Axo-Somatic and Apical Dendritic Kv7/M Channels Differentially Regulate the Intrinsic Excitability of Adult Rat CA1 Pyramidal Cells

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Submitted 19 December 2005; accepted in final form 22 February 2006

Yue, Cuiyong and Yoel Yaari. Axo-somatic and apical dendritic Kv7/M channels differentially regulate the intrinsic excitability of adult rat CA1 pyramidal cells. J Neurophysiol 95: 3480–3495, 2006. First published February 22, 2006; doi:10.1152/jn.01333.2005. Kv7/KCNQ/M channel subunits are widely expressed in peripheral and central neurons, where they give rise to a muscarinic-sensitive, subthreshold, and noninactivating K⁺ current (M current). Immunohistochemical data suggest that Kv7/M channels are expressed in both axons, somata, and dendrites, but their distinctive roles in these compartments are not known. Here we used intracellular microelectrode recordings to monitor the effects of selective Kv7/M channel modulators focally applied to the axo-somatic region and to the apical dendrites of adult rat CA1 pyramidal cells. We show that both compartments express functional Kv7/M channels that synergistically control intrinsic neuronal excitability, albeit in different ways. Axo-somatic Kv7/M channels activate during the spike afterdepolarization and counteract the depolarizing drive furnished by conjointly activated persistent Na⁺ channels. Thereby they limit the size and duration of the spike ADP and prevent its escalation into a somatic spike burst. Apical dendritic Kv7/M channels do not ordinarily regulate the somatic spike ADP and spike output. In hyperexcitable conditions that promote Ca²⁺ electrogenesis in these dendrites, they elevate the threshold for initiating Ca²⁺ spikes and associated downstream spike bursts. Thus the concerted activity of Kv7/M channels in both compartments serves to reduce the propensity to generate self-sustained burst responses and fosters a regular, stimulus-graded spike output of the neuron. Given that the activity of Kv7/M channels is regulated by multiple neurotransmitters, they may provide a substrate for neuromodulation of neuronal input/output relations at both the axo-somatic and apical dendritic regions.

INTRODUCTION

The M-type K⁺ current (M-current or Iₘ) is a slow, subthreshold, nonactivating K⁺ current that is found in many peripheral and central neurons (Brown 1988), including CA1 pyramidal cells (Halliwell and Adams 1982). Because of its particular properties, it has been assumed since its discovery that Iₘ serves to limit sustained neuronal excitation (Madison and Nicoll 1984). Iₘ is suppressed by muscarinic agonists, although many other neurotransmitters converge to up- or downregulate it by a variety of second-messenger cascades (Marriott 1997). A large body of evidence now suggests that native neuronal M-channels are formed by heteromeric assemblies of Kv7 (previously designated KCNQ) subunits, particularly Kv7.2 with Kv7.3, and perhaps also with Kv7.5 (Peters et al. 2005; Schroeder et al. 2000; Shah et al. 2002; Wang et al. 1998).

Several drugs that selectively modulate both heterologously expressed and native Kv7/M channel have recently been developed (Brown and Yu 2000). Most notably, the ‘cognition enhancers’ linopirdine and XE991 were shown to block Iₘ (Aiken et al. 1995; Schnee and Brown 1998; Wang et al. 1998), whereas the anticonvulsant drug retigabine was found to increase Iₘ (Rundfeldt and Netzer 2000; Tatulian et al. 2001; Wickenden et al. 2000). The availability of these drugs has permitted a more precise examination of the role of Iₘ in controlling neuronal excitability (Aiken et al. 1995; Hu et al. 2002; Yue and Yaari 2004; Gu et al. 2005). Using these drugs to modify Iₘ in CA1 pyramidal cells, we showed recently that this current normally controls the size and duration of the somatic spike afterdepolarization (ADP) (Yue and Yaari 2004). Blocking Iₘ caused uncontrolled growth of the spike ADP, leading to bursting in most neurons. Conversely, augmenting this current reduced the spike ADP and induced regular firing in bursting neurons. These observations, however, did not disclose the location of the Kv7/M channels that regulate the somatic spike ADP and thus the firing mode of these neurons. Although a recent immunohistochemical study has suggested that Kv7.2 subunits aggregate in the axon initial segment of CA1 pyramidal cells (quite often in conjunction with Kv7.3; Devaux et al. 2004), diffuse staining for Kv7.2, Kv7.3, and Kv7.5 was also found throughout the somatodendritic membrane of these neurons (Devaux et al. 2004; Shah et al. 2002). Yet, whether these subunits assemble to form functional Kv7/M channels in both proximal and distal portions of the neuron has not been clearly determined to date.

Here we used intracellular recordings combined with local application of Kv7/M channel modulators to the axo-somatic region and apical dendrites of adult CA1 pyramidal cells. We sought to determine the presence and characterize the function of Kv7/M channels in these two neuronal compartments. We report that both compartments express functional Kv7/M channels that synergistically control the spike output of these neurons, albeit in different ways.

METHODS

Slice preparation

All experimental protocols were approved by the Hebrew University Animal Care and Use Committee. Transverse hippocampal slices were prepared from adult Sabra rats (8–12 wk old). Animals were anesthetized with ether or isoflurane (3–4%) and decapitated with a guillotine. The brain was removed and immediately immersed in

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ice-cold oxygenated (95% O₂-5% CO₂) dissection artificial cerebrospinal fluid (ACSF). The caudal two thirds of one hemisphere (containing one hippocampus) were glued to the stage of a vibratome (Leica, Wetzlar, Germany). Transverse brain slices (400 μm thick) were cut from the region of the hemisphere containing the anterior hippocampus and the hippocampal portion was dissected out of each brain slice. Routinely, the CA3 region was cut away. This was done to avoid spread of spontaneous burst activity from CA3 to CA1 in experiments using K⁺ channel blockers, although this problem was also prevented in most experiments by adding blockers of synaptic transmission to the ACSF (see following text). The residual hippocampal slices (consisting mainly of CA1 and dentate gyrus) were transferred to an incubation chamber containing oxygenated saline at room temperature (21–24°C), where they were allowed to recover for ≥1 h. The slices were transferred one at a time to an interface slice chamber and perfused from below with oxygenated ACSF at 33.5°C. The upper surface of the slices was exposed to the humidified gas mixture.

The standard ACSF contained (in mM): NaCl 124, KCl 3.5, MgCl₂ 2, CaCl₂ 1.6, NaHCO₃ 26, and d-glucose 10 (pH 7.3). For some experiments 1 mM NiCl₂ was added to the ACSF, and the concentration of MgCl₂ was reduced to 1 mM to maintain a constant concentration of divalent cations. In most experiments, the ACSFs also contained the glutamate receptor antagonists 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 15 μM) and 2-amino-5-phosphono-valeric acid (APV; 50 μM) to block fast excitatory postsynaptic potentials, and the γ-aminobutyric acid type A (GABAA) receptor antagonist picrotoxin (100 μM) to block fast inhibitory postsynaptic potentials. Other drugs were added to the ACSF as indicated.

Electrophysiological methods

Current-clamp recordings from the somata of pyramidal cells in the CA1 pyramidal layer were made using sharp, K⁺-acetate-filled (4 M) glass microelectrodes (70–100 MΩ). An active bridge circuit in the amplifier (Axoclamp 2A, Axon Instruments) allowed simultaneous injection of current and measurement of membrane potential. The bridge balance was carefully monitored and adjusted before each measurement. The pyramidal cells accepted for this study had stable resting potentials of at least −60 mV and overshooting action potentials.

Drug applications

Drugs were applied to the whole slice by bath application or to discrete locations in the slice using a puff application system. Because the spread of drugs in slices maintained in an interface chamber is predominantly by diffusion, the onset and offset of drug effects are slow (in the order of tens of minutes). For all bath-applied drugs used in this study, maximal effects were obtained within 20–30 min of adding them to the ACSF.

For focal drug applications we used a puffing system consisting of a pneumatic pump (Picospritzer III, General Valve, Fairfield, NJ) connected to a patch pipette (tip diameter about 10 μm) filled with ACSF and the drugs intended for focal application. The tip of the pipette touched the upper surface of the slice. In control experiments the intensity and duration of the pressure pulses were adjusted to produce a drop that covered a circular area about 50 μm in diameter when ejected onto the surface of the slice (visualized by including fast-green dye in the pipette solution). This yielded pressure pulses of 5 bars for 20 ms, which were used for all applications. For somatic drug applications, the puffing pipette was positioned in stratum pyramidale about 25 μm from the recording microelectrode (lodged in the soma of the neuron). To apply drugs to the distal apical dendrites, the puffing pipette was positioned in stratum radiatum about 200–300 μm away from, and vertical to, the stratum pyramidale (the experimental arrangement is shown in Fig. 1A). The effects of focally applied drugs, when present, appeared usually after 3 min of puffing, and attained maximum within 8–10 min. These delays undoubtedly arise from the slow spread of the drugs from their site of application into the slice. We found that to replicate effects of bath-applied drugs, the optimal drug concentrations in our pressure application pipettes generally should be tenfold higher than those used in bath applications.

Chemicals

All chemicals and drugs were purchased from Sigma Chemicals (Rehovot, Israel) except for CNQX (RBI, Natick, MA), XE991 (Tocris Cookson, Bristol, UK), and retigabine (kind gift of Dr. J. B. Jensen, NeuroSearch, Ballerup, Denmark). Stock solutions (10 mM) of linopirdine (in ethanol), XE991, retigabine, and 4β-phorbol 12,13-dibutyrate (PDB) (in dimethylsulfoxide) were stored at −20°C. Ethanol and dimethylsulfoxide added to the standard ACSF to a concentration of 0.001% had no effects on the measured spike parameters.

**FIG. 1.** Changes in input resistance ($R_i$) induced by Kv7/M channel modulators focally applied to apical dendrites or axo-soma. A: scheme of experimental arrangement (see METHODS). B: neurons were stimulated with 200-ms-long negative square pulses of increasing intensity (in 50-pA steps). In each panel here and in all figures below the top trace represents the intracellular voltage record and the bottom trace depicts the current pulses injected into the cell. DC current injected to counteract the depolarizing or hyperpolarizing effects of the drugs is shown only in this figure (Bc and Cc; dashed line reflects 0 DC current). Resting membrane potential is denoted to the left of the voltage trace. Applying linopirdine to the apical dendrites had no detectible effects on $R_i$ (a to b), whereas applying it to the soma caused $R_i$ increase and also reduced the voltage sag and rebound depolarization (c). C: applying retigabine to the apical dendrites had no detectible effects on $R_i$ (a to b), whereas applying it to the soma caused a marked decrease in $R_i$ and also enhanced the voltage sag and rebound depolarization (c).
Data measurement and analysis

The intracellular signals were digitized, stored, and analyzed using a Pentium computer and pCLAMP9 data acquisition system (Axon Instruments). Apparent input resistance (R_{m}) was measured from voltage deflections induced by small (50- to 100-pA) 200-ms negative current pulses. It should be remembered, however, that R_{m} measurements in CA1 pyramidal cells are confounded by the presence of other conductances that are active around the resting potential, particularly the hyperpolarization-activated cation conductance (I_{h}; Surges et al. 2004).

The size of the spike ADP was measured as the integrated “area under the curve” between the fast afterhyperpolarization (fAHP) and the point at which membrane voltage returned to resting potential. The amplitude of Ca^{2+} spikes was measured as the voltage difference between the peak of the spike and the resting membrane potential. For measuring changes in R_{m}, spike waveform and neuronal discharge behavior after bath or local drug applications, drug-induced changes in resting membrane potential were counteracted by steady injections of appropriate negative or positive currents. Averaged data were expressed as mean ± SE. The significance of the differences between the measured parameters was evaluated using, as required, Student’s unpaired or paired t-test. Multiple comparisons were done with two-way ANOVAs, followed by Bonferroni post hoc tests. The significance level in all tests was set to P < 0.05.

RESULTS

Effects of focally applied Kv7/M channel modulators on “passive” membrane properties

To characterize the distinct roles of axo-somatic and apical dendritic Kv7/M channels in control of neuronal excitability, we first examined the effects of the Kv7/M channel modulators applied focally to either the distal or proximal parts of CA1 pyramidal cells. In separate experiments we examined the effects of the Kv7/M channel blockers linopirdine and XE991 and of the Kv7/M channel opener retigabine. The application pipette contained either 100 μM linopirdine, 30 μM XE991, or 100 μM retigabine dissolved in standard ACSF. The experimental arrangement is illustrated in Fig. 1A. In these and in later experiments, the effects of the focally applied drugs were monitored for >10 min. If a single puff of a drug yielded no obvious effect within the first 3 min, the drug was puffed twice again in immediate succession at the same region (altogether three puffs). If a clear response was observed within the first 3 min, no more puffs were applied in that region. In six control experiments, puffing ACSF without drugs onto either proximal or distal portions of the neurons had no effects on their passive (resting potential and R_{m}) and active membrane properties (spike threshold, amplitude, and ADP).

Applications of linopirdine to the apical dendrites did not significantly affect resting membrane potential and R_{m} recorded in the soma (Table 1; Fig. 1, Ba and Bb). However, when applied to the axo-somatic region (one puff), linopirdine depolarized most neurons (five of six) by 1 to 6 mV. On average, this effect amounted to 3.3 ± 0.6 mV (P < 0.05; Table 1). Concurrently, linopirdine applied to the axo-soma increased R_{m} (measured from control resting potential; see METHODS) by 37.0 ± 12.6% (P < 0.05; Table 1; Fig. 1, Bb and Bc). Similar results were obtained with XE991. Its application to the distal dendrites affected neither resting membrane potential nor R_{m} (P < 0.05; Table 1). However, when applied to the axo-somatic region (one puff), XE991 depolarized the neurons by 1 to 10 mV. On average, this effect amounted to 4.3 ± 0.8 mV (P < 0.05; Table 1). Concurrently, XE991 increased R_{m} by 30 ± 10.9% (P < 0.05; Table 1).

Interestingly, linopirdine or XE991 applied to the axo-soma not only increased R_{m}, but also reduced the voltage sags in the hyperpolarizing responses evoked by negative current pulses, as well as the rebound depolarizations that occur at the termination of these stimuli (Fig. 1B, compare voltage traces in c to those of similar size in a and b). Both the voltage sags and the rebound depolarizations have been attributed to activation of the hyperpolarization-activated cationic current (I_{h}; Gasparini and DiFrancesco 1997). Our observations suggest that deactivation of I_{h} during hyperpolarization from resting potential also contributes appreciably to these phenomena.

Retigabine applied to the apical dendrites also had no effect on resting potential and R_{m} (P < 0.05; Table 1; Fig. 1, Ca and Cb). When applied to the axo-soma (one puff), however, retigabine hyperpolarized the neurons by 1 to 9 mV; the average hyperpolarization was 5.0 ± 0.8 mV (P < 0.05; Table 1). Concurrently, R_{m} decreased by 49.1 ± 4.7% (P < 0.05; Table 1; Fig. 1, Cb and Cc). In addition to that, retigabine applied to the axo-soma markedly augmented the voltage sags during hyperpolarizations and rebound depolarizations (Fig. 1C, compare traces in c to those of similar size in a and b). These effects are expected given that retigabine shifts the I_{h} activation curve in the hyperpolarizing direction by about 20 mV (Main et al. 2000; Tatulian et al. 2001).

The ineffectiveness of distal applications of Kv7/M channel modulators in altering resting membrane potential and R_{m} conceivably may arise from insufficient drug delivery to the apical dendrites. However, in another series of experiments (described below; Figs. 9 and 10), we found that similar distal applications of

| TABLE 1. Effects of Kv7/M channel modulators focally applied to apical dendrites or axo-soma on resting potential (V_{m}) and input resistance (R_{m}) |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                   | Linopirdine       |                  | XE991             |                  | Retigabine        |                  |
|                   | Apical dendrites  | Axo-soma         | Apical dendrites  | Axo-soma         | Apical dendrites  | Axo-soma         |
|                   | Control           | Drug             | Control           | Drug             | Control           | Drug             |
| V_{m}, mV         | (n = 6)           | (n = 9)          | (n = 6)           | (n = 9)          | (n = 6)           | (n = 9)          |
| Resting potential | -66.7±1.0         | -66.0±1.1        | -67.9±1.2         | -64.6±1.2        | -67.0±0.9         | -66.6±1.2        |
| R_{m}, MO         | 38.0±3.0          | 37.9±2.9         | 42.9±3.0          | 38.9±6.3         | 36.3±3.2          | 37.4±3.5         |

Values are means ± SE. *Statistically significant difference between drug and control groups (P < 0.05).
linopirdine, XE991, and retigabine modulate dendritic Ca\(^{2+}\) spikes and Ca\(^{2+}\)-dependent bursting in the same way they do when applied in the bath in micromolar concentrations. In those experiments also, the drugs did not alter resting membrane potential or \(R_{N}\). Notwithstanding, we repeated the dendritic application experiments (three puffs) with even higher concentrations of linopirdine (500 \(\mu\)M), XE991 (300 \(\mu\)M), and retigabine (500 \(\mu\)M) in the application pipette. Each drug was tested in three neurons. Again, there was no change in resting membrane potential and \(R_{N}\) within the 10-min observation period after puffing the drugs onto the apical dendrites.

Together, our findings indicate that distal Kv7/M channels do not play a part in determining the “passive” membrane properties of the axo-somatic membrane. The small but significant changes in resting potential imposed by linopirdine and XE991 applied to the axo-soma suggest that proximal Kv7/M channels are partially active at resting potential. Similar changes were described previously after bath application of these drugs (Yue and Yaari 2004).

### Effects of focally applied Kv7/M channel modulators on somatic spike ADP

We previously showed that linopirdine, XE991, and retigabine strongly modify action potential generation in CA1 pyramidal cells when applied in the bath (Yue and Yaari 2004). Most remarkably, linopirdine (0.3–10 \(\mu\)M) and XE991 (0.1–3 \(\mu\)M) dose-dependently augmented the somatic spike ADP, leading to the generation of spike bursts at the higher concentrations. Retigabine (1–10 \(\mu\)M) exerted the converse effect, reducing the size of the spike ADP, while augmenting a partially overlapping afterhyperpolarization (AHP) of medium duration (mAHP). The effects of these drugs on the spike ADP were not secondary to changes in membrane potential because they also occurred when the membrane potential was manually clamped to its natural resting value (Yue and Yaari 2004). Together, these findings suggested that Kv7/M channels control the spike output of CA1 pyramidal cells by limiting the size of the somatic spike ADP.

To evaluate the contribution of axo-somatic and dendritic Kv7/M channels to the control of the somatic spike ADP, we examined the effects of the three drugs when applied focally to axo-soma or apical dendrites. Solitary spikes were evoked by brief (4-ms) positive current pulses from the resting membrane potential recorded in control conditions. Representative results obtained in two experiments with linopirdine are illustrated in Fig. 2, A and B. In the first experiment (Fig. 2A), linopirdine was first applied repetitively (three puffs) to the apical dendrites and 15 min later to the axo-soma (one puff). Dendritic application of linopirdine had no effect on the spike ADP (Fig. 2, Aa and Ab, and traces a and b in Fig. 2Ae), whereas its application to the axo-soma (one puff) appreciably augmented the spike ADP until it triggered a burst (Fig. 2, Ac and Ad, and traces c and d in Fig. 2Ae). In the second experiment (Fig. 2B) the order of applications was reversed. Again, axo-somatic application (one puff) of linopirdine caused considerable augmentation of the spike ADP and induced bursting (Fig. 2, Ba–Bc, and traces a–c in Fig. 2Be). Dendritic application of linopirdine after recovery of this effect (trace not shown) did not modify the spike ADP (Fig. 2Bd and trace d in Fig. 2Be). In a series of 16 experiments, the spike ADP size (measured by integrating the voltage trajectory over time; see METHODS) was 151.8 ± 13.9 mV · ms in control, 151.0 ± 19.5 mV · ms after dendritic application, and 318.7 ± 16.1 mV · ms (\(P < 0.05\)) after axo-somatic application of linopirdine. The average increase in ADP size after axo-somatic linopirdine application (measured before the appearance of bursting) was 141.6 ± 25.5%. In all but two neurons (14 of 16) the augmented ADPs ultimately progressed to full-blown bursts consisting of three to eight spikes. The mean number of intraburst spikes in the 14 bursting neurons was 4.1 ± 0.3.

Similar results were obtained with XE991, as illustrated in Fig. 2C. When applied to the distal dendrites, XE991 had no noteworthy effect on the spike ADP (Fig. 2, Ca and Cb, and traces a and b in Fig. 2Ce). However, a single puff of XE991 to the axo-soma markedly augmented the spike ADP (Fig. 2, Cc and Cd and traces c and d in Fig. 2Ce). In a series of 10 experiments, the spike ADP size was 180.7 ± 12.1 mV · ms in control, 170.1 ± 9.6 mV · ms after dendritic application (three puffs), and 341.1 ± 41.6 mV · ms (\(P < 0.05\)) after axo-somatic application (one puff) of XE991. The average increase in ADP size after axo-somatic application of XE991 was 93.4 ± 24.8% and all neurons eventually became bursters. When XE991 was applied first to the axo-soma (one puff) it also caused spike ADP augmentation and bursting in all cases (\(n = 4\); data not shown). The mean number of intraburst spikes in the 14 bursting neurons was 3.7 ± 0.6.

Finally, we tested the effects of focally applied retigabine. Application of retigabine to the apical dendrites (three puffs) had variable effects. In some neurons the spike ADP was not affected (Fig. 2, Da and Db and traces a and b in Fig. 2De), whereas in others it was somewhat reduced. On average, dendritic application of retigabine reduced the spike ADP from 171.5 ± 28.4 to 140.3 ± 24.4 mV · ms (\(n = 8\); \(P < 0.05\)). When subsequently applied to the axo-somatic region (one puff), however, retigabine consistently and robustly reduced the spike ADP to 65.1 ± 14.5 mV · ms (\(n = 8\); \(P < 0.05\)), converting it to a conspicuous mAHP (Fig. 2, Dc and Dd and traces c and d in Fig. 2De). The average decreases in ADP size after dendritic and axo-somatic applications were 18.5 ± 4.7 and 62.4 ± 6.2%, respectively.

The effects of focally applied Kv7/M modulators on the size of the somatic spike ADP are summarized in Fig. 2F. Together, they suggest that the somatic spike ADP is tightly regulated by Kv7/M channels residing in the axo-somatic region of the neuron. During the spike ADP the activity of these channels slowly increases, thereby facilitating the repolarization of the neuron. The small but significant reduction of somatic spike ADP by distally applied retigabine (Fig. 2, Da, Db, and E) suggests that functional Kv7/M channels are expressed in apical dendrites and may be recruited by the backpropagating spike. These channels, however, do not appear to limit the somatic spike ADP in ordinary conditions, as evident from the complete lack of effect of distally applied linopirdine and XE991 (Fig. 2, Aa, Ab, Ca, Cb, and E).

The increases in spike ADP size and in \(R_{N}\) induced by axo-somatic applications of linopirdine or XE991 were not positively correlated with each other. The relationship between these two variables obtained in 20 experiments with either linopirdine (\(n = 11\)) or XE991 (\(n = 9\)) is provided by the scatterplot in Fig. 2F. Assuming a linear correlation between the two variables yielded a negative and small Pearson corre-
FIG. 2. Effects of Kv7/M channel modulators focally applied to apical dendrites or axo-soma on the somatic spike afterdepolarization (ADP). A: applying linopirdine to the apical dendrites had no detectible effects on the spike ADP (a to b), whereas applying it to the soma caused ADP growth (c) until it triggered a burst (d). Traces in a–d are expanded in size and overlaid in e to facilitate comparison. B: same as in A but in the reversed order. Linopirdine was first applied to the soma and caused ADP growth (a to b) until it triggered a burst (c). A few minutes later the neuron reverted to regular firing mode and the spike ADP diminished back to control size (not shown). Applying linopirdine to the apical dendrites had no effect on the spike ADP (d). Traces in a–d are overlaid in e. C: applying XE991 to the apical dendrites had no detectible effects on the spike ADP (a to b), whereas applying it to the soma caused ADP growth (c) until it triggered a burst (d). Traces in a–d are overlaid in e. D: applying retigabine to the apical dendrites had no detectible effects on the spike ADP (a to b), whereas applying it to the soma progressively curtailed the ADP (c) while enhancing an overlapping medium-duration afterhyperpolarization (mAHP) (d). Traces in a–d are overlaid in e. E: bar diagram summarizing the effects of the 3 Kv7/M channel modulators. For each drug used, the bars depict ADP size (means ± SE) in control (black) and after dendritic (gray) and somatic (white) applications. *Statistically significant difference between drug and control groups (P < 0.05). F: plot of the change in ADP size vs. the change in $R_n$ in 20 neurons exposed to Kv7 channel blockers. Linear regression line drawn has a slope of $-0.19$. 
lation coefficient \((r = -0.12; P = 0.63)\). Thus even though both effects of linopirdine are mediated by axo-somatic Kv7/M channel block, ADP growth is not merely the end result of the increase in \(R_N\). Given that ADP growth is mediated by a regenerative recruitment of \(I_{NaP}\) unleashed by blocking Kv7/M channels (Yue et al. 2004, 2005), the variation in the amount of ADP increase across different neurons may reflect more closely the variation in \(I_{NaP}\) densities.

Effects of focally applied Kv7/M channel modulators on spike output

In normal conditions, CA1 pyramidal cells fire trains of independent spikes when stimulated with prolonged supra-threshold positive current pulses (see, for example, Fig. 3Aa), although a tendency for early spike clustering is evident in some neurons, particularly at high stimulus intensity (Jensen et al. 1994; Sue et al. 2001). The number of evoked spikes increases with stimulus intensity and decreases with time. Here we examined how modulation of Kv7/M channel activity in the axo-soma and apical dendrites affects this spike output/stimulus intensity relationship. Representative experiments in which linopirdine, XE991, and retigabine were focally applied are shown in Fig. 3, A–C. In normal ACSF, increasing stimulus intensity (from 0 to 450 pA in 50-pA steps) caused a gradual and quite linear increase in spike output (Fig. 3, Aa, Ba, and Ca). Application of linopirdine (Fig. 3Ab) or XE991 (Fig. 3Bb) to the apical dendrites (three puffs) had no noticeable effect on this monotonic relationship. In contrast, a single puff of linopirdine (Fig. 3Ac) or XE991 (Fig. 3Bc) to the axo-soma substantially modified this relationship by inducing an all-or-none burst of three to six spikes as the neuron’s minimal response to threshold-straddling stimuli. The burst was followed by a conspicuous mAHP, which caused a cessation of firing (Fig. 3, Ac and Bc). With stronger stimuli, however, the

![FIG. 3. Effects of Kv7/M channel modulators focally applied to apical dendrites or axo-soma on spike output/stimulus intensity relationship. Three representative neurons depicted in A–C were injected with 180-ms-long positive current pulses of increasing intensity (in 100-pA steps). They all fired initially in a regular spiking mode (in some traces the spikes are truncated, as indicated by the dashed lines). A: applying linopirdine to the apical dendrites had no detectible effects on the spike output/stimulus intensity relationship (a to b), whereas applying it to the axo-soma converted the regular spiking mode to a bursting mode (b to c). B: XE991 applications mimicked the effects of linopirdine (a to c). C: applying retigabine to the apical dendrites also did not affect the spike output/stimulus intensity relationship (a to b), whereas applying it to the axo-soma increased the stimulus intensity needed to evoke a spike and suppressed spike output to maximally 2 spikes (b to c). D: summary plots depicting the spike output/stimulus intensity relationships in control conditions (squares) and after linopirdine application to apical dendrites (white circles) and to axo-soma (black circles). Each plot is an average of 8 experiments. E: same as in D, but for 9 experiments with XE991. F: same as in D, but for 8 experiments with retigabine. In D–F, error bars represent SE; *P < 0.05.](http://jn.physiology.org/)

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burst–mAHP complex was followed by one or more solitary spikes. Figure 1, D and E depicts the averaged plots of spike output (the number of evoked spikes) versus stimulus intensity obtained, respectively, in the experiments with linopirdine ($n = 8$) and XE991 ($n = 9$). When delivered to the axo-soma, both drugs significantly augmented spike output at all stimulus intensities ($P < 0.05$). However, the strongest effects were exerted at stimulus intensities that were only slightly above threshold.

Retigabine applied to the apical dendrites also had no detectible effects on spike output (Fig. 3Cb). A single puff of retigabine onto the axo-soma, however, appreciably suppressed spike generation, limiting it to maximally two to three spikes at the highest stimulus intensity (Fig. 3Cc). Similar results were obtained in all experiments with retigabine ($n = 8$), as shown also in the averaged spike output/stimulus intensity plots for these experiments (Fig. 3F). The effects of retigabine were significant at all stimulus intensities $>50$ pA ($P < 0.05$).

These data show that Kv7/M channels in the axo-somatic region of CA1 pyramidal cells impose a graded spike output/stimulus intensity relationship, whereas their dendritic counterparts do not influence this relationship in ordinary conditions.

Effects of focally applied 4-aminopyridine on somatic spike ADP and spike output

For comparison, we also examined the effects of focally applied 4-aminopyridine (4AP) on the somatic spike ADP and discharge behavior of CA1 pyramidal cells. It was previously shown that millimolar 4AP induces intrinsic bursting in these neurons by blocking A-type $K^+$ channels (A channels) in the apical dendrites (Hoffman et al. 1997). Blocking these channels facilitates spike backpropagation into the distal dendrites, thereby initiating a local dendritic $Ca^{2+}$ spike. This, in turn, spreads back to the axo-soma where it augments the spike ADP to the point of bursting (Magee and Carruth 1999). Because of differences in experimental techniques, we first sought to replicate the finding that 4AP induces somatic bursting by blocking $K^+$ channels in apical dendrites. The pressure application pipette contained 30 mM 4AP. A representative experiment ($n = 3$) is shown in Fig. 4A. A single puff of 4AP delivered to the apical dendrites consistently augmented the spike ADP and induced bursting (Fig. 4, Aa–Ac). In contrast, somatic application of 4AP after recovery of this effect (trace not shown) did not modify the spike ADP (Fig. 4Ad and trace d in Fig. 4Ae). We also performed similar 4AP applications in the reversed order ($n = 3$). When applied to the axo-soma, 4AP had no effect on the somatic spike ADP (Fig. 4, Ba and Bb). When applied to the apical dendrites (one puff), however, it consistently induced ADP growth and bursting (Fig. 4, Ba and Bb and traces c and d in Fig. 4Bc). When applied to the apical dendrites (one puff), however, it consistently induced ADP growth and bursting (Fig. 4, Bc and Bd and traces c and d in Fig. 4Be). The mean number of spikes in 4AP-induced bursts was $3.2 \pm 0.8$. Applications of 4AP had no detectible effects on resting potential and $R_N$. These findings corroborate the notion that blocking A channels in apical dendrites, but not in the axo-soma, markedly enhances the somatic spike ADP, as shown previously (Magee and Carruth 1999). Thus the somatic spike ADP is ordinarily maintained at subthreshold voltages by

![FIG. 4. Effects of 4-aminopyridine (4AP) locally applied to apical dendrites or axo-soma on the somatic spike ADP. A: applying 4AP to the apical dendrites caused ADP growth (a to b) until it triggered a burst (c). A few minutes later the neuron reverted to regular firing mode and the spike ADP diminished back to control size (not shown). Applying 4AP to the axo-soma had no effect on the spike ADP (d). Traces in a–d are expanded in size and overlaid in e. B: same as in A but in the reversed order. 4AP had no effect on the spike ADP when first applied to the axo-soma (a to b), but caused progressive ADP growth (c) and eventually bursting (d) when applied to the dendrite. Traces in a–d are expanded in size and overlaid in e. C: effects of locally applied 4AP on spike output. Applying 4AP to the apical dendrites converted the regular spiking mode to a bursting mode (a to b). After recovery of this effect (c), applying 4AP to the axo-soma had only a mild effect, enhancing the tendency for bursting at the onset of strong stimuli (d; top trace).]
the synergistic action of Kv7/M channels in the axo-soma and A channels in the apical dendrites.

We also examined the effects of focally applied 4AP on the spike output/stimulus intensity relationship \((n = 6)\). As illustrated in Fig. 4C, 4AP applied to the apical dendrites (one puff) converted the regular firing mode of the neurons to a bursting mode (Fig. 4, Ca and Cb). This effect reversed after washing 4AP away (Fig. 3Cc). In half of the neurons, application of 4AP to the axo-soma (three puffs) slightly increased the tendency for spike clustering in responses evoked by strong stimuli (Fig. 4Cd), whereas in the other half of the neurons there was no noticeable effect.

Effects of bath-applied 4AP blockers

We have shown above that Kv7/M channels provide inhibitory feedback at the axo-soma that maintains the somatic spike ADP at subthreshold potentials. But what is the source for the positive feedback underlying ADP growth once these channels are blocked? In adult CA1 pyramidal cells, the depolarizing drive for the spike ADP is thought to be provided mainly by a persistent Na\(^+\) current \((I_{\text{NaP}})\) (Azouz et al. 1996; Su et al. 2001; Yue et al. 2005), generated by noninactivating Na\(^+\) channels (French et al. 1990). It is thus likely that blocking axo-somatic Kv7/M channels results in growth of the somatic spike ADP by unleashing the depolarizing action of \(I_{\text{NaP}}\). This hypothesis predicts that blocking axo-somatic persistent Na\(^+\) channels would reverse the action of Kv7/M channel blockers. To test this hypothesis, we first examined the effects of three drugs that effectively block \(I_{\text{NaP}}\) in CA1 pyramidal cells—the anticonvulsant phenytoin, the neuroprotective drug riluzole, and the protein kinase C activator PDB (Yue et al. 2005)—on linopirdine-induced bursting. However, at doses that completely block \(I_{\text{NaP}}\), these drugs also attenuate the transient Na\(^+\) current (by nearly 20%; Yue et al. 2005). Therefore we also monitored their effects on the rate of rise of the spike (which reflects the maximal transient Na\(^+\) conductance; Hodgkin and Katz 1949) as well as on other spike parameters.

Representative experiments are shown in Fig. 5, A–C. In all three neurons, 10 \(\mu\)M linopirdine added to the ACSF converted their single spike response to a prominent burst of five to six spikes (Fig. 5, Aa and Ab, Ba and Bb, and Ca and Cb). Bath application of phenytoin (100 \(\mu\)M; Fig. 5Ac), riluzole (10 \(\mu\)M; Fig. 5Bc), and PDB (5 \(\mu\)M; Fig. 5Cc) progressively suppressed the bursts. In all cases the bursts were reduced to a single spike before any changes in rise time or amplitude of the first spike were detected (insets in Fig. 5, Ac, Bc, and Cc), indicating that these drugs suppress bursting without substantially reducing transient Na\(^+\) current. The same results were consistently

**Fig. 5.** Effects of bath-applied persistent Na\(^+\) current \((I_{\text{NaP}})\) blockers on intrinsic bursting induced by linopirdine and 4AP. A–C: in each part, \(a\) and \(b\) illustrate the firing of the neuron in response to brief stimuli before (Control) and about 30 min after adding 10 \(\mu\)M linopirdine to the artificial cerebrospinal fluid (ACSF), respectively. In all 3 experiments, linopirdine converted the single spike to a burst of 5–6 spikes. Adding 100 \(\mu\)M phenytoin (Ac), 10 \(\mu\)M riluzole (Bc), or 5 \(\mu\)M 4β-phorbol 12,13-dibutyrate (PDB) (Cc) to the linopirdine-containing ACSFs completely reversed the effects of linopirdine and restored regular firing in all cases. Insets, Ac, Bc, Cc: rise time of the first fast spike in linopirdine before (continuous trace) and at the time at which the \(I_{\text{NaP}}\) blockers suppressed the burst (dotted trace, slightly shifted horizontally to facilitate comparison). All 3 drugs suppressed bursting without grossly affecting the rise time of the first spike. D–F: effects of blocking persistent Na\(^+\) channels on intrinsic bursting induced by linopirdine or 4AP. In each part, \(a\) and \(b\) illustrate the firing of the neuron in response to brief stimuli before (Control) and about 30 min after adding 3 mM 4AP to the ACSF. In all 3 experiments, 4AP converted the single spike to a burst of 3–4 spikes. Adding phenytoin (Dc) or riluzole (Ec) to the 4AP-containing ACSFs had no effect on 4AP-induced bursts, whereas adding PDB prolonged the bursts (Fc).
obtained in 17 similar experiments in which phenytoin (n = 8), riluzole (n = 4), and PDB (n = 5) were bath applied after linopirdine.

One caveat in using phenytoin, riluzole, and PDB as $I_{\text{NaP}}$ blockers is that they were also shown to reduce some voltage-activated Ca$^{2+}$ currents (e.g., Doerner et al. 1988; Stefani et al. 1997; Yaari et al. 1987). If such an effect is exerted in adult CA1 pyramidal cells, then these drugs should also suppress 4AP-induced bursting, which is driven by T/R- and L-type Ca$^{2+}$ channels in apical dendrites of CA1 pyramidal cells (Magee and Carruth 1999). Therefore we examined how these drugs affect 4AP-induced bursting. Representative results are illustrated in Fig. 5, D–F. Using the same concentrations that readily blocked linopirdine-induced bursting, neither phenytoin (100 μM; Fig. 5D; n = 4), nor riluzole (10 μM; Fig. 5E; n = 5), nor PDB (5 μM; Fig. 5F; n = 5) blocked bursting induced by 3 mM 4AP. Whereas the first two drugs had no detectible effects on this activity, PDB caused prolongation of 4AP-induced bursts (Fig. 5F). The latter effect may stem from suppression by PDB of the Ca$^{2+}$-dependent slow AHP (sAHP) that underlies spike frequency adaptation (Malenka et al. 1986).

Another possible caveat in interpreting the action of riluzole is that it was shown to open big (BK) and small (SK) conductance Ca$^{2+}$-activated K$^+$ channels (e.g., Cao et al. 2002; Wu and Li 1999). Such an action, if exerted, might contribute to burst suppression by riluzole. However, riluzole (10 μM) also consistently suppressed linopirdine-induced bursting in conditions of blocked BK and SK channels (achieved by adding 100 nM iberiotoxin and 100 nM apamin to the ACSF; see Yue and Yaari 2004) (n = 3; data not shown).

Together, our findings support the notion that the growth of the spike ADP into a burst response after blockade of Kv7/M channels critically depends on recruitment of $I_{\text{NaP}}$.

Effects of focally applied $I_{\text{NaP}}$ blockers

We further examined the distribution of persistent Na$^+$ channels that mediate bursting when Kv7/M channels are blocked. To that end, we monitored the effects of focally applied riluzole and PDB on this activity. The application pipette contained 100 μM riluzole or 50 μM PDB (phenytoin was not used in these experiments because the tenfold concentration required for local application was beyond its solubility in ACSF). Representative results are shown in Fig. 6. Dendritic applications (three puffs) of riluzole (Fig. 6A) or PDB (Fig. 6B) had no effect on linopirdine-induced bursting (Fig. 6, Aa and Ab and Ba and Bb). Thus the number of intraburst spikes both before and after distal drug applications were 4.3 ± 0.5 (n = 4) for riluzole and 4.7 ± 0.7 (n = 6) for PDB. In contrast, single axo-somatic applications of these drugs consistently diminished the bursts to a single spike (Fig. 6, Ac and Bc; $P < 0.05$), without modifying the rise time of the first spike (Fig. 6, Ac and Bc, insets).

Finally, we tested the effects of focally applied tetrodotoxin (TTX; 50 nM in the application pipette). We previously showed that application of TTX to the apical dendrite blocks natural bursting in developing CA1 pyramidal cells, presumably by blocking spike backpropagation that is essential for evoking a dendritic Ca$^{2+}$ spike, which, in turn, depolarizes the soma and triggers a burst of Na$^+$ spikes (see Fig. 8 in Chen et al. 2005). Applying TTX to the apical dendrite (three puffs) had no effect on
bursting induced by linopirdine (Fig. 6, Ca and Cb). The number of intraburst spikes both before and after distal TTX applications was $4.2 \pm 0.5$ ($n = 4$). Thus spike backpropagation is not essential for linopirdine-induced bursting. When TTX was similarly applied to the axo-soma, it completely blocked the burst (Fig. 6Cc; $P < 0.05$) before noticeably affecting the upstroke of the first spike (Fig. 6Cc, inset). Several minutes later the first spike was also depressed by TTX. Figure 6D summarizes the effects of distally versus proximally applied Na$^{+}$ channel blockers on linopirdine-induced bursting.

For comparison, we tested the effects of locally applied TTX on 4AP-induced bursting ($n = 3$). Expectedly (Magee and Carruth 1999), TTX applied to the apical dendrites (one puff) blocked bursting without affecting the first spike in all of these experiments (Fig. 6, Ea and Eb).

Effects of blocking Ca$^{2+}$ currents

The above findings clearly show that recruitment of proximal persistent Na$^{+}$ channels plays a critical role in ADP growth and bursting when Kv7/M channels are inoperative. However, they do not exclude the participation of voltage-activated Ca$^{2+}$ channels in this process. To assess the contribution of the latter channels, we examined the effects of Ni$^{2+}$ on linopirdine-induced bursting. As illustrated in Fig. 7A, adding 1 mM Ni$^{2+}$ to the ACSF (by equimolar substitution with Mg$^{2+}$) caused progressive augmentation of the burst by reducing the repolarization capacity of the neuron (Fig. 7, Aa–Ac). Ultimately each burst transformed to a plateau depolarization lasting more than 100 ms (Fig. 7Ac, inset). Similar results were obtained in 11 experiments. The Ni$^{2+}$-induced plateau potentials, as well as the initial bursts, were consistently reduced to a solitary spike by bath-applied phenytoin (100 µM; $n = 3$), riluzole (10 µM; $n = 3$), and PDB (5 µM; $n = 5$). An example for the latter action is shown for phenytoin in Fig. 7Ad. Applying 1 mM Ni$^{2+}$ to neurons superfused with normal ACSF caused reduction in fAHP and spike broadening (Fig. 7, Bb and Bb) but did not induce bursting, as previously described (Su et al. 2001). However, subsequent addition of 10 µM linopirdine to the Ni$^{2+}$-containing ACSF induced bursting followed by the development of plateau potentials (Fig. 7, Bc and Bb; $n = 3$). Again, the burst–plateau potential complexes were suppressed by phenytoin (Fig. 7Bc).

Finally, we examined the effects of Ni$^{2+}$ focally applied from an application pipette containing 5 mM Ni$^{2+}$. As illustrated in Fig. 7C, application of Ni$^{2+}$ to the apical dendrites (three puffs) had no notable effect on linopirdine-induced bursting (Fig. 7, Cu and Cb; $n = 3$). When applied to the axo-soma, however, one puff of Ni$^{2+}$ augmented the bursts and prevented their repolarization, thereby inducing prolonged plateau potentials lasting hundreds of milliseconds (Fig. 7, Ce and Cd; $n = 3$).

In contrast to the marked facilitatory effects of Ni$^{2+}$ on bursting induced by linopirdine, bursting induced by millimolar 4AP was completely suppressed by 1 mM Ni$^{2+}$ applied in the bath (Fig. 7, Du and Db; $n = 5$) or by puffing Ni$^{2+}$ only. 

![Figure 7](http://jn.physiology.org/)
Effects of focally applied Kv7/M channel modulators on dendritic Ca\(^{2+}\) spikes

The data gathered thus far indicated that axo-somatic Kv7/M channels critically regulate the spike output of CA1 pyramidal cells, but did not disclose any distinct function of apical dendritic Kv7/M channels. It is possible, however, that the latter channels play an important role in dendritic electrical events that arise only when the dendrites themselves are strongly depolarized, such as Ca\(^{2+}\) spikes (Benardo et al. 1982; Wong et al. 1979). These slow spikes do not propagate actively into the axo-soma, but may cause sufficient depolarization of that region to initiate a spike burst (Golding et al. 1999). Because the results of our experiments with retigabine suggested that apical dendrites may express functional Kv7/M channels (Fig. 2, D and E), we investigated whether these channels modulate dendritic Ca\(^{2+}\) electrogensis.

Figure 8 illustrates the method we used to evoke slow Ca\(^{2+}\) spikes without intervening fast Na\(^{+}\) spikes in the apical dendrites of CA1 pyramidal cells. Perfusing the slices with ACSF containing 0.5 \(\mu\)M TTX and 3 mM 4AP blocked Na\(^{+}\) electrogensis. In this condition strong, 180-ms-long, positive current pulses applied at the soma evoked regenerative slow spikes (Fig. 8, Aa and Ab), which were consistently and entirely blocked by adding 1 mM Ni\(^{2+}\) to the ACSF (Fig. 8Ac; \(n = 6\)). To validate the notion that these Ca\(^{2+}\) spikes recorded in the soma arise in the apical dendrites, we examined the effects of Ni\(^{2+}\) locally applied from a pipette containing 5 mM Ni\(^{2+}\). Indeed, when applied to the axo-soma, Ni\(^{2+}\) did not affect the Ca\(^{2+}\) spikes (Fig. 8, Ba and Bb), but blocked them entirely when applied (one puff) to the apical dendrites (Fig. 8Bc; \(n = 5\)). The dendritic Ca\(^{2+}\) spikes were resistant to 10 \(\mu\)M riluzole (Fig. 8, Ca and Cb). Thus Ca\(^{2+}\) spike amplitudes in control versus 30 min in riluzole were 66.1 \(\pm\) 12.4 versus 67.8 \(\pm\) 13.0 (\(n = 4\)) mV. These results further support our conclusion that riluzole suppresses linopirdine-induced bursting by blocking persistent Na\(^{+}\) channels. A previous study has shown that PDB also does not block dendritic Ca\(^{2+}\) spikes in CA1 pyramidal cells (Malenka et al. 1986).

We first tested the effects of bath applied Kv7/M channel blockers on dendritic Ca\(^{2+}\) electrogensis. Representative experiments are illustrated in Fig. 9, A–C. In all these experiments the current intensity required for evoking a maximal Ca\(^{2+}\) spike was first determined in control conditions (i.e., in ACSF containing TTX and 4AP) and then again during exposure to the drug. In the experiment described in Fig. 9A, a stimulus intensity of 600 pA was required to evoke a Ca\(^{2+}\) spike in control conditions (Fig. 9Aa), but only 300 pA was necessary for evoking a Ca\(^{2+}\) spike during exposure to 10 \(\mu\)M linopirdine (Fig. 9Ab). Moreover, stimuli of larger intensity evoked maximally a single Ca\(^{2+}\) spike in control conditions, but two Ca\(^{2+}\) spikes in linopirdine-containing ACSF (Fig. 9, Aa and Ab, topmost traces). Although threshold current values for evoking Ca\(^{2+}\) varied with the \(R_S\) of the neurons, similar effects of linopirdine were obtained in all experiments (\(n = 8\)). Thus the mean threshold current required for evoking a Ca\(^{2+}\) spike decreased from 566.7 \(\pm\) 49.4 to 383.3 \(\pm\) 30.7 pA (\(P < 0.05\)) by adding 10 \(\mu\)M linopirdine to the ACSF. Likewise, adding 3 \(\mu\)M XE991 to the ACSF reduced the stimulus intensity required for evoking Ca\(^{2+}\) spikes from 500.0 \(\pm\) 57.7 to 333.3 \(\pm\) 33.3 pA (\(n = 3\); \(P < 0.05\)) and facilitated the generation of multiple Ca\(^{2+}\) spikes at high stimulus intensities (Fig. 9B). Conversely, adding 10 \(\mu\)M retigabine to the ACSF consistently suppressed the generation of all-or-none Ca\(^{2+}\) spikes even at the highest stimulus intensities (Fig. 9, Ca and Cb; \(n = 5\)).

The simplest explanation for these effects of linopirdine and XE991 is that the drug-induced increase in \(R_S\) facilitates the depolarization of apical dendrites by currents applied at the soma. The converse action of retigabine is similarly accounted for by the drug-induced reduction in \(R_S\). To examine whether dendritic Kv7/M channels themselves regulate the transduction of depolarization to Ca\(^{2+}\) spike generation, we applied the three drugs focally to the apical dendrites. Indeed, as illustrated in Fig. 9D, dendritic applications (one puff) of linopirdine...

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Once onto the apical dendrites (Fig. 7, Ea and Eb; \(n = 3\)), as shown previously (Magee and Carruth 1999).

From the experiments with Ni\(^{2+}\) we conclude that recruitment of voltage-activated Ca\(^{2+}\) channels during linopirdine-induced bursts is mandatory for burst termination, but not for burst initiation and growth. The combined block of Kv7/M and Ca\(^{2+}\)-activated K\(^{+}\) channels deprives the neuron from major repolarizing currents, leading to the development of plateau potentials driven predominantly by I\(_{\text{Na-p}}\).
Retigabine applied to the apical dendrites consistently suppressed Ca²⁺ spikes to the apical dendrites. Neurons were stimulated with 180-ms positive current pulses and current intensity was varied (in 100-pA steps) to evoke subthreshold and spike responses. A: in the 1st neuron stimulus intensity of 600 pA was required to evoke a Ca²⁺ spike in control conditions (a). Adding 10 µM linopirdine to the ACSF reduced threshold current intensity to 500 pA and facilitated the generation of multiple Ca²⁺ spikes (b). B: in the 2nd neuron, adding 3 µM XE991 to the ACSF reduced threshold current intensity from 600 to 300 pA and facilitated the generation of multiple Ca²⁺ spikes (a, b). C: in the 3rd neuron, a 500-pA stimulus was required to evoke a Ca²⁺ spike in control conditions (a). Adding 10 µM retigabine to the ACSF suppressed the generation of Ca²⁺ spikes even at the highest stimulus intensities (b). D: in the 4th neuron, applying linopirdine to the apical dendrites reduced threshold current intensity from 500 to 400 pA (a, b). E: in the 5th neuron, applying XE991 to the apical dendrites reduced threshold current intensity from 700 to 400 pA and facilitated the generation of multiple Ca²⁺ spikes (a, b). F: in the last neuron, stimuli of 600 pA or more evoked Ca²⁺ spikes in this neuron (a). Retigabine applied to the apical dendrites suppressed the generation of Ca²⁺ spikes at all stimulus intensities (b).

Finally, we examined the effects of the Kv7/M channel modulators on spike output in slices perfused with ACSF containing 3 mM 4AP. In this condition the spike output/stimulus intensity relationship depends on both somatic and dendritic electrical events (Magee and Carruth 1999). In control conditions (4AP-containing ACSF), stimulating the neurons with 180-ms-long positive current pulses usually evoked a single burst as the minimal neuronal responses to threshold-straddling stimuli (Fig. 10, Aa, Ba, and Ca); increasing stimulus intensity evoked additional solitary spikes (Fig. 10, Aa and Ca) or an extra burst (Fig. 10Ba). Linopirdine focally applied to the apical dendrites (one to three puffs) substantially augmented the number of spikes evoked at all stimulus intensities (n = 5; P < 0.05; Fig. 10Ab). A similar action was exerted by distally applied XE991 (Fig. 10, Ba and Bb; n = 3). Conversely, retigabine puffed onto the apical dendrites (one puff) unfailingly suppressed spike output at all stimulus intensities (Fig. 10C; n = 4). Figure 8, D–F depicts the averaged spike output/stimulus intensity plots obtained in these experiments. The marked effects of distally applied linopirdine, XE991, and retigabine on spike output in 4AP-containing ACSF contrast sharply with the lack of any effect when similarly applied in normal ACSF (Fig. 3, D–F).

In conclusion, our results indicate the presence of functional Kv7/M channels in the apical dendrites. These channels, together with their axo-somatic counterparts, regulate the spike output of CA1 pyramidal cells in conditions of enhanced dendritic excitability.

**DISCUSSION**

The main finding of this study is that Kv7/M channels having distinct functions are present in axo-soma and in apical
dendrites of CA1 pyramidal neurons. These channels critically control the intrinsic excitability of these neurons. Kv7/M channels in the axo-somatic region counteract the depolarizing drive provided by persistent Na⁺ channels, thereby preventing the spike ADP from escalating into a burst. The impact of dendritic Kv7/M channels on intrinsic excitability may be overshadowed by other inhibitory currents in the dendrites (e.g., I_A), but once these currents are reduced and dendritic excitability rises, Kv7/M channels are recruited to lessen the hyperexcitable state that otherwise would cause sustained burst discharge at the axo-soma. The concerted activity of Kv7/M channels in both compartments serves to reduce the propensity to generate self-sustained burst responses and fosters a regular, stimulus-graded spike output of the neuron.

**Kv7/M channels in axo-soma**

Using local application of Kv7/M channel modulators, we show here that Kv7/M channels in the axo-soma of CA1 pyramidal cells tightly control the size of the somatic spike ADP. Blocking these channels unleashes a regenerative depolarization that causes growth of the spike ADP until it attains

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**FIG. 10. Effects of Kv7/M channel modulators applied to hyperexcitable apical dendrites on spike output/stimulus intensity relationship.** In the 3 experiments shown in A–C, the ACSFs contained 3 mM 4AP, which induced somatic bursting originating in the apical dendrites. A: in the 1st neuron, applying linopirdine to the apical dendrites reduced threshold current intensity from 400 to 300 pA and enhanced the spike output at all suprathreshold stimuli (a, b). B: in the 2nd neuron, applying XE991 to the apical dendrites reduced threshold current intensity from 400 to 300 pA and substantially facilitated the spike output at all suprathreshold stimuli (a, b). C: in the last neuron, stimuli of ≥400 pA evoked multiple spike responses in this neuron (a). Applying retigabine to the apical dendrites suppressed spike output at all stimulus intensities (b). D: summary plots depicting the spike output/stimulus intensity relationships in control conditions (squares) and after linopirdine application to apical dendrites (white circles). Each plot is an average of 5 experiments. E: same as in D, but for 3 experiments with XE991. F: same as in D, but for 4 experiments with retigabine. In D–F, error bars represent SE; *P < 0.05.
spike threshold and triggers an additional spike. This process repeats itself several times, thereby producing a spike burst. Blocking $I_{\text{NaP}}$ in the axo-somatic region of the neuron reverses this process, suggesting that the latter current furnishes the main depolarizing drive for the regenerative growth of the ADP. In contrast, blocking Ca$^{2+}$ currents does not affect this process, indicating that recruitment of Ca$^{2+}$ channels is not obligatory for burst electrogenesis. On the contrary, blocking Ca$^{2+}$ currents causes protraction of the burst into a plateau depolarization lasting hundreds of milliseconds, indicating that Ca$^{2+}$-activated K$^+$ currents are essential for burst termination in condition of blocked axo-somatic Kv7/M channels.

A large body of evidence now suggests that the spike ADP in adult CA1 pyramidal cells is generated by recruitment of axo-somatic persistent Na$^+$ channels (Azouz et al. 1996; Su et al. 2001; Yue et al. 2005). These channels activate quickly at subthreshold potentials and do not inactivate substantially over a period of hundreds of milliseconds (French et al. 1990). Because fast repolarization of the spike is incomplete, ending at a potential nearly 10 mV more depolarized than resting potential (i.e., at about $-55$ mV; Su et al. 2001), these channels are likely to generate a substantial inward spike aftercurrent that would tend to repolarize the neuron again to spike threshold (Yue et al. 2005). Here we show that this action of $I_{\text{NaP}}$ is counteracted by activation of nearby Kv7/M channels that generate a repolarizing current, $I_{\text{K}}$. Other slow K$^+$ channels that may generate an outward spike aftercurrent, such as SK Ca$^{2+}$-activated K$^+$ channels and D-type K$^+$ channels, do not exert control over the spike ADP, as blocking them selectively does not substantially modify the spike ADP, provided Kv7/M channels are active (Yue and Yaari 2004). In contrast, even a 10% reduction in $I_{\text{M}}$ causes a significant increase of the spike ADP. Thus the size of the somatic spike ADP and its propensity to trigger a burst are determined largely by a balance between $I_{\text{NaP}}$ and $I_{\text{M}}$ in the proximal portions of the neuron. An imbalance between these two currents in favor of $I_{\text{NaP}}$ facilitates the spike ADP and converts the output of the neuron from a solitary spike to a high-frequency spike burst.

Although our technique for local application of Kv7/M channel modulators could not discern between Kv7/M channels in axon initial segment, soma, or proximal dendrites, recent immunohistochemical evidence suggests that these channels may aggregate in the axon initial segment, as well as in the nodes of Ranvier (Devaux et al. 2004). Interestingly, the axon initial segment and nodal membranes in many neurons also express a high density of Na$_{\text{v}}$1.6 channels (Boiko et al. 2003). Of all the $\alpha$-subunit isoforms of voltage-activated Na$^+$ channels expressed in brain neurons (Na$_{\text{v}}$1.1, Na$_{\text{v}}$1.2, Na$_{\text{v}}$1.3, and Na$_{\text{v}}$1.6; Goldin 1999), Na$_{\text{v}}$1.6 is the main subunit underly- ing $I_{\text{NaP}}$ (Burbidge et al. 2002; Smith et al. 1998). Therefore it is conceivable that the two principal species of ion channels that shape the waveform of the somatic spike ADP; that is, Na$_{\text{v}}$1.6 and Kv7/M channels are coexpressed at high densities in the axon initial segment. Thus the axon initial segment may be a strategic site not only for spike initiation (Spruston et al. 1995; Turner et al. 1991), but also for generating the active spike ADP and associated bursting.

**Kv7/M channels in apical dendrites**

Although it is well established that dendrites of CNS neurons express multiple types of K$^+$ channels that contribute to the complexity and heterogeneity of neuronal discharge behavior (Chen and Johnston 2004; Golding et al. 1999; Johnston et al. 2000), a functional role for Kv7/M channels in these neuronal processes has not been demonstrated before. The evidence that these channels are expressed in apical dendrites of CA1 pyramidal cells is twofold. First immunohistochemical studies have demonstrated moderate staining for Kv7.2, Kv7.3, and Kv7.5 in apical dendrites of CA1 pyramidal cells (Roche et al. 2002; Shah et al. 2002). Second, recent single-channel recordings from these structures (170–250 µm away from the soma) encountered, albeit infrequently, putative M-type K$^+$ channels (Chen and Johnston 2004). Our findings that in some conditions (e.g., 4AP-containing ACSF) apical dendrites are very responsive to directly applied modulators of Kv7/M channels are congruent with these findings and further show that, despite their scarcity, dendritic Kv7/M channels may be of functional significance.

Distal applications of linopirdine or XE991 to block Kv7/M channels in the apical dendrites did not affect “passive” membrane properties of CA1 pyramidal cells recorded in the soma, nor did they modify the somatic spike ADP or the spike output/stimulus intensity relationship in slices perfused with normal ACSF. These findings suggest that in normal conditions dendritic Kv7/M channels do not tightly regulate the overall intrinsic excitability of these neurons. This may be attributable to a low Kv7/M channel density in the apical dendrites (Chen and Johnston 2004); to the restriction of these channels to dendritic branches of higher order, where they would be electrotonically isolated from the soma; and/or to the overwhelming inhibitory effects of other currents, such as $I_{\text{L}}$ or $I_{\text{h}}$, whose densities in the apical dendrites are severalfold higher than in the soma (Hoffman et al. 1997; Magee 1998). Indeed, blocking $I_{\text{L}}$ in the apical dendrites with millimolar 4AP augments the somatic spike ADP and induces somatic bursting, whereas blocking $I_{\text{h}}$ in the axo-soma does not produce these effects (Magee and Carruth 1999). Interestingly, we found that once $I_{\text{L}}$ was blocked, dendritic hyperexcitability came to be tightly controlled by Kv7/M channels. Perhaps in this condition of enhanced spike invasion into the apical dendrites and Ca$^{2+}$ spike electrogenesis, distal dendritic Kv7/M channels are more effectively recruited and therefore exercise enhanced inhibitory control over dendritic excitability.

**Functional implications**

Hippocampal pyramidal cells in vivo were shown to alternate between regular firing (“simple” spikes) and burst firing (“complex” spikes), depending on the behavioral state of the animal (Otto et al. 1991; Ranck 1973). It is not yet known how intrinsic factors contribute to “complex” spike bursting in vivo. However, with respect to the number and frequency domain of intraburst spikes, “complex” spikes in CA1 pyramidal cells (Harris et al. 2001) are remarkably similar to intrinsic bursts recorded in vitro after blocking axo-somatic Kv7/M channels. It is thus possible that transitions from “simple” to “complex” spiking and vice versa involve modulation of Kv7/M channels in the axo-soma. Multiple hippocampal neurotransmitters were
shown to down- or upmodulate Kv7/M channels (Brown and Yu 2000; Marrion 1997) and the release of these modulators onto CA1 pyramidal cells may mediate transitions between "simple" and "complex" spiking in vivo. Such a transition may occur during behavioral states of learning, at which "complex" spiking predominates (Otto et al. 1991; Ranck 1973). The association of "complex" spiking with learning may have a causative component because several studies have shown that postsynaptic bursting greatly enhances long-term synaptic plasticity (Pike et al. 1999; Thomas et al. 1998). Furthermore, Kv7 channel blockers that induce axo-somatic bursting were shown to improve performance of cognitively impaired rodents in several tests of learning and memory (Fontana et al. 1994). Thus neurotransmitter modulation of axo-somatic Kv7/M channels in vivo may regulate synaptic plasticity, and thus learning and memory.

Neurromodulation of Kv7/M channels in apical dendrites is not expected to greatly affect neuronal excitability in ordinary conditions, but may do so in conditions of enhanced dendritic excitability. During both postnatal ontogenesis (Chen et al. 2005; Costa et al. 1991) and adult epileptogenesis (Sanabria et al. 2005; Costa et al. 1991) and adult epileptogenesis (Sanabria et al. 2002) CA1 pyramidal cells manifest an enhanced propensity to burst. In both conditions bursts are mediated by dendritic Ca\(^{2+}\) spikes that are triggered by back-propagating spikes, similar to the burst mechanism invoked by 4AP (Magee and Carruth 1999). It would be interesting to explore whether in these normal developmental and pathological hyperexcitable conditions dendritic Kv7/M channels play a critical role in the control of neuronal spike output.

**References**


