J Neurophysiol 100: 2589–2604, 2008.
First published August 6, 2008; doi:10.1152/jn.90433.2008.

SK ($K_{Ca}2$) Channels Do Not Control Somatic Excitability in CA1 Pyramidal Neurons But Can Be Activated by Dendritic Excitatory Synapses and Regulate Their Impact

Ning Gu, Hua Hu, Koen Vervaeke, and Johan F. Storm
Institute of Basal Medicine, Department of Physiology, and Centre of Molecular Biology and Neuroscience, University of Oslo, Norway

Submitted 2 February 2008; accepted in final form 31 July 2008

Gu N, Hu H, Vervaeke K, Storm JF. SK ($K_{Ca}2$) channels do not control somatic excitability in CA1 pyramidal neurons but can be activated by dendritic excitatory synapses and regulate their impact. *J Neurophysiol* 100: 2589–2604, 2008. First published August 6, 2008; doi:10.1152/jn.90433.2008. Calcium-activated K^+ channels of the $K_{Ca}2$ type (SK channels) are prominently expressed in the mammalian brain, including hippocampus. These channels are thought to underlie neuronal excitability control and have been implicated in plasticity, memory, and neural disease. Contrary to previous reports, we found that somatic spike-evoked medium afterhyperpolarizations (mAHPs) and corresponding excitability control were not caused by SK channels but mainly by Kv7/KCNQ/M channels in CA1 hippocampal pyramidal neurons. Thus apparently, these SK channels are hardly activated by somatic Na^+ spikes. To further test this conclusion, we used sharp electrode, whole cell, and perforated-patch recordings from rat CA1 pyramidal neurons. We found that SK channel blockers consistently failed to suppress mAHPs under a range of experimental conditions: mAHPs following single spikes or spike trains, at −60 or −80 mV, at 20–30°C, in low or elevated extracellular [K^+], or spike trains triggered by synaptic stimulation after blocking *N*-methyl-D-aspartic acid receptors (NMDARs). Nevertheless, we found that SK channels in these cells were readily activated by artificially enhanced Ca^{2+} spikes, and an SK channel opener (1-ethyl-2-benzimidazolinone) enhanced somatic AHPs following Na^+ spikes, thus reducing excitability. In contrast to CA1 pyramidal cells, bursting pyramidal cells in the subiculum showed a Na^+ spike-evoked mAHP that was reduced by apamin, indicating cell-type-dependent differences in mAHP mechanisms. Testing for other SK channel functions in CA1, we found that field excitatory postsynaptic potentials mediated by NMDARs were enhanced by apamin, supporting the idea that dendritic SK channels are activated by NMDAR-dependent calcium influx. We conclude that SK channels in rat CA1 pyramidal cells can be activated by NMDAR-mediated synaptic input and cause feedback regulation of synaptic efficacy but are normally not appreciably activated by somatic Na^+ spikes in this cell type.

INTRODUCTION

SK channels are calcium-activated potassium (K^+) channels of small conductance (10–20 pS) that are widely expressed in vertebrate neurons and other tissues (Kohler et al. 1996). These channels are composed of proteins of the $K_{Ca}2$ family, the three members ($K_{Ca}2.1$–2.3, also called SK1-3) of which are all expressed in the mammalian brain, including the hippocampus (Kohler et al. 1996; Sailer et al. 2002; Stocker and Pedarzani 2000). The selective SK channel blocker, apamin, a bee venom peptide toxin, has been reported to improve learning and memory retention of mice, facilitate the induction of hippocampal long-term synaptic potentiation (LTP), and increase immediate early gene expression (Behnisch and Reymann 1998; Heurteaux et al. 1993; Kramar et al. 2004; Messier et al. 1991; Norris et al. 1998; Stackman et al. 2002). Dysfunction of SK channels may be linked to psychiatric disease, including schizophrenia and bipolar disorder (Chandy et al. 1998), and these channels may also be targets of antipsychotic drugs (Terstappen et al. 2001). It is therefore of considerable interest to determine the physiological roles at the cellular level of neuronal SK channels in the brain.

The main functions usually ascribed to SK channels in neurons, are generation of afterhyperpolarizations (AHPs) and feed-back regulation of neuronal excitability, including spike-frequency adaptation (Bond et al. 1999; Hille 2001; Sah 1996; Sah and Faber 2002; Vergara et al. 1998). Rodent hippocampal pyramidal cells have been widely used as prototype neurons for studying mechanisms and functions of AHPs in general and SK channels in particular (for review, see e.g., Bond et al. 2005; Faber and Sah 2003, 2007; Storm 1990). In these cells, action potentials are followed by a sequence of three AHPs: fast (fAHP), medium (mAHP), and slow (sAHP) (Storm 1987a, 1989). A similar pattern is found in many other mammalian central neurons (Pape and Driesang 1998; Pineda et al. 1992; Schwindt et al. 1988; Takahashi 1990; Viana et al. 1993).

Until a few years ago, the hippocampal sAHP, which is modulated by a wide variety of transmitter and second-messenger systems, was generally believed to be caused by SK channels (Hille 2001; Kohler et al. 1996; Vergara et al. 1998). In particular, channels formed by SK1 subunits, which initially were reported to be rather insensitive to apamin (Kohler et al. 1996), were considered likely generators of the apamin-resistant sAHP (Hille 2001; Vergara et al. 1998). However, recent findings indicate that SK channels do not underlie the sAHP after all (for review, see Vogalis et al. 2003): SK1 channels were found to be far more sensitive to apamin than the sAHP (Shah and Haylett 2000; Strobaek et al. 2000), overexpression of SK1 or SK2 in neocortical pyramidal cells did not enhance I_{sAHP} and expression of a dominant negative SK3 subunit, capable of suppressing SK1, SK2, and SK3 channels, did not reduce I_{sAHP} (Villalobos et al. 2004), and CA1 pyramidal cells of transgenic mice lacking functional SK1, SK2 and SK3 genes, still showed robust sAHPs (Bond et al. 2004).

Address for reprint requests and other correspondence: J. F. Storm, Dept. of Physiology at IMB and Centre for Molecular Biology and Neuroscience (CMBN), University of Oslo., PB 1103 Blindern, N-0317 Oslo, Norway (E-mail: jstorm@medisin.uio.no).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

TABLE 1. *Different recording conditions used for testing the effects of SK channel blockers on the mAHP in CA1 pyramidal cells*

Age (wk)			Temperature, °C			Recording Method/IC Solution					$[Ca^{2+}]_o$, mM	
						Sharp Electrode		Whole Cell		Perforated Patch		
2–4	4–8	8–24	23.5	26.0	30.0	K-Ac	K-MeS.	K-Gluco.	K-MeS.	gramici. K-MeS.	2	2.5–3
A÷(40)	A÷(31) T÷(6)	A÷(13)	A÷(8) T÷(6)	A÷(12)	A÷(64)	A÷(66) T÷(6)	A÷(4)	A÷(2)	A÷(7)	A÷(5)	A÷(79)	A÷(5) T÷(6)

n values are in parentheses. A÷, application of apamin 100nM had no detectable effect; T÷, application of d-tubocurarine 100μM had no detectable effect; K-Ac, potassium acetate; K-Gluco, potassium gluconate; K-MeS, potassium methylsulfate; gramici., gramicidine; mAHP, medium afterhyperpolarization.

In view of the highly probable conclusion that the SK channels do not generate the sAHP and that these channels nevertheless can generate a considerable K^+ current in hippocampal pyramidal cells (Bond et al. 2004; Sah and Faber 2002; Sailer et al. 2002; Stackman et al. 2002; Stocker et al. 1999; Vogalis et al. 2003), it is important to determine the functions of this channel type.

Almost 20 years ago, the SK channel blocker apamin was first used to test the hypothesis that SK channels generate somatic AHPs and excitability control in hippocampal pyramidal cells, but the results were consistently negative (Storm 1989; Williamson and Alger 1990). Instead these studies indicated that the mAHP in these cells was generated by M channels when the cell is depolarized and by h channels when it is hyperpolarized (Storm 1989). This conclusion was supported by a recent study of transgenic mice, where the mAHP of CA1 pyramidal cells was strongly reduced when M/KCNQ channels were suppressed by expression of dominant negative KCNQ2 subunits (Peters et al. 2005). Furthermore Gu et al. (2005) confirmed and extended the conclusions from (Storm 1989) that the somatic mAHP in rat CA1 pyramidal cells is not caused by SK channels but is mainly generated by Kv7/KCNQ/M channels at depolarized levels and by HCN/h channels at hyperpolarized levels. Also Yue and Yaari (2004), studying the roles of potassium currents in control of excitability, afterdepolarizations, and bursting in rat CA1 pyramidal cells, found clear effects of Kv7/KCNQ/M channel blockers but no effect of SK channel blockers.

In spite of these convergent lines of evidence, there is still a persistent and widespread view that SK channels contribute substantially to the mAHP in rat CA1 hippocampal pyramidal neurons (Bildl et al. 2004; Bond et al. 1999, 2004; Bowden et al. 2001; Faber and Sah 2003; Melyan et al. 2002; Sah and Faber 2002; Sailer et al. 2002; Stocker 2004; Stocker et al. 1999, 2004). Thus until very recently, several studies have claimed that an apamin-sensitive current contribute to the mAHP and excitability control in these neurons (Bond et al. 2005; Faber and Sah 2007; Kaczorowski et al. 2007; Shah et al. 2006). Resolving this issue seems essential for understanding the functions of this prominent channel family in an important and widely studied class of neurons. Therefore we have now re-examined the functions of SK channels in hippocampal pyramidal cells under a variety of experimental conditions. Consistently we found no detectable contribution to the mAHP or excitability control from SK channels under any of the conditions tested.

Next we asked the question if the SK channels in CA1 pyramidal cells are not generating the sAHP or the mAHP or excitability control or spike frequency adaptation, as accumulating evidence now indicates (Gu et al. 2005; Shah and Haylett 2000; Storm 1989; Strobaek et al. 2000; Villalobos et al. 2004; Vogalis et al. 2003), what else is their function in these cells?

Interestingly, recent studies of pyramidal cells from both hippocampus and amygdala indicate that dendritic SK channels are activated by stimulation of glutamate receptors in the dendrites by glutamatergic synaptic input or artificially applied glutamate; these SK channels are activated presumably via calcium influx through NMDA receptors and/or voltage-gated calcium channels; and the SK current can limit calcium influx by repolarizing the cell and reinstate the Mg^{2+} block of the *N*-methyl-D-aspartate receptor (NMDAR) channels, thus forming a negative feedback loop (Bloodgood and Sabatini 2007; Stackman et al. 2002). These and other data indicate that SK channels may regulate and even contribute to hippocampal synaptic plasticity (Faber et al. 2005; Lin et al. 2008; Ngo-Anh et al. 2005; Stackman et al. 2002; Tzounopoulos and Stackman 2003). Our data support such postsynaptic functions of dendritic SK channels.

We conclude that postsynaptic SK channels in rat CA1 pyramidal cells can be activated by dendritic calcium influx through NMDA receptors and/or voltage-gated Ca^{2+} channels but cannot be sufficiently activated by normal somatic sodium action potentials to affect somatic responses in this cell type under a variety of conditions. Our data seem to indicate that in this cell type somatic depolarization can substantially activate SK channels only under artificial or extreme conditions, such as following Ca^{2+} spikes facilitated by potassium channel blockers or in the presence of an SK channel opener drug.

METHODS

Slice electrophysiology

Transverse hippocampal slices (400 μm thick) were prepared from male Wistar rats (2.5–8 wk of age). Also older Wistar rats ($\leq$6 mo) were used in some experiments (Table 1). The experimental procedures were approved by the responsible veterinarian of the Institute, in accordance with the statute regulating animal experimentation (Norwegian Ministry of Agriculture 1996). Briefly, the rats were deeply anesthetized with Suprane before decapitation. Hippocampal slices (400 μm) were cut with a vibratome (Campden Instruments, UK) and maintained in an interface chamber filled with artificial cerebral spinal fluid (ACSF) containing (mM) 125 NaCl, 25 $NaHCO_3$, 1.25 KCl, 1.25 KH_2PO_4, 1.5 $MgCl_2$, 1.0 $CaCl_2$, and 16 glucose and saturated with 95% O_2-5% CO_2. During recording, the slices were kept submerged and perfused with ACSF of the composition described in the preceding text, except that [$CaCl_2$] was 2.0 mM. To block Ca^{2+} influx, the ACSF was sometimes replaced by Ca^{2+}-free medium containing (in mM) 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.5 $MgCl_2$, 2.0 $MnCl_2$, and 16

TABLE 1. *Continued*

$[K^+]_o$, mM		pH_0		Holding Potential, mV			Train Duration, ms				Spike Number			
2.5	3.5	7.3–7.4	7.7	−55 to −60	−70	−80	50	100–200	400	2000	1	5	10	>10
A÷(79) T÷(6)	A÷(5)	A÷(77) T÷(6)	A÷(7)	A÷(79) T÷(6)	A÷(6)	A÷(5)	A÷(26)	A÷(46)	A÷(15) T÷(6)	A÷(8)	A÷(8)	A÷(47)	A÷(7) T÷(6)	A÷(15)

glucose. In some experiments, 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) was added to the ACSF to block spontaneous excitatory synaptic transmission. The ACSF was saturated with 95% O_2-5% CO_2, and the temperature was kept constant (within ±0.5°C), normally at 30°C.

Either an Axoclamp 2A (Molecular Devices, Union City, CA) or a Dagan BVC 700A (Dagan Corporation, Minniapolis) was used for all recordings. Intracellular recordings were obtained with sharp electrodes filled with 2 M potassium acetate or 2 M $KMeSO_4$ (resistance: 80–140 MΩ, pH 7.3–7.4). Whole cell recordings were obtained under IR-DIC microscopy or with the "blind" method. The patch pipettes were filled with a solution containing (in mM) 140 $KMeSO_4$ or K gluconate, 10 HEPES, 10 phosphocreatine Na salt, 2 ATP Na salt, 0.4 GTP Na salt, and 2 $MgCl_2$ (pipette resistance, 4–7 MΩ; series-resistance, R_{series}, 10–40 MΩ). All potentials were corrected for the junction potential. For perforated-patch recording, the pipette solution contained (in mM) 140 KMeSO4, 10 HEPES, 2 ATP, 0.4 GTP, 2 MgCl2, 10 phosphocreatine, and 10 tetraethylammonium chloride (TEA) and 50 μg/ml gramicidin. After seal formation (>1 GΩ seal resistance), we saw a gradual reduction in R_{series}, to <100 MΩ. The recording was discarded if we observed a precipitous drop in R_{series}, considered to indicate a break-in to whole cell configuration. By using TEA in the pipette and monitoring spike half-width, we obtained an additional assessment of subtle perforated patch rupture. Only cells with a stable resting membrane potential negative to −60 mV and stable action potential duration and amplitude (>80 mV) were used for recording.

To evoke excitatory synaptic potentials in the CA1 pyramidal cells in whole cell mode, presynaptic fibers were electrically stimulated with a sharpened tungsten electrode placed in the middle of stratum radiatum ~100 μm from the cell recorded (stimulus: 100 μs, 70–250 μA). Trains of five stimuli at 100 Hz were delivered once every 30 s. Field potentials were recorded by stimulation of presynaptic fibers with a tungsten electrode and recording in the middle of the st. radiatum with a low-resistance pipette. During whole cell recordings and field potential recordings in Figs. 3 and S4,[1] DL-2-amino-5-phosphonopentanoic acid (DL-AP5, 100 μM) was routinely added to the ACSF to prevent long-term potentiation (LTP) or depression (LTD). In the experiments illustrated in Fig. 7, the extracellular Mg^{2+} concentration was reduced to 1.0 mM, and DNQX (40 μM) and picrotoxin (100 μM) were added to the bath solution to block the non-NMDAR-mediated excitatory postsynaptic potentials (EPSPs) and GABAR-mediated inhibitory postsynaptic potentials (IPSPs), to isolate NMDAR-mediated EPSPs. Short trains of stimuli (3 stimuli at 100 Hz) were delivered once every 20 s. D-AP5 (100 μM) was added before the end of every experiment (n = 12) to confirm that the EPSPs were mediated by NMDA type glutamate receptors.

Data acquisition, storage, and analysis

The data were acquired with pCLAMP 7.0 or 9.0 (Molecular Devices) at a sampling rate of 5–20 kHz and were measured and plotted with pCLAMP 9.0 and Origin 7.0 (Microcal). Values are expressed as means ± SE. Two-tailed Student's t-test was used for statistical analysis (α = 0.05). The P values are given in the figure legends.

The mAHP and sAHP amplitudes were measured by averaging the values within a time window (20 and 50 ms, respectively) around the peak of each AHP (20–100 ms and 0.1–3.0 s after a spike or spike train, respectively). The cell input resistance was measured by injecting weak negative current pulses and dividing the steady-state voltage response by the current pulse amplitude ($R_{input} = \Delta V/\Delta I$).

Chemicals and drugs

Apamin was obtained from Latoxan (France), XE991 [10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone] from DuPont, DNQX and 1-ethyl-2-benzimidazolinone (1-EBIO) from Tocris Cookson, and the remaining chemicals from Sigma-Aldrich Norway AS (Oslo, Norway). Substances were bath-applied by adding them to the perfusion medium.

RESULTS

Somatic intracellular recordings were obtained from 111 pyramidal cells in the CA1 st. pyramidale of rat hippocampal slices. To control for possible effects of different recording methods, both sharp electrode intracellular recordings (n = 90), whole cell patch-clamp recordings (n = 16) and perforated-patch recordings (n = 5) were used (see METHODS). To also control for possible effects of differences in animal age, temperature, intracellular medium, and extracellular potassium ($[K^+]_o$) and calcium concentrations ($[Ca^{2+}]_o$), these factors were systematically varied in some experiments (see following text; Table 1). None of these factors, however, were found to affect the main conclusions of this study. Most of the results and examples presented in the following text (i.e., unless other conditions are explicitly indicated), were obtained from CA1 pyramidal cells in hippocampal slices from young adult male rats (5–8 wk of age), in normal ACSF (with $[K^+]_o$ = 2.5 mM and $[Ca^{2+}]_o$ = 2.0 mM) at 30–35°C.

The resting membrane potential (V_{rest}) and input resistance (R_{input}) were −64.5 ± 0.7 mV and 44.6 ± 1.8 MΩ for sharp electrode recordings (n = 30), −72.8 ± 1.4 mV and 50.8 ± 2.6 MΩ for whole cell recording (n = 10), and −74.3 ± 1.2 mV and 79.1 ± 4.1 MΩ for perforated patch recordings (n = 5), respectively.

SK channels are selectively activated by Ca^{2+} action potentials but not by Na^+ spikes

To ensure that the SK channels were available for activation under our experimental conditions and that our apamin or

[1] The online version of this article contains supplemental data.

application procedure was effective, we first used experimental paradigms that are known to strongly activate Ca^{2+}-activated K^+ channels (Fig. 1*A*).

Figure 1*A* shows current-clamp recordings from a CA1 pyramidal cell in which Na^+ channels and some K^+ channels have been blocked by TTX (1 μM) and tetraethyl-ammonium (TEA, 5 mM). Under these conditions, a brief depolarizing current step evoked a calcium spike followed by AHPs with medium and slow kinetics (Fig. 1*A,* control) (Madison and Nicoll 1982, 1986; Pedarzani and Storm 1993). Bath application of 100 nM apamin fully blocked the medium AHP (△, mAHP), whereas the slow AHP was resistant to apamin (▲, sAHP) in agreement with previous reports (Gu et al. 2005; Lancaster and Nicoll 1987; Stocker et al. 1999; ; Storm 1989). Apamin also strongly increased the duration of the Ca^{2+} spike and caused a prominent afterdepolarization (ADP, *) with additional Ca^{2+} spikes to appear, indicating blockade of an SK current that contributed to repolarization (Fig. 1*A*). Similar effects of apamin were found in all cells that showed a prominent mAHP (>2.0 mV) following the Ca^{2+} spike ($n = 5$; see summary data in Fig. 1*C*). In some cells, the mAHPs following Ca^{2+} spikes were small (<2.0 mV) or absent, apparently being partly occluded or replaced by an afterdepolarization (ADP), which may be due to slow decay of the inward Ca^{2+} current, and/or activation of the Ca^{2+}-activated nonselective cation current (I_{CAN}); (Knox et al. 1996). Never-

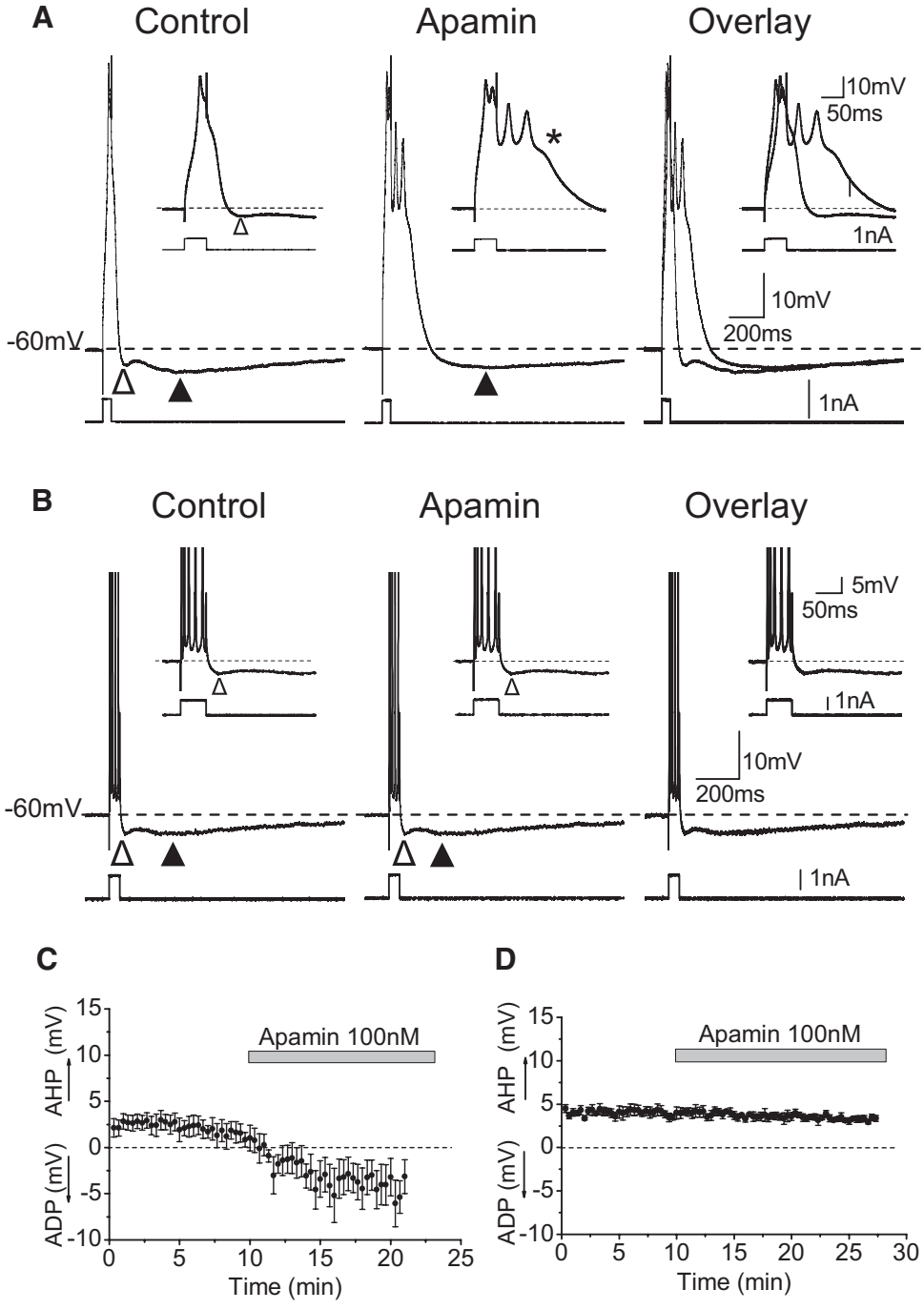

FIG. 1. Apamin supressed the medium afterhyperpolarization (mAHP) following a calcium spike but not the mAHP following a train of sodium spikes in CA1 pyramidal neurons. *A*: typical recording of Ca^{2+} spikes evoked by current injection in the presence of 1 μM TTX and 5 mM TEA. Bath application of 100 nM apamin broadened the Ca^{2+} spike and fully suppressed the ensuing mAHP (△) but not the slow AHP (sAHP, ▲). Following apamin application, the mAHP was inhibited and replaced by an afterdepolarization (ADP, *). *B*: typical recording of AHPs following a train of 5 Na^+ spikes evoked by current injection in normal medium (no TTX or TEA). Application of 100 nM apamin had no measurable effect on the mAHP (△) or sAHP (▲). The *insets* in *A* and *B* are magnifications of the larger trace. *C*: summary plot of the mAHP amplitude following a Ca^{2+} spike before and after apamin application in all cells tested that showed a prominent mAHP (peak amplitude, >2 mV) following Ca^{2+} spikes before apamim application ($n = 5$). [See also Supplementary Fig. S1.] *D*: summary plot of the mAHP amplitude following 5 Na^+ spikes before and after apamin application in all cells tested ($n = 4$). No significant effect on the mAHP was observed ($P > 0.05$). All data in Figs. 1–5 were obtained by intracellular somatic current-clamp recording with sharp electrodes. Each Ca^{2+} spike (*A* and *C*) or Na^+ spike train (*B* and *D*) was evoked by injection of a 50-ms depolarizing current pulse after maintaining the background potential at −60 mV with DC current injection.

theless apamin showed clear effects also in these cells, by suppressing the mAHP and/or enhancing the ADP. Supplementary Fig. S1 shows the average time course of apamin effect in all cells tested (including cells with and without a prominent mAHP: $n = 10$).

In contrast, when cells were exposed to normal extracellular ACSF (without TTX and TEA), apamin failed to affect the mAHPs that followed 50-ms high-frequency Na^+ spike trains (Fig. 1, *B* and *D*; $n = 6$, $P > 0.05$).

The effect of apamin on Ca^{2+} spike-mAHPs was seen also when our recording prior to apamin lasted longer (10–12 min; $n = 5$) than in the sharp electrode recordings without TTX or TEA where apamin failed to affect the mAHP (8–10 min). This, as well as our previous recordings of robust apamin-sensitive tail currents under similar conditions (Sailer et al. 2002), indicates that channel rundown was not the cause of the negative results. Evidently, SK channels are available for activation in our recording conditions, but they require exceptionally strong Ca^{2+} influx to noticeably affect somatic electrical activity.

Taken together, our results indicate that functional SK channels are available in CA1 pyramidal cells and can be activated by the massive Ca^{2+} influx provided by Ca^{2+} spikes evoked in the presence of Na^+ and K^+ channel blockers, but that these SK channels are not significantly activated by Na^+ spike trains under normal conditions at least not to an extent that appreciably contributes to the somatic mAHP.

Do SK channels contribute to the mAHP under other experimental conditions?

Although several studies have indicated that the somatic mAHPs in CA1 pyramidal neurons are not caused by SK channels, but mainly by M current at depolarized potentials and h current at hyperpolarized potentials (Gu et al. 2005; Peters et al. 2005; Storm 1989), apparently conflicting results have been reported and widely cited by others (Bildl et al. 2004; Bond et al. 1999, 2004, 2005; Bowden et al. 2001; Faber and Sah 2003, 2007; Kaczorowski et al. 2007; Melyan et al. 2002; Sah and Faber 2002; Shah et al. 2006; Stocker 2004; Stocker et al. 2004). Therefore we found it necessary to test the roles of SK channels systematically under a variety of experimental conditions. Hence we conducted an extensive search for an SK-channel contribution to the mAHP ($n = 77$ cells; Table 1), while varying parameters that might have caused different results: age of the animal, temperature, recording technique (whole cell gigaseal, sharp electrode intracellular, and perforated-patch recording), extracellular $[K^+]$, $[Ca^{2+}]$, and pH, background membrane potential, duration of the depolarizing pulse, number and frequency of action potentials, and used different SK channel blockers. All tests were performed in the absence of bicuculline because some bicuculline salts are known to suppress SK channels (Johnson and Seutin 1997).

The results of these tests are summarized in Table 1. Figure 2 shows typical recordings and averaged time courses obtained with sharp electrodes in slices from 3- to 8-wk-old rats, in the presence of 2 mM $[Ca^{2+}]_o$. Application of apamin produced no detectable effect on the mAHP and sAHP in any of the conditions tested. For example, apamin failed to affect the mAHP and sAHP following a high-frequency train of five spikes evoked by a depolarizing current pulse (50 ms) in 3.5 mM $[K^+]_o$ at 30°C with the background membrane potential (V_m) held at −60 mV (Fig. 2*A*). A high concentration of the SK channel blocker *d*-tubocurarine (d-TC, 100 μM) had no detectable effect on the mAHP or sAHP following a longer spike train (400 ms) at room temperature in 2.5 mM $[K^+]_o$ (Fig. 2*B*). This protocol resembles closely to the one used by Stocker et al. (1999), e.g., their Fig. 5. Apamin had no significant effect on the mAHP following a high-frequency (100 Hz) train of 20 spikes evoked by 20 brief (2 ms) pulses in 2.5 mM $[K^+]_o$ at 30°C (Fig. 2*C*). Apamin also failed to affect the mAHP following five spikes in perforated-patch recordings in 2.5 mM $[K^+]_o$ at 30°C (Fig. 2*D*). Finally, apamin had no effect on the mAHP following a single spike in 2.5 mM K^+ at 26°C (Fig. 2*E*). In contrast, the M-channel blocker XE991 consistently abolished the mAHP at this membrane potential (Gu et al. 2005; Peters et al. 2005) (Fig. 2*E*).

As summarized in Table 1, no effect of SK channel blockade was apparent in the 77 cells tested under a variety of conditions. Thus high concentrations of apamin (100 nM, Table 1, "A") or d-TC (100 μM, Table 1, "T") consistently failed to significantly reduce the mAHP amplitude ("A÷", "T÷"). Even when the extracellular pH was raised to 7.7—a treatment that has been reported to enhance SK channel activity (Kelly and Church 2004), apamin failed to affect the mAHP ($n = 7$, Table 1, pH_o).

However, in some experiments, we observed a slow, time-dependent rundown of the mAHP amplitude, independently of drug application, in particular during whole cell recording. A typical example of such rundown (from a cell that was excluded from analysis for this reason) is illustrated in Supplementary Fig. S2. Such rundown may perhaps explain why others have reported an apparent reduction in mAHP following apamin application during whole cell recording.

Taken together, these results support the conclusion that SK channels do not contribute noticeably to the mAHP in rat CA1 pyramidal cells.

Ionic mechanisms of mAHPs following excitatory synaptic activation

Although SK channels do not seem to contribute appreciably to the normal mAHP evoked by somatic current injection, it is still possible that excitatory synaptic input could activate these channels. This seems an interesting possibility because the apical dendrites that receive most of the excitatory synaptic input are also prone to generate Ca^{2+} spikes or Ca^{2+} channel-mediated plateau potentials (Andreasen and Nedergaard 1996; Cai et al. 2004; Magee and Carruth 1999; Magee and Johnston 1995).

To test this hypothesis, presynaptic axons in st. radiatum were activated by high-frequency trains of stimuli, each evoking an EPSP. First, we tested whether SK channels are activated by EPSPs caused by non-NMDA-type glutamate receptors by stimulating in the presence of the NMDA receptor blocker APV (Fig. 3*A*, control). To maximize the postsynaptic Ca^{2+} influx and, thus the likelihood that SK channels should be activated, the stimulation intensity was increased until each EPSP evoked an action potential. The train of five EPSPs with spikes was followed by a mAHP; but application of 100 nM apamin had no detectable effect (Fig. 3, *A,* apamin, and *B, left*). In contrast, XE991 readily

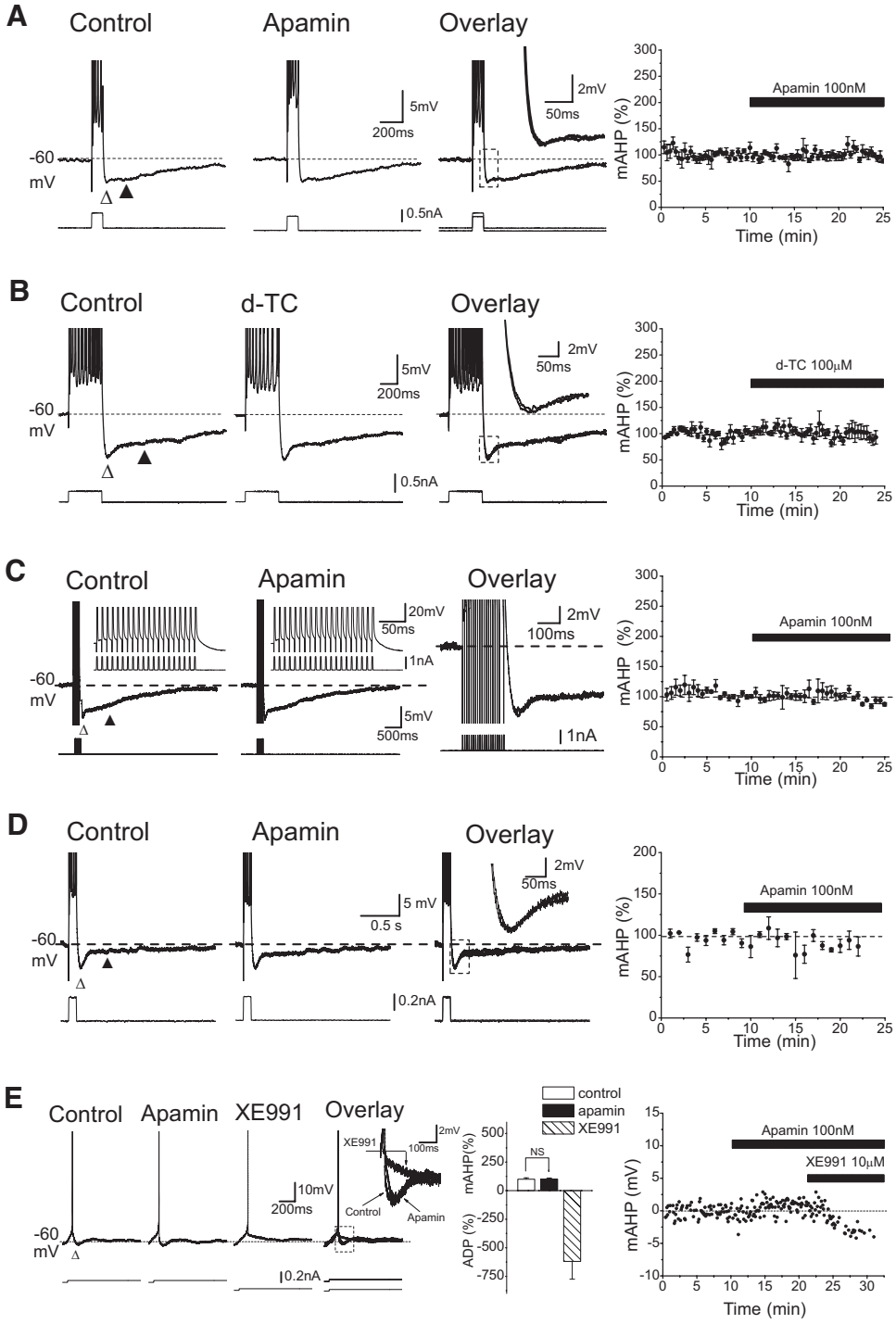

FIG. 2. The mAHP in CA1 pyramidal neurons was resistant to SK channel blockers in various recording conditions. During recording, each cell was kept at −60 mV by steady current injection. The effect of 100 nM apamin (*A* and *C–E*) or 100 μM *d*-tubocurarine (d-TC, in *B*) on the mAHP was tested under the following conditions: *A*: mAHP following a train of 5 spikes at 100 Hz with extracellular [K^+] raised to 3.5 mM and recording temperature set to 30°C; *B*: mAHP following a train 10 spikes at 25 Hz, at 23°C; *C*: mAHP following a train of 20 spikes, each evoked by a brief (2 ms) depolarizing current pulse at 100 Hz and 30°C. The data in *A–C* were obtained by somatic intracellular recording with sharp electrodes. *D*: perforated-patch recording of mAHPs following a train of 5 spikes at 50 Hz at 30°C. Summary plots of mAHP amplitude time courses before and after drug application are shown to the right in *A–D*. *E*: mAHP following a single action potential at 26°C. Apamin or *d*-TC failed to produce any significant reduction of the mAHP amplitude in each of the conditions tested ($P > 0.05$ in all groups). In contrast, the KCNQ/M-channel blocker XE991 (10 μM) inhibited the mAHP following single spike (*E*; $P < 0.01$). Summary data and sample time course are shown in *E, right*.

blocked the synaptically evoked mAHP, and uncovered an afterdepolarization (ADP *; Fig. 3, *A, B, right,* and *C*). Similar negative results with apamin were obtained in all five cells tested (see the summary graphs in Fig. 3, *D* and *E*).

Although apamin had no convincing effect on the somatic mAHP following a high-frequency EPSP train in the presence of APV, it still seems possible that an SK-mediated mAHP component might be recruited with intact NMDA receptors. Thus the slow kinetics of an NMDAR-mediated EPSP might recruit more dendritic Ca^{2+} channels, leading to a greater influx of Ca^{2+} into the dendrites, which might spread to the soma and activate somatic SK channels during the mAHP. To test for this, we repeated the experiments described in the preceding text in the absence of NMDAR blockade. Still, bath application of apamin (100 nm) again failed to affect the amplitude of the mAHP that followed the 100-Hz train of five EPSPs (Supplementary Fig. S3, *A* and *C*). In contrast, subsequent application of the Kv7/KCNQ/M-channel blocker XE991 (10 μM) significantly reduced the mAHP amplitude (Supplementary Fig. S3, *B* and *D*). Supplementary Fig. S3*E* shows the summary results of apamin and XE991 ($n = 4$, paired *t*-test, NS: $P > 0.05$; *:

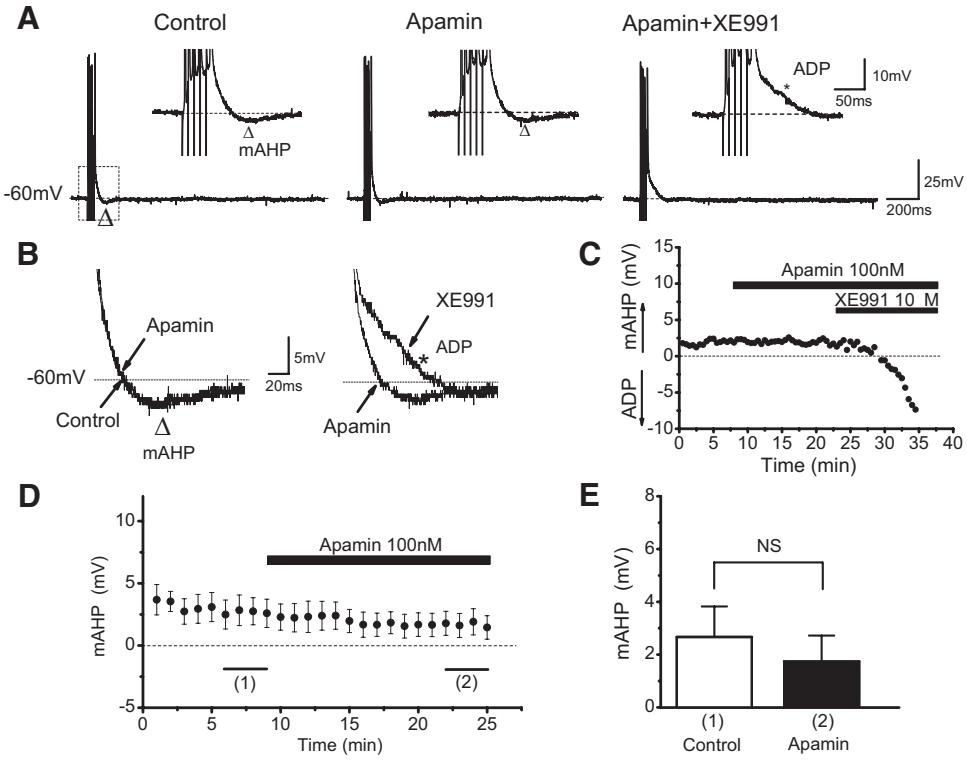

FIG. 3. Apamin failed to inhibit the mAHP following excitatory synaptic potentials with action potentials. *A*: representative somatic voltage response to stimulation of presynaptic fibers in stratum radiatum. A series of five stimuli at 100 Hz evoked 5 excitatory postsynaptic potentials (EPSPs), each triggering an action potential (truncated), followed by a mAHP (△). *Insets*: the mAHP on expanded scales. Application of 100 nM apamin had no apparent effect, whereas subsequent application of 10 μM XE991 blocked the mAHP. *B*: mAHP traces from *A*, shown expanded and superimposed. *C*: time course of the effects of apamin and XE991 on the synaptically evoked mAHP amplitude (same experiment as in *A* and *B*). *D*: time course of the effect of apamin on the synaptically evoked mAHP amplitude in all cells tested ($n = 5$). *E*: summary plot of synaptically evoked mAHP amplitudes, before and after apamin application. The mAHP amplitude was measured at the times marked with 1 and 2 in *D* ($n = 5$, NS: $P > 0.05$).

$P < 0.05$). The average numbers of action potentials evoked by EPSPs were 4.25 ± 0.4 before and 4.15 ± 0.4 after application of apamin. ($n = 4$, $P > 0.05$).

To avoid possible side effects of intracellular recording, including possible rundown of Ca^{2+} channel or SK channel activity, we also performed extracellular field potential recordings from st. pyramidale in CA1 (Supplementary Fig. S4). Stimulation of axons in st. radiatum with high-frequency (100 Hz) trains of six pulses at high stimulus intensity evoked large field EPSPs and population spikes. Bath application of 100 nM apamin had no detectable effect on these field potential responses (Supplementary Fig. S4). Taken together, these results indicate that not even the spikes and subsequent mAHPs triggered by excitatory dendritic synapses are appreciably modulated by SK channels when the NMDA-receptors are blocked (see following text).

Excitability control and spike frequency adaptation

To further test how K^+ currents underlying the mAHP affect excitability, we evoked repetitive firing by injecting 1-s depolarizing pulses. As illustrated in Fig. 4*A, top,* apamin (100 nM) had no significant effect on the number, frequency, or pattern of action potentials, whereas XE991 (10 μM) strongly increased the spike frequency, often causing an initial high-frequency spike cluster, followed by a pause in the firing and an enhanced sAHP (Fig. 4*A, right*). Similar results were obtained for different current intensities and spike frequencies (Fig. 4*A, middle* and *bottom*). As illustrated in Fig. 4*B,* when the spike frequency was plotted as a function of injected current intensity (*f/I* plots) for all the five cells tested, no obvious effect of apamin could be detected, whereas XE991 clearly increased the slopes of the *f/I* curves, indicating a significant increased excitability (Fig. 4*B, right*). This was seen both for the instantaneous frequency of the three first interspike intervals (1/ISI) as well as for steady-state discharge frequency toward the end of each pulse (Fig. 4*B*). Likewise, when the average *f/I* plots were compared before and after apamin and XE991 applications (Fig. 4*D*), no significant effect of apamin could be detected, whereas XE991 increased the action potential (AP) frequency at all current intensities tested (Fig. 4*D, right*). Finally, when the instantaneous discharge frequency was plotted as a function of time during the spike train (Fig. 4*C,* current pulses: 0.15 nA), no effect of apamin was apparent, whereas XE991 clearly increased the firing frequency (Fig. 4*C, right*).

Whereas excitability reflects the overall tendency to generate action potentials, spike frequency adaptation specifically refers to time-dependent decline in AP frequency during a spike train. To determine the possible roles of SK channels in spike frequency adaptation, we evoked repetitive firing by injecting 50- to 100-ms depolarizing pulses, while varying the pulse intensity to ensure that each pulse always triggered five APs (see Supplementary Fig. S5). Application of 100 nM apamin had no detectable effect on the adaptation, neither when the background V_m was maintained at −60 mV (Supplementary Fig. S5*A*, $n = 5$) nor when it was −80 mV (Fig. S5*B*, $n = 5$). This lack of an apamin effect was observed in all cells tested ($n = 5$ for each condition).

These results indicate that SK channels do not contribute appreciably to excitability control or spike frequency adaptation in CA1 pyramidal cells.

Effects of the SK-channel opener 1-EBIO

To further test whether SK channels in CA1 pyramidal cells were available for activation under our experimental conditions and how they might contribute if they were

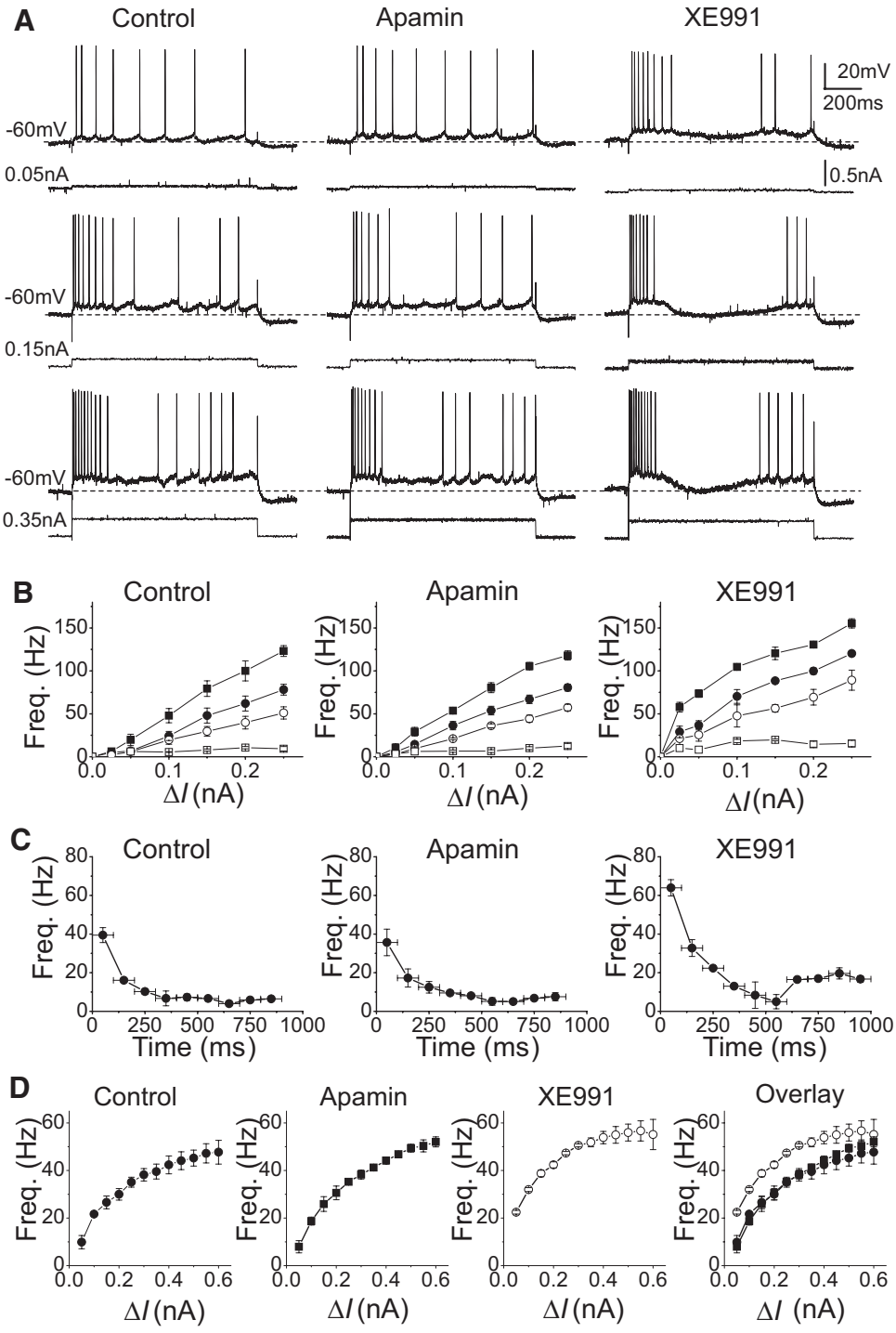

FIG. 4. Apamin had no apparent effect on excitability during repetitive discharge in CA1 pyramidal neurons. *A*: typical voltage responses to 1-s current pulses of different amplitudes (*top*: 0.05 nA, *middle*: 0.15 nA; *bottom*: 0.35 nA) before and after application of apamin (100 nM), followed by the M channel blocker XE991 (10 μM). *B*: instantaneous firing frequencies of the 2nd (■), 3rd (●), and 4th (○) spike, and the steady-state firing frequency (□) during the 1-s spike train, plotted against the amplitude of the current pulse. The steady-state firing frequency was calculated as the average frequency during the last 400 ms of each current pulse; the instantaneous frequency of a given spike is defined as the inverse of the interspike interval (ISI) between that spike and the 1 preceding it. Apamin had no apparent effect on the firing frequencies, whereas XE991 increased the firing frequency ($n = 5$). *C*: averaged firing frequencies measured within 100-ms time windows during a 1-s, 0.15-nA current pulse. No significant effect of apamin was detected. In contrast, XE991 increased the early firing frequencies within the first 300 ms. *D*: average firing frequency of the 1-s spike train plotted against the current pulse amplitude. Apamin had no significant effect, whereas XE991 caused a strong increase in the average firing frequency in response to current pulses <0.5 nA.

activated, we used the SK-channel opener 1-EBIO (Pedarzani et al. 2001). As shown in Fig. 5, application of 10 μM 1-EBIO more than doubled the amplitude of the mAHP following a five-spike train (Fig. 5, *A–C*). Subsequent application of apamin, in the continued presence of 1-EBIO (Fig. 5, *A–C*), exactly reversed the effect of 1-EBIO, thus returning the mAHP to the same amplitude as it has in control medium (Fig. 5, *A–C*). In parallel, 1-EBIO, reduced the excitability of the cell, so that a stronger current pulse was needed to evoke five spikes in the presence of 1-EBIO than in control medium or after apamin had been added (Fig. 5*A, bottom*). By comparing the response to the same current pulse intensity before and after adding 1-EBIO, the 1-EBIO-induced reduction in excitability was clearly seen as a reduction in the number of spikes (Fig. 5*E*). This effect was also reversed by adding apamin (Fig. 5*F*). However, no resting membrane potential (RMP) change was detected after application of 1-EBIO, and the input resistance (calculated by dividing the steady-state voltage response by the current pulse amplitude: $R_{input} = \Delta V/\Delta I$) was also the same before (55.5 ± 6.25 MΩ) and after (55.6 ± 5.9 MΩ) application of 1-EBIO (paired *t*-test, $P > 0.05$, $n = 4$). The

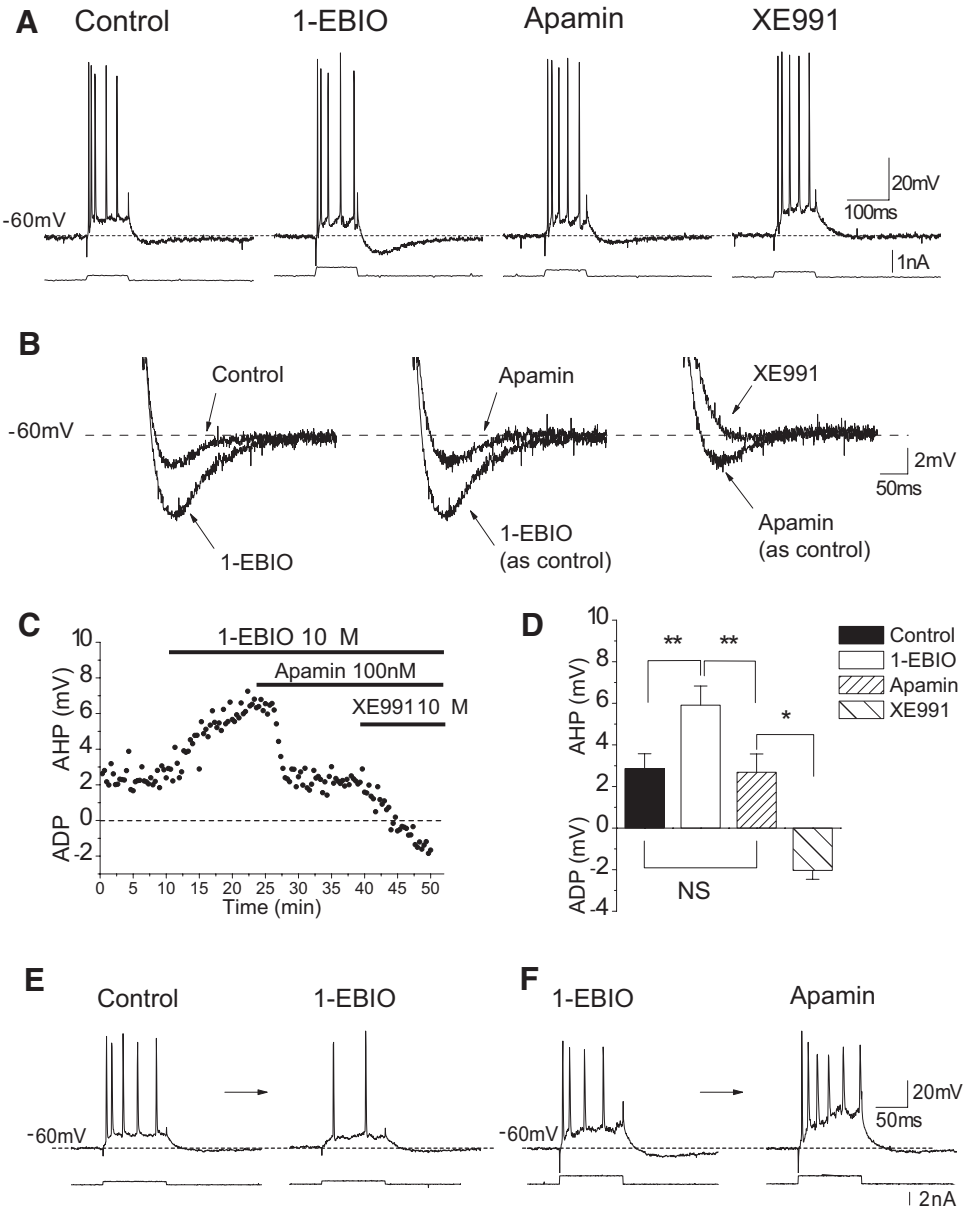

FIG. 5. The SK channel opener 1-ethyl-2-benzimidazolinone (1-EBIO) activated an apamin-sensitive component in the mAHP and reduced neuronal excitability. *A*: representative voltage responses to 100-ms current pulses before and after application of 10 μM 1-EBIO, followed by 100 nM apamin, and by 10 μM XE991. 1-EBIO caused a strong increase of mAHP amplitude. Apamin fully reversed the effect of 1-EBIO, reducing the mAHP to the level similar to that of the control condition. Subsequent application of XE991 fully blocked the mAHP. *B*: The mAHP traces from *A* shown expanded and superimposed. *C*: time course of the mAHP during the experiment shown in *A*. *D*: summary plot of mAHP amplitudes before and after 1-EBIO, followed by apamin, then by XE991, in all cells tested ($n = 4$, **: $P < 0.01$, *: $P < 0.05$, NS: $P > 0.05$). *E*: 1-EBIO reduced the number of action potentials evoked by a 100-ms current pulse (0.5 nA), indicating reduced excitability ($n = 4$). *F*: the inhibitory effect of 1-EBIO was reversed by apamin ($n = 4$).

latter result is probably due to lack of SK channel activation at resting intracellular [Ca^{2+}] levels, even in the presence of 1-EBIO.

Effects of apamin in bursting pyramidal neurons in subiculum

Because our present and previous data (Gu et al. 2005; Storm 1989) indicate that SK channels do not contribute appreciably to the mAHP following Na^+ spikes in CA1 pyramidal cells, we wished to test whether this is generally true also for other classes of hippocampal pyramidal cells. In a parallel study, we have tested CA3 pyramidal cells and found that apamin has no obvious effect on the Na^+-spike-induced mAHP also in these cells (Sharifullina et al. 2008). In addition, we tested pyramidal neurons in the subiculum area of the rat hippocampus (Fig. 6). Most subicular pyramidal cells show bursting behavior, firing clusters of action potentials at high frequency (>200 Hz) (Staff et al. 2000). Only such bursting cells were included in this study. Figure 6*A* shows the typical firing patterns of such a bursting subicular neuron. We found that bath application of apamin (100 nM) significantly reduced the mAHP following the burst in these subicular pyramidal neurons (Fig. 6*B*). Thus an apamin effect was observed in all cells tested (Fig. 6, *C* and *D*, $n = 8$, $P < 0.01$), in clear contrast to the lack of effect in CA1 pyramidal cells. (A preliminary report of these results was published by Hu et al. 2004.) These results indicate that the functional roles of SK channels differ between bursting subicular and other hippocampal (CA1, CA3) pyramidal cells. In addition, these results indicate that there is nothing about our recording methods, conditions or slice preparations that prevent us from observing an apamin effect on the mAHP when it occurs. Thus the CA1 cells and subicular cells were recorded in identical horizontal hippocampal slice preparations under identical conditions. In a previous study, parallel recordings of apamin effects on AHPs in frog

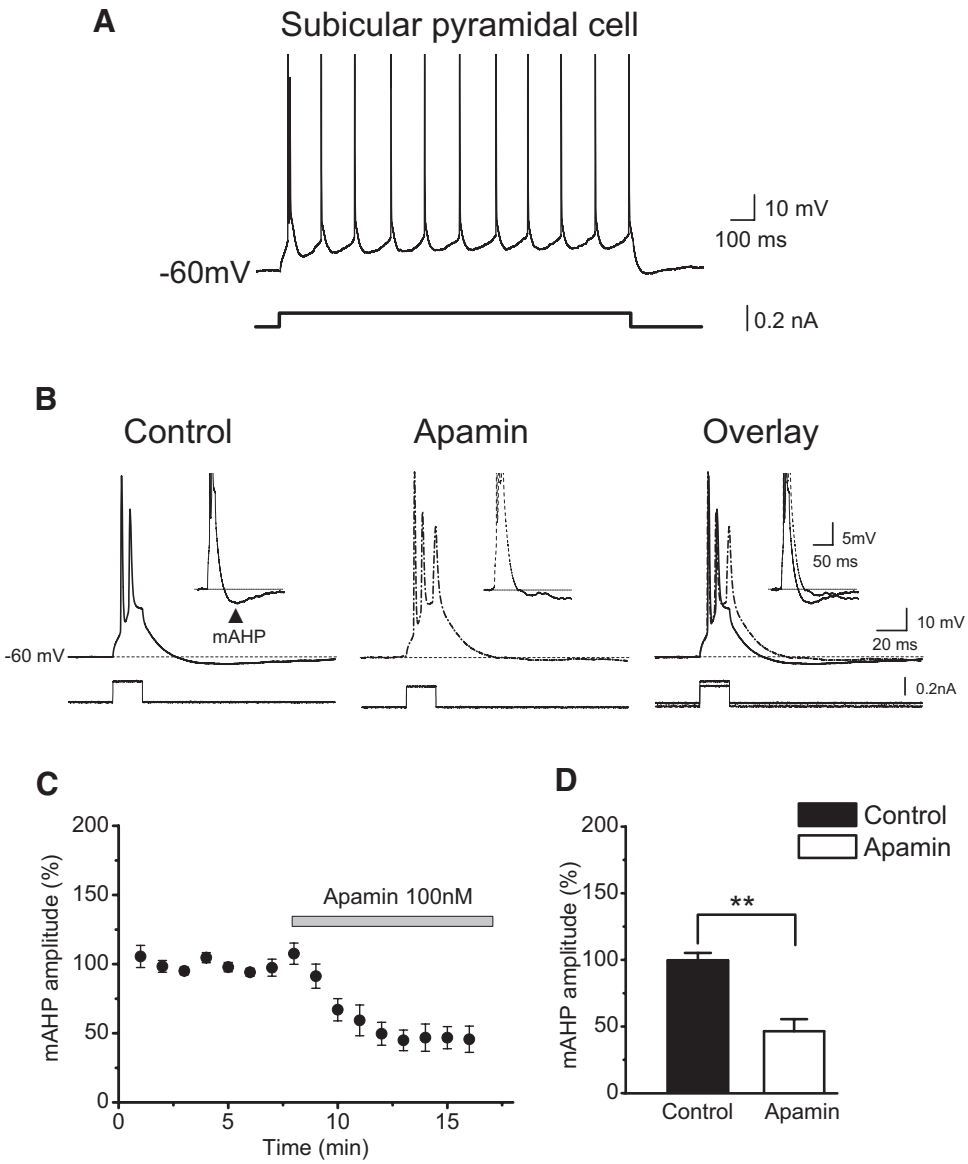

FIG. 6. In busting subicular neurons, apamin reduces the mAHP following a spike burst. *A*: typical response of a bursting pyramidal cell from rat subiculum to a depolarizing pulse of 1.5-s duration. (Whole cell recording from the pyramidal cell layer of subiculum in a horizontal hippocampal slice. The background membrane potential prior to testing was held at −60 mV by steady current injection.) The cell fired a burst at the beginning of the current pulse, followed by a train of single action potentials. *B*: in normal extracelluar medium (control), a short burst consisting of 2 spikes was elicited by a brief pulse (2-ms duration) and was followed by a mAHP) (see *inset* ▲). Application of 100 nM apamin significantly reduced the mAHP amplitude and converted the 2-spike burst into a 3-spike burst. *C*: the average time course for all busting subicular neurons tested with apamin. The effect of apamin on the mAHPs following a 2- or 3-spike burst is plotted. *D*: statistics for all busting subicular neurons tested, showing that apamin significantly reduced the mAHP amplitude in these cells ($n = 8$, paired t-test $P < 0.01$).

sympathetic neurons served as a similar positive control (Storm 1989).

SK channel regulate NMDAR-mediated EPSP

Because the SK channels in CA1 pyramidal cells appear not to generate the mAHP or the sAHP (Gu et al. 2005; Shah and Haylett 2000; Storm 1989; Strobaek et al. 2000; Villalobos et al. 2004; Vogalis et al. 2003), we next asked what else their function might be. Previous studies have provided evidence that dendritic SK channels activated by Ca^{2+} influx through NMDA receptors or voltage-gated Ca^{2+} channels can regulate dendritic Ca^{2+} signals and modulate synaptic efficacy and plasticity (Bloodgood and Sabatini 2007; Faber et al. 2005; Ngo-Anh et al. 2005; Stackman et al. 2002). In particular, Ngo-Anh et al. (2005) found that apamin increased the peak EPSP amplitude in mouse CA1 pyramidal cells by 70% (in the presence of 1 mM Mg and picrotoxin), and subsequent application of APV reversed the apamin-induced increase. Thus it appears that SK channels, by generating a repolarizing conductance in response to increased intracellular Ca^{2+}, form an effective Ca^{2+}-mediated feedback loop that regulate postsynaptic Ca^{2+} influx. These conclusions are based on results from hippocampal slices from mice (Bloodgood and Sabatini 2007; Stackman et al. 2002), rat hippocampal slice cultures (Cai et al. 2004), acute slices from rat hippocampus (Ngo-Anh et al. 2005) and rat amygdala (Faber et al. 2005).

To test, in a noninvasive manner, whether SK channels are activated by NMDAR-mediated synaptic transmission and can regulate its impact, we performed extracellular field potential recordings in st. radiatum of CA1. Figure 7*A* shows NMDAR-mediated postsynaptic responses (EPSPs) in normal medium with 1 mM [Mg^{2+}] evoked by a stimulation electrode in st. radiatum, which contains the Shaffer collateral pathway from CA3 to CA1. The responses were induced by a series of three stimuli at 100 Hz in the presence of the AMPA receptor antagonist DNQX (40 μM) and the $GABA_A$ receptors blocker picrotoxin (100 μM). Subsequent application of the SK channel blocker apamin (100 nM) significantly increased the amplitude and area of the EPSPs (red trace). Finally, application

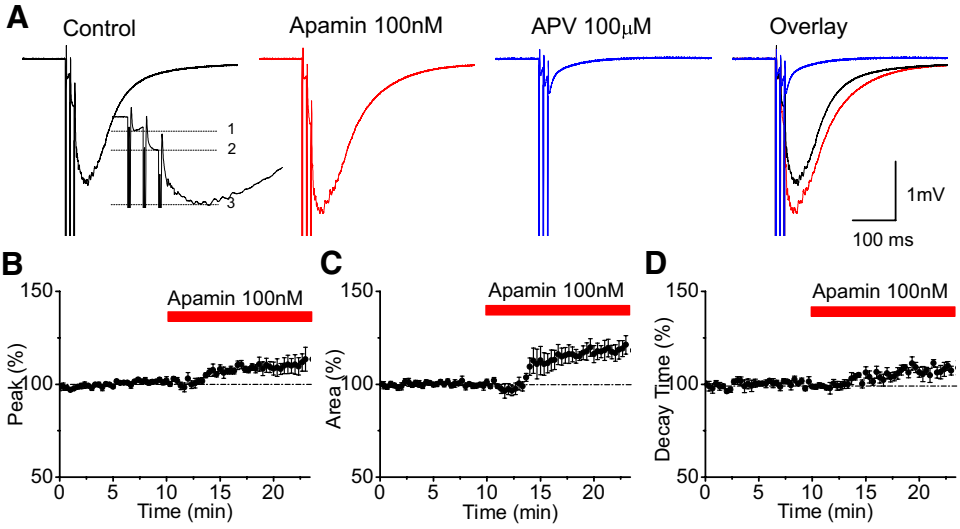

FIG. 7. Apamin enhanced the $NMDA_R$-mediated EPSPs. *A*: Sample traces from a field potential recording in CA3–CA1 Shafer-collateral pathway. A train of high-frequency stimuli were applied in the presence of AMPA receptor anagnist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 40 μM) and GABA receptor blocker picrotoxin (100 μM). *Insets*: the same trace in expanded scale with dashed line marking the peak of the amplitude of EPSP 1, 2 and 3. SK channel blocker apamin (100 nM) increased the amplitude and area of the *N*-methyl-D-aspartate (NMDA) EPSPs (red trace). NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (100 μM) strongly inhibited the excitatory responses (blue trace). *B–D*: averaged time courses of the effects of apamin on peak amplitude, area and 90–10% decay time of the last NMDA-EPSPs ($n = 6$, $P < 0.05$).

of the NMDA receptor antagonist DL-AP5 blocked the EPSPs (blue trace). The average time courses of the apamin effects on the peak amplitude, the area, and the decay time of the last NMDAR-EPSPs are shown in Fig. 7, *B–D,* respectively ($n = 6$, $P < 0.05$). Note, however, that the apparent temporal summation of NMDAR-mediated EPSPs is highly nonlinear, both before and after apamin application (see Fig. 7*A, inset*). Thus the EPSP component evoked by the third stimulus is far larger than the two previous ones, and it also shows a remarkably slow rise and a "noisy" peak. Although the increased amplitude of the late EPSPs may be partly due to presynaptic facilitation, the effect is so large that it seems likely to involve activation of voltage-dependent dendritic conductances, such as regenerative depolarizations caused by voltage-gated calcium and/or NMDAR channels (Andreasen and Nedergaard 1996; Magee and Johnston 1995). Thus the SK channel activation seen in these experiments (Fig. 7) may have mechanisms in common with the calcium spike-evoked SK channel activation shown in Fig. 1*A*.

Because synapses onto hippocampal pyramidal cells are also equipped with postsynaptic metabotropic glutamate receptors (mGluR), which might activate SK channels by triggering release of Ca^{2+} ions from intracellular stores (Nakamura et al. 1999), we also repeated this type of experiment after blocking mGluRs. In the presence of the two mGluR blockers MPEP (10 μM) and LY341495 (50 μM), we obtained results that were indistinguishable from those shown in Fig. 7 ($n = 6$, $P < 0.05$, data not shown), indicating that mGlu receptors were not important for activating SK channels under these conditions.

These results are compatible with those of Ngo-Anh et al. (2005) and Bloodgood and Sabatini (2007) and also readily explain the lack of effect of apamin on the AHP following a train of synaptically evoked spikes during NMDAR blockade (Fig. 3). Thus if the activation of postsynaptic SK channels is dependent on NMDAR currents, directly or indirectly (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005), an apamin-induced enhancement of postsynaptic responses Fig. 7 can only occur in the absence of NMDAR blockade.

So far, we tested the roles of SK channels during synaptic transmission in the presence of NMDAR blockers (Fig. 3) or non-NMDAR blockers (Fig. 7). It is obviously of interest to examine the roles of SK channels also in the absence of any glutamate receptor blockers. Whereas previous studies tested the roles of SK channels in subthreshold postsynaptic events (Bloodgood and Sabatini 2007; Faber et al. 2005; Ngo-Anh et al. 2005; Stackman et al. 2002), we used high-frequency stimulation in st. radiatum, of sufficient strength to evoke a train of population spikes, to achieve optimal activation of SK channels and for comparison with somatically evoked spike trains and AHPs (Figs. 1, 2, 4, and 5) as well as postsynaptic responses in the presence of NMDAR blockers (Supplementary Fig. S4). The responses were measured in somatic field potential recordings (Supplementary Fig. S6), and the stimulation strength was adjusted so that the amplitudes of the population spikes were less than maximal to allow room for an increase. However, this stimulation paradigm tends to induce NMDAR-dependent plasticity in the non-NMDA glutamate receptors (LTP and LTD) (Bear 1996; Bliss and Lomo 1973). Therefore we allowed a long stabilization period for the LTP induction and expression to saturate (Bliss and Lomo 1973), thus achieving a stable baseline before apamin was applied. Under these conditions, bath application of apamin (100 nM) had no detectable effect on the train of population spikes or any other part of the postsynaptic responses ($n = 6$; Supplementary Fig. S6, *A* and *E–H*). In contrast, subsequent application of the Kv7/KCNQ/M-channel blocker XE-991 (10 μM; blue trace in Supplementary Fig. S6*B*), caused a clear increase in the first two population spikes (Supplementary Fig. S6, *C* and *D*). (Later in the train of population spikes, more complex effects of XE-991 were observed, including reduced population spike amplitudes, probably due to secondary effects of the enhanced initial spiking.) These effects of XE991, which we have previously found are purely postsynaptic under these conditions (Vervaeke et al. 2006), demonstrate that the population spikes could be increased by blockade of postsynaptic K^+ channels under these conditions, thus indicating that the lack of an apamin effect was not due to trivial experimental factors.

The lack of an apamin effect may seem surprising in view of recent results indicating that dendritic spines of mouse CA1 pyramidal neurons contain SK channels that are specifically activated by Ca^{2+} influx through voltage-gated Ca^{2+} channels and/or NMDA channels in the spines (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005). Because Na^+ action potentials backpropagate (bAPs) into the dendrites and spines (Blood-

good and Sabatini 2007; Ngo-Anh et al. 2005), they should help opening the voltage-gated Ca^{2+} and/or NMDA channels and hence activate the SK channels in the spines (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005). This would be expected to attenuate the EPSPs, thus reducing the population spike amplitudes. However, very recently, after the experiments of this study were completed, Lin et al. (2008) reported that LTP induction strongly downregulates the SK channel activity in the spines of mouse CA1 pyramidal neurons by removing SK2 channels from the postsynaptic membrane (PSD). This may explain the lack of apamin effect seen in Supplementary Fig. S6. According to this hypothesis, when LTP is induced by high-frequency stimulation during the long stabilization period, the SK channels in the spine are downregulated (Lin et al. 2008), precluding a subsequent effect apamin. In addition, as discussed in the following text, a local apamin effect in the spines or dendrites may have too weak impact in the soma to be detected there due to enhanced filtering by the dendritic cable during high-frequency discharge.

DISCUSSION

The main conclusion from this study is that SK (also called $K_{Ca}2$) channels in the dendrites of rat CA1 hippocampal pyramidal cells can be activated by excitatory synaptic input in st. radiatum through activation of NMDA-type glutamate receptors, but there is little or no contribution from SK channels to the somatic mAHP and excitability regulation in these cells. This conclusion seems to hold both for the mAHP following a single action potential and for the mAHP that follows a spike train. Furthermore, no contribution of SK channels to excitability control or spike frequency adaptation could be detected in any of the experimental conditions or paradigms tested although artificial activation of SK channels by the SK-channel opener 1-EBIO significantly enhanced the mAHP amplitude and reduced cell excitability. Thus apparently, the SK channels in CA1 hippocampal pyramidal cells are available for activation by NMDAR-mediated, dendritic synaptic input or by artificially enhanced spike-evoked calcium influx (Sailer et al. 2002) but are normally not activated by somatic Na^+ spikes to an extent that appreciably influences the somatic response pattern, not even during high-frequency discharge.

These results may seem unexpected in view of the widespread idea that the main functions of SK channels in CA1 pyramidal cells and other neurons is action potential-triggered feedback control of excitability, including spike frequency adaptation and generation of afterhyperpolarizations (Faber and Sah 2003; Stackman et al. 2002; Stocker 2004; Stocker et al. 1999). Our results may also seem surprising because functional SK channels are evidently expressed at high density in the somatodendritic membrane of CA1 pyramidal cells and can generate a robust outward current (Sailer et al. 2002; Stocker et al. 1999). Furthermore, it is clear that normal action potentials in these cells cause a sufficient rise in intracellular calcium concentration to readily activate two other classes of Ca^{2+}-activated K^+ channels: the BK channels and the sAHP channels (Alger and Nicoll 1980; Faber and Sah 2003; Storm 1987a,b, 1990).

However, our results are fully compatible with other reports, including our previous studies showing no detectable SK channel contribution to the mAHP in CA1 pyramidal cells (Gu et al. 2005; Storm 1989). In particular, our results agree with previous evidence that the somatic mAHP in rat and mouse CA1 hippocampal pyramidal neurons are mainly caused by Kv7/M/KCNQ channels at depolarized potentials (approximately −60 mV) and by HCN/h channels at hyperpolarized potentials (approximately −80 mV) (Gu et al. 2005; Hu et al. 2007; Peters et al. 2005; Storm 1989); evidence that artificial enhancement of SK channel activity by application of an SK channel opener can effectively reduce the excitability of hippocampal pyramidal neurons (Pedarzani et al. 2001), although SK channels do not normally contribute appreciably to excitability control and adaptation in these cells (Gu et al. 2005; Storm 1989); and evidence that SK channels can be activated by calcium influx through NMDA-type glutamate receptor channels (Cai et al. 2004; Faber et al. 2005; Ngo-Anh et al. 2005; Stackman et al. 2002) or dendritic voltage-gated Ca channels (Bloodgood and Sabatini 2007; Cai et al. 2004; Ngo-Anh et al. 2005). This supports the possibility that SK channels may regulate, and even contribute to the expression of, LTP and LTD and perhaps other forms of synaptic plasticity that may underlie memory encoding (Cai et al. 2004; Faber et al. 2005; Ngo-Anh et al. 2005; Stackman et al. 2002).

Comparison with previous results on somatic excitability and AHPs

As already mentioned, our results appear to contradict several previous reports (Norris et al. 1998; Stackman et al. 2002; Stocker et al. 1999) and the widespread perception that SK channels are important generators of the mAHP in hippocampal pyramidal cells (Bildl et al. 2004; Bond et al. 1999, 2004; Bowden et al. 2001; Faber and Sah 2003; Melyan et al. 2002; Sah and Faber 2002; Sailer et al. 2002; Stocker 2004; Stocker et al. 2004).

It has been suggested (Stocker et al. 1999) that an SK channel component might have been overlooked in previous studies (Storm 1989) because bicuculline then was used routinely to block inhibitory synaptic input and various bicuculline salts have later been shown to be efficient SK channel blockers (Johnson and Seutin 1997). However, this explanation is clearly invalid for two reasons. First, in all our previous studies of AHPs (Pedarzani and Storm 1993, 1996; Storm 1987a,b, 1989), only the free base of bicuculline was always used for the explicit reason to avoid the risk of possible unknown side effects of the anions of bicuculline salts even before such side effects were discovered (Johnson and Seutin 1997). Therefore all salts of bicuculline that later were shown to block SK channels (Johnson and Seutin 1997) were always avoided in our previous studies; and bicuculline-free base, which was used in our studies, has later been shown to not block SK channels to any appreciable extent (Johnson and Seutin 1997) or block them by less than ~10% (Khawaled et al. 1999) at the concentration used previously (5 μM) (Storm 1989). Second, we show in this study, like that of Gu et al. (2005), that an SK channel contribution could not be detected even in the complete absence of bicuculline.

Another tentative explanation for the discrepancies might be related to the use of different recording techniques and intracellular media. Whereas the early studies used KCl-filled sharp microelectrodes, which was commonly used at that time to

achieve low, constant electrodes resistance for current- and voltage-clamp recording (Storm 1989; Williamson and Alger 1990), more recent studies that reported SK-channel involvement used whole cell recording with K-methylsulphate- or K-gluconate-based intracellular media (Stackman et al. 2002; Stocker et al. 1999). Because artificially high intracellular Cl^- concentration has been reported by some groups to cause severe rundown of Ca^{2+}-dependent K^+ channel activity (Zhang et al. 1994); but see (Storm 1987a, 1989), one might worry that the early studies failed to detect a significant SK component because KCl suppressed SK channel activity. However, this hypothesis was disproved in the present study. Thus we tested a wide range of recording conditions (Table 1), including conditions identical to those used by Stocker et al. (1999) (Fig. 5) and Stackman et al. (2002) (whole cell; K-methylsulphate-based intracellular media) and found that a SK component could still not be detected (Table 1).

A more likely explanation for the discrepancy is the following: several ionic currents, in particular I_M and Ca^{2+}-dependent K^+ currents in CA1 pyramidal cells, are prone to rundown during *whole cell* recording with a variety of intracellular media (see e.g., Fig. 2 in Zhang et al. 1994; Fig. 5*C* in Melyan et al. 2002; Fig. 6*E* in Sailer et al. 2002). It is therefore essential to study the time course of the effects of any kind of drug or manipulation as was done in our previous studies of AHPs (Pedarzani and Storm 1993, 1996; Sailer et al. 2002). This is particularly important in the absence of wash-out and recovery from SK channel blockade (Stackman et al. 2002; Stocker et al. 1999). Unfortunately, when time course plots are lacking (Stackman et al. 2002; Stocker et al. 1999), a steady rundown of channel activity, which occurs independently of drug application, might be mistaken for an effect of SK channel blockade.

Our results agree well with those of Yue and Yaari (2004), who showed that SK channel blockade by apamin failed to facilitate the somatic afterdepolarization (ADP) in these cells, whereas M-channel blockade had a strong effect. Our conclusion is also fully compatible with the finding that SK channel openers can enhance the mAHP in these cells (Pedarzani et al. 2001) as we also found (Fig. 5). The point is that the appearance of an SK component after artificial enhancement of SK channel activity does not indicate that these channels are involved in the mAHP under *normal* (drug-free) conditions. Our results are of course also compatible with the finding of Villalobos et al. (2004) that overexpression of SK1 or SK2 subunits in mice enhanced the mAHP in *neocortical* pyramidal cells. Furthermore, a recent study by Yoshida and Alonso (2007) showed that apamin had no significant effect on neuronal excitability, spike frequency adaptation, AHPs or ADPs in layer 5 pyramidal neurons in the rat entorhinal cortex, whereas M-channel blockade had clear effects on all these parameters (Yoshida and Alonso 2007). These findings closely parallel our results in rat and mouse hippocampal CA1–CA3 pyramidal cells in this and previous studies (Gu et al. 2005; Hu et al. 2007; Peters et al. 2005; Storm 1989) and suggest that the lack of SK channel impact on somatic spiking combined with strong M-channel effects applies to several, but not all (Fig. 6), classes of mammalian, cortical pyramidal cells.

An apamin-insensitive form of the human SK3-channel has been described (Wittekindt et al. 2004), and other toxin-insensitive forms may exist. However, it is unlikely that any of these, or any other Ca^{2+}-activated current (Alger et al. 1994; Brown and Griffith 1983), are important for the mAHP because the mAHP is largely resistant to Ca^{2+}-free medium and Ca^{2+} channel blockade (Gu et al. 2005; Storm 1989) as well as to the SK-channel blocker d-TC (Table 1 and Fig. 2*B*). Besides no orthologous form of the apamin-insensitive SK3 was found in rat (Wittekindt et al. 2004).

Although SK channels in CA1 pyramidal cells are readily activated by Ca^{2+} spikes evoked during blockade of Na^+ and some K^+ channels, as have been verified both in sharp electrode current-clamp (Fig. 1, *A* and *B*) and in whole cell voltage-clamp recordings (Sailer et al. 2002), they do not appear to be activated by the Ca^{2+} influx provided by somatic Na^+ action potentials not even by high-frequency bursts. There seem to be at least four, not mutually exclusive, possible explanations. First, the SK channels in these cells may need a more massive Ca^{2+} influx (Cai et al. 2004) than either the BK or sAHP channels, which both are activated by a single spike (Lancaster and Nicoll 1987; Marrion and Tavalin 1998; Storm 1987a, 1990). Second, the SK channels, unlike BK channels, may not be efficiently co-localized with the voltage-gated Ca^{2+} channels that open during a normal Na^+ spike (Lancaster and Nicoll 1987; Storm 1987b, 1990). Third, the sAHP channels may be more sensitive to $[Ca^{2+}]_i$ than are SK channels, or fourth, may be targeted to a different subcellular compartment (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005).

Recently Bloodgood and Sabatini (2007) and Ngo-Anh et al. (2005) reported evidence that SK channels in dendritic spines are specifically activated by CaV2.3 (R-type) voltage-gated Ca^{2+} channels in the spines. Because Na^+ action potentials backpropagate (bAPs) into the dendrites and spines (Bloodgood and Sabatini 2007; Koch and Zador 1993; Ngo-Anh et al. 2005), they open CaV2.3 channels and, hence, SK channels in the spines (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005). This would be expected to cause a local AHP, at least in the spine. However, because no apamin-sensitive AHP was observed in our somatic recording (Fig. 3), it seems likely that such local AHPs have too weak impact in the soma to be detected there due to filtering by the dendritic cable. Such filtering is probably strongly enhanced by the increased membrane conductance caused by other spike-evoked K^+ channels, e.g., Kv7/M and delayed rectifier (DR) channels. In contrast, the Ca^{2+}-spikes evoked in the presence of the K^+ channels blocker TEA may cause sufficient depolarization of the somatodendritc membrane to open more voltage-gated Ca^{2+} channels, probably of several types, thus causing stronger SK channel activation. In addition, TEA (which partly blocks M and DR channels) will reduce the spike-evoked K^+ conductance, thus reducing the filtering by the dendritic cable. These factors may explain why SK channels in CA1 pyramidal cells are readily activated by Ca^{2+} spikes and Ca^{2+} plateau potentials but not by Na^+ spikes.

However, our results from CA1 pyramidal cells obviously do not preclude that SK channels can generate Na^+ spike-evoked mAHPs in other types on neurons (Abel et al. 2004; Sah 1996; Sah and Faber 2002; Savic et al. 2001; Schwindt et al. 1988; Vogalis et al. 2003). On the contrary, we found that bursting pyramidal cells in the rat subiculum generate a prominent mAHP that is substantially reduced by SK channel blockade by apamin (Hu et al. 2004), and we have detected SK-dependent mAHPs also in rat neocortical pyramidal cells

(Abel et al. 2004; Foehring et al. 1989; Hu and Storm, unpublished data) and cerebellar Purkinje cells (Cingolani et al. 2002; Hu and Storm, unpublished results) but not in rat CA3 pyramidal cells (Sharifullina et al. 2008). Thus it appears that the hippocampal CA1 and CA3 pyramidal cells differ from several other cell types by having a Kv7/KCNQ/M-channel-dependent mAHP rather than an SK-channel dependent mAHP (Gu et al. 2005; Storm 1989).

Impact of SK channel activation during synaptic transmission

While the SK channels could evidently be activated by NMDAR-mediated EPSPs (Fig. 7), we observed no detectable effect of SK channel blockade on EPSP-evoked spike trains and mAHPs in the presence of the NMDAR blocker D-AP5 (Fig. 3 and Supplementary Fig. S4). In the latter experiments, D-AP5 was used both to prevent NMDAR-dependent synaptic plasticity (LTP and LTD) and to test whether SK channels could be activated by synaptically evoked action-potentials as opposed to NMDAR-mediated EPSPs alone. Again these results indicate that SK channels in CA1 pyramidal cells do not substantially contribute to feed-back regulation or AHPs of somatic spikes, whether triggered by somatic or dendritic, non-NMDAR-mediated input.

However, under normal conditions, without NMDAR blockers, it seems likely that the SK channels are activated by EPSPs, whether spikes are triggered or not (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005). The spikes probably help to remove the magnesium block of the NMDARs, thus increasing their contribution to Ca^{2+} influx and SK activation. Nevertheless, the occurrence of action potentials may secondarily tend to mask the SK-channel effects. Thus once action potentials occur, they activate several voltage- and calcium-gated currents, including the M current (Fig. 3, *A–C*), which dominate the subsequent response and may thus swamp the effects of EPSP- and NMDAR-activated SK channels. Therefore the SK channel effect may, paradoxically, be relatively more important for subthreshold EPSPs than for EPSP-evoked spike trains.

How can our finding that apamin had no detectable effect on the train of population spikes (Supplementary Fig. S6) be reconciled with the recent finding that dendritic spines contain SK channels that are specifically activated by local voltage-gated Ca^{2+} channels and/or NMDA channels (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005)? One possibility is that SK channels in spines were downregulated by prior LTP induction as recently shown by Lin et al. (2008). However, in the experiments shown in Fig. 7, SK channels would be expected to undergo a similar downregulation due to massive opening of NMDA channels. A second possibility is that our method of stimulation was not able to evoked sufficient local dendritic depolarization to strongly activate the high-threshold R-type Ca^{2+} channels and/or NMDA channels that have been reported to be necessary for SK channel activation (Bloodgood and Sabatini 2007). To strongly activate these channels, there may be a need for near coincident and spatially close synaptic activation. Thus ~15 to ~20 synapses on the same dendrite branch need to be activated within a few milliseconds to evoke a substantial dendritic Ca^{2+} response (Losonczy and Magee 2006). Although bAPs are also able to activate R-type Ca^{2+} channels under certain conditions (Sabatini and Svoboda 2000), this has only been shown for very proximal spines in the proximal dendritic branches (Losonczy and Magee 2006; Sabatini and Svoboda 2000), whereas more distal branches are likely to receive a much more attenuated bAPs. Our stimulation electrode was positioned in the middle of st. radiatum and >100 μm laterally displaced from the main dendritic axis of the neurons from which we recorded the population spikes, to avoid direct activation of the dendrites. Thus it is possible that our stimulation protocol activated synapses that were too dispersed along the dendritic tree to strongly activate high-threshold Ca^{2+} channels and/or unblock NMDA channels and, hence, open dendritic SK channels.

Implications for SK channel functions

We conclude that the SK channels appear to have no obvious function in feed-back regulation of somatic spiking in CA1 hippocampal pyramidal cells in response to non-NMDAR-mediated excitatory input. First, the hypothesis that they underlie the sAHP has been undermined by several convergent results (Stocker et al. 2004; Vogalis et al. 2003). Second, several lines of evidence, including the present study, indicate that they also do not contribute appreciably to the somatic mAHP and excitability control (Gu et al. 2005; Peters et al. 2005; Storm 1989).

However, the presence of a robust SK current in the CA1 pyramidal cells suggests that these channels are of functional importance in these cells. Convergent evidence from several recent studies, including the present study, supports the hypothesis that SK channels can be activated by glutamatergic synaptic input through activation of NMDARs or voltage-gated Ca^{2+} channels in CA1 pyramidal dendrites (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005). Thus SK evidence indicates that channels in dendritic spines are directly activated by Ca^{2+} influx through NMDAR channels or Cav2.3 (R-type) Ca^{2+} channels (Faber et al. 2005; Ngo-Anh et al. 2005; Stackman et al. 2002; Tzounopoulos and Stackman 2003). Thereby they curtail the postsynaptic depolarization, rapidly reinstate the magnesium block of the NMDAR channels, and limit the Ca^{2+} influx. This SK channel-mediated negative feed-back control of postsynaptic Ca^{2+} may explain how these channel regulate the induction of synaptic plasticity (Faber et al. 2005; Ngo-Anh et al. 2005; Stackman et al. 2002; Tzounopoulos and Stackman 2003).

These ideas are supported by the findings that overexpression of SK2 channels in the hippocampus decreased synaptically evoked glutamatergic EPSPs, attenuated LTP induced by 50-Hz stimulation in hippocampal slices and drastically impaired learning and memory in two hippocampal-dependent behavioral tasks (Hammond et al. 2006). Recent data from Lin et al. (2008) also indicate that SK2 channels are themselves downregulated by LTP and that this modulation contributes to LTP expression in hippocampal CA1 cells. Thus they found that pairing of synaptically evoked EPSPs with backpropagating action potentials abolished SK2 channel activity, thus contributing to the enhanced EPSPs that characterize LTP expression. Indications that apamin can improve learning and memory retention and increase immediate early gene expression in the hippocampus of mice (Heurteaux et al. 1993; Messier et al. 1991) further support the idea that hippocampal

SK channels have important functions in plasticity. Such functions may also be partly mediated by SK channels expressed in GABAergic interneurons (Savic et al. 2001; Zhang and McBain 1995).

That SK channels are also involved in other forms of neural plasticity is suggested by a recent study by Cai et al. (2007) that indicates that posttranslational downregulation of SK channel function in the distal dendrites of CA1 pyramidal cells contributes to deafferentation-induced reactive hyperexcitability. This new insight may open new possibilities for prevention or treatment of such conditions, e.g., in posttraumatic epilepsy. In addition, it is likely that SK channels also can regulate other forms of dendritic electrogenesis and may become engaged in different response patterns than those tested here.

ACKNOWLEDGMENTS

G. Nu performed all the intracellular and whole cell current-clamp experiments included in this study (including those presented Figs. 1–7, S1–S3, S5, and S6 and Table 1); H. Hu performed the experiments illustrated in Figs. 2*D* and 6 ($n = 5$) and pilot whole cell current-clamp experiments (data not shown); K. Vervaeke performed the extracellular field potential recordings illustrated in Fig. S4; J. F. Storm performed pilot intracellular current-clamp experiments (data not shown). We thank Dr. Jonathan Couey for technical assistance to N. Gu during visual patch recording. The paper was mainly written by N. Gu and J. F. Storm.

GRANTS

This study was supported by the Norwegian Research Council (NFR) via the Functional Genomics in Norway and Norwegian Centre of Excellence programs.

REFERENCES

Abel HJ, Lee JC, Callaway JC, Foehring RC. Relationships between intracellular calcium and afterhyperpolarizations in neocortical pyramidal neurons. *J Neurophysiol* 91: 324–335, 2004.

Alger BE, Nicoll RA. Epileptiform burst afterhyperolarization: calcium-dependent potassium potential in hippocampal CA1 pyramidal cells. *Science* 210: 1122–1124, 1980.

Alger BE, Sim JA, Brown DA. Single-channel activity correlated with medium-duration, Ca-dependent K current in cultured rat hippocampal neurones. *Neurosci Lett* 168: 23–28, 1994.

Andreasen M, Nedergaard S. Dendritic electrogenesis in rat hippocampal CA1 pyramidal neurons: functional aspects of Na^+ and Ca^{2+} currents in apical dendrites. *Hippocampus* 6: 79–95, 1996.

Bear MF. A synaptic basis for memory storage in the cerebral cortex. *Proc Natl Acad Sci USA* 93: 13453–13459, 1996.

Behnisch T, Reymann KG. Inhibition of apamin-sensitive calcium dependent potassium channels facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus in vitro. *Neurosci Lett* 253: 91–94, 1998.

Bildl W, Strassmaier T, Thurm H, Andersen J, Eble S, Oliver D, Knipper M, Mann M, Schulte U, Adelman JP, Fakler B. Protein kinase CK2 is coassembled with small conductance ca(2+)-activated k(+) channels and regulates channel gating. *Neuron* 43: 847–858, 2004.

Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232: 331–356, 1973.

Bloodgood BL, Sabatini BL. Nonlinear regulation of unitary synaptic signals by CaV(2.3) voltage-sensitive calcium channels located in dendritic spines. *Neuron* 53: 249–260, 2007.

Bond CT, Herson PS, Strassmaier T, Hammond R, Stackman R, Maylie J, Adelman JP. Small conductance Ca^{2+}-activated K^+ channel knock-out mice reveal the identity of calcium-dependent afterhyperpolarization currents. *J Neurosci* 24: 5301–5306, 2004.

Bond CT, Maylie J, Adelman JP. Small-conductance calcium-activated potassium channels. *Ann N Y Acad Sci* 868: 370–378, 1999.

Bond CT, Maylie J, Adelman JP. SK channels in excitability, pacemaking and synaptic integration. *Curr Opin Neurobiol* 15: 305–311, 2005.

Bowden SE, Fletcher S, Loane DJ, Marrion NV. Somatic colocalization of rat SK1 and D class (Ca(v) 1.2) L-type calcium channels in rat CA1 hippocampal pyramidal neurons. *J Neurosci* 21: RC175, 2001.

Brown DA, Griffith WH. Calcium-activated outward current in voltage-clamped hippocampal neurones of the guinea pig. *J Physiol* 337: 287–301, 1983.

Cai X, Liang CW, Muralidharan S, Kao JP, Tang CM, Thompson SM. Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* 44: 351–364, 2004.

Cai X, Wei DS, Gallagher SE, Bagal A, Mei YA, Kao JP, Thompson SM, Tang CM. Hyperexcitability of distal dendrites in hippocampal pyramidal cells after chronic partial deafferentation. *J Neurosci* 27: 59–68, 2007.

Chandy KG, Fantino E, Wittekindt O, Kalman K, Tong LL, Ho TH, Gutman GA, Crocq MA, Ganguli R, Nimgaonkar V, Morris-Rosendahl DJ, Gargus JJ. Isolation of a novel potassium channel gene hSKCa3 containing a polymorphic CAG repeat: a candidate for schizophrenia and bipolar disorder? *Mol Psychiatry* 3: 32–37, 1998.

Cingolani LA, Gymnopoulos M, Boccaccio A, Stocker M, Pedarzani P. Developmental regulation of small-conductance Ca^{2+}-activated K^+ channel expression and function in rat Purkinje neurons. *J Neurosci* 22: 4456–4467, 2002.

Faber ES, Delaney AJ, Sah P. SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala. *Nat Neurosci* 8: 635–641, 2005.

Faber ES, Sah P. Calcium-activated potassium channels: multiple contributions to neuronal function. *Neuroscientist* 9: 181–194, 2003.

Faber ES, Sah P. Functions of SK channels in central neurons. *Clin Exp Pharmacol Physiol* 34: 1077–1083, 2007.

Foehring RC, Schwindt PC, Crill WE. Norepinephrine selectively reduces slow Ca^{2+}- and Na^+-mediated K^+ currents in cat neocortical neurons. *J Neurophysiol* 61: 245–256, 1989.

Gu N, Vervaeke K, Hu H, Storm JF. Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. *J Physiol* 566: 689–715, 2005.

Hammond RS, Bond CT, Strassmaier T, Ngo-Anh TJ, Adelman JP, Maylie J, Stackman RW. Small-conductance Ca^{2+}-activated K^+ channel type 2 (SK2) modulates hippocampal learning, memory, and synaptic plasticity. *J Neurosci* 26: 1844–1853, 2006.

Heurteaux C, Messier C, Destrade C, Lazdunski M. Memory processing and apamin induce immediate early gene expression in mouse brain. *Brain Res Mol Brain Res* 18: 17–22, 1993.

Hille B. *Ion Channels of Excitable Membranes.* Sunderland, MA: Sinauer, 2001.

Hu H, Gu N, Storm JF. Differential contribution of apamin-sensitive SK potassium channels to afterhyperpolarizations (mAHP) in subicular and CA1 rat hippocampal pyramidal neurons. *FENS Forum Abstr* 2: A082.7, 2004.

Hu H, Vervaeke K, Storm JF. M-channels (Kv7/KCNQ channels) that regulate synaptic integration, excitability, and spike pattern of CA1 pyramidal cells are located in the perisomatic region. *J Neurosci* 27: 1853–1867, 2007.

Johnson SW, Seutin V. Bicuculline methiodide potentiates NMDA-dependent burst firing in rat dopamine neurons by blocking apamin-sensitive Ca^{2+}-activated K^+ currents. *Neurosci Lett* 231: 13–16, 1997.

Kaczorowski CC, Disterhoft J, Spruston N. Stability and plasticity of intrinsic membrane properties in hippocampal CA1 pyramidal neurons: effects of internal anions. *J Physiol* 578: 799–818, 2007.

Kelly T, Church J. pH Modulation of currents that contribute to the medium and slow afterhyperpolarizations in rat CA1 pyramidal neurons. *J Physiol* 554: 449–466, 2004.

Khawaled R, Bruening-Wright A, Adelman JP, Maylie J. Bicuculline block of small-conductance calcium-activated potassium channels. *Pfluegers* 438: 314–321, 1999.

Knox RJ, Jonas EA, Kao LS, Smith PJ, Connor JA, Kaczmarek LK. Ca^{2+} influx and activation of a cation current are coupled to intracellular Ca^{2+} release in peptidergic neurons of *Aplysia californica. J Physiol* 494: 627–639, 1996.

Koch C, Zador A. The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *J Neurosci* 13: 413–422, 1993.

Kohler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, Maylie J, Adelman JP. Small-conductance, calcium-activated potassium channels from mammalian brain. *Science* 273: 1709–1714, 1996.

Kramar EA, Lin B, Lin CY, Arai AC, Gall CM, Lynch G. A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. *J Neurosci* 24: 5151–5161, 2004.

Lancaster B, Nicoll RA. Properties of two calcium-activated hyperpolarizations in rat hippocampal neurons. *J Physiol* 389: 187–203, 1987.

Lin MT, Lujan R, Watanabe M, Adelman JP, Maylie J. SK2 channel plasticity contributes to LTP at Schaffer collateral-CA1 synapses. *Nat Neurosci* 11: 170–177, 2008.

Losonczy A, Magee JC. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* 50: 291–307, 2006.

Madison DV, Nicoll RA. Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature* 299: 636–638, 1982.

Madison DV, Nicoll RA. Cyclic adenosine 3′,5′-monophosphate mediates beta-receptor actions of noradrenaline in rat hippocampal pyramidal cells. *J Physiol* 372: 245–259, 1986.

Magee JC, Carruth M. Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. *J Neurophysiol* 82: 1895–1901, 1999.

Magee JC, Johnston D. Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *J Physiol* 487: 67–90, 1995.

Marrion NV, Tavalin SJ. Selective activation of Ca^{2+}-activated K^+ channels by co-localized Ca^{2+} channels in hippocampal neurons. *Nature* 395: 900–905, 1998.

Melyan Z, Wheal HV, Lancaster B. Metabotropic-mediated kainate receptor regulation of IsAHP and excitability in pyramidal cells. *Neuron* 34: 107–114, 2002.

Messier C, Mourre C, Bontempi B, Sif J, Lazdunski M, Destrade C. Effect of apamin, a toxin that inhibits Ca(2+)-dependent K^+ channels, on learning and memory processes. *Brain Res* 551: 322–326, 1991.

Nakamura T, Barbara JG, Nakamura K, Ross WN. Synergistic release of Ca^{2+} from IP3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. *Neuron* 24: 727–737, 1999.

Ngo-Anh TJ, Bloodgood BL, Lin M, Sabatini BL, Maylie J, Adelman JP. SK channels and NMDA receptors form a Ca^{2+}-mediated feedback loop in dendritic spines. *Nat Neurosci* 8: 642–649, 2005.

Norris CM, Halpain S, Foster TC. Reversal of age-related alterations in synaptic plasticity by blockade of L-type Ca^{2+} channels. *J Neurosci* 18: 3171–3179, 1998.

Pape HC, Driesang RB. Ionic mechanisms of intrinsic oscillations in neurons of the basolateral amygdaloid complex. *J Neurophysiol* 79: 217–226, 1998.

Pedarzani P, Mosbacher J, Rivard A, Cingolani LA, Oliver D, Stocker M, Adelman JP, Fakler B. Control of electrical activity in central neurons by modulating the gating of small conductance Ca^{2+}-activated K^+ channels. *J Biol Chem* 276: 9762–9769, 2001.

Pedarzani P, Storm JF. PKA mediates the effects of monoamine transmitters on the K^+ current underlying the slow spike frequency adaptation in hippocampal neurons. *Neuron* 11: 1023–1035, 1993.

Pedarzani P, Storm JF. Interaction between alpha- and beta-adrenergic receptor agonists modulating the slow Ca(2+)-activated K^+ current I_{AHP} in hippocampal neurons. *Eur J Neurosci* 8: 2098–2110, 1996.

Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. *Nat Neurosci* 8: 51–60, 2005.

Pineda JC, Galarraga E, Bargas J, Cristancho M, Aceves J. Charybdotoxin and apamin sensitivity of the calcium-dependent repolarization and the afterhyperpolarization in neostriatal neurons. *J Neurophysiol* 68: 287–294, 1992.

Sabatini BL, Svoboda K. Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* 408: 589–593, 2000.

Sah P. Ca(2+)-activated K^+ currents in neurons: types, physiological roles and modulation. *Trends Neurosci* 19: 150–154, 1996.

Sah P, Faber ES. Channels underlying neuronal calcium-activated potassium currents. *Prog Neurobiol* 66: 345–353, 2002.

Sailer CA, Hu H, Kaufmann WA, Trieb M, Schwarzer C, Storm JF, Knaus HG. Regional differences in distribution and functional expression of small-conductance Ca^{2+}-activated K^+ channels in rat brain. *J Neurosci* 22: 9698–9707, 2002.

Savic N, Pedarzani P, Sciancalepore M. Medium afterhyperpolarization and firing pattern modulation in interneurons of stratum radiatum in the CA3 hippocampal region. *J Neurophysiol* 85: 1986–1997, 2001.

Schwindt PC, Spain WJ, Foehring RC, Stafstrom CE, Chubb MC, Crill WE. Multiple potassium conductances and their functions in neurons from cat sensorimotor cortex in vitro. *J Neurophysiol* 59: 424–449, 1988.

Shah M, Haylett DG. The pharmacology of hSK1 Ca^{2+}-activated K^+ channels expressed in mammalian cell lines. *Br J Pharmacol* 129: 627–630, 2000.

Shah MM, Javadzadeh-Tabatabaie M, Benton DC, Ganellin CR, Haylett DG. Enhancement of hippocampal pyramidal cell excitability by the novel selective slow-afterhyperpolarization channel blocker 3-(triphenylmethyl-aminomethyl)pyridine (UCL2077). *Mol Pharmacol* 70: 1494–1502, 2006.

Sharifullina E, Le Q, Isbrandt D, Storm JF. Kv7/KCNQ/M channels underlie control of excitability, subthreshold oscillations and medium after-hyperpolarization in rat and mouse CA3 hippocampal pyramidal cells. *FENS Forum Abstr* 4: 078.29, 2008.

Stackman RW, Hammond RS, Linardatos E, Gerlach A, Maylie J, Adelman JP, Tzounopoulos T. Small conductance Ca^{2+}-activated K^+ channels modulate synaptic plasticity and memory encoding. *J Neurosci* 22: 10163–10171, 2002.

Staff NP, Jung HY, Thiagarajan T, Yao M, Spruston N. Resting and active properties of pyramidal neurons in subiculum and CA1 of rat hippocampus. *J Neurophysiol* 84: 2398–2408, 2000.

Stocker M. Ca(2+)-activated K(+) channels: molecular determinants and function of the SK family. *Nat Rev Neurosci* 5: 758–770, 2004.

Stocker M, Hirzel K, D'hoedt D, Pedarzani P. Matching molecules to function: neuronal Ca(2+)-activated K(+) channels and afterhyperpolarizations. *Toxicon* 43: 933–949, 2004.

Stocker M, Krause M, Pedarzani P. An apamin-sensitive Ca^{2+}-activated K^+ current in hippocampal pyramidal neurons. *Proc Natl Acad Sci USA* 96: 4662–4667, 1999.

Stocker M, Pedarzani P. Differential distribution of three Ca(2+)-activated K(+) channel subunits, SK1, SK2, and SK3, in the adult rat central nervous system. *Mol Cell Neurosci* 15: 476–493, 2000.

Storm JF. Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. *J Physiol* 385: 733–759, 1987a.

Storm JF. Intracellular injection of a Ca^{2+} chelator inhibits spike repolarization in hippocampal neurons. *Brain Res* 435: 387–392, 1987b.

Storm JF. An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells. *J Physiol* 409: 171–190, 1989.

Storm JF. Potassium currents in hippocampal pyramidal cells. *Prog Brain Res* 83: 161–187, 1990.

Strobaek D, Jorgensen TD, Christophersen P, Ahring PK, Olesen SP. Pharmacological characterization of small-conductance Ca(2+)-activated K(+) channels stably expressed in HEK 293 cells. *Br J Pharmacol* 129: 991–999, 2000.

Takahashi T. Membrane currents in visually identified motoneurons of neonatal rat spinal cord. *J Physiol* 423: 27–46, 1990.

Terstappen GC, Pula G, Carignani C, Chen MX, Roncarati R. Pharmacological characterisation of the human small conductance calcium-activated potassium channel hSK3 reveals sensitivity to tricyclic antidepressants and antipsychotic phenothiazines. *Neuropharmacology* 40: 772–783, 2001.

Tzounopoulos T, Stackman R. Enhancing synaptic plasticity and memory: a role for small-conductance Ca(2+)-activated K^+ channels. *Neuroscientist* 9: 434–439, 2003.

Vergara C, Latorre R, Marrion NV, Adelman JP. Calcium-activated potassium channels. *Curr Opin Neurobiol* 8: 321–329, 1998.

Vervaeke K, Gu N, Agdestein C, Hu H, Storm JF. Kv7/KCNQ/M-channels in rat glutamatergic hippocampal axons and their role in regulation of excitability and transmitter release. *J Physiol* 576: 235–256, 2006.

Viana F, Bayliss DA, Berger AJ. Multiple potassium conductances and their role in action potential repolarization and repetitive firing behavior of neonatal rat hypoglossal motoneurons. *J Neurophysiol* 69: 2150–2163, 1993.

Villalobos C, Shakkottai VG, Chandy KG, Michelhaugh SK, Andrade R. SKCa channels mediate the medium but not the slow calcium-activated afterhyperpolarization in cortical neurons. *J Neurosci* 24: 3537–3542, 2004.

Vogalis F, Storm JF, Lancaster B. SK channels and the varieties of slow after-hyperpolarizations in neurons. *Eur J Neurosci* 18: 3155–3166, 2003.

Williamson A, Alger BE. Characterization of an early afterhyperpolarization after a brief train of action potentials in rat hippocampal neurons in vitro. *J Neurophysiol* 63: 72–81, 1990.

Wittekindt OH, Visan V, Tomita H, Imtiaz F, Gargus JJ, Lehmann-Horn F, Grissmer S, Morris-Rosendahl DJ. An apamin- and scyllatoxin-insensitive isoform of the human SK3 channel. *Mol Pharmacol* 65: 788–801, 2004.

Yoshida M, Alonso A. Cell-type specific modulation of intrinsic firing properties and subthreshold membrane oscillations by the m(kv7)-current in neurons of the entorhinal cortex. *J Neurophysiol* 98: 2779–2794, 2007.

Yue C, Yaari Y. KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. *J Neurosci* 24: 4614–4624, 2004.

Zhang L, McBain CJ. Potassium conductances underlying repolarization and after-hyperpolarization in rat CA1 hippocampal interneurons. *J Physiol* 488: 661–672, 1995.

Zhang L, Weiner JL, Valiante TA, Velumian AA, Watson PL, Jahromi SS, Schertzer S, Pennefather P, Carlen PL. Whole-cell recording of the Ca(2+)-dependent slow afterhyperpolarization in hippocampal neurons: effects of internally applied anions. *Pfluegers* 426: 247–253, 1994.