Role of Small Conductance Ca^{2+} -Activated K^{+} Channels in Controlling CA1 Pyramidal Cell Excitability

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Small-conductance Ca^{2+} -activated K^{+} (SK or $\text{K}_{\text{Ca}2}$) channels are widely expressed in the CNS. In several types of neurons, these channels were shown to become activated during repetitive firing, causing early spike frequency adaptation. In CA1 pyramidal cells, SK channels in dendritic spines were shown to regulate synaptic transmission. However, the presence of functional SK channels in the somata and their role in controlling the intrinsic firing of these neurons has been controversial. Using whole-cell voltage-clamp and current-clamp recordings in acute hippocampal slices and focal applications of irreversible and reversible SK channel blockers, we provide evidence that functional SK channels are expressed in the somata and proximal dendrites of adult rat CA1 pyramidal cells. Although these channels can generate a medium duration afterhyperpolarizing current, they play only an auxiliary role in controlling the intrinsic excitability of these neurons, secondary to the low voltage-activating, noninactivating $\text{K}_{\text{V}7/\text{M}}$ channels. As long as $\text{K}_{\text{V}7/\text{M}}$ channels are operative, activation of SK channels during repetitive firing does not notably affect the spike output of CA1 pyramidal cells. However, when $\text{K}_{\text{V}7/\text{M}}$ channel activity is compromised, SK channel activation significantly and uniquely reduces spike output of these neurons. Therefore, proximal SK channels provide a “second line of defense” against intrinsic hyperexcitability, which may play a role in multiple conditions in which $\text{K}_{\text{V}7/\text{M}}$ channels activity is compromised, such as hyposmolarity.

Key words: Bursting; hippocampus; Kv7 channels; mAHP; pyramidal cell; SK channels

Introduction

Brain neurons express several types of voltage- and Ca^{2+} -gated K^{+} channels that underlie spike frequency adaptation and afterhyperpolarization (AHP). Among these channels are the small conductance Ca^{2+} -activated K^{+} (SK or $\text{K}_{\text{Ca}2}$) channels, of which three isoforms have been identified: SK1, SK2, and SK3 (Köhler et al., 1996). All three isoforms are expressed in hippocampal CA1 pyramidal cells, SK2 being the most abundant (Stocker and Pedarzani, 2000; Bond et al., 2004; Sailer et al., 2004). SK2 channels are highly expressed in dendritic spines of CA1 pyramidal cells, where they become activated by Ca^{2+} entry through nearby synaptic NMDA receptor channels (Ngo-Anh et al., 2005; Wang et al., 2014) and R-type voltage-gated Ca^{2+} channels (Bloodgood and Sabatini, 2007). Through this coupling, SK2 channels modulate the waveform of EPSPs. Recent analyses of SK2 protein expression in CA1 pyramidal cells *in situ* (Ballesteros-Merino et al., 2012) and in culture (Maciaszek et al., 2012) suggest that SK2 channels are also present in

dendrites proper, where they may control Ca^{2+} spiking (Gu et al., 2008) and spike-induced Ca^{2+} entry (Tonini et al., 2013). In contrast, the density of SK2 protein in the plasma membrane of adult CA1 pyramidal somata is considered relatively low (Ballesteros-Merino et al., 2012; Maciaszek et al., 2012).

SK channels are selectively and irreversibly blocked by apamin (Blatz and Magelby, 1986; Köhler et al., 1996; Ishii et al., 1997). Using whole-cell voltage-clamp recordings from somata of rodent CA1 pyramidal cells, an apamin-sensitive Ca^{2+} -activated K^{+} current (I_{SK}), attributable mainly to SK2 channels, can be readily evoked by prolonged depolarizing current pulses (Stocker et al., 1999; Bond et al., 2004). However, the contribution of somatic SK channels to this current is unknown. In addition, the issue of I_{SK} activation during actual spike discharge and its effect on the spike output of these neurons has been controversial. Earlier studies suggested that SK channels in CA1 pyramidal cells mediate a Ca^{2+} -gated K^{+} current that is pharmacologically and kinetically distinct from the slow K^{+} current producing the slow AHP (sAHP) and that this I_{SK} may contribute to the generation of the medium duration AHP (mAHP) and associated spike frequency adaptation (Stocker et al., 1999; Stocker et al., 2004). However, later studies contended that, at rest potential, both the mAHP and spike frequency adaptation are underlain exclusively by activation of the low voltage-activated, noninactivating, muscarinic-sensitive K^{+} current (I_{M} ; Gu et al., 2005; Gu et al., 2008). In CA1 pyramidal cells, the latter current is generated by $\text{K}_{\text{V}7/\text{M}}$ channels consisting primarily of the $\text{K}_{\text{V}7.2}$ and $\text{K}_{\text{V}7.3}$ isoforms of the $\text{K}_{\text{V}7}$ (KCNQ) family (Wang et al., 1998; Shah et al., 2002).

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Here, we reinvestigated the role of I_{SK} in controlling spike output of CA1 pyramidal cells. We show that this current can be generated at or near the somata of these neurons, but blocking this current in ordinary or even hyperexcitable conditions has no impact on their spike output. However, when K_v7/M channel activity is compromised, conjointly activated I_{SK} exerts a strong and unique self-inhibitory action limiting spike discharge in these neurons.

Materials and Methods

Animals. Experiments were conducted in accordance with the guidelines of the Animal Care Committee of the Hebrew University using adult (125–150 g) male Sabra rats.

Whole-cell patch-clamp recordings. Under deep anesthesia with ketamine (100 mg/kg; Pfizer) and xylazine (15 mg/kg; Medical Market), rats were perfused through the heart with 1–3°C cold sucrose-based artificial CSF (aCSF) containing the following (in mM): 56 NaCl, 100 sucrose, 2.5 KCl, 1.25 NaH_2PO_4 , 30 NaHCO_3 , 1 CaCl_2 , 5 MgCl_2 , and 20 glucose, oxygenated with 95% O_2 /5% CO_2 . After complete perfusion, rats were decapitated, the brain was quickly removed, and 300- μm -thick transverse hippocampal slices were prepared with a vibratome (VT 1200 S; Leica) and gradually warmed to 34°C over 30 min in a storage chamber perfused with oxygenated aCSF identical to the one above except for the addition of 26 mM NaHCO_3 and 60 mM NaCl. Slices were then transferred to a holding chamber and equilibrated at room temperature (21–22°C) for at least 30 min with oxygenated aCSF containing the following (in mM): 125 NaCl, 3.5 KCl, 1.25 NaH_2PO_4 , 2 MgCl_2 , 2 CaCl_2 , 26 NaHCO_3 , and 15 glucose, pH 7.4, 310 mOsmol. For recording, slices were placed one at a time in a submerged recording chamber at room temperature (21–22°C) and continuously perfused with oxygenated aCSF, pH 7.4, osmolarity 305 mOsmol, containing the following (in mM): 125 NaCl, 1.25 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 2.5 CaCl_2 , 1.5 MgCl_2 , 16 glucose, and 1 tetraethyl ammonium chloride (TEA), along with 0.5 μM TTX. The latter aCSF also contained the glutamate receptor antagonists 6-cyano-7-nitro-quinolaxaline-2,3-dione (CNQX; 15 μM) and 2-amino-5-phosphono-valeric acid (APV; 50 μM) to block fast EPSPs and the GABA $_A$ receptor antagonist picrotoxin (100 μM) to block fast IPSPs. Pyramidal cells in the CA1 field were visualized at 60 $\times$ magnification using a Nikon FN1 microscope and an infrared video camera (Hamamatsu). Recording patch pipettes (2–4 M Ω) were pulled from borosilicate glass on a vertical puller (Narishige). Intracellular solution contained the following (in mM): 135 K-gluconate, 10 KCl, 10 HEPES, 1 MgCl_2 , 2 Na_2ATP , 0.3 GTP, pH 7.3. 8-(4-chlorophenylthio) adenosine 3,5-cyclic monophosphate (8CPT-cAMP; 50 μM) was included to inhibit the slow Ca^{2+} -activated AHP current (sI_{AHP} ; Madison, Nicoll, 1986). In many experiments, Lucifer yellow (200 μM) was added to the intracellular solution to visualize the soma and the dendritic tree of the patched neuron using a high-sensitivity cooled CCD camera (DS-Qi1; Nikon) and NIS-Elements Advanced Research software (Nikon). Tight-seal whole-cell recordings were obtained using a patch-clamp amplifier (Axopatch 200A; Molecular Devices). Neurons were voltage clamped at –55 mV. The voltage command for eliciting I_{SK} comprised a 1-s-long hyperpolarizing prepulse to –70 mV, followed by an 80-ms-long depolarizing pulse to 20 mV. Series resistance compensation was used to improve the voltage-clamp control (>70%) so that the maximal residual voltage error did not exceed 5 mV. A liquid junction potential of 3 mV was measured between the intracellular and extracellular solutions and corrected offline.

For focal application of SK channel blockers, a second patch pipette containing aCSF and one of the blockers was placed in the immediate proximity of soma or dendrite. Pressure injection pulses (20–40 psi; 5–20 ms) were applied using a Picospritzer II (General Valve). They were triggered by the pClamp software applied 10–30 ms before the depolarizing command to influence I_{SK} effectively. Parameters of the pressure injection pulses were constant during a single experiment.

Sharp microelectrode recordings. Rats were decapitated under isoflurane anesthesia and transverse hippocampal slices (400 μm) were prepared with a vibratome and transferred to a storage chamber perfused

with oxygenated aCSF containing the following (in mM): 124 NaCl, 3.5 KCl, 1 MgCl_2 , 1.6 CaCl_2 , 26 NaHCO_3 , and 10 glucose, pH 7.4, osmolarity 305 mOsm, at room temperature. For recording, slices were placed one at a time in an interface chamber and superfused with warmed (33.5°C) oxygenated aCSF of the same composition to which 15 μM CNQX, 50 μM APV, and 100 μM picrotoxin were added. Intracellular recordings were obtained using sharp glass microelectrodes containing 4 M K^+ -acetate (90–110 M Ω) and an amplifier (Axoclamp 2A; Molecular Devices), allowing simultaneous injection of current and measurement of membrane potential. The bridge balance was monitored carefully and adjusted before each measurement. The pyramidal cells included in this study had stable resting potentials of –60 mV or more and overshooting action potentials. The intracellular signals were filtered on line at 10 kHz, digitized at a sampling rate of 10 kHz or more, and stored on a personal computer using a data acquisition system (Digidata 1322A; Molecular Devices) and pCLAMP9 software (Molecular Devices).

Chemicals and drugs. All chemicals and drugs were obtained from Sigma. Stock solutions were diluted 1:1000 when added to the aCSF from aqueous stock solutions. Measurements of drug effects were usually performed after 30 min of slice perfusion with the drug-containing aCSF.

Data analysis. Solitary spikes or burst responses were evoked by injecting threshold-straddling, brief (4 ms) depolarizing current pulses. The size of the spike afterdepolarization (ADP) of solitary spikes, as well as the size of the depolarization shift (DS) underlying an intrinsic spike burst, were measured (in mV $\cdot$ ms) as the integrated “area under the curve” between the fast AHP ($fAHP$) of the first spike and the point at which membrane voltage returned to resting potential. Results are presented as the mean $\pm$ SEM. Assessment of statistical significance of differences between means was performed with paired two-tailed Student’s t test or, for multiple comparisons (see Fig. 3), with two-way repeated-measures ANOVA. In all tests, the significance level was set to $p < 0.05$.

Results

SK channels are expressed at or near the soma in CA1 pyramidal cells

In CA1 pyramidal cells *in situ*, I_{SK} can be recorded in isolation at the soma of CA1 pyramidal cells in response to prolonged depolarizing pulses delivered via a somatically attached patch pipette (Stocker et al., 1999). Figure 1 demonstrates an outward K^+ current evoked by an 80-ms-long depolarizing current pulse to 20 mV. The holding potential was –55 mV and the depolarizing pulse was preceded by a 1-s-long hyperpolarizing pulse to –70 mV (Fig. 1A, inset). As expected, adding 100 nM apamin to the aCSF blocked almost all (92%) of this current (from 374.9 ± 28.3 to 31.5 ± 9.4 pA; $n = 8$; $p = 0.0004$). This effect was irreversible (32.8 ± 8.9 pA after 5 min drug washout; $n = 8$; $p = 0.482$; Fig. 1A,B). Likewise, 100 μM D-tubocurarine (D-TC), a reversible SK channel antagonist (Ishii et al., 1997), blocked most (84%) of the current (from 408.7 ± 30.6 to 66.2 ± 17.9 pA; $n = 8$; $p = 0.0007$) and this effect was almost completely reversible upon drug washout (355.5 ± 52.0 pA after 5 min drug washout; $n = 8$; $p = 0.253$; Fig. 1C,D).

Although I_{SK} can be recorded in somata of CA1 pyramidal cells, the apamin-sensitive SK channels generating this current (Bond et al., 2004) might be localized remotely from the site of recording. Indeed, immunohistochemical findings suggest that these channels are more abundantly expressed in the dendritic spines of these neurons (Ngo-Anh et al., 2005; Lin et al., 2008). To determine whether a component of I_{SK} originates at or near the soma, we tested the effects of apamin or D-TC applied focally to the somata (in stratum pyramidale) or to the proximal apical dendrites (in stratum radiatum) of visualized CA1 pyramidal cells (see Materials and Methods). The puff pipette (“puffer”) contained 500 nM apamin or 200 μM D-TC, as well as 200 μM Lucifer yellow for better visualization of the puffer. A scheme of the experimental arrangement is depicted in Figure 2A. During

Figure 1. Isolation of I_{SK} in CA1 pyramidal cells. **A**, Representative recordings from a CA1 pyramidal cell of outward current, in experimental conditions that isolate I_{SK} , evoked by an 80-ms-long depolarizing current pulse to 20 mV. The holding potential was -55 mV and the depolarizing pulse was preceded by a 1-s-long hyperpolarizing pulse to -70 mV (inset). Addition of 100 nM apamin to the aCSF irreversibly blocked almost all (92%) of the current. **B**, Bar diagram summarizing the irreversible inhibitory effect of apamin on the outward current ($n = 8$). **C**, Same as **A**, but showing the effect of 100 μ M D-TC, which reversibly blocked most (84%) of the outward current. **D**, Bar diagram summarizing the reversible inhibitory effects D-TC on the outward current ($n = 8$). * $p < 0.05$ (statistical significance).

continuous recordings of I_{SK} from the soma, the puffer was placed under visual control first on the soma (Fig. 2*Bac*) and then on the proximal apical dendrite 30–60 μ m from the soma (Fig. 2*Bbc*) or vice versa. Bath flow of the aCSF was kept at ~ 5 ml/min in a direction parallel to the pyramidal cell layer perpendicular to the orientation of the dendritic arbor.

A representative experiment of focal application of apamin is shown in Figure 2*C*. After a quite stable baseline recording of I_{SK} , apamin was puffed onto the soma (puff duration 5 ms), causing an immediate reduction of I_{SK} (Fig. 2*Cab*). This effect did not recover and another puff applied at the same site several minutes later had only a negligible additive effect (Fig. 2*Cb*). However, subsequent application of apamin to the proximal dendrite caused a further significant reduction of I_{SK} , which also did not recover (Fig. 2*Cab*). The bar diagram in Figure 2*D* summarizes the results from 11 similar experiments. Somatic apamin application reduced I_{SK} by $29.5 \pm 7.4\%$ ($p = 0.0007$) without any recovery ($28.7 \pm 7.5\%$ inhibition after 3 min; $n = 11$; $p = 0.21$). A subsequent dendritic puff of apamin reduced I_{SK} further, by another $27.3 \pm 6.3\%$ ($n = 10$; $p = 0.006$), causing an overall $48.2 \pm 6.3\%$ inhibition of I_{SK} , again without recovery ($48.0 \pm 6.4\%$ of control; $n = 10$; $p = 0.37$).

To minimize the possibility of a spillover effect, we also applied apamin in the reverse sequence: first to the proximal apical dendrite and then to the soma. Apical dendritic apamin application reduced I_{SK} by $29.0 \pm 8.2\%$ ($p = 0.009$; $n = 7$). This effect is similar to the reduction obtained by applying apamin to the dendrite after somatic application ($p = 0.17$). Likewise, applying apamin to the soma after applying it to the proximal apical dendrite further reduced I_{SK} by $30.6 \pm 5.9\%$ ($p < 0.008$; $n = 6$). Again, this effect is similar to the reduction obtained by applying apamin to the soma without prior apical dendritic application ($p = 0.28$). These results suggest that each of the two neuronal

compartments individually contain functional SK channels. Applying apamin to basilar dendrites in stratum oriens had no notable effect on I_{SK} , but this current was reduced when the drug was subsequently applied to the soma (data not shown).

Similar experiments were performed with focal applications of D-TC. Somatically applied D-TC reduced I_{SK} by $22.9 \pm 3.1\%$ ($n = 12$; $p = 0.0003$; Fig. 2*Eab*). The currents almost completely recovered within 15 s after each application ($97.1 \pm 1.6\%$ of control; $n = 12$; $p = 0.053$; Fig. 2*Eab*). Subsequent application of D-TC to the dendrites reduced I_{SK} by $30.7 \pm 1.4\%$; $n = 12$; $p = 0.001$, followed by almost complete recovery ($93.8 \pm 3.6\%$ of control; $n = 12$; $p = 0.11$; Fig. 2*Eab*). As in the case of apamin, D-TC application to the dendrites reduced I_{SK} to the same extent whether applied before ($29.2 \pm 6.9\%$) or after ($32.2 \pm 7.5\%$) its application to the soma ($p = 0.28$; $n = 6$). The bar diagram in Figure 2*F* summarizes the results from 12 similar experiments.

Together, these data indicate the presence of functional SK channels at both the somata and proximal dendrites of CA1 pyramidal cells. Moreover, the experiments with apamin suggest that $\sim 50\%$ of the SK channels activated by somatic depolarizations reside in these compartments. The remaining channels likely are expressed in the distal dendritic compartments of these neurons (Ngo-Anh et al., 2005; Lin et al., 2008).

SK channels do not control spike output in ordinary conditions

In CA1 pyramidal cells, spikes are initiated at the axon initial segment and backpropagate into the soma and apical dendrites, causing substantial increases in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). Single spikes can raise $[Ca^{2+}]_i$ by ~ 60 nM, whereas short spike trains can cause an increase of 0.6 μ M or more (Spruston et al., 1995). The latter increases in $[Ca^{2+}]_i$ likely activate SK channels, because their EC_{50} values for Ca^{2+} activation are 0.3–0.5 μ M (Köhler et al., 1996; Xia et al., 1998). Such activation would be expected to produce apamin-sensitive spike frequency adaptation. Indeed, it was originally argued that application of apamin increases spike output in CA1 pyramidal cells (Stocker et al., 1999). However, later studies failed to confirm this finding (Gu et al., 2005; Gu et al., 2008). We repeated this experimental paradigm, as illustrated in Figure 3*A*. Spikes were evoked by injecting a series of 180-ms-long depolarizing current pulses incrementing in steps of 50 pA up to 400 pA. Typically, the evoked spike activity displayed marked frequency adaptation during the pulses (Madison and Nicoll, 1984). However, no significant effect on spike output was observed during 30–45 min applications of 100 nM apamin (Fig. 3*Aab*). This finding was reproduced in 7 experiments, as summarized in Figure 3*B* ($p = 0.558$).

Auxiliary control of spike output by SK channels

The fact that apamin does not enhance spike output may indicate that the increase in somatic $[Ca^{2+}]_i$ is not sufficient to activate I_{SK} to an extent that will inhibit firing. An alternative explanation is that the concurrent activation of I_M occludes the inhibitory effect of I_{SK} . Recent studies suggest that I_M is extremely potent in down-regulating CA1 pyramidal cell excitability even after a solitary spike (Yue and Yaari, 2004, 2006). These neurons manifest a prominent spike ADP driven largely by persistent Na^+ current (I_{NaP} ; Azouz et al., 1996; Yue et al., 2005; Chen and Yaari, 2008). Because of its low threshold of activation and lack of inactivation, I_M is recruited during the spike ADP, inhibiting its escalation into a spike burst (Yue and Yaari, 2004, 2006). Another factor contributing to the marked self-inhibitory efficacy of I_M is the high

Figure 2. Functional localization of SK channels in CA1 pyramidal cells. **A**, Scheme of the experimental arrangement. Point 1 indicates the somatic and point 2 the apical dendritic location of the puff pipette in relation to the recording point (near point 1). **B**, Photos showing the location of puff applications with respect to the location of the recording patch pipette as follows: **a**, with the puff pipette aimed at the soma; **b**, with the puff pipette aimed at the proximal dendrite; and **c**, an overlay of two fluorescent photos of the same cell, which has been filled with Lucifer yellow via the patch pipette, showing the two locations of the puff pipette, which also is filled with Lucifer yellow. **C**, Focal apamin application. **a**, Overlaid traces from a representative experiment showing the blocking effects of apamin focally applied first to the soma (trace 1) and subsequently to the proximal dendrite (trace 2). **b**, Time course of the experiment in **a** showing the cumulative block of I_{SK} by apamin focally applied to the soma (1) or to the proximal dendrite (2). **D**, Bar diagram summarizing the effects apamin illustrated above ($n = 11$). **E**, Focal d-TC application. **a**, Overlaid traces from a representative experiment showing the blocking effects of d-TC focally applied first to the soma (trace 1) and subsequently to the proximal dendrite (trace 2). **b**, Time course of the experiment in **a** showing the cumulative block of I_{SK} by apamin focally applied to the soma (1) or to the proximal dendrite (2). **F**, Bar diagram summarizing the effects d-TC illustrated above ($n = 12$).

density of $K_{V7/M}$ channels expressed at the axon initial segment (AIS; Lai and Jan, 2006; Pan et al., 2006; Rasmussen et al., 2007), the site of spike initiation (Spruston et al., 1995). Therefore, it is possible that activation of $K_{V7/M}$ channels during discharge of a single spike or a train of spikes may shunt other coactivated currents, such as I_{SK} , diminishing their influence on spike output.

We tested this hypothesis by monitoring the effects of apamin in slices superfused for at least 50 min with aCSF containing the I_M blockers linopirdine or XE991 (Aiken et al., 1995; Wang et al., 1998; Schnee, Brown, 1998). As shown previously (Yue, Yaari, 2004, 2006), linopirdine (10 μ M) converted the intrinsic neuronal firing pattern from regular firing (nonbursting) to burst firing. Each of these neurons fired a tight cluster of 2 or more spikes (typically 4–5 spikes) as its threshold response to the 180-ms-long depolarizing pulses. Stronger pulses evoked an initial burst response followed after a short gap in firing by several solitary

spikes (Fig. 3Ca). Interestingly, apamin significantly enhanced spike output in all linopirdine-treated neurons ($p = 0.0002$; Fig. 3Cab,D). Typically, apamin caused the neuron to generate additional spikes during the previously defined gap in firing (Fig. 3Cab). Similar results were obtained in five neurons treated with 10 μ M XE991, which also converted to bursting mode (data not shown). For comparison, we also tested the effects of 100 nM apamin on spike output in CA1 pyramidal cells rendered intrinsically bursting by superfusing the slices with high- K^+ (7.5 mM) aCSF (Jensen et al., 1994). As in linopirdine-treated slices, exciting CA1 pyramidal cells evoked an initial burst followed by solitary spikes (Fig. 3Ea). However, no effect of apamin on spike output was seen during the 30–45 min of recordings in these experiments ($n = 5$; $p = 0.639$; Fig. 3Eab,F).

We also tested the effects of apamin on spike output of CA1 pyramidal cells superfused with aCSF containing 3 mM

Figure 3. In CA1 pyramidal cells blocking SK channels has no effect on spike discharge unless K_v7M channels are blocked. **A**, Representative recordings from one neuron showing firing evoked by long (180 ms) depolarizing current pulses at two stimulation intensities (50 and 400 pA, bottom and top traces, respectively), before (**a**, control) and >50 min after adding apamin (100 nM) to the aCSF (**b**, + Apamin). **B**, Summary plots of the number of evoked spikes versus stimulation intensity before (squares) and after (circles) exposure to apamin. Each data point represents the mean $\pm$ SEM. The two plots were not significantly different from each other. **C**, **D**, Same as **A** and **B**, but all aCSFs contained $10 \mu M$ linopirdine, which caused the neurons to fire an initial burst followed by solitary spikes upon depolarization. The two plots in **D** were significantly different from each other. **E**, **F**, Same as **A** and **B**, but all

4-aminopyridine (4-AP). Previous studies have shown that 4-AP, likely by blocking dendritic A-type K^+ current (I_A), causes spike broadening (Storm, 1987) and facilitates the generation of slow dendritic Ca^{2+} spikes subsequent to their invasion by somatic backpropagating fast Na^+ spikes (Magee et al., 1998). These in turn depolarize the soma, causing somatic bursting (Magee and Carruth, 1999). Exciting the neurons in this condition evoked repetitive bursting activity (Fig. 3*Ga*). Although, in this situation, Ca^{2+} influx into the apical dendrites and associated activation of SK channels were likely enhanced, adding 100 nM apamin to the 4-AP-containing aCSF again had absolutely no effect on spike output ($n = 6$; $p = 0.66$; Fig. 3*Gab,H*).

Together, these data show that I_{SK} is activated during repetitive firing in CA1 pyramidal cells, but it limits their spike output only when I_M is compromised.

Auxiliary control of intrinsically generated bursts by SK channels

We next examined more closely whether recruitment of SK channels affects the magnitude of intrinsically generated bursts in CA1 pyramidal cells. Apamin (100 nM) consistently augmented bursts produced by inhibiting I_M with $10 \mu M$ linopirdine, as evidenced by the increase in the number of intraburst spikes (linopirdine: 4.8 ± 0.5 spikes; with apamin: 7.8 ± 0.6 spikes; $n = 12$ cells; $p = 0.00007$; Fig. 4*Aabc,D*), as well as in the size of the underlying DS (measured as “area under the curve”; linopirdine: 1167.8 ± 173.5 mV $\cdot$ ms; with apamin: 2179.0 ± 232.5 mV $\cdot$ ms; $n = 12$; $p = 0.000004$; Fig. 4*Aabc,E*). For comparison, we investigated whether apamin affects bursts invoked by raising the K^+ content of the aCSF (Jensen et al., 1994; Fig. 4*Ba*). Intrinsic bursting of CA1 pyramidal cells in high- K^+ aCSF is driven by I_{NaP} (Azouz et al., 1996), as is the case of bursting produced by I_M blockers in these neurons (Yue, Yaari, 2006). Adding apamin to the high- K^+ aCSF (7.5 mM) had no effect on the number of intraburst spikes (high- K^+ : 3.0 ± 0.3 ; with apamin: 2.8 ± 0.7 ; $p = 0.704$; Fig. 4*Babc,D*) or on the size of the

aCSFs contained 7.5 mM K^+ , which also caused the neurons to fire an initial burst followed by solitary spikes upon depolarization. The two plots in **F** were not significantly different from each other. **G**, **H**, Same as **A** and **B**, but all aCSFs contained 3 mM 4-AP, which caused the neurons to generate repetitive bursts in response to depolarization. The two plots in **H** were not significantly different from each other.

underlying DS (measured as area under the curve; high- K^+ : 723.5 ± 146.5 ; with apamin: 729.6 ± 297.6 mV·ms; $n = 5$; $p = 0.974$; Fig. 4*Babc,E*).

We also investigated the effect of apamin on bursts invoked in CA1 pyramidal cells by 3 mM 4-AP, in which case bursts are driven predominantly by apical dendritic Ca^{2+} currents (Magee and Caruth, 1999). Addition of apamin did not increase the number of intraburst spikes (4-AP: 3.0; with apamin: 3.0; $n = 6$; Fig. 4*Cab,D*) or DS size any further (4-AP: 1013.6 ± 76.2 mV·ms; 4-AP with apamin: 1154.3 ± 75.0 mV·ms; $n = 6$; $p = 0.151$; Fig. 4*Cc,E*). It is noteworthy that, despite the difference in ionic mechanism, the DS of bursts invoked by 4-AP and by linopiridine as recorded in the somata were the same size ($p = 0.55$). However, only bursts invoked by linopiridine were facilitated by apamin.

The fact that apamin did not enhance bursts invoked by 4-AP is seemingly at odds with recent findings of Gu et al. (2008) showing that Ca^{2+} spikes in apical dendrites of CA1 pyramidal cells are broadened by apamin. Those investigators concluded that, during a dendritic Ca^{2+} spike, Ca^{2+} influx through voltage-gated Ca^{2+} channels is sufficiently large to activate local SK channels that in turn curtail the Ca^{2+} spike. However, in that study, Ca^{2+} spikes were invoked by adding 5 mM TEA to the aCSF. TEA at this concentration blocks several types of voltage-gated K^+ channels, as well as heteromeric $K_v7.2/3$ channels ($IC_{50} = 3.5$ mM; Wang et al., 1998) that generate I_M in CA1 pyramidal cells (Shah et al., 2002). Indeed, using voltage-clamp recordings (Caspi et al., 2009), we found that 5 mM TEA markedly reduces I_M in these neurons by 66.6% (A. Caspi and Y.Y., unpublished observations). This suggested that the apamin-induced broadening of dendritic Ca^{2+} spikes in 5 mM TEA (Gu et al., 2008) might be enabled by the concurrent inhibition of I_M rather than by the mere increase in Ca^{2+} influx. Indeed, adding 5 mM TEA to the aCSF invoked bursts that were dramatically enhanced by subsequent addition of 100 nM apamin (Fig. 4*F*). The DS size increased from 2974.7 ± 140.0 mV·ms in TEA to 6216.0 ± 532.9 mV·ms in TEA and apamin ($n = 3$; $p = 0.035$; Fig. 4*H*). An even stronger facilitatory effect of apamin was observed in neurons pretreated with both 3 mM 4-AP and 10 μ M XE991 to conjointly block both I_A and I_M (Fig. 4*G*). The DS size increased from 4558.3 ± 1539.2 mV·ms in 4-AP and XE991 to 15634.7 ± 4739.0 mV·ms after adding apamin ($n = 5$; $p = 0.046$; Fig. 4*H*).

Figure 4. Blocking SK channels facilitates only bursts induced by blocking K_v7/M channels in CA1 pyramidal cells. **A**, Representative recordings from a neuron in a slice superfused with aCSF containing 10 μ M linopiridine. **a**, Applying brief (4 ms) stimuli elicited bursts of six spikes. **b, c**, Addition of 100 nM apamin to the aCSF prolonged the bursts to 9 spikes. **B**, Representative recordings from a neuron in a slice superfused with high- K^+ aCSF (7.5 mM). **a**, Applying brief stimuli elicited bursts of three spikes. **b, c**, Addition of 100 nM apamin to the aCSF did not intensify the burst. **C**, Representative recordings from a neuron superfused with aCSF containing 3 mM 4-AP. **a**, Applying brief stimuli elicited bursts of three spikes, the latter spike likely being a Ca^{2+} spike. **b, c**, Addition of 100 nM apamin to the aCSF did not intensify the burst. **D**, Bar histogram summarizing the effects of apamin on the number of intraburst spikes in bursting neurons produced by linopiridine ($n = 12$), high K^+ ($n = 5$), and 4-AP ($n = 6$). **E**, Bar histogram summarizing the effects of apamin on the DS in the bursting neurons shown in **D**. **F**, Recordings from a bursting neuron in a slice superfused with aCSF containing 5 mM TEA. Addition of 100 nM apamin markedly enhanced the burst response (traces are overlaid). **G**, Recordings from another bursting neuron in a slice superfused with aCSF containing 3 mM 4-AP and 10 μ M XE991. Addition of 100 nM apamin markedly enhanced the burst response (traces are overlaid). **H**, Bar histogram summarizing the effects of apamin on the DS in the bursting neurons produced by TEA ($n = 3$) or 4-AP and XE991 ($n = 5$).

These data indicate that, in CA1 pyramidal cells, I_{SK} plays a role in curtailing bursts only when I_M is reduced. Even bursts driven by Ca^{2+} spikes are not modulated by I_{SK} as long as I_M is operative.

Figure 5. SK channels enhancing excitability are expressed at or near the soma of CA1 pyramidal cells. **A**, Representative experiment showing the effects of d-TC ($100\ \mu\text{M}$) applied in the aCSF on the firing of an XE911-treated neuron. In aCSF containing $10\ \mu\text{M}$ XE911, the neuron fired a burst of four spikes (**a**), which was increased to seven spikes by d-TC (**b**) in a reversible manner (**c**). **B**, **C**, Bar histograms summarizing the effect of d-TC applied in the aCSF on the number of intraburst spikes and DSs, respectively ($n = 5$). **D**, Scheme of a hippocampal slice showing points of focal d-TC applications (1–4) in relation to the recording point (3). **E**, Data from a representative experiment in which d-TC was focally applied to distal (1), proximal apical dendritic (2), and somatic regions (3) of the recorded neuron. The neuron was superfused with aCSF containing $10\ \mu\text{M}$ XE911. The graph depicts the number of intraburst spikes at each time point. **F**, Selected recordings from the experiment described in **E**. The letters depict the time points at which the recordings were obtained (triangles in **E**). Note extra spikes in **c** and **e**. **G**, Data from a representative experiment in which d-TC was focally applied to basal dendritic (4) and somatic regions (3) of the recorded neuron. Otherwise, similar to **E**. **H**, Selected recordings from the experiment described in **G**. Note extra spikes only in **c**. **I**, Bar histogram summarizing the number of intraburst spikes generated by XE911-treated neurons 5 min after focally applying d-TC to soma ($n = 7$), proximal apical dendrites ($n = 7$), distal dendrites ($n = 6$), and basal dendrites ($n = 4$). The number of neurons in the control group was seven. Significant increase was achieved only when d-TC was applied to the soma or to the proximal dendrites.

Somatic localization of SK channels exerting auxiliary control of bursting

As in the case of apamin, applying D-TC in the aCSF caused a marked enhancement of bursts produced by I_M blockers in CA1 pyramidal cells (Fig. 5*Aab*). Therefore, in neurons treated with 10 μ M XE991, D-TC (100 μ M) augmented both the number of intraburst spikes (from 4.4 ± 0.2 to 8.4 ± 0.7 spikes; $p = 0.0009$; Fig. 5*B*) and DS size (from 1265.5 ± 100.0 to 2768.4 ± 189.5 mV·ms; $n = 5$; $p = 0.0005$; Fig. 5*C*). These effects were completely reversible upon drug washout (Fig. 5*Ac*). We therefore used focal applications of D-TC to characterize the subcellular localization of the SK channels responsible for the auxiliary control of bursting in CA1 pyramidal cells.

The puffer contained 5 mM D-TC in aCSF. In the first series of experiments, we sequentially applied D-TC to the apical dendrites at two locations in stratum radiatum, ~ 150 and ~ 400 μ m from the soma of the impaled neuron, after which D-TC was applied to the somatic region. The experimental arrangement is illustrated in Figure 5*D*. The neurons were perfused with aCSF containing 10 μ M XE991 and stimulated at 1 per minute with a suprathreshold brief pulse. The results of a representative experiment are shown in Figure 5, *E* and *F*. In response to each stimulus, the neuron fired consistently a burst of five spikes (Fig. 5*F*, trace *a*). Application of D-TC to the distal apical dendrite (Fig. 5*E*, time marked by arrow 1) had no effect on the number of intraburst spikes (Fig. 5*E,F*, trace *b*). However, applying D-TC more proximally to the soma (Fig. 5*E*, time marked by arrow 2) reversibly increased the number of intraburst spikes to six (Fig. 5*E,F*, traces *c* and *d*). Subsequent application of D-TC to the somatic region (Fig. 5*E*, time marked by arrow 3) increased the number of intraburst spikes to seven (Fig. 5*E,F*, trace *e*). In the second set of experiments, we focally applied D-TC in the stratum oriens at ~ 100 μ m from the soma of the impaled neuron and then moved the puffer to the somatic region. A representative experiment is shown in Figure 5, *G* and *H*. The neuron generated a burst of five spikes in control (Fig. 5*Ha*) and after applying D-TC to the stratum oriens (Fig. 5*G*, time marked by arrow 4, Fig. 5*Hb*). However, subsequently applying D-TC to the somatic region prolonged the burst and increased the number of intraburst spikes to seven (Fig. 5*G*, time marked by arrow 3, Fig. 5*Hc*).

Figure 5*I* summarizes the effects of focally applied D-TC. This SK channel blocker prolonged linopirdine-induced bursts only when applied to the soma or proximal apical dendrites. These data indicate that the auxiliary control of bursts by I_{SK} is mediated predominantly by SK channels localized to these compartments.

SK channels do not control the spike ADP

In CA1 pyramidal cells, the fast spike is followed by a prominent ADP lasting tens of milliseconds (Schwartzkroin, 1975; Jensen et al., 1994, 1996). The size of the spike ADP is strongly controlled by $K_{V7/M}$ channels (Yue and Yaari, 2004). We have reported previously that apamin (100 nM) does not modify the size of the ADP (Yue, Yaari, 2004). We have repeated these experiments and obtained the same results (control: 190.5 ± 23.7 mV·ms; with apamin: 158.6 ± 12.4 mV·ms; $n = 7$; $p = 0.079$; Fig. 6*Aabc,D*). To determine whether $K_{V7/M}$ channels activation occludes an inhibitory effect of I_{SK} on the spike ADP, we monitored the effects of apamin after blocking $K_{V7/M}$ channels with 10 μ M linopirdine. As shown previously, linopirdine consistently facilitated the spike ADP, converting the entire response into a burst in $\sim 80\%$ of the neurons (Yue, Yaari, 2004). In neurons in which the facilitated ADP was not sufficient to trigger a burst, further exposure to 10 mM apamin in the aCSF did not significantly affect the spike

Figure 6. SK channels do not control the spike ADP. **A**, Representative recordings from a neuron in a slice superfused with normal aCSF. **a**, Applying brief (4 ms) stimuli elicited single spikes followed by an ADP. Addition of 100 nM apamin to the aCSF had no effect on the spike ADP (**b**, overlaid and enlarged traces in **c**). **B**, Recordings from a neuron in a slice superfused with aCSF containing 10 μ M linopirdine. In this cell, applying brief stimuli elicited single spikes followed by an ADP (**a**). Addition of 100 nM apamin to the aCSF had no effect on the spike ADP (**b**, overlaid and enlarged traces in **c**). **C**, Recordings from a neuron in a slice superfused with aCSF containing 10 μ M linopirdine. In this cell, applying brief stimuli elicited a burst of six spikes (**a**, inset). An appropriately timed hyperpolarizing pulse blocked the burst component of the response, thereby unmasking a spike ADP (**a**). Addition of 100 nM apamin to the aCSF minimally increased the spike ADP (**b**, overlaid and enlarged traces in **c**). **D**, A bar histogram summarizing the effects of apamin on ADP size in control neurons ($n = 7$), in linopirdine-treated nonbursters (NB; $n = 6$), and in linopirdine-treated bursters (B; $n = 5$). No significant effects were found.

ADP (linopirdine: 214.5 ± 29.5 mV·ms; with apamin: 234.4 ± 35.0 mV·ms; $n = 6$; $p = 0.185$; Fig. 6*Aabc,D*). In neurons that transformed to bursting mode, the burst hindered the measurement of the first spike ADP. Therefore, we unmasked the ADP by applying a brief (4 ms) negative current pulse immediately after the positive current pulse that initiated firing in the neuron (Jensen et al., 1996; Chen and Yaari, 2008). The strength of the second pulse was carefully raised until the burst response was suppressed in $\sim 50\%$ of the trials (Fig. 6*Ca*). Apamin had no significant effect on the size of the unmasked ADP (linopirdine: 385.4 ± 129.5 mV·ms; with apamin: 446.8 ± 172.3 mV·ms; $n = 5$; $p = 0.243$; Fig. 6*Cabc,D*). Together, these data indicate that regardless of whether I_M is blocked, a solitary spike does not activate I_{SK} sufficiently to produce inhibition of the spike ADP.

Figure 7. Role of different K^+ channels in controlling the excitability of CA1 pyramidal cells with blocked K_v7/M channels. **A**, Representative experiment showing the consequences of adding $10 \mu M$ paxilline (a selective blocker of $BK Ca^{2+}$ -activated K^+ channels; I_C) to the XE991-containing aCSF on bursting of CA1 pyramidal cells. The neuron fired five spikes before (**a**) and after (**b**) adding paxilline, but subsequent addition of $100 nM$ apamin enhanced the burst to 10 spikes (**c**). Expectedly, paxilline caused spike broadening (**d**). **B**, Representative experiment showing the consequences of adding $10 \mu M$ α -dendrotoxin (a selective blocker of D -type K^+ channels; I_D) to the XE991-containing aCSF on bursting of CA1 pyramidal cells. The neuron fired five spikes before (**a**) and after (**b**) adding α -dendrotoxin, but subsequent addition of $100 nM$ apamin enhanced the burst to 14 spikes (**c**). Expectedly, α -dendrotoxin caused spike broadening (**d**). **C**, Representative experiment showing the consequences of adding $1 mM$ 8-Br cAMP (an inhibitor of K^+ channels producing the slow AHP current; I_{sAHP}) to the XE991-containing aCSF on bursting of CA1 pyramidal cells. The neuron fired five spikes before (**a**) and after (**b**) adding 8-Br cAMP, but subsequent addition of $100 nM$ apamin enhanced the burst to eight spikes or more, followed by a long plateau potential (**c**). Expectedly, 8-Br cAMP markedly reduced the sAHP after a 500-ms-long, suprathreshold depolarizing pulse (**d**). **D**, **E**, Bar histograms summarizing the effects of paxilline ($n = 5$), α -dendrotoxin ($n = 5$), and 8-Br cAMP ($n = 5$) on the number of intraburst spikes (**D**) and DS size (**E**), respectively.

Comparing SK channels with other K^+ channels modulating excitability

We next investigated whether the auxiliary control of bursting is uniquely mediated by SK channels by testing, in XE991-treated neurons, how reducing other Ca^{2+} - and voltage-gated K^+ channels affects their excitability. In CA1 pyramidal cells, the Ca^{2+} - and voltage-gated K^+ current generated by the big conductance K^+ (BK) channels (I_C) is responsible for the late phase of spike repolarization; blocking I_C causes spike broadening and decreases the fAHP (Lancaster and Nicoll, 1987; Storm, 1987). Exposing the neurons to $10 \mu M$ paxilline, a selective blocker of BK channels (Shao et al., 1999), expectedly caused spike broadening (Fig.

7Ad), but had no effect on the number of intraburst spikes (XE991: 5.0 ± 0.1 ; with paxilline: 4.8 ± 0.2 ; $n = 5$; $p = 0.513$; Fig. 7Aab,D) or on DS size (XE991: 1527.2 ± 61.1 ; with paxilline: 1418.6 ± 82.2 mV·ms; $p = 0.421$; Fig. 7Aab,E). In contrast, subsequent addition of apamin to the aCSF markedly enhanced the burst response (Fig. 7Ac).

The D-type potassium current (I_D) was shown previously to modulate the excitability of CA1 pyramidal neurons (Storm, 1988; Golding et al., 1999; Metz et al., 2007). Exposing the neurons to $2 \mu M$ α -dendrotoxin, a selective blocker of I_D (Grissmer et al., 1994), also caused spike broadening (Fig. 7Bd), but had no effect on the number of intraburst spikes (XE991: 4.8 ± 0.2 ; with α -dendrotoxin: 5.2 ± 0.2 ; $n = 5$; $p = 0.513$; Fig. 7Bab,D) or on DS size (XE991: 1418.7 ± 53.1 ; with α -dendrotoxin: 1480.9 ± 85.5 mV·ms; $p = 0.731$; Fig. 7Bab,E). Again, subsequent addition of apamin to the aCSF enhanced the burst response (Fig. 7Bc).

Finally, we investigated whether the Ca^{2+} -dependent slow AHP current (I_{sAHP}) controls bursting invoked by blocking I_M . In CA1 pyramidal cells, I_{sAHP} activates during sustained repetitive firing and causes spike frequency adaptation and a sAHP that is readily suppressed by c-AMP (Madison and Nicoll, 1986). Application of $1 mM$ 8-bromo-cAMP, a cell-permeable analog of cAMP, strongly reduced the sAHP induced by long depolarizing current pulses and strongly facilitated the repetitive discharge in these neurons (Fig. 7Cd). This drug also suppressed the sAHP after each burst, but had no effect on the number of intraburst spikes (XE991: 5.0 ± 0.3 ; after 8-bromo-cAMP; 5.2 ± 0.4 ; $p = 0.555$; $n = 5$; Fig. 7Cab,D) or DS size (XE991: 1623.6 ± 197.9 ; 1660.9 ± 184.4 mV·ms; after 8-bromo-cAMP; $p = 0.389$; Fig. 7C,Cab,E). Further addition of apamin enhanced the burst response (Fig. 7Cc). These findings indicate that I_{SK} is a critical K^+ current for curtailing short spike bursts once K_v7/M channels are blocked.

Role of SK channels in hyposmotic bursting

The results of the comparative pharmacological experiments, summarized in Figure 7, D and E, point to a unique, albeit auxiliary, role of SK channels in the control of bursting in CA1 pyramidal cells. We wondered under what physiological or pathophysiological conditions this role may come into effect. One such condition is brain hyposmolarity, which may be caused, for example, by excessive water consumption (polydipsia) or enhanced release of antidiuretic hormone and is clinically expressed in brain hyperexcitability and epileptiform seizures (Adrogué and Madias, 2000). Intriguingly, CA1 pyramidal cells

Figure 8. Blocking SK channels enhances hypotonic bursting in CA1 pyramidal cells. **A**, Representative experiment showing the effects of apamin on the hypotonic bursting of a CA1 pyramidal cell. In hypotonic aCSF (240 mOsm), the neuron fired bursts of three spikes in response to brief stimuli (**a**). Addition of 100 nM apamin prolonged the burst response to 7 spikes (**b**). **B**, **C**, Bar histograms summarizing, respectively, the effects of apamin on the number of intraburst spikes and DS in hypotonic aCSF ($n = 6$).

readily convert to bursting mode when extracellular osmolarity is moderately reduced (Azouz et al., 1997) and this conversion is underlain by hypotonic suppression of I_M (Caspi et al., 2009). We therefore tested the effects of apamin in CA1 pyramidal cells maintained in hypotonic aCSF (240 mOsm). As illustrated in Figure 8, application of 100 nM apamin markedly increased the number of intraburst spikes (hypotonic aCSF: 3.3 ± 0.6 ; with apamin: 5.3 ± 1.0 , $n = 6$; $p = 0.01$; Fig. 8Aa,b), as well as DS size (hypotonic aCSF: 907.3 ± 216.7 ; with apamin: 1397.6 ± 236.4 mV·ms; $p = 0.01$; Fig. 8Ac,c). These results further indicate that somatic and perisomatic SK channels may be important in constraining intrinsic neuronal hyperexcitability in pathophysiological conditions that compromise I_M .

Discussion

In the present study, we investigated the role of somatic and perisomatic SK channels in CA1 pyramidal cells, the major principal neurons relaying information from hippocampus to cortex (van Groen and Wyss, 1990). Our data show explicitly that these channels are functionally expressed in somata and proximal dendrites of CA1 pyramidal cells, yet they play only an auxiliary role secondary to K_V7/M channels in controlling the intrinsic excitability of these neurons. As long as K_V7/M channels are operative, activation of SK channels during repetitive firing does not notably affect spike output of CA1 pyramidal cells. However, when K_V7/M channel activity is compromised, SK channel activation significantly and uniquely reduces spike output in these neurons.

Expression of functional SK channels in CA1 pyramidal cell somata

Apamin-sensitive K^+ currents are readily evoked by depolarizing pulses in whole-cell patch-clamp recordings at somata of adult rat CA1 pyramidal cells (Fig. 1; Stocker et al., 1999), indicating expression of functional SK channels. However, these recordings

do not disclose the precise subcellular localization of these channels. By inference from SK knock-out mice, these channels likely comprise SK2 subunits, because SK2-null mice do not manifest apamin-sensitive currents (Bond et al., 2004). Studies of SK2 protein distribution using immunohistochemistry (Sailer et al., 2002, 2004; Ngo-Anh et al., 2005; Ballesteros-Merino et al., 2012) and force nanoscopy (Maciaszek et al., 2012) suggest a steep gradient from soma to distal apical dendrites, with maximal expression in dendritic spines. However, we show here that focal somatic application of apamin produced an $\sim 30\%$ reduction in I_{SK} recorded at the soma using the whole-cell recording configuration regardless of whether apamin was applied previously to the proximal apical dendrites or to the basal dendrites. A similar reduction of I_{SK} was produced by focal apamin application to the proximal apical dendrites regardless of whether apamin was pre-applied to the soma (Fig. 2). Because apamin blocks SK channels irreversibly (Ishii et al., 1997), the I_{SK} reduction observed upon somatic apamin application is unlikely to be due to spillover of apamin to the dendrites and vice versa. Therefore, our data show conclusively that functional SK channels are expressed in both somata and proximal apical dendrites of adult rat CA1 pyramidal cells and account for approximately half of the somatically recorded I_{SK} .

Role of SK channels in controlling spike output

Despite the indubitable expression of functional SK channels at the soma, our results indicate that SK channels do not modulate the intrinsic excitability of CA1 pyramidal cells in ordinary or hyperexcitable conditions unless K_V7/M channels are blocked (Figs. 3, 4). It might be argued that blocking K_V7/M leads to bursting underlain by a large DS, which causes a greater Ca^{2+} influx, thereby recruiting a larger I_{SK} than a short train of independent spikes. However, apamin had no effect on bursts induced by high K^+ or 4-AP, which also were underlain by large DS and augmented Ca^{2+} influx (Fig. 4). Therefore, these findings suggest that burst firing evokes a functionally relevant I_{SK} in the proximal compartment of the neuron (Fig. 5), but its inhibitory impact is ordinarily occluded by the overwhelming inhibitory effect of coactivated K_V7/M channels.

Apamin had no effects on the waveform of solitary spikes, even in presence of K_V7/M channel blockers (Fig. 6), indicating that a single spike cannot evoke an inhibitory I_{SK} . This contrasts with the ability of a single spike to evoke a strong inhibitory I_M that prevents the regenerative growth of somatic spike ADPs to spike bursts (Yue and Yaari, 2004, 2006). The modulation by I_{SK} of spike-induced Ca^{2+} transients in proximal processes of cultured hippocampal neurons also requires a train of several spikes, whereas a single spike is ineffective (Tonini et al., 2013). This dependence on the number of spikes is likely due to the fact that spike trains induce much larger and spatially more extensive Ca^{2+} transients than a solitary spike (Spruston et al., 1995), thus activating a larger fraction of SK channels.

Role of SK channels in controlling Ca^{2+} -dependent bursting

Application of 4-AP invokes intrinsic bursting in CA1 pyramidal cells, likely by blocking the A-type K^+ current in apical dendrites, allowing for the generation of Ca^{2+} spikes by back-propagating Na^+ spikes (Magee and Carruth, 1999). Here, we show that these Ca^{2+} -dependent bursts are not affected by apamin as long as K_V7/M channels are operative (Fig. 4C–F). Therefore, SK channels play only a secondary role in controlling burst discharges evoked by dendritic Ca^{2+} spikes, in which I_{SK} is expected to be particularly large due to enhanced Ca^{2+} entry. Our findings ap-

pear incongruent with the findings by Gu et al. (2008) showing that dendritic Ca^{2+} spikes are significantly broadened by apamin. In that study, however, Ca^{2+} spikes were evoked by exposing neurons to 5 mM TEA, which blocks multiple types of K^+ channels, including $\text{K}_{\text{V}}7/\text{M}$ channels (Wang et al., 1998). Indeed, we found that 5 mM TEA blocks 80% of I_{M} in CA1 pyramidal cells (A. Caspi and Y.Y., unpublished observations). In our experiments also, Ca^{2+} -dependent bursts invoked by TEA (or a combination of 4-AP and the $\text{K}_{\text{V}}7/\text{M}$ channel blocker XE991) were profoundly augmented by apamin. That apamin had no effect on bursting under 4-AP alone indicates that $\text{K}_{\text{V}}7/\text{M}$ channels occlude the inhibitory impact of I_{SK} even in conditions producing Ca^{2+} spikes.

Unique auxiliary role of SK channels

Why does activation of proximal SK channels impact the intrinsic excitability of CA1 pyramidal cells only when $\text{K}_{\text{V}}7/\text{M}$ channels are inoperative? Activation of a sufficient number of SK channels requires repetitive firing or bursting, which also strongly activate $\text{K}_{\text{V}}7/\text{M}$ channels. The latter low-threshold, noninactivating channels are densely packed at the AIS (Lai and Jan, 2006; Pan et al., 2006; Rasmussen et al., 2007). Therefore, the AIS hyperpolarization produced by somatic and perisomatic I_{SK} is shunted by the large $\text{K}_{\text{V}}7/\text{M}$ conductance, occluding its inhibitory effect on spike generation. It is noteworthy that, in CA1 pyramidal cells treated with 1-EBIO, which profoundly augments I_{SK} by increasing SK2 channels sensitivity to Ca^{2+} (Pedarzani et al., 2001), repetitive firing is attenuated by I_{SK} in neurons with operative $\text{K}_{\text{V}}7/\text{M}$ channels, as evident from the excitatory effect of apamin in this condition (Gu et al., 2008).

Our data show that the auxiliary role of SK channels in controlling CA1 pyramidal cell excitability is unique, because it is not mimicked by inhibiting other types of K^+ currents (I_{C} , I_{D} , and I_{SAHP} ; Fig. 7). The inactivating properties of I_{C} and I_{D} (Storm, 1988; Shao et al., 1999) may explain why they do not contribute to the curtailment of bursts. A larger number of spikes may be required by the K^+ channels underlying the I_{SAHP} to generate stronger inhibition than that produced by I_{SK} .

Functional implications

In contrast to their auxiliary role in CA1 pyramidal cells, SK channels were shown to decrease spike output in other types of CNS neurons without a prerequisite for blocking $\text{K}_{\text{V}}7/\text{M}$ channels (Faber and Sah, 2007; Adelman et al., 2012). It would be interesting to explore whether, in these neurons, $\text{K}_{\text{V}}7/\text{M}$ channels play a lesser role in controlling spike output than the one exercised in CA1 pyramidal cells. Conversely, it is possible that availability of SK channels is larger in the latter neurons due to a larger SK channel density or to a more efficient coupling between Ca^{2+} influx and activation of proximal SK channels.

What might be the role of somatic and proximal dendritic SK channels in CA1 pyramidal cells? $\text{K}_{\text{V}}7/\text{M}$ channels provide the primary negative feedback mechanism in CA1 pyramidal cells by potentially curtailing the spike ADP (Yue and Yaari, 2004). Here, we show that SK channels furnish a “second line of defense” against intrinsic hyperexcitability that may play an important role in some conditions, such as low extracellular osmolarity (Fig. 8). This mechanism may be recruited *in vivo* in pathophysiological conditions of hyposmolarity to elevate the threshold for epileptiform seizures. Furthermore, multiple neurotransmitters and gliotransmitters are known to reduce $\text{K}_{\text{V}}7/\text{M}$ conductance (Brown and Passmore, 2009) and their facilitation of intrinsic excitability might be controlled by SK channels. It is also note-

worthy that hereditary loss-of-function mutations in $\text{K}_{\text{V}}7.2$ and $\text{K}_{\text{V}}7.3$ channel subunits cause seizures in neonates (known as benign familial neonatal convulsions; BFNCs), which disappear within a few months after birth (Plouin and Kaminska, 2013). The reason for this remission is not known. However, animal experiments show that SK2 protein expression in CA1 neurons increases during early postnatal development (Ballesteros-Merino et al., 2012). If this reflects an increase in functional SK channels, it may eventually provide the brain of BFNC patients with negative feedback compensation for the $\text{K}_{\text{V}}7/\text{M}$ channel loss of function.

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