Na\textsuperscript{+}-Activated K\textsuperscript{+} Current Contributes to Postexcitatory Hyperpolarization in Neocortical Intrinsically Bursting Neurons

Silvana Franceschetti, Tatiana Lavazza, Giulia Curia, Patrizia Aracri, Ferruccio Panzica, Giulio Sancini, Giuliano Avanzini, and Jacopo Magistretti. Na\textsuperscript{+}-activated K\textsuperscript{+} current contributes to postexcitatory hyperpolarization in neocortical intrinsically bursting neurons. J Neurophysiol 89: 2101–2111, 2003; 10.1152/jn.00695.2002. The ionic mechanisms underlying the termination of action-potential (AP) bursts and postburst afterhyperpolarization (AHP) in intrinsically bursting (IB) neocortical neurons were investigated by performing intracellular recordings in thin slices of rat sensorimotor cortex. The blockade of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents enhanced postburst depolarizing afterpotentials, but had inconsistent and minor effects on the amplitude and duration of AHPs. On the contrary, experimental conditions resulting in reduction of voltage-dependent Na\textsuperscript{+} entry into the cells caused a significant decrease of AHP amplitude. Slice perfusion with a modified artificial cerebrospinal fluid in which LiCl (40 mM) partially replaced NaCl had negligible effects on the properties of individual APs, whereas it consistently increased burst length and amplitude and duration of AHPs. On the contrary, experimental depolarizing afterpotentials, but had inconsistent and minor effects on the amplitude and duration of AHPs. On the contrary, experimental conditions resulting in reduction of voltage-dependent Na\textsuperscript{+} entry into the cells caused a significant decrease of AHP amplitude. Slice perfusion with a modified artificial cerebrospinal fluid in which LiCl (40 mM) partially replaced NaCl had negligible effects on the properties of individual APs, whereas it consistently increased burst length and amplitude and duration of AHPs. Experiments performed by partially replacing Na\textsuperscript{+} ions with choline revealed a comparable reduction in AHP amplitude associated with an inhibition of bursting activity. Moreover, in voltage-clamp experiments carried out in both in situ and acutely isolated neurons, partial substitution of extracellular NaCl with LiCl significantly and reversibly reduced the amplitude of K\textsuperscript{+} currents evoked by depolarizing stimuli above-threshold for Na\textsuperscript{+}-current activation. The above effect of Na\textsuperscript{+}-to-Li\textsuperscript{+} substitution was not seen when voltage-gated Na\textsuperscript{+} currents were blocked with TTX, indicating the presence of a specific K\textsuperscript{+}-current component activated by voltage-dependent Na\textsuperscript{+} (but not Li\textsuperscript{+}) influx. The above findings suggest that a Na\textsuperscript{+}-activated K\textsuperscript{+} current recruited by the Na\textsuperscript{+} entry secondary to burst discharge significantly contributes to AHP generation and the maintenance of rhythmic burst recurrence during sustained depolarizations in neocortical IB neurons.

INTRODUCTION

Neocortical pyramidal neurons can generate diverse patterns of firing activity, which depend on neuronal morphology, passive properties, and active ion currents. These different intrinsic properties interact with synaptically driven inputs in setting the complex integrative function of the neocortical mantle. In the deep layers of both sensorimotor and visual cortical areas, projecting pyramidal neurons display remarkably heterogeneous firing behaviors (Connors et al. 1982; Connors and Gutnick 1990; Franceschetti et al. 1998; Kasper et al. 1994; Mason and Larkman 1990; McCormick et al. 1985; Montoro et al. 1988; Silva et al. 1990; Tseng and Prince 1993) and include a large proportion of intrinsically bursting (IB) neurons that significantly contribute to physiological and epileptic intracortical synchronization (Chagnac-Amitai and Connors 1989; Silva et al. 1990).

Subpopulations of neurons endowed with intrinsically bursting behavior and suberving different integrative functions (Galarreta and Hestrin 2000; Lisman 1997; Snider et al. 1998; Williams and Stuart 1999) are also found in other cortical structures (e.g., the hippocampal CA3 region), as well as in various subcortical nuclei, such as thalamic or brain stem nuclei. However, the fine mechanisms underlying this particular firing behavior in different structures are probably uneven, depending on different inward currents upholding burst discharge and different control mechanisms of burst termination or recurrence. During constant membrane depolarization, neocortical IB neurons generate quasirhythmic bursts or a single initial burst followed by a rhythmic discharge of single APs, each followed by a prominent depolarizing afterpotential (DAP) (Franceschetti et al. 1998; McCormick et al. 1985; Montoro et al. 1988; Silva et al. 1990; Tseng and Prince 1993). In both of the above cases, each excitatory event (i.e., the complex consisting of burst or single AP plus DAP) is followed by a robust hyperpolarizing afterpotential (AHP) that has a relatively constant shape and depth. The frequency of rhythmic discharges in IB neurons shows no tendency to decline during long-lasting depolarizing stimuli but, as a result of the pronounced AHPs, the rate of repetition of individual excitatory events is lower (Silva et al. 1990) than the discharge frequencies observed in adapting, regular spiking (RS) neurons (Baranyi et al. 1993; Stafstrom et al. 1984).

In a previous series of experiments made on rat neocortical neurons we noticed that pharmacological manipulations strongly reducing Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents, which substantially contribute to the firing properties and AHP generation in adapting, RS neurons (Madison and Nicoll 1982 1984; Schwindt et al. 1992; see also Sah and Davies 2000 for review), have only minor effects on spike discharge and AHPs in...
IB neurons. On the basis of these preliminary observations, we undertook a study aimed at investigating the ionic mechanisms accounting for the postexcitatory hyperpolarizing potentials in IB neurons. We performed intracellular and patch-clamp recordings on in situ and acutely isolated pyramidal neurons from rat sensorimotor cortex and found that the AHPs produced by IB neurons critically depend on normal Na\(^{+}\) influx during AP discharge. Our data are in favor of a key role for a Na\(^{+}\)-activated K\(^{+}\) current in generating AHPs and controlling burst length and rhythmic discharge in neocortical IB neurons.

**Methods**

Slice and cell preparation

Sprague-Dawley rats (Charles River, Italy) aged 12–45 postnatal days were deeply anesthetized with ether vapors and decapitated. Their brains were removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 26.5 NaHCO\(_3\), 2 CaCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 2 MgSO\(_4\), 3.5 KCl, and 10 glucose (bubbled with 95% O\(_2\)-5% CO\(_2\)). Coronal slices 300- to 350-μm thick were cut from the sensorimotor cortex using a vibratome. Those used for conventional intracellular recordings were immediately transferred to an interface chamber, perfused with ACSF, and allowed to equilibrate for 1–2 h before the electrophysiological recordings were started. The slices used to prepare dissociated neurons were kept for 5–15 min in a modified ACSF (bubbled with pure O\(_2\); pH 7.4) in which NaHCO\(_3\) was replaced by 25 mM HEPES-NaOH and containing 1 mg/ml pronase (protease type XIV, Sigma–Aldrich) to digest the extracellular matrix and 1 mM kynurenic acid to prevent excitotoxic cell damage. After the enzymatic treatment, the slices were washed and stored for variable times in an enzyme-free solution. The dissociated neurons thus obtained were plated on a petri dish (Falcon) coated with concanavalin A (Sigma–Aldrich), allowed to settle and attach for 2–3 min, and then bath perfused with modified ACSF (see following text). Only the pyramid-shaped neurons were selected for patch-clamp recordings.

All of the experimental procedures were carried out in compliance with the 86/609/UE law on animal research and the guidelines for animal care and management of the Ethics Committee of the C. Besta Institute.

Intracellular recordings

Intracellular recordings from layer V pyramidal neurons were carried out on slices using an IR-283 amplifier (Neurodata Instrument Corp., New York). The bath temperature was kept at 35°C. Sharp electrodes were prepared using borosilicate glass capillaries (Harvard Apparatus) and filled with 2 M K-acetate (resistance 80–90 MΩ). Voltage and current signals were stored on magnetic tape and digitized using an AT-MIO-16-E A/D converter (National Instruments, Austin, TX) at a sampling rate of 4–10 kHz.

The electrophysiological characteristics of the impaled neurons were tested under control conditions (i.e., standard ACSF). Kynurenic acid (2 mM) was routinely added to the perfusing solution to block glutamatergic neurotransmission. In control experiments, ouabain was added at concentrations of 1–10 μM.

Various strategies were adopted to block the Ca\(^{2+}\) currents and Ca\(^{2+}\)-activated K\(^{+}\) currents. Most often, the CaCl\(_2\) concentration in the ACSF was lowered to 0.2 mM, and CoCl\(_2\) (2 mM) or MnCl\(_2\) (2.4 mM) was added (low-Ca\(^{2+}\) ACSF). In a few experiments, CaCl\(_2\) was lowered to 1.8–1 mM, and NiCl\(_2\) (200–500 μM) and CdCl\(_2\) (400 μM) were added. In all of the ACSF prepared in this way, MgCl\(_2\) was raised to a final concentration of 5–7 mM, NaH\(_2\)PO\(_4\) was omitted, and NaHCO\(_3\) was lowered to 15 mM and partially replaced with HEPES-NaOH (10 mM). The data were collected 45–60 min after starting perfusion with low-Ca\(^{2+}\) ACSF. Postsynaptic potentials were monitored in several experiments to confirm the abolition of synaptic transmission in the slices perfused with low-Ca\(^{2+}\) ACSF. In some experiments, the calcium chelator BAPTA (100–200 mM) was added to the intrapipette solution.

In the experiments in which extracellular LiCl, choline-Cl, or tetraethylammonium (TEA)-Cl were used, the same salts substituted equimolar amounts of NaCl.

**Patch-clamp experiments**

Whole cell, patch-clamp experiments on isolated neurons were performed at room temperature using an Axopatch 200B amplifier (Axon Instruments). In the experiments aimed at recording Na\(^{+}\) (or Li\(^{+}\)) currents, borosilicate glass electrodes were filled with a solution containing (in mM) 120 CsCl, 1 MgCl\(_2\), 10 EGTA–CsOH, 10 HEPES–CsOH, 2 ATP–Na\(_2\), 10 phosphocreatine-diTris, 0.3 GTP–Na\(_2\), and 20 U/ml creatine phosphokinase, pH 7.2. In experiments performed to study voltage-activated K\(^{+}\) currents, patch pipettes were filled with a solution containing (in mM) 110 K-aspartate, 13 KCl, 1 MgCl\(_2\), 10 EGTA–KOH, 2 ATP–Na\(_2\), 10 HEPES–KOH, 0.3 GTP–Na\(_2\), 10 phosphocreatine-diTris, and 20 U/ml creatine phosphokinase, pH 7.2. When filled with the above solutions, the patch pipettes had a resistance of 3–6 MΩ.

In experiments performed to study Na\(^{+}\) currents, the neurons were bath perfused with a solution containing (in mM) 15 NaCl (or LiCl), 1.3 CaCl\(_2\), 2 MgCl\(_2\), 0.4 CdCl\(_2\), 0.3 NiCl\(_2\), 20 TEA-Cl, 110 choline-Cl, 10 HEPES–NaOH, and 10 glucose, pH 7.3, bubbled with pure O\(_2\). In experiments on K\(^{+}\) currents, the following external solution was perfused (mM): 135 NaCl, 1.3 CaCl\(_2\), 0.4 CdCl\(_2\), 0.3 NiCl\(_2\), 2 MgCl\(_2\), 3 KCl, 10 HEPES–NaOH, and 10 glucose, pH 7.4. TTX (1 μM) was also added to block Na\(^{+}\) currents (I_{Na}). LiCl equimolarly replaced NaCl at a final concentration of 135 or 40 mM.

After seal formation and cell membrane rupturing, capacitive currents were minimized by means of the amplifier circuitry. A 70–90% series resistance compensation was routinely achieved, and the estimated maximum voltage-clamp error did not exceed 4 mV. Data were digitized by means of a Digidata 1200 interface, and the pClamp 8.2 software (Axon Instruments) was used to generate stimulus protocols and to acquire signals. When appropriate, residual transients and leakage currents were eliminated using an on-line P/4 subtraction protocol. Junction potential errors were not corrected. Recordings showing signs of bad clamp control were excluded from the analysis.

**Data analysis**

**INTRACELLULAR RECORDINGS.** The voltage traces were analyzed using a dedicated homemade software. AP duration was measured as the AP width at −40 mV. The first derivative was used to assess the slope of the AP depolarizing and hyperpolarizing branches. Membrane input resistance (R_{m}) was measured at the peak of the negative membrane-voltage deflection elicited by 0.2- to 0.25-nA current steps. The membrane time constant (τ_m) was evaluated using a single exponential function to fit the onset phase of the same voltage responses.

The amplitude and duration of the AHPs were measured on the hyperpolarizing deflections following individual bursts elicited by means of brief (20–40 ms) depolarizing pulses or short (200 ms) 100- to 200-Hz trains of 2-ms stimuli, the amplitude of which was adjusted to obtain consistent AP activation.

**VOLTAGE-CLAMP RECORDINGS.** Data were analyzed using pClamp 8.2 (Axon Instruments) and Origin 6.0 software (Microcal Incorporated) on a Pentium PC. Conductances were calculated as G = I/(V – V_{rev}), where G is conductance, I is current, V is the membrane voltage, and V_{rev} is the theoretical reversal potential. V_{rev} estimated according
to the Nernst law was \( +42.9 \) mV for \( \text{Na}^+ \) currents (with 15 mM NaCl plus about 7 mM NaOH in the external solution) and \( -94.0 \) mV for \( \text{K}^+ \) currents.

Plots of the voltage dependence of activation were fitted using a Boltzmann function in the form: \( G/G_{\text{max}} = 1/[1 + \exp(V_{1/2} - V/k)] \), where \( G_{\text{max}} \) is the maximal peak conductance, \( G \) is the peak conductance at each test voltage, \( V_{1/2} \) is the voltage at which half-maximal activation is reached, and \( k \) is the slope factor.

The data are expressed as mean values \( \pm \) SE and were statistically analyzed using Student’s two-tailed \( t \)-test for paired data or Wilcoxon’s nonparametric test.

**RESULTS**

**AHP in intrinsically bursting neurons**

Intracellular current-clamp recordings were obtained in 38 IB pyramidal neurons and, for comparison, in 9 adapting RS neurons. As shown in Fig. 1, IB neurons at their spontaneous resting potential \( (V_{\text{rest}}) \) \( (-65.5 \pm 0.6 \) mV; \( n = 27 \) typically responded to sustained depolarizing current pulses with recurrent rhythmic bursts of two to five APs arising from a depolarizing envelope (Fig. 1, A\text{1}, and B\text{1}). Each burst was followed by a robust and quite uniform AHP. The depth of these AHPs appeared to depend mainly on the characteristics of each individual burst, being poorly influenced by the duration of the foregoing discharge. The AHPs were definitely more robust after bursts that included a long subthreshold depolarization sustaining three-four APs (peak amplitude: \( 7.9 \pm 0.2 \) mV, \( n = 8 \)) than after bursts consisting of shorter depolarizing envelopes and AP doublets (\( 6.0 \pm 0.4 \) mV, \( n = 14 \); \( P < 0.001 \)) (Fig. 1, A\text{1}, A\text{2}, B\text{1}, and B\text{2}). In several IB neurons, during depolarizing-current injection burst firing turned into recurrent discharge of single APs, each of which was followed by a prominent DAP (Fig. 1B\text{1}, arrows). In this case, the postexcitatory hyperpolarization was smaller than that following a burst, but still robust and invariant over time. Figure 1, C\text{1} and C\text{2}, shows the firing pattern and AHPs observed in a representative adapting RS neuron: a rather weak AHP followed the response to a brief (40 ms) depolarizing pulse adjusted to evoke a triplet of APs, similar to that observed in most of the spontaneous bursts of IB neurons. During longer step depolarizations, AHPs gradually increased in duration, and an enlarged AHP came into view at the end of the discharge evoked by a 400-ms depolarizing pulse.

To investigate the ionic mechanisms responsible for the firing behavior and postburst AHPs observed in IB neurons, we first examined the \( \text{K}^+ \) dependence of AHPs. Three neurons were perfused with ACSF containing variable \( \text{K}^+ \) concentrations \((2.5, 7, \) and \( 10 \) mM) and stimulated with 40-ms depolarizing pulses (adjusted to evoke individual bursts) or depolarizing pulse trains. The reversal potential of the AHPs was about \(-105 \) mV in the presence of 2.5 mM extracellular \( \text{K}^+ \) and shifted in the positive direction by about 25 and 35 mV in the presence of 7 and 10 mM \( \text{K}^+ \), respectively. These shifts are in good agreement with those expected on the basis of Nernst’s law for \( \text{K}^+ \) conductances. In neurons recorded at their \( V_{\text{rest}} \), the increase in extracellular \( \text{K}^+ \) concentration consistently led to a prolongation of individual bursts and reduced the amplitude of postburst and posttrain AHPs (data not shown).

We then analyzed the possible roles of \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) currents. To evaluate the effects of a broad blockade of these currents, we perfused 10 IB neurons with a modified low-\( \text{Ca}^{2+} \) ACSF in which extracellular \( \text{Ca}^{2+} \) was largely replaced by divalent cations unable to permeate \( \text{Ca}^{2+} \) channels (see methods). Long-lasting (1 h) perfusion with low-\( \text{Ca}^{2+} \) ACSF led to
TABLE 1. Effects of perfusion with low-Ca\(^{2+}\) ACSF on the main electrophysiological parameters of IB neurons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Low Ca(^{2+})</th>
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<tbody>
<tr>
<td>AP Slope, V/s</td>
<td>318.4 ± 28.3</td>
<td>315.3 ± 22.3*</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>108.5 ± 4.7</td>
<td>109.5 ± 5.0*</td>
</tr>
<tr>
<td>Repolarization, V/s</td>
<td>119.7 ± 7.7</td>
<td>104.7 ± 11.6*</td>
</tr>
<tr>
<td>(R_{\text{IN}}, \text{M}\Omega)</td>
<td>35.8 ± 2.3</td>
<td>39.2 ± 2.5*</td>
</tr>
<tr>
<td>(\tau_{\text{m}}, \text{ms})</td>
<td>14.9 ± 2.6</td>
<td>16.8 ± 2.8*</td>
</tr>
<tr>
<td>Postburst AHP Amplitude, mV</td>
<td>6.0 ± 0.6</td>
<td>5.1 ± 0.5*</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>137.2 ± 16.2</td>
<td>145.0 ± 16.2*</td>
</tr>
<tr>
<td>Area, mV × ms</td>
<td>389.1 ± 28.2</td>
<td>340.6 ± 21.6*</td>
</tr>
<tr>
<td>Postrain AHP Amplitude, mV</td>
<td>11.4 ± 0.7</td>
<td>10.4 ± 0.6*</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>189.2 ± 18.5</td>
<td>175.4 ± 20.0*</td>
</tr>
<tr>
<td>Area, mV × ms</td>
<td>1023.3 ± 117.9</td>
<td>915.3 ± 114.9*</td>
</tr>
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</table>

Values are means ± SE; the number of neurons was 10. AP, action potential; AHP, afterhyperpolarization. * Not significantly different from control. † Significantly different from control, \(P < 0.05\).

A moderate increase in input resistance (Table 1), thus leading to slightly larger membrane-potential deflections in response to both hyperpolarizing and depolarizing stimuli (Fig. 2A). The repolarizing phase of the APs was slightly slowed (Table 1), thus increasing the duration of individual APs from 1.7 ± 0.5 to 2.3 ± 0.1 ms (\(P < 0.005\)). In most neurons (8/10) these effects were accompanied by a broadening of the burst discharges and an increased magnitude of the DAP (Fig. 2A, inset), without any significant changes in the characteristics of rhythmic burst repetition. Similar effects were observed in the IB neurons recorded using intracellular electrodes containing 100–200 mmol/l of the Ca\(^{2+}\) chelator BAPTA (n = 5, data not shown). The slight increase in input resistance found during low-Ca\(^{2+}\) perfusion was not accompanied by any consistent increase in the frequency of burst repetition. This was probably due to a delay in the late repolarization following the enhanced DAPs (see following text), which would compensate for the increased depolarizing drive thereby resulting in a relatively unchanged interburst interval.

In the adapting RS neurons recorded in control experiments, perfusion with low-Ca\(^{2+}\) ACSF led to an increased firing rate in response to the injection of depolarizing current steps. The AHP following each AP decreased in amplitude and duration, thus leading to a decline in late firing adaptation (Fig. 2B).

To quantitatively evaluate the contribution of Ca\(^{2+}\)-activated K\(^{+}\) currents to burst-dependent hyperpolarization, we measured the amplitude and duration of the AHPs following individual bursts elicited by injecting brief depolarizing stimuli (postburst AHP) (Fig. 3A). We also examined the AHPs following sequences of APs elicited by injecting brief trains (200 ms, 100–200 Hz) of depolarizing stimuli (posttrain AHPs) (Fig. 3A). Stimulus trains were used to induce completely reproducible high-frequency firing outlasting the duration of individual spontaneous bursts and to evaluate the characteristics of the enhanced AHP resulting from a prolonged high-frequency discharge. In the presence of low-Ca\(^{2+}\) ACSF, the postexcitatory depolarization following both individual bursts and pulse trains became more prominent and increased in duration, thus correspondingly delaying the onset of the AHP. However, AHP peak amplitude was only slightly reduced (n = 7) or unchanged (n = 3) (Fig. 3, A1 and A2), and no changes were observed in AHP duration (Table 1). Figure 3B shows the conspicuous effects of perfusion with low-Ca\(^{2+}\) ACSF in a RS adapting neuron, in which the same treatment led to a marked reduction in both early and late postrain AHPs.

**Na\(^{+}\) dependence of hyperpolarizing afterpotentials**

To explore the possibility that AHP generation in IB neurons mainly relies on Na\(^{+}\) rather than Ca\(^{2+}\) entry through the membrane, we performed a series of experiments in which...
extracellular Na+ was partially replaced with Li+ or choline. NaCl replacement with LiCl was employed in most experiments because it is known that Li+ ions, like choline, have no effects on K+ channels activated by increased intracellular Na+ concentrations (Bishoff et al. 1998; Dryer et al. 1989; Haimann et al. 1990, 1992; Safronov and Vogel 1996), but, unlike choline, can permeate Na+ channels nearly as well as Na+ itself (Hille 1972). As shown in Fig. 4, A1–A4 and B1–B4, in the presence of either 40 mM LiCl (n = 8) or 40 mM choline-Cl (n = 3), the AHPs following each of the recurrent bursts elicited by moderate membrane depolarization, as well as the AHP occurring at the end of depolarizing-pulse injection (Fig. 4, A4 and B4, arrows), were clearly reduced in amplitude. The same was true for the AHPs following single bursts evoked by brief depolarizing steps (Fig. 4, A3 and B3).

The stimulus amplitude needed to reach the firing threshold was similar in the normal ACSF and in the presence of Li+ (Fig. 4A1), but it was increased in the presence of choline (Fig. 4B1), probably because of a reduction in the depolarizing drive due to near-threshold Na+ currents. Li+ but not choline enhanced burst firing and DAPs (Fig. 4, A1, A2, B1, and B2). To evaluate the net contribution of Na+ influx to AHP generation in more detail, we examined the effects of the partial substitution of Na+ with Li+ in 11 neurons that had previously undergone a prolonged treatment aimed at preliminarily abolishing or reducing the contribution of Ca2+-activated K+ currents. To this end, perfusion with low-Ca2+ ACSF, followed by the partial (40 or 100 mM) substitution of NaCl with choline-Cl. The bursting activity remaining during low-Ca2+ medium perfusion tended to fade in the presence of 40 mM choline-Cl and was suppressed by 100 mM choline-Cl (Fig. 6A). In the presence of choline, the slope and (to a lesser extent) the amplitude of APs decreased (Table 2). Furthermore, AP repolarization was slowed, and the postexcitatory hyperpolarizations following AP trains and individual burst discharges evoked by brief stimuli were greatly reduced (Fig. 6, B, C1, C2, and D; Table 2). An obvious decrease in AHP amplitude also occurred following prolonged depolarizations sustaining the repetitive discharge of broadened APs (Fig. 6A,

During prolonged periods of LiCl perfusion (>15 min, a time interval longer than that during which all of the data presented here were collected), many neurons showed gradual membrane depolarization with respect to their control Vrest, which needed to be counteracted by negative-current injection, and completely reversed during washout. We interpreted this trend toward membrane depolarization as the consequence of a possible delayed “side-effect” of Li+ ions on the electrogenic Na+-K+ pump. To compare the effect of LiCl substitution with that due to a direct Na+-K+ pump blockade, we perfused four neurons with ouabain (1–10 μM) and found that spontaneous membrane depolarization and hyperexcitability phenomena ultimately leading to spreading depression occurred in a delayed time window (20–45 min, depending on ouabain concentration). However, before the occurrence of these spontaneous depolarizing events we never observed any changes in firing behavior or AHP characteristics similar to those induced by LiCl substitution (data not shown).

To evaluate the effect of a simple reduction of Na+ current amplitude on the firing properties of IB neurons, we performed further experiments in which Na+ was substituted with choline (which does not permeate Na+ channels) after abolishing or minimizing Ca2+ and Ca2+-activated K+ currents (n = 5). Figure 6 summarizes the effects of perfusion with low-Ca2+ ACSF, followed by the partial (40 or 100 mM) substitution of NaCl with choline-Cl. The bursting activity remaining during low-Ca2+ medium perfusion tended to fade in the presence of 40 mM choline-Cl and was suppressed by 100 mM choline-Cl (Fig. 6A). In the presence of choline, the slope and (to a lesser extent) the amplitude of APs decreased (Table 2). Furthermore, AP repolarization was slowed, and the postexcitatory hyperpolarizations following AP trains and individual burst discharges evoked by brief stimuli were greatly reduced (Fig. 6, B, C1, C2, and D; Table 2). An obvious decrease in AHP amplitude also occurred following prolonged depolarizations sustaining the repetitive discharge of broadened APs (Fig. 6A,

FIG. 4. Effect of partial extracellular NaCl substitution with 40 mM LiCl (A1–A4) or 40 mM choline-Cl (B1–B4) on the firing properties and AHPs of IB neurons. Both treatments led to a clear reduction of the AHPs following each recurrent burst elicited by moderate membrane depolarization (A1, A2, B1, and B2) as well as the AHPs that developed at the end of the discharge (A3 and B3 show the superimposition of the traces individually shown in A1 and B1). There was a similar reduction in the AHPs following the single bursts evoked by brief depolarizing current steps (A1 and B1).

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rightmost panel), which could still be elicited by increasing the depolarizing stimulus’ amplitude in the presence of 100 mM choline-Cl.

Effects of Na\(^{+}\) substitution with Li\(^{+}\) on voltage-dependent Na\(^{+}\) and K\(^{+}\) currents

To exclude the possibility that the above-described effects of Li\(^{+}\) on the firing properties and AHPs of IB neurons were the consequence of an inhibitory action of the same ion on voltage-dependent K\(^{+}\) conductances, we evaluated the effects of LiCl on both total and 4-AP–resistant K\(^{+}\) currents recorded in acutely dissociated neocortical neurons with patch-clamp, whole cell experiments (n = 9). As shown in Fig. 7, A and B, for two representative neurons, LiCl, at the same concentration used for the slice experiments (corresponding to an approximately 30% substitution of extracellular NaCl), was ineffective on both total and 4-AP–insensitive K\(^{+}\) currents, whereas a minimal reduction in K\(^{+}\) current amplitude was sometimes observed in the currents evoked after the complete substitution of NaCl with LiCl (Fig. 7A). Moreover, as shown in Fig. 7, C\(_1\) and C\(_2\), for a representative neuron, the voltage-activated Na\(^{+}\) and Li\(^{+}\) currents completely overlapped, and the current-voltage (I-V) relationship and activation kinetics of the inward transient (Na\(^{+}\) or Li\(^{+}\)) current was very similar in normal NaCl and after the complete equimolar substitution of NaCl with LiCl (n = 3) (Fig. 7C\(_2\)). To directly evaluate the presence of K\(^{+}\) current(s) activated by voltage-dependent Na\(^{+}\) entry in the neurons under study,

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Effect of partial extracellular NaCl substitution with 40 mM LiCl on the firing properties and AHPs of IB neurons recorded in low-Ca\(^{2+}\) ACSF. A: representative IB neuron recorded after 1 h of perfusion with low-Ca\(^{2+}\) (Co\(^{2+}\)-substituted) ACSF and at 2 different times after starting perfusion with Li\(^{+}\)-substituted ACSF. In the presence of LiCl, continuous high-frequency firing progressively replaced the broad bursts observed during low-Ca\(^{2+}\) perfusion in response to sustained depolarizing-current pulses. B: the same ionic change greatly reduced the AHPs following trains of APs in slices perfused with low-Ca\(^{2+}\) ACSF. C\(_1\) and C\(_2\), graphic representation of the changes in AHP peak amplitude and AHP integral (describing the area under AHP) during perfusion with low-Ca\(^{2+}\) and with low-Ca\(^{2+}\) plus 40 mM Li\(^{+}\) (*P < 0.05, Wilcoxon test)

<table>
<thead>
<tr>
<th>TABLE 2.</th>
<th>Effects of partial substitution of extracellular Na(^{+}) with Li(^{+}) or choline on the main electrophysiological parameters of IB neurons</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>AP</td>
<td></td>
</tr>
<tr>
<td>Slope, V//s</td>
<td>315.4 ± 22.3</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>114.6 ± 6.8</td>
</tr>
<tr>
<td>Repolarization, V//s</td>
<td>115.6 ± 10.3</td>
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<tr>
<td>Duration, ms</td>
<td>2.1 ± 0.2</td>
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<tr>
<td>R(_{\text{IN}}), MΩ</td>
<td>37.7 ± 3.3</td>
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<tr>
<td>t(_{\text{m}}), ms</td>
<td>16.7 ± 4.4</td>
</tr>
<tr>
<td>Postburst AHP</td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>139.0 ± 24.1</td>
</tr>
<tr>
<td>Area, mV × ms</td>
<td>334.2 ± 21.9</td>
</tr>
<tr>
<td>Postrain AHP</td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>173.0 ± 12.4</td>
</tr>
<tr>
<td>Area, mV × ms</td>
<td>834.2 ± 98.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of neurons for LiCl was 6 and for choline-Cl was 5. Control solution was the low-Ca\(^{2+}\) ACSF (artificial cerebrospinal fluid) LiCl (40 mM) and choline-Cl (40 mM) replaced equimolar concentrations of NaCl in the same low-Ca\(^{2+}\) ACSF. AP, action potential; AHP, afterhyperpolarization. * Not significantly different from control. † Significantly different from control, P < 0.05.
patch-clamp experiments were carried out in both acutely dissociated neurons \( (n = 5) \) and in situ pyramidal neurons \( (n = 6) \), in which total voltage-dependent Na\(^+\) and K\(^+\) currents were simultaneously recorded (Fig. 8). In these experiments, cells and slices were perfused with intra- and extracellular solutions identical to those used to record total K\(^+\) currents, except for TTX, which was omitted from the extracellular solution. The amplitude of outward currents was measured 20–30 ms after the peak of Na\(^+\) or Li\(^+\) inward currents evoked by depolarizing steps at −30 mV, starting from a holding potential of −70 mV. Under control conditions, outward-current amplitude \( (I_{\text{out}}) \) was 136.0 ± 48.7 pA in dissociated neurons and 556.2 ± 80.0 pA in experiments on slices. After complete substitution of Na\(^+\) with Li\(^+\), \( I_{\text{out}} \) decreased to 117.2 ± 47.7 and 487.8 ± 71.2 pA \( (P < 0.05 \) in both cases) in dissociated and in situ neurons, respectively, and recovered to 175.6 ± 60.1 and 555.4 ± 85.8 pA, respectively, during Li\(^+\) washout (Fig. 8, A–C). No reduction in outward-current amplitude after Na\(^+\) substitution with Li\(^+\) was ever observed in the presence of 1 \( \mu \)M TTX (Fig. 8D).

**D I S C U S S I O N**

The present data suggest that a K\(^+\) current activated by AP-dependent Na\(^+\) entry critically participates in postexcitatory membrane hyperpolarization in IB neurons, thus contributing to the maintenance of the rhythmic rebursting activity that characterizes this neuronal phenotype. Indeed, experimental manipulations aimed at reducing voltage-dependent Na\(^+\) entry consistently led to a marked reduction in postburst AHP and, ultimately, the disruption of the regular repetition of burst firing.

**Roles of Ca\(^{2+}\)-activated K\(^+\) currents in IB neurons’ firing activity**

In neocortical adapting, RS neurons, Ca\(^{2+}\)-activated K\(^+\) currents play a major role in regulating repetitive firing and generating both the fast and slow components of AHPs (Schwindt et al. 1992). Indeed, in our experiments on adapting RS cells, substitution of extracellular Ca\(^{2+}\) with nonpermeant divalent cations obviously attenuated spike frequency adaptation, greatly reduced early AHPs, and almost completely suppressed slow AHPs, as it is known to occur in a number of neuronal types in various cortical and subcortical structures of the mammalian brain (see review by Sah and Davies 2000). The same procedure had definitely less prominent effects on the patterns of activity of IB neurons. In particular, burst generation, postexcitatory hy-
perpolarizations, and rebursting frequency during sustained depolarizations were basically unaffected, thus suggesting that a set of K⁺ currents different from Ca²⁺-activated K⁺ currents give a predominant contribution in determining and regulating the excitable properties typical of this physiological neuronal phenotype.

Nevertheless, some role did appear to be played by Ca²⁺-activated K⁺ currents in IB neurons’ firing activity. First, the blockade of Ca²⁺-activated K⁺ currents slowed the repolarization following individual spontaneous bursts (as well those following stimulus-induced AP trains), thus generating enhanced DAPs. Moreover, the repolarization of individual APs became slightly slower. This effect probably accounts for the broadening of individual bursts and the elongation of the interval between APs in each burst that we observed in many IB neurons perfused with low-Ca²⁺ ACSF. These findings suggest that the fast-activating Ca²⁺-dependent K⁺ currents flowing through big (BK) channels participate in shaping the firing activity of neocortical IB neurons, particularly in shortening DAPs. On the contrary, the “slow” Ca²⁺-activated K⁺ currents flowing through either small (SK) channels or subfamilies of BK channels (Sah 1996) appeared to play a minor role in regulating and terminating burst firing.

**AHPs and burst firing: a role for a K⁺ current activated by Na⁺ entry**

The possibility that a Na⁺-dependent K⁺ current significantly contributes to the patterns of activity characterizing IB neurons was explored because it was clear that the amplitude of postburst AHPs observed in this cell type was influenced by the magnitude of the foregoing depolarizing events (including the number of Na⁺-dependent APs and the subthreshold depolarizing envelope). These postexcitatory hyperpolarizations were due to the activation of a K⁺ current, since they were greatly reduced by raising the extracellular K⁺ concentration, and their reversal potential followed the predicted changes of the equilibrium potential for K⁺ ions. Altogether, the results we obtained are in favor of a K⁺ current activated by an AP-induced Na⁺ influx largely contributing to AHP generation in IB neurons.

First, we found that, in IB neurons, substitution of extracel-
lular Na$^+$ with Li$^+$, which leaves voltage-dependent Na$^+$ currents and single APs largely unaffected, results in substantial inhibition of AHPs following not only spike trains, but also short excitatory events such as individual bursts (often lasting <100 ms). Moreover, the same procedure consistently induced an increase in the frequency of rebursting during the application of prolonged depolarizing stimuli. These findings suggested that a K$^+$ current activated by Na$^+$, but not Li$^+$, entry plays a key role in generating postexcitatory AHPs and regulating bursting frequency in IB neurons. On the other hand, our voltage-clamp experiments directly excluded major aspecific, inhibitory effects of Li$^+$ on voltage-dependent K$^+$ currents. More importantly, they also demonstrated that a small, yet measurable component of total K$^+$ currents evoked by “above-threshold” depolarizing pulses depends on the foregoing activation of normal Na$^+$, but not Li$^+$, currents generated by voltage-gated Na$^+$ channels. These results are consistent with a Na$^+$-activated K$^+$ current ($I_{KNa}$) being expressed in IB neurons and being responsive to short “pulses” of Na$^+$ influx through voltage-gated Na$^+$ channels. They also support the idea that activation of such a $I_{KNa}$ by AP-induced Na$^+$ influx critically contributes to postburst AHPs and rhythmic bursting activity in IB neurons.

Functional properties of the Na$^+$-activated K$^+$ current

Our experiments indicated that Li$^+$, although nearly as permeant through voltage-gated Na$^+$ channels as Na$^+$ itself, cannot substitute for Na$^+$ in activating the Na$^+$-dependent K$^+$-current component and sustaining normal activity-dependent AHPs. Consistently with this finding, it is known that neuronal Na$^+$-activated K$^+$ channels ($K_{Na}$) are insensitive to Li$^+$ as an intracellular substitute for Na$^+$ (Bishoff et al. 1998; Dryer et al. 1989; Haimann et al. 1990; Safronov and Vogel 1996).

A problem raised by the attribution to $I_{KNa}$ of a physiological role in shaping neuronal firing activity is represented by the fact that activation of $K_{Na}$ channels has been found to require very high, potentially pathological levels of intracellular Na$^+$ ([Na$^+$])$_i$ in several cell systems (e.g., Dryer et al. 1989; Dryer 1991; Haimann et al. 1990; Kameyama et al. 1984). Various studies have emphasized the role of $I_{KNa}$ in hyperpolarizing excitable membranes after prolonged discharges of APs or long-lasting depolarizing plateaus capable of causing substantial cell loading with Na$^+$ (reviewed by Dryer 1994). It has been reported that $K_{Na}$ channels show a higher sensitivity to intracellular Na$^+$ in the presence of low concentrations of intracellular K$^+$ ($[K^+]_i$) (Haimann et al. 1992; Niu and Meech 2000). Such cooperative effect of decreased $[K^+]_i$ with increased $[Na^+]_i$ on $K_{Na}$ channels may underlie the marked shortening of the AP duration observed in cardiac myocytes undergoing hypoxia (Carmeliet 1978; Isenberg et al. 1983), a condition during which $K_{Na}$ channels have indeed been found to substantially contribute to increased K$^+$ efflux (Mitani and Shattock 1992). On the other hand, $I_{KNa}$ has been reported to modulate physiological neuronal firing and contribute to postexcitatory hyperpolarization in various neuronal types (Safronov and Vogel 1996; Santos et al. 1998). Data previously obtained in nonbursting neocortical neurons (Schwindt et al. 1989) suggested that Na$^+$-activated K$^+$ conductances are widely expressed in neocortical neurons and can induce hyperpolarization after repetitive firing or moderate depolarizations unable to significantly activate Ca$^{2+}$-dependent K$^+$ conductances. It has also been found that a Na$^+$-activated slow depolarization participates in shaping “spindle-like”bursting activity in perigeniculate GABAergic neurons (Kim and McCormick 1998) and contributes to slowing the hyperpolarization following spike trains in visual cortex (Sanchez-Vives et al. 2000). The above evidence would suggest that normal patterns of AP discharge can induce [Na$^+$]$_i$, increases high enough to activate the relatively insensitive Na$^+$-dependent K$^+$ channels in several neurons. This could imply that neuronal $K_{Na}$ channels are located in close proximity to Na$^+$-inflow sites, where high degrees of [Na$^+$], are locally reached, perhaps by colocalizing and clustering with voltage-gated Na$^+$ channels. This hypothesis is supported by evidence obtained in Xenopus myelinated axons indicating that $K_{Na}$ channels colocalize with Na$^+$ channels expressed at high density in nodal regions (Koh et al. 1994).

Na$^+$-dependent processes and excitability in IB neurons

In previous studies, we found that the bursting properties of neocortical IB neurons essentially rely on voltage-dependent Na$^+$ currents, which account for both fast, high-frequency AP bursts and the underlying depolarizing envelope (Franceschetti et al. 1995; Guateto et al. 1996; Mantegazza et al. 1998). Furthermore, an increasing amount of evidence supports the idea that, in various types of cortical IB neurons, a sustained Na$^+$ current centrally contributes to up-
holding bursts and DAPs and is also involved in setting the threshold for AP generation (Azouz et al. 1996; Brumberg et al. 2000) or, as suggested by Williams and Stuart (1999), in amplifying back-propagating dendritic APs. On the basis of these findings, we can assume that IB neurons are endowed with a particularly high density of Na\(^+\) channels sustaining fast APs and prominent Na\(^+\)/H\(^+\)dependent subthreshold depolarizations. Each excitatory event may thus cause a transient but sufficiently large Na\(^+\) influx capable of activating \(K_{\text{Na}}\) channels. Again, this hypothesis supports the idea of a colocalization of voltage-gated Na\(^+\) channels and \(K_{\text{Na}}\) channels, as it has already been found to be the case in nodal regions of Xenopus myelinated axons (Koh et al. 1994), where the high Na\(^+\)-channel density would allow for large increases of local [Na\(^+\)], during firing.

In conclusion, our data suggest that a Na\(^+\)-activated K\(^+\) current significantly contributes to postburst hyperpolarization in neocortical IB neurons and that it participates in controlling the time course of burst recurrence by interplay with classical voltage-dependent K\(^+\) currents and near-threshold “rhythmogenic” conductances (such as anomalous rectifying currents) (Foehring and Waters 1991; Schwindt et al. 1988). The balance between Na\(^+\) and K\(^+\) currents therefore appears to play a predominant role in burst behavior, whereas Ca\(^+\)-activated K\(^+\) currents provide a more limited contribution. Neocortical IB neurons are endowed with physiological and anatomical characteristics (Chagnac-Amitai et al. 1990; Kasper et al. 1994) that confer on them the ability to promote and sustain physiological and pathological synchrony (Chagnac-Amitai and Connors 1989; Silva et al. 1990). Furthermore, it has been found that neocortical layer V critically contributes to seizure generation and propagation (Hoffman and Prince 1995; Pasikova et al. 2001; Telfeian and Connors 1998). It can be expected that the particular setting of the intrinsic properties responsible for intrinsic burst generation and termination in IB neurons of layer V underlies specific epileptogenic mechanisms taking place in the neocortex and probably influence the effectiveness of antiepileptic drugs.

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REFERENCES


