the rates of calcium entry and buffering/extrusion (Jahromi et al. 1999; Kaiser et al. 2001; Lasser Ross et al. 1997; Schiller et al. 1995). Although measured somatic calcium levels never truly plateaued, they asymptotically approached plateau levels (Fig. 2E).

Previously, Helmchen et al. (1996) observed that the dendritic plateau \([Ca^{2+}]_i\) in layer V pyramidal cells was linearly dependent on firing frequency (tested up to about 30 Hz). They suggested that this linearity could be functionally important because it would allow the cell to “remember” its recent firing behavior by means of a simple calculation. Using 100 μM fura-2, we confirmed the findings of Helmchen et al. (1996) and found that a similar linear relationship held for the somatic calcium peaks as well (11 nM/Hz for soma, 11.2 nM/Hz for proximal apical dendrite). For higher frequencies, however, the relationship between plateau \([Ca^{2+}]_i\) and firing frequency was clearly nonlinear (Fig. 3A; 5 Hz, n = 2; 10 Hz, n = 4; 20 Hz, n = 13; 50 Hz, n = 12; 100 Hz, n = 15 cells) (see also Jackson and Redman 2003; Jahromi et al. 1999; Lasser Ross et al. 1997).

We used the lower-affinity calcium-indicator dyes fura-4f and fura-6f (Molecular Probes, Eugene, OR) to test whether this nonlinearity was a physiological phenomenon or an artifact of saturation of \(Ca^{2+}\) binding to fura-2 (\(K_D = 222\) nM, in vitro; 140 nM without Mg\(^{2+}\): Molecular Probes). With the intermediate-affinity dye, fura-4f (100 μM; \(K_D = 770\) nM without Mg\(^{2+}\): Molecular Probes), the relationship between plateau calcium levels and firing frequency approximated linearity over a wider range of \(\% \Delta F/F\) (Fig. 3B; n = 6). Finally, fura-6f...
proximal dendrite versus the soma (Table 2) (Jahromi et al. 1999; Lasser Ross et al. 1997; Sah and Clements 1999). It is important to note that the kinetics of the calcium transients were altered by the exogenous calcium buffer fura-2 (cf. Helmchen et al. 1996; Holthoff et al. 2002; Jackson and Redman 2003; Lasser-Ross et al. 1997; Neher and Augustine 1992; Schiller et al. 1995; Tank et al. 1995). For comparison, we measured dendritic decay time courses with 20 or 100 µM fura-2 in the intracellular recording solution. With 20 µM fura-2, the calcium transient induced by a 200-ms step in voltage-clamp mode from −60 to +10 mV decayed with a τ = 578 ± 50 (n = 2; 31°C), compared with τ = 686 ± 34 (n = 7) for the same stimulus with 100 µM fura-2.

We also examined dendritic τ values for rise and decay of [Ca²⁺], using the low-affinity dye, fura-6f. With 100-Hz spiking, the τ_rise with fura-6f was 307 ± 101 ms (n = 3 cells) versus 114 ± 17 ms in fura-2 (Table 2). The τ_decay with fura-6f was 409 ± 39 ms (n = 3 cells) versus 619 ± 77 ms in fura-2 (Table 2). Somatic τ_decay with fura-6f was 586 ± 28 ms (n = 3 cells) versus 2076 ± 275 ms in fura-2 (Table 2). Thus rise times were faster and decay times slower with the higher-affinity dye, fura-2.

KINETICS OF TRANSIENTS. Peak [Ca²⁺], always occurred coincident with the end of the spike train (Figs. 2 and 4). Peak [Ca²⁺], increased exponentially and the rate of increase of calcium transients (both soma and dendrites) depended on firing frequency (Fig. 4, B–D; Table 2). The time constant for the increase in Ca²⁺ decreased with increasing firing frequency (Fig. 4D). The decay of [Ca²⁺], was also exponential. Plots of [Ca²⁺], decay versus firing frequency for soma and dendrite varied between cells (Fig. 4E), with no clear correlation with firing frequency. Rates of both rise and fall were higher in the proximal dendrite versus the soma (Table 2) (Jahromi et al. 1999; Lasser Ross et al. 1997; Sah and Clements 1999). It is important to note that the kinetics of the calcium transients were altered by the exogenous calcium buffer fura-2 (cf. Helmchen et al. 1996; Holthoff et al. 2002; Jackson and Redman 2003; Lasser-Ross et al. 1997; Neher and Augustine 1992; Schiller et al. 1995; Tank et al. 1995). For comparison, we measured dendritic decay time courses with 20 or 100 µM fura-2 in the intracellular recording solution. With 20 µM fura-2, the calcium transient induced by a 200-ms step in voltage-clamp mode from −60 to +10 mV decayed with a τ = 578 ± 50 (n = 2; 31°C), compared with τ = 686 ± 34 (n = 7) for the same stimulus with 100 µM fura-2.

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FIG. 3. Relationship between [Ca²⁺], and firing frequency increasingly approximated linearity with lower-affinity dyes. In many cases, the errors bars fell within the squares. A: with 100 µM fura-2 as the Ca²⁺ indicator, we observed nonlinear relationships between firing frequency and ΔF/ΔF in the soma (black) and proximal apical dendrite (gray) (n = 15). Error bars indicate SE. B: these relationships become more linear with the lower-affinity Ca²⁺ indicator, fura-4f (n = 6; 100 µM). C: with 100 µM fura-6f, the relationships approximate straight lines (n = 4).

KINETICS OF TRANSIENTS. Peak [Ca²⁺], always occurred coincident with the end of the spike train (Figs. 2 and 4). Peak [Ca²⁺], increased exponentially and the rate of increase of calcium transients (both soma and dendrites) depended on firing frequency (Fig. 4, B–D; Table 2). The time constant for the increase in Ca²⁺ decreased with increasing firing frequency (Fig. 4D). The decay of [Ca²⁺], was also exponential. Plots of [Ca²⁺], decay versus firing frequency for soma and dendrite varied between cells (Fig. 4E), with no clear correlation with firing frequency. Rates of both rise and fall were higher in the proximal dendrite versus the soma (Table 2) (Jahromi et al. 1999; Lasser Ross et al. 1997; Sah and Clements 1999).

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Table 2. Rise and decay time constants of calcium transients

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Number of Cells</th>
<th>$\tau_{\text{rise}, \text{soma}}, \text{ms}$</th>
<th>$\tau_{\text{rise}, \text{dendrite}}, \text{ms}$</th>
<th>$\tau_{\text{fall}, \text{soma}}, \text{ms}$</th>
<th>$\tau_{\text{fall}, \text{dendrite}}, \text{ms}$</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>1690 ± 141</td>
<td>842 ± 49</td>
<td>1974 ± 125</td>
<td>1036 ± 104</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>345 ± 25</td>
<td>120 ± 11</td>
<td>3021 ± 375</td>
<td>543 ± 36</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>355 ± 13</td>
<td>114 ± 17</td>
<td>2076 ± 275</td>
<td>619 ± 77</td>
</tr>
<tr>
<td>100</td>
<td>4</td>
<td>325 ± 46</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. All data were obtained with 100 μM fura-2.

**Pharmacology of AHPs**

To separate the 2 afterhyperpolarizations, the β-adrenergic agonist isoproterenol (Foehring et al. 1989) and the bee venom peptide apamin (Schwindt et al. 1988a) were used to block the sAHP and the mAHP, respectively (Sah and Faber 2002). For the following data, AHPs were in response to 20 spikes at 50 Hz (the result of repeated suprathreshold 5-ms current injections). Generally 10 μM isoproterenol completely blocked the sAHP. When isoproterenol (10 μM) was added to the ACSF bath, the isolated mAHP was found to decay with a time constant of 157 ± 11 ms (n = 3; Fig. 5A, inset). Apamin (30–100 nM) blocked the mAHP to reveal the slow rise of the sAHP, as well as the sAHP’s slow exponential decay (τ of 1.18 ± 0.64 s; n = 4; Fig. 5B). The time-to-peak (TTP; from the last spike) for the rise of the isolated sAHP was 343 ± 84 ms (n = 7).

In CA1 pyramidal cells, $I_h$ and $I_d$ contribute to the mAHP (Storm 1989). We tested for contributions of $I_h$ (the hyperpolarization-activated inward cation current; Spain et al. 1987) or $I_{\text{M-current}}$ (a slow, nonactivating-depolarization-dependent K+ current; McCormick and Prince 1986) to the AHPs in our cells. Hyperpolarizing current steps (500 ms, 100–200 pA) from a resting membrane potential of −65 mV revealed the presence of a small $I_{\text{h}}$, as evidenced by a 4.3 ± 1.4% (Fig. 5C; n = 3) voltage sag (measured as the percentage decrease in voltage between peak and steady state). This sag disappeared when the relatively specific $I_{\text{h}}$ blocker ZD 7288 (10 μM; Tocris, Ellisville, MO; Sciancalepore and Constanti 1998) was added to the bath. Steady-state input resistance increased from the control value of 159 ± 3 to 190 ± 7 MΩ (n = 3) on treatment with ZD7288 (Fig. 5C). Blockade of $I_h$ produced no significant change in the AHP elicited from a resting membrane potential of −65 mV by either 3 (almost entirely mAHP) or 20 spikes (mAHP plus sAHP) at 50 Hz (Fig. 5D), likely because $I_h$ becomes prominent only at potentials more hyperpolarized than −70 mV (Sciancalepore and Constanti 1998; Spain et al. 1987).

Similarly, 3 cells were treated with the M-current blocker linopirdine (10 μM; Costa and Brown 1997) and no AHP changes were evident (data not shown). Thus for these resting potentials and stimuli, $I_h$ and M-current made little contribution to the mAHP or sAHP.

**AHP amplitude versus number and frequency of spikes**

The level of $[\text{Ca}^{2+}]_i$ attained was proportional to the number and frequency of spikes. We next examined the relationships between AHPs and firing. A mAHP could be observed after a single action potential in all cells (Fig. 6A; Fig. 1C). With increased numbers of spikes, the mAHP increased in size and a slower component, the sAHP, became apparent (Fig. 6B; Fig. 1). For a given number of spikes, the AHPs were larger at higher firing frequencies (Fig. 6, C vs. B). The amplitude of the mAHP (isolated with 10–100 μM isoproterenol) increased steadily with the number of spikes before attaining a maximum after about 20 spikes (Fig. 6D). This corresponded to about 170–230 nM peak $[\text{Ca}^{2+}]_i$ in the soma (Table 3).

Although the mAHP generally appeared after one action potential, several spikes (typically 3–5 at 10–100 Hz) were required to elicit the sAHP (Fig. 6D). After this “delay,” the isolated mAHP (50–100 nM apamin) also increased with spike number until reaching a plateau amplitude, requiring about 20 spikes to attain its maximum amplitude at 50 Hz (Fig. 6D). This corresponded to about 250–350 nM peak $[\text{Ca}^{2+}]_i$ at the soma (Fig. 6E; Table 3). These data agree with previous findings in human (Lorenzon and Foehring 1992) and cat neocortex (Schwindt et al. 1988a).

We observed no significant changes in $[\text{Ca}^{2+}]_i$ in response to apamin or isoproterenol. We found similar dependency of the mAHP and sAHP amplitude on the number of spikes at 10, 20, and 100 Hz (data not shown). One possible interpretation of these data is that the sAHP is less sensitive to calcium than the mAHP, such that greater calcium entry is required for its activation. However, after the initial delay, the sAHP increased with spike number at about the same rate as the mAHP (Fig. 6D).
APs, AHPs, AND CALCIUM

I sAHP and I mAHP

The time course of the mAHP (τ about 150 ms) was faster, and the sAHP similar (τ bout 1–2 s) to the measured decay of [Ca$^{2+}$]$_i$ in soma (τ about 2 s; Table 2) or proximal apical dendrite (τ about 500–700 ms; Table 2). In addition, despite the $\tau_{\text{decay}}$ of the Ca$^{2+}$ transient being faster with the low-affinity 100 μM furo-6f (see above) than with 10 μM fura-2, the $\tau_{\text{decay}}$ for the sAHP elicited by 100 spikes at 50 Hz did not differ statistically for the 2 buffers: 2133 ± 142 ms for fura-6f (n = 9 cells) versus 2419 ± 233 ms for fura-2 (n = 12 cells) (data not shown). These data suggest that there is not a close match between the time course of either AHP component and [Ca$^{2+}$]$_i$ (Jahromi et al. 1999; Lasser Ross et al. 1997).

We performed voltage-clamp experiments to directly study the relationships between AHP currents and [Ca$^{2+}$]$_i$. AHP currents were elicited as tail currents after voltage steps to +0 mV from a holding potential between −60 and −70 mV. Space clamp on pyramidal cells with intact dendrites is problematic, and voltage was clearly not well controlled during the voltage steps. We used these steps as a means of elevating [Ca$^{2+}$]$_i$, which in turn activates the mAHP and sAHP tail currents (which lack voltage dependency: Schwindt et al. 1988). The outward AHP tail currents are small and very slow. Low-frequency signals show little dendritic attenuation; thus slow K$^+$ tail currents approaching the DC condition should exhibit minimal space-clamp error (Surmeier et al. 1994). The similarity we observed in time course between current-clamp (AHPs) and voltage-clamp (tail currents) data suggest that the space-clamp error lies within acceptable limits. Series resistance for all voltage-clamp data ranged between 16 and 30 MΩ. Cells with greater series resistance (or that showed a substantial increase in series resistance over the course of the recording) were discarded. Typical series resistance errors for the combined mAHP and sAHP currents were about 2 mV (100 pA × 20 MΩ). The reversal potentials for both AHP (tail) currents were close to $E_K$ (see following text), further suggesting acceptable voltage control.

Steps of 100- to 250-ms duration gave rise to a tail current consisting of several different components. After block by Cd$^{2+}$ (400 μM), only a transient current, with $\tau = 16.9 ± 1.7$ ms (n = 3), remained (Fig. 7A). To avoid contaminating our data with this fast, Ca$^{2+}$-independent current (likely in part a voltage-gated K$^+$ current), all fits to $I_{\text{AHP}}$ decays began at 55 ms after step repolarization. We operationally defined the $I_{\text{mAHP}}$ amplitude as the measurement at 55 ms. Because $I_{\text{mAHP}}$ decays with a time constant of about 155 ms, <5% should remain after 500 ms. Thus a measurement 555 ms after the last spike was taken as an index of $I_{\text{mAHP}}$ amplitude. Using those time points, Cd$^{2+}$ (400 μM) blocked 80 ± 4% (n = 6) of the current at 55 ms ($I_{\text{mAHP}}$) and 91 ± 3% (n = 6) of $I_{\text{AHP}}$.

Whereas short steps of 10–20 ms gave rise to a current whose decay matched a single exponential of time constant of about 150 ms (similar to mAHP), longer steps (~250 ms)

![FIG. 6. AHPs follow spike trains elicited by 5-ms suprathreshold current steps at a constant frequency of 20 or 50 Hz. A: AHP after a single action potential. B: AHP after 20 spikes (or 2 spikes, inset) at 20 Hz. Note the slow component present after 20 spikes, but not 2 spikes. C: AHP after 20 spikes (or 2 spikes, inset) at 50 Hz. Again, the mAHP and sAHP follow 20 spikes, but only the mAHP is seen after 2 spikes. Spikes were truncated to emphasize afterpotentials. D: mAHP and sAHP amplitudes isolated with isoproterenol and apamin, respectively, vs. number of spikes at constant frequency (50 Hz). Data presented as means ± SE. E: $[\text{Ca}^{2+}]_i$, vs. number of spikes at 50 Hz (n = 5 cells).

$I_{\text{sAHP}}$ and $I_{\text{mAHP}}$

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>3</th>
<th>5</th>
<th>20</th>
<th>30</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Spikes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>115 ± 6</td>
<td>151 ± 8</td>
<td>176 ± 20</td>
<td>160 ± 28</td>
<td>261 (one cell)</td>
</tr>
<tr>
<td>40</td>
<td>134 ± 12</td>
<td>159 ± 14</td>
<td>219 ± 19</td>
<td>242 ± 22</td>
<td>—</td>
</tr>
<tr>
<td>100</td>
<td>136 ± 5</td>
<td>166 ± 6</td>
<td>231 ± 11</td>
<td>265 ± 37</td>
<td>311 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of cells (unless otherwise stated): 10 Hz, 4; 40 Hz, 4; 100 Hz, 12.
produced a tail current that decayed as a double exponential, with one time constant similar to the time constant of the mAHP and the second r to that of the sAHP (Fig. 7B) (n = 9 cells). The faster-decaying current component was blocked by apamin (30–100 nM), confirming that it was I_{mAHP} (Fig. 7D) and the slower component was blocked by isoproterenol (10 μM), confirming that its I_{sAHP} (Fig. 7C). Under the conditions of our experiments, neither 30–100 nM apamin nor 10 μM isoproterenol significantly altered [Ca^{2+}].

At −70 mV, we observed tail currents of average amplitude 126 ± 15 pA (I_{mAHP}, n = 9) and 77.3 ± 14.6 pA (I_{sAHP}, n = 9). We determined exponential fits to the decay of pharmacologically isolated mAHP and sAHP currents, as well as multiple exponential fits to whole AHP currents. The data obtained by these methods did not differ significantly, so the results were pooled. Similar to our current-clamp findings, I_{mAHP} decayed with time constant τ = 121 ± 26 ms (n = 12), I_{sAHP} decayed with τ = 1,910 ± 340 ms (n = 10). The third (faster) current decayed with τ = 18 ± 2.0 ms (n = 8). The τ for the rise of the isolated I_{sAHP} was 94 ± 35 ms (n = 5).

I_{AHP} reversal potentials

The reversal potentials of I_{mAHP} and I_{sAHP} were determined by measuring tail current amplitudes (at the holding potential) after a 200-ms voltage step to +0 mV (holding potentials ranged between −60 and −110 mV). Again, I_{mAHP} was measured as the current 55 ms after the step, and I_{sAHP} was measured as the current 500 ms later. The mAHP current reversed at −93 ± 3 mV (n = 4), consistent with an E_{K} of about −100 mV (calculated from the Nernst equation: Fig. 8). In 2 of 4 cells, I_{AHP} reversed at −95 mV. In the remaining 2 cells tested, I_{sAHP} asymptotically approached zero current at −90 to −95 mV, but never truly reversed. One possible explanation for this latter observation would be that the sAHP channels are located remotely from the recording electrode. We compared the reversal potential of the sAHP in 2.5 mM K⁺ (−103, −102 mV) to that in 7.5 mM K⁺ (−71, −71 mV; shifts of 31 and 32 mV) (n = 2; data not shown). The Nernst potential predicts a shift of the reversal potential of 29 mV. These data further confirm that the sAHP current is carried by K⁺ ions. E_{Ca} for our recording solutions was near our holding potential (about −55 mV); thus Cl⁻ currents made little contribution to I_{mAHP} and I_{sAHP}.

I_{AHP} versus [Ca^{2+}]

In *Xenopus* oocytes, both SK1 and SK2 channels exhibit a sigmoidal dependency on [Ca^{2+}], with a Hill coefficient between 3.9 and 4.8 (Köhler et al. 1996), implying that cooperative binding of Ca^{2+} ions is required for channel activation. In CA1 pyramidal neurons, putative SK channels had a similar Ca^{2+} sensitivity (EC_{50} about 500 nM; Hill coefficient about 4.6: Hirschberg et al. 1999; Sellyanko et al. 1998). The apamin sensitivity of I_{mAHP} indicates that it is attributed to SK channels. If we could accurately measure the Ca^{2+} signal that activates the SK channels, we should thus find a sigmoidal relationship between I_{mAHP} and [Ca^{2+}], and the Hill coefficient and affinity should be similar to that seen in expression systems. Likewise, if the sAHP is also attributable to SK channels, we should find a similar relationship to [Ca^{2+}], for I_{mAHP} and I_{sAHP}.

To test this, we isolated I_{mAHP} or I_{sAHP} with isoproterenol and apamin, respectively, and plotted the resulting tail currents versus simultaneously obtained [Ca^{2+}], to determine the relationships I between I_{mAHP} and [Ca^{2+}] (Fig. 9), and 2 between I_{mAHP} and [Ca^{2+}] (Fig. 10). This method allowed examination of a wide range of [Ca^{2+}], in a single trace ([Ca^{2+}], peaked at the end of the step and decreased thereafter). An underlying assumption is that the AHP kinetics are slow relative to Ca^{2+} entry so that at the relationship is essentially memoryless and “instantaneous.” The first 55 ms after step repolarization were omitted from the plots because of the large contribution of voltage-gated K⁺ channels to the current (see above). We found the average relationships for I_{mAHP} versus [Ca^{2+}], (Fig. 10, C and D; n = 5) and for I_{sAHP} versus [Ca^{2+}], (Fig. 9C; n = 5). We then used a Kaleidagraph to determine the best fit to the data (least squares criterion).
relationships to \([\text{Ca}^{2+}]\) into the properties of the unknown sAHP channels and their
1994). We extend the observation that this plateau (Helmchen et al. 1996; Maravall et al. 2000; Regehr et al.
with increasing numbers of spikes until a plateau was attained
in any of 8 cells tested. The plots in Figs. 9C and 10C suggest
that the apparent affinity for \([\text{Ca}^{2+}]\), is similar for both AHP currents (or \(I_{\text{mAHP}}\) has a lower apparent affinity). These \(I_{\text{mAHP}}\)
data were similar to those described for \(I_{\text{AHP}}\) versus \([\text{Ca}^{2+}]\), in

**DISCUSSION**

We recorded from layer II/III pyramidal cells from rat sen-
rorimotor cortex to examine the effects of discharge history on
global changes in \([\text{Ca}^{2+}]\). We quantified the changes in
\([\text{Ca}^{2+}]\), in response to trains of APs. Further, we examined the
relationships between APs, AHPs, and \([\text{Ca}^{2+}]\) to gain insight
into the properties of the unknown sAHP channels and their
relationships to \([\text{Ca}^{2+}]\). Somatic current injections elicited APs that induced AHPs and \([\text{Ca}^{2+}]\) transients in the soma and
proximal dendrites (Markram et al. 1995; Yuste et al. 1994). At
a given firing frequency, both \([\text{Ca}^{2+}]\), and AHPs increased with increasing numbers of spikes until a plateau was attained
(Helmchen et al. 1996; Maravall et al. 2000; Regehr et al.
1994). We extend the observation that this plateau \([\text{Ca}^{2+}]\), was
linearly related to firing frequency (Helmchen et al. 1996) to
somatic \([\text{Ca}^{2+}]\), and this relationship holds throughout the
physiological firing range (to about 100 Hz). These findings are
consistent with \([\text{Ca}^{2+}]\), providing the cell with a simple and
precise indicator of its recent activity (Helmchen et al. 1996;
see also Engel et al. 1999; Maravall et al. 2000), as well as
acting as a negative feedback system by activating AHP con-
ductances (Wang 1998). Because of the observed nonlinearity
of fluorescence changes attributed to \(\text{Ca}^{2+}\) binding to fura-2,
we restricted our subsequent analyses to cells whose response
was \(\leq 40\% \Delta F/F\). In addition, we tested a lower dose of fura-2
and the lower affinity dyes fura-4f and fura-6f.

For \(I_{\text{AHP}}\), these averaged plots were fit by the \(n\)-th order
sigmoid

\[
I_{\text{AHP}} = \frac{d(\text{Ca}^{2+})}{[\text{Ca}^{2+}] + (K_D)} + b
\]

(2)

In these 5 cells, the average best fit for \(K_D\) was 201 ± 12 nM
and \(n = 4.51 ± 0.7\). \(I_{\text{mAHP}}\) showed a dependency on \([\text{Ca}^{2+}]\);
however we could not fit \(I_{\text{mAHP}}\) versus \([\text{Ca}^{2+}]\), with a sigmoid
in any of 8 cells tested. The plots in Figs. 9C and 10C suggest
that the apparent affinity for \([\text{Ca}^{2+}]\), is similar for both AHP
currents (or \(I_{\text{mAHP}}\) has a lower apparent affinity). These \(I_{\text{mAHP}}\)
data were similar to those described for \(I_{\text{AHP}}\) versus \([\text{Ca}^{2+}]\), in

**SK channels and AHP pharmacology**

Pyramidal cells provide an opportunity to 1) examine rela-
tionships between APs and \([\text{Ca}^{2+}]\), for SK channels in a native

![FIG. 9](image-url) There was a cooperative relationship between \(I_{\text{AHP}}\)
and \([\text{Ca}^{2+}]\), A: plot of somatic (red) and proximal apical
dendritic (blue) \([\text{Ca}^{2+}]\), transient, corresponding in time to tail
currents after a 150-ms voltage-clamp step (−70 to 0 mV). B: tail
currents from the same cell, corresponding to the \(\text{Ca}^{2+}\) trace
in A. Black trace: control tail current. Gray trace: current in the
presence of 10 μM isoproterenol (iso) to block the sAHP current.
Red trace: sAHP current isolated by subtraction (control — isoproterenol). C: isolated \(I_{\text{mAHP}}\) was plotted vs. \([\text{Ca}^{2+}]\),
for the cell illustrated in A and B. Data were well fit by a
4th-power sigmoid. For 5 cells tested, the \(K_D\) was 201 ± 12 nM
and the Hill coefficient, \(n = 4.51 ± 0.7\) (see RESULTS).

![FIG. 10](image-url) Relationship between \(I_{\text{mAHP}}\) and \([\text{Ca}^{2+}]\), was not sigmoidal. A–C:
current isolated by subtraction of apamin trace (100 nM). A: plot of somatic
\([\text{Ca}^{2+}]\), transient corresponding in time to tail currents after a 200-ms voltage-
clamp step from −70 to 0 mV. B: tail currents from the same cell, correspond-
ing to the \(\text{Ca}^{2+}\) trace in A. Black trace: control tail current. Gray trace: current
in the presence of 100 nM apamin to block the mAHP current. Red trace: mAHP current isolated by subtraction (control — apamin). C: isolated \(I_{\text{mAHP}}\) was plotted vs. \([\text{Ca}^{2+}]\), for the cell illustrated in A and B. Eight cells were
tested. In no case could the data be fit by a sigmoid.

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membrane and 2) gain insight into the nature of the sAHP channels by comparing the mAHP and sAHP. The apamin sensitivity of the mAHP indicates that it is mediated by SK channels (Sah and Faber 2002). SK1 and SK2 subunits are expressed in neocortex (Stocker and Pedarzini 2000) and SK2 channels are a prime candidate for the mAHP current. The basis for the sAHP is less clear.

**mAHP versus sAHP**

Both the mAHP and the sAHP are Ca$^{2+}$-dependent (Connors et al. 1984; Lorenzon and Foehring 1992, 1993; Pineda et al. 1998; Schwindt et al. 1988b) and increase in amplitude with number and frequency of spikes (as do increases in [Ca$^{2+}$]). High doses of Ca$^{2+}$ chelators (EGTA, BAPTA) also block both the mAHP and the sAHP (Lorenzon and Foehring 1995; Schwindt et al. 1992a; Velumian et al. 1999). Both AHP currents reverse at potentials near $E_K$, confirming that they are K$^+$ currents (Lorenzon and Foehring 1992, 1993; Schwindt et al. 1988b).

There were key differences between the mAHP and the sAHP. The mAHP follows a single action potential, whereas the sAHP rises and falls slowly, even with rapid changes in intracellular calcium (but see Lancaster and Zucker 1994; Lancaster et al. 1991; Zhang et al. 1995). Many neurotransmitter systems and signaling pathways modulate the sAHP (Knöpfel et al. 1990; Nicoll 1988; Sah and Clements 1999; Schwindt et al. 1988b). Our data for $I_{\text{AHP}}$ Versus [Ca$^{2+}$] (see following text) suggest that the sAHP channels may require an elevation of [Ca$^{2+}$] in the cytoplasm, rather than at the membrane, consistent with a role for a cytoplasmic intermediate between Ca$^{2+}$ and the K$^+$ channels.

**sAHP activation**

The sAHP current activates slowly after a spike train, despite the Ca$^{2+}$ transient peaking at the end of the spike train (cf. Jahromi et al. 1999; Lasser Ross et al. 1997) (Figs. 9 and 10). Why is the sAHP slow? The slow rising phase of the sAHP current (cf. Fig. 9B) has been interpreted as being attributed to Ca$^{2+}$ diffusion and a greater separation between sAHP channels and their source of Ca$^{2+}$ (Jahromi et al. 1999; Lancaster and Zucker 1994; Lancaster et al. 1991; Zhang et al. 1995), although in some cell types the Q$_{10}$ of this rising phase appears too high for simple diffusion (Sah and McLachlan 1992). In addition, experiments by Sah and Clements (1999) showed that the sAHP rises and falls slowly, even with rapid changes in intracellular calcium (but see Lancaster and Zucker 1994).

The mAHP could be mediated by a channel with intrinsically slow kinetics (Lancaster et al. 1991; Sah and Clements 1999) or could be dependent on calcium-induced calcium release (CICR). CICR contributes to the sAHP in peripheral neurons (Davies et al. 1996; Sah and McLachlan 1992; Vogalis et al. 2001). However, pharmacological experiments have shown that, although CICR is a factor in the firing behavior of immature pyramidal cells, it contributes little to the AHP in mature, repetitively firing pyramidal neurons (Pineda et al. 1998, 1999; Zhang et al. 1995). Another possibility is delayed facilitation of Ca$^{2+}$ channels (Bowden et al. 2001; Clouès et al. 1997). In CA1 pyramidal neurons, L-type Ca$^{2+}$ channels are the primary source of Ca$^{2+}$ for the sAHP (Marriott and Tavalan 1998; Moyer et al. 1992; Rascal et al. 1991). It has been proposed that the slow kinetics of the sAHP are attributed to delayed facilitation of L-type channels of the alpha1D (Ca$^{2+}$,L.3) type (Bowden et al. 2001). However, delayed facilitation has not been described in mature neocortical pyramidal neurons, and L-type channels do not couple to the sAHP (Pineda et al. 1998).

It is also possible that there is an intervening messenger or buffer (Hocherman et al. 1992; Sah and Faber 2002; Schwindt et al. 1992b; Zhang et al. 1995). Many neurotransmitter systems and signaling pathways modulate the sAHP (Knöpfel et al. 1990; Nicoll 1988; Sah and Clements 1999; Schwindt et al. 1988b). Our data for $I_{\text{AHP}}$ Versus [Ca$^{2+}$] (see following text) suggest that the sAHP channels may require an elevation of [Ca$^{2+}$] in the cytoplasm, rather than at the membrane, consistent with a role for a cytoplasmic intermediate between Ca$^{2+}$ and the K$^+$ channels.

$I_{\text{AHP}}$ Versus [Ca$^{2+}$]

Because Ca$^{2+}$-dependent SK channels underlie the mAHP, one would expect a precise temporal match between the decay of [Ca$^{2+}$], and the decay of $I_{\text{mAHP}}$ (Knöpfel and Nahwiler 1992; Knöpfel et al. 1990). There was no precise match between the $t_{\text{decay}}$ of $I_{\text{mAHP}}$ with $t_{\text{decay}}$ of the bulk [Ca$^{2+}$], in soma or dendrite. We found general agreement between the decay time courses of $I_{\text{AHP}}$ and somatic [Ca$^{2+}$]. However, this relationship was not precise, as previously reported by Lasser Ross et al. (1997) and Jahromi et al. (1999) in hippocampal pyramidal cells. Furthermore, AHP decay times were not different between cells recorded with fura-6f and fura-2, despite faster $t_{\text{decay}}$ for [Ca$^{2+}$], using fura-6f. Similarly, BAPTA prolongs $I_{\text{AHP}}$ in CA1 pyramidal neurons without parallel changes in the decay time course of the Ca$^{2+}$ transient (Jahromi et al. 1999; see also Lasser-Ross et al. 1997).

$I_{\text{AHP}}$ exhibited a cooperative relationship with [Ca$^{2+}$], similar to SK channels in expression systems (Hirshberg et al. 1998; Kohler et al. 1996). Thus the Ca$^{2+}$ sensor for $I_{\text{AHP}}$ has properties similar to the Ca$^{2+}$ sensor of SK channels. This suggests that either 1) SK channels are responsible for the sAHP, or 2) the sAHP is attributed to non-SK channels, but the same Ca$^{2+}$ sensor (e.g., calmodulin; Xia et al. 1998), or one with similar Ca$^{2+}$ binding affinity and cooperativity, is used.

It is likely that there is a distinction between the bulk [Ca$^{2+}$], that we are able to image and [Ca$^{2+}$] in the immediate vicinity of the AHP channels (or Ca$^{2+}$ sensor). We first observed the sAHP after 3–5 spikes, which corresponds to a bulk [Ca$^{2+}$] of about 140–160 nM. The sAHP was maximal by 30–50 spikes, corresponding to [Ca$^{2+}$] of about 250–350 nM. These data agree well with the foot and plateau, respectively, of the $I_{\text{AHP}}$ versus [Ca$^{2+}$] plots, providing an independent estimate of Ca$^{2+}$ sensitivity of $I_{\text{AHP}}$.

Wilson and Callaway (2000) combined experiments and modeling to examine the relationship between the apamin-sensitive (SK) $I_{\text{AHP}}$ versus [Ca$^{2+}$], in dopaminergic cells (substantia nigra). They determined that a sigmoidal relationship between $I_{\text{AHP}}$ and bulk cytoplasmic [Ca$^{2+}$], would occur only if cytoplasmic Ca$^{2+}$ was well mixed. Immediately after Ca$^{2+}$ entry, [Ca$^{2+}$] would be highest at the membrane and lower in the cytoplasm. On termination of Ca$^{2+}$ entry, this gradient should dissipate. If average [Ca$^{2+}$], concentration is not proportional to Ca$^{2+}$ at the interior surface of the membrane (e.g., because of Ca$^{2+}$ depletion by Ca$^{2+}$ removal), the apparent
Ca$^{2+}$ dependency of the tail current is shifted positive and the sigmoidal shape distorted. In dopamine cells, Wilson and Callaway (2000) observed such a distorted relationship, similar to $I_{\text{mAHP}}$ versus [Ca$^{2+}$], in neocortical pyramidal cells.

Our data suggest that the sAHP channels in neocortical pyramidal cells (but not mAHP channels) respond to a Ca$^{2+}$ signal that is proportional to that measured in the bulk cytoplasm. This results in a relationship between current and [Ca$^{2+}$], that is similar in form to SK channels in expression systems, but with an apparent affinity that is higher (about 200 nM vs. 400–500 nM in expression systems; Hirschberg et al. 1998). In contrast, our data also suggest that the apamin-sensitive (SK) mAHP channels respond to restricted domains of Ca$^{2+}$ not accurately reflected by our measurement of bulk [Ca$^{2+}$].

Ca$^{2+}$ entry and AHPs

Pineda et al. (1998) demonstrated specificity in the relationships between Ca$^{2+}$ channel types and AHPs in rat neocortex. Consistent with sAHP channels responding to the bulk cytoplasmic Ca$^{2+}$ and mAHP channels responding to a restricted Ca$^{2+}$ domain, there is greater specificity in the relationship between mAHP channels and the Ca$^{2+}$ source. Only P-type currents activated the mAHP in repetitively firing neurons; N-, P-, and Q-type currents coupled to the sAHP, Ca$^{2+}$ entering through L-type channels contributed to neither AHP.

These data indicate separation between mAHP and sAHP channels. The separation could be physical, or it could be more subtle. For instance, Schwindt et al. (1992a; see also Lorenzen and Foehring 1995; Velumyan and Carlen 1999; Zhang et al. 1995) showed that, whereas perfusion of BAPTA or EGTA into pyramidal cells blocked the mAHP as expected, low doses of these exogenous mobile calcium buffers potentiated and slowed the sAHP. Intervention of a mobile Ca$^{2+}$ sensor might explain why the mAHP appears after one spike, but the sAHP only after several. An interposed binding protein might also underlie the sensitivity of the sAHP to internal anions (Zhang et al. 1999).

Further, Pineda et al. (1999) described developmental differences in the coupling specificity of Ca$^{2+}$-channels and AHPs that correlated with differences in the sAHP and firing behavior. Thus the secrets of the sAHP may be revealed, not by a hunt for a novel channel type, but through an understanding of the intricacies of intracellular calcium dynamics.

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