Calcium-Activated Afterhyperpolarizations Regulate Synchronization and Timing of Epileptiform Bursts in Hippocampal CA3 Pyramidal Neurons

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Submitted 25 April 2006; accepted in final form 30 August 2006

Fernández de Sevilla, David, Julieta Garduño, Emilio Galván, and Washington Buño. Calcium-activated afterhyperpolarizations regulate synchronization and timing of epileptiform bursts in hippocampal CA3 pyramidal neurons. J Neurophysiol 96: 3028–3041, 2006. First published September 13, 2006; doi:10.1152/jn.00434.2006. Calcium-activated potassium conductances regulate neuronal excitability, but their role in epileptogenesis remains elusive. We investigated in rat CA3 pyramidal neurons the contribution of the Ca$$^{2+}$$-activated K$$^-$$-mediated afterhyperpolarizations (AHPs) in the genesis and regulation of epileptiform activity induced in vitro by 4-aminopyridine (4-AP) in Mg$$^{2+}$$-free Ringer. Recurring spike bursts terminated by prolonged AHPs were generated. Burst synchronization between CA3 pyramidal neurons in paired recordings typified this interictal-like activity. A downregulation of the medium afterhyperpolarization (mAHP) paralleled the emergence of the interictal-like activity. When the mAHP was reduced or enhanced by apamin and EBIO bursts induced by 4-AP were increased or blocked, respectively. Inhibition of the slow afterhyperpolarization (sAHP) with carbachol, t-ACPD, or isoproterenol increased bursting frequency and disrupted burst regularity and synchronization between pyramidal neuron pairs. In contrast, enhancing the sAHP by intracellular dialysis with KMeSO$$\text{$_4$}$ reduced burst frequency. Block of GABA$$\text{$_A$}$$-A$$\text{$_H$}$$ inhibition did not modify the abnormal activity. We describe novel cellular mechanisms where 1) the inhibition of the mAHP plays an essential role in the genesis and regulation of the bursting activity by reducing negative feedback, 2) the sAHP sets the interburst interval by decreasing excitability, and 3) bursting was synchronized by excitatory synaptic interactions that increased in advance and during bursts and decreased throughout the subsequent sAHP. These cellular mechanisms are active in the CA3 region, where epileptiform activity is initiated, and cooperatively regulate the timing of the synchronized rhythmic interictal-like network activity.

INTRODUCTION

Changes in neuronal excitability and synaptic efficacy contribute to induce the coordinated network activity needed to generate abnormal epileptiform bursting. In hippocampal pyramidal neurons a key role in the control of excitability is carried out by Ca$$^{2+}$$-activated K$$^-$$ currents with medium (mI$$\text{AHP}$$) and slow (sI$$\text{AHP}$$) deactivation kinetics that mediate the medium AHP (mAHP) and the slow AHP (sAHP), respectively (Borde et al. 1995, 2000; Carrer et al. 2003; Sah and Bekkers 1996; reviewed in Sah and Faber 2002; Stocker 2004; Storm 1987; Vogalis et al. 2003). It has been recognized that an abnormal regulation of the sI$$\text{AHP}$/mAHP$ may contribute to epileptogenesis (Alger and Nicoll 1980; Alger and Williamson 1988; Martín et al. 2001; Matsumoto and Ajmone-Marsan 1964; Traub et al. 1993; reviewed in de Curtis and Avanzini 2001; McCormick and Contreras 2001). However, important aspects of the contribution of the sAHP to abnormal hyperexcitable states remain under debate. In addition, little attention has been paid to the contribution of the mI$$\text{AHP}$/mAHP$ to epileptogenesis (Alger and Williamson 1988; Empson and Jefferys 2001; Garduño et al. 2005; McCown and Breese 1990; Verma-Ahuja et al. 1998).

We centered our analysis on the contribution of both I$$\text{AHP}$/mAHP$ to epileptogenesis in CA3 pyramidal neurons, the region where hippocampal epileptiform activity is initiated (Colom and Saggau 1994; Luhmann et al. 2000; MacVicar and Dudek 1982; Miles and Wong 1983; Schwartzkroin and Prince 1978). We show that 1) a downregulation of the mI$$\text{AHP}$/mAHP$ paralleled the emergence of epileptiform bursting; 2) when the mI$$\text{AHP}$/mAHP$ was reduced or enhanced by pharmacological manipulations, bursts were increased or blocked, respectively; 3) manipulations that decreased the sI$$\text{AHP}$/mAHP$ increased bursting frequency and decreased network synchronization; 4) in contrast, increasing the sI$$\text{AHP}$/mAHP$ reduced bursting frequency; and 5) bursting in pyramidal neuron pairs was synchronized by excitatory synaptic interaction that increased shortly in advance and during bursts and decreased throughout the subsequent sAHP. The rhythmic bursting network activity that characterizes CA3 epileptogenesis is regulated by intrinsic cellular mechanisms where the mAHP and the sAHP play different roles, but nevertheless act cooperatively to regulate the synchronized bursting that characterizes the interictal-like network activity in the CA3 region. These cellular mechanisms may also be an integral part in the normal function of hippocampal region by regulating networks dynamics, such as the theta rhythm, where bursts of synchronous population activity occur (e.g., Buño et al. 1978) and are reset by interictal spikes in vivo (Lerma et al. 1984).

METHODS

Slice preparation

Procedures of animal care, surgery, and slice preparation were in accordance with the guidelines laid down by the European Communities Council. Juvenile Wistar rats (12–15 days) were decapitated and their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). The composition of the ACSF was (in mM): 124 NaCl, 2.69 KCl, 1.25 KH$$\text{PO}_4$, 2 Mg$$\text{SO}_4$, 26 NaHCO$$\text{$_3$}$, 2 CaCl$$\text{$_2$}$, and 10 glucose. The ACSF was continuously gassed with a 95% O$$\text{$_2$}$/5% CO$$\text{$_2$}$ mixture to attain a pH of 7.3–7.4. Transverse hippocampal slices (400$$\mu$$m thick) were prepared using a Vibratome (Pelco 101, Series 1000, St. Louis, MO), incubated >1 h at room temperature (20–22°C), and were transferred to a recording chamber (about 1 ml) placed on an inverted (Nikon TMS, Tokyo, Japan).

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Electrophysiology

Whole cell recordings from pyramidal cells placed in the ventral branch of the CA3 region (Fig. 1A) were both in the current- and voltage-clamp modes with (4–8 Ω) patch-pipettes connected to an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA). Pipettes were filled with a solution that contained (in mM): 135 K-glucuronate, 1 EGTA, 10 KCl, 10 HEPES, 2 ATP, 0.4 GTP, and 1 MgCl₂, buffered to pH 7.2–7.3 with KOH.

In some experiments a pipette solution designed to block K⁺-mediated conductances was used that contained (in mM): 107.5 Cs-glucuronate, 1 EGTA, 20 HEPEP, 8 NaCl, 1 MgCl₂, 2 ATP, and 0.4 GTP. Paired recordings were performed with the same methodology except that an additional EPC-7 amplifier (List Electronic; Darmstadt, Germany) was used. In some cases one of the electrodes used for paired recordings was filled with a solution that contained (in mM): J) Cs-glucuronate that substituted K-glucuronate; 2) 150 KMeSO₄ (ICN Pharmaceuticals, Costa Mesa, CA), 1 EGTA, 10 HEPEP, 4 ATP, and 0.4 GTP; or 3) 40 mM 2-bis-(2-aminophenoxy)-ethane-N,N',N'-tetraacetic acid (BAPTA). Experiments started after a 15- to 20-min stabilization period after establishing the whole-cell configuration and both current- and voltage-clamp recordings were used as needed to analyze the abnormal activity and the underlying currents, respectively. In voltage-clamp experiments, the holding potential (Vₜ) was adjusted to −60 or −50 mV and current-clamp recordings were made at the resting membrane potential (Vₘ), except if indicated otherwise (e.g., voltage- and current-clamp measurements of the mAHP/sAHPs were made at a Vₜ of −50 mV and by setting the Vₘ at −50 mV by continuous current injection, respectively). The mAHP and sAHP were activated under voltage clamp by a depolarizing voltage command pulse (duration 200 ms, from the Vₚ to 10 mV) and the mAHP and sAHP were activated under current clamp by a 200-ms depolarizing current pulse at intensities that evoked a spike burst. Experiments were rejected if the series resistance (10–20 MΩ) changed >20% or the membrane resting Vₚ dropped to <−50 mV during recordings. pClamp software (Axon Instruments) was used for stimulus generation, data display, acquisition, storage, and analysis.

Stimulation

Synaptic responses were evoked by mossy fiber (MF) stimulation through a pair of nichrome wires (Ø 60 μm) separated about 100 μm, insulated except at the tips, and placed in the stratum lucidum about 500 μm away from the recorded neuron (Fig. 1A). Electrodes were connected to a stimulator unit (Cibermed, Madrid, Spain) driven by the Clampex program (Axon Instruments). Stimulation was delivered at a rate of 3–5 Hz and included three or four parallel pulses per 60 s (500 Hz). Synaptic responses were evoked by brief (20 ms) current injection, respectively. The mAHP and sAHP were activated under voltage clamp by a depolarizing voltage command pulse (duration 200 ms, from the Vₚ to 10 mV) and the mAHP and sAHP were activated under current clamp by a 200-ms depolarizing current pulse at intensities that evoked a spike burst. Experiments were rejected if the series resistance (10–20 MΩ) changed >20% or the membrane resting Vₚ dropped to <−50 mV during recordings. pClamp software (Axon Instruments) was used for stimulus generation, data display, acquisition, storage, and analysis.

Induction of epileptiform activity

Epileptiform activity was induced with 50–100 μM 4-AP added to a modified Mg²⁺-free ACSF that contained (in mM): 124 NaCl, 2.69 KCl, 1.25 KH₂PO₄, 26 NaHCO₃, 2 CaCl₂, and 10 glucose. 4-AP increases excitability and presynaptic glutamate release by block of the transient A-type K⁺-mediated current (Iₐ). Responses mediated by release glutamate by N-methyl-d-aspartate (NMDA)–receptor activation are enhanced in Mg²⁺-free solutions by relieving the voltage-dependent block by extracellular Mg²⁺. We previously showed that 4-AP + Mg²⁺-free and 4-AP in control ACSF had identical epileptogenic effects in the CA1 region (Martín et al. 2001). We used the Mg²⁺-free ACSF because the induction of epileptiform activity was faster and stable for a longer period of time than with 4-AP per se (as tested in six cells not included in this study).

Pharmacology

All the following drugs were added to the solutions and superfused in some experiments: Picrotoxin (PTX; 40 μM), to block γ-aminobutyric acid (GABA)–mediated synaptic inhibition, and saclofen (100 μM), to block GABAₐ inhibition. Bicuculline (50 μM), to block GABAₐ inhibition; the drug also inhibits the mAHP/mAHP (Debarbieux et al. 1998; Stocker et al. 1999). Apamin (100 nM), which specifically blocks small conductance (SK) Ca²⁺-activated K⁺-mediated channels and the mAHP/mAHP (see DISCUSSION). EBIO (1-ethyl-2-benzimidazolinone), which enhances channel activity and the Ca²⁺-activated K⁺ current (Debarbieux et al. 1998; Garduño et al. 2004). EBIO was prepared as a stock solution in DMSO, stored at −18°C, diluted before use, and added at concentrations between 200 μM and 1 mM. The DMSO at the concentrations used had no effect on membrane properties or synaptic potentials (n = 4; Garduño et al. 2005). t-ACPD [(±)-1-amino cyclopentane-trans-1,3-dicarboxylic acid, 20 μM], a nonselective metabotropic glutamate receptor (mGluR) agonist; carbacol (CCh, 10 μM), a wide-spectrum nonhydrolysable cholinergic agonist; or isoproterenol (5–10 μM), a β-adrenergic agonist—all three are unspecific blockers of the mAHP/sAHPs. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 20 μM), which specifically blocks non-NMDA glutamate receptors. In addition, atropine (10 μM) that inhibits muscarinic receptors, MCGP ([S]-α-methyl-4-carboxyphenylglycine, 0.5–1.0 mM), or LY341495 (20 μM), group I and group II mGluR antagonists, were superfused throughout the experiment and starting ≥10 min before switching to the 4-AP
solution. Chemicals were purchased from Sigma (St. Louis, MO), Tocris Cookson (Bristol, UK), and Alomone Labs (Jerusalem, Israel).

RESULTS

Results are based on 137 pyramidal neurons from the ventral branch of the CA3 region (Fig. 1A) that were recorded at room temperature and exhibited a stable mean \( V_m \) of \(-69.3 \pm 1.9 \) mV and an input resistance \( (R_m) \) of 195.5 \pm 33.3 \( \Omega \). In addition, experiments \((n = 18)\) were also performed at 32–34°C and the mean \( V_m \) was \(-62.3 \pm 2.1 \) mV and the \( R_m \) 175.5 \pm 45.8 \( \Omega \), respectively. Neurons were silent in control conditions and either an initial burst followed by a silence \((n = 48\) or roughly 35\%\) or a sustained response with little frequency adaptation \((n = 89\) or roughly 65\%) were evoked by suprathreshold depolarizing pulses. The \( V_m \) and \( R_m \) were not statistically different in both neuron types and responses evoked by hyperpolarizing pulses always displayed repolarizing sag that was essentially identical in bursting and slowly adapting neurons (Fig. 1A).

Evolution of epileptiform activity

The AHP terminates epileptiform bursts. In current-clamp conditions pyramidal neurons did not show spontaneous spike activity in control ACSF (i.e., were “silent”), did not reveal \( V_m \) oscillation, and spontaneous synaptic activity was scarce (Current-clamp, Fig. 1C). Superfusion with 4-AP (50–100 \( \mu \)M) induced epileptiform activity in all the CA3 pyramidal neurons analyzed \((n = 104)\). The abnormal activity started after nearly 10–15 min of superfusion with the 4-AP solution and was initially characterized by repetitive single spikes and spike bursts. This mixed activity rapidly changed to continuous spike bursts \((3.3 \pm 0.1\) spikes, 258.2 \pm 30.9 ms duration; at 0.23 \pm 0.03 s\(^{-1}\); \( n = 35\), selected at random from the sample) and stabilized in about 10–20 min (Luhmann et al. 2000; Perreault and Avoli 1992). Each burst rode on a large and prolonged depolarizing wave termed paroxysmal depolarization shift (PDS) that was always terminated by an AHP (Fig. 1E) (Garduno et al. 2005; Goldensohn and Purpura 1963; Martin et al. 2001; Matsumoto and Ajmone-Marsan 1964; reviewed in Avoli et al. 2002; de Curtis and Avanzini 2001; McCormick and Contreras 2001). Brief volleys of synaptic activity could precede or follow spike bursts but were absent or markedly reduced during the sAHP (Voltage-clamp, Fig. 1E). The postburst AHP could display an initial faster and a subsequent slower component (Current-clamp, Fig. 1E). The AHP component with faster decay kinetics \((30–90\) ms; 5.1 \pm 1.8 mV; \( n = 35\)) in control conditions, measured at \(-50\) mV could be reduced in amplitude (by 72.2 \pm 9.3\% from control values; 10/35 cells or roughly 29\% measured at \(-50\) mV) or disappear altogether \((25/35\) neurons or roughly 71\%) during the evolution of the epileptiform activity (continuous arrows, Current-clamp, Fig. 1E). In contrast, the AHP with slower decay kinetics was always present, it decayed to baseline in 3.7 \pm 0.2 s, had a peak amplitude of 10.1 \pm 0.3 mV \((n = 35)\), and did not change \((97 \pm 5.7\%\) of control; \( P > 0.05\); same cells, measured at \(-50\) mV) with time during the epileptiform activity (interrupted arrows, Current-clamp, Fig. 1E). The epileptogenic effects of the 4-AP reverted after a prolonged washout \((>20\) min; \( n = 12)\). We centered our analysis on this type of bursting that has been termed interictal-like activity (Avoli et al. 1993; reviewed in Avoli et al. 2002; de Curtis and Avanzini 2001; McCormick and Contreras 2001). In some cases \((10/35\) cells or roughly 29\%), ictal-like activity was also generated, characterized by bursts at higher frequency, riding on a sustained depolarization (Schiller 2004; Traub et al. 1993; reviewed in Avoli et al. 2002; de Curtis and Avanzini 2001; McCormick and Contreras 2001), and not followed by AHPs (see following text).

It is noteworthy that both during the mixed initial bursting—single-spke activity and the subsequent interictal-like activity bursts were essentially identical and always synchronized in paired recordings, indicating that similar population activity was occurring in the network during both periods (see following text).

We also calculated autocorrelation functions that provide an estimation of the membrane potential oscillations during the control and abnormal interictal-like activity \((n = 10)\). In control conditions autocorrelations were flat (Current-clamp, Fig. 1D), consistent with the absence of oscillations. During the interictal-like activity autocorrelations revealed periodic peaks separated by slow waves (Current-clamp, Fig. 1F), in harmony with the rhythmic repetitive PDS topped by bursts followed by AHPs.

There were no significant differences between the above-described activity recorded at room temperature and the one induced by 4-AP at 32–34°C \((3.8 \pm 0.6\) spikes, 300.4 \pm 42.8-ms duration; at 0.33 \pm 0.06 s\(^{-1}\); \( n = 10\) and the faster \((30–90\) ms; 4.5 \pm 1.5 mV; \( n = 10\) and slower \((2.8 \pm 0.4 s; 5.6 \pm 0.9 mV)\) AHPs. In addition, both the mAHP and sAHP showed similar behaviors, with the former decreasing or disappearing with the evolution of the abnormal activity and the latter remaining unchanged, respectively.

Under voltage-clamp mode cells were silent in control conditions (Voltage-clamp, Fig. 1D) and the abnormal activity evoked in all cells \((n = 33)\) by the 4-AP challenge \((100\) \( \mu \)M) was initially \((about 10–15 min)\) typified by single or bursts of “unclamped” action currents riding on an inward current wave followed by a long-lasting outward “tail” current. The inward and outward currents correspond to the PDS and the sAHP recorded under current-clamp mode, respectively. The activity then stabilized at a frequency of 0.18 \pm 0.02 s\(^{-1}\) after about 20 min of superfusion with the 4-AP solution. Burst duration was 170.7 \pm 23.9 ms and the number of action currents per burst was 2.7 \pm 0.2, respectively \((n = 33)\). Differences in the characteristics of the abnormal activity under current- and voltage-clamp modes may be explained by the distinct recording methods.

The large prolonged outward current that followed bursts could show \((12/33\) cells or roughly 36\%) an early, briefer higher-amplitude \((50–150\) ms, 39.8 \pm 9.8 pA, measured at \(-50\) mV in control conditions) component that usually disappeared with the evolution of the epileptiform activity \((9/12 or 75\%)\) or was substantially reduced in amplitude \((by 65 \pm 9.3\%\) from control values; 3/12 cells or 25\%, measured at \(-50\) mV) (filled arrows in Voltage-clamp, Fig. 1E). A slower current \((25.7 \pm 1.3 pA,\) decay \( t_\alpha = 5.6 \pm 2.6 s; n = 33,\) measured at \(-50\) mV) was always present and did not change \((102 \pm 9.1\% ; P > 0.05; same cells)\) during epileptiform activity (Voltage-clamp, Fig. 1E). Accordingly, the corresponding autocorrelation functions were flat in control conditions (Voltage-clamp, Fig. 1D).
and showed periodic peaks at the bursting frequency during epileptiform activity (Voltage-clamp, Fig. 1F). The spontaneous synaptic activity was clearly reduced during the slow outward currents (open arrows, Voltage-clamp, Fig. 1E).

**Synaptic inhibition does not contribute to the AHPs**

Voltage- and Ca\(^{2+}\)-activated K\(^+\) conductances or synaptic inhibition could contribute to the afterhyperpolarization. Block of GABA\(_A\) inhibition with PTX (40 \(\mu M\)) did not change the frequency of epileptiform burst (0.16 ± 0.01 s\(^{-1}\); \(P > 0.05\); \(n = 8\)), burst duration (179.4 ± 22.1 ms; \(P > 0.05\); same cells), number of action currents per burst (3.0 ± 0.1 \(P > 0.05\); same cells), nor the amplitude and duration (26.5 ± 0.3 pA; 5.2 ± 0.9 s; \(P > 0.05\), same neurons) of the outward current that follows epileptiform bursts (Fig. 2, A–C). Moreover, block of GABA\(_B\) inhibition with saclofen (100 \(\mu M\)) did not modify the epileptiform activity, the peak amplitude (89.6 ± 10.7% of control; \(P > 0.05\); \(n = 5\)) of the outward current, or the area under the outward current (91.3 ± 13% of control; \(P > 0.05\); \(n = 5\); Fig. 2D). The values shown correspond to measurements performed when the interictal-like activity had stabilized under the outward current (91.3 ± 13% of control; \(P > 0.05\); same cells).

Voltage- and Ca\(^{2+}\)-activated K\(^+\) conductances or synaptic inhibition could not contribute to the slow outward currents. Note the absence of medium Ca\(^{2+}\)-mediated currents without contribution of Cl\(^-\)-mediated GABA\(_A\) inhibition. An inward current (IC) (19.8 ± 7.9 pA, measured 50 ms after burst termination; IC, Fig. 3A) or an afterdepolarization (ADP) that followed bursts (15.9 ± 8.3 mV, measured 50 ms after burst termination) and that decay kinetics similar to that of the mI\(_{AHP}\)/mAHP was usually unmasked (7/11 or roughly 64%) with intracellular Cs\(^+\) (see following text).

With the conventional K-gluconate intracellular solution the kinetics of the postburst I\(_{AHP}\) may show two components with decay time constants of approximately 200 ms and >2 s, suggesting that they were mediated by the Ca\(^{2+}\)-activated K\(^+\) currents mI\(_{AHP}\) and sI\(_{AHP}\), respectively (Control, Fig. 3, B and C). Both conductances were isolated pharmacologically because apamin blocked the mI\(_{AHP}\) without modifying the sI\(_{AHP}\) (Fig. 3B; \(n = 9\)) and could disclose an early inward current or an ADP that was followed by the sI\(_{AHP}\)/mAHP (4/9 cells or 44%).

The molecular identity of the Ca\(^{2+}\)-activated K\(^+\) channels mediating the mI\(_{AHP}\) is known (Bond et al. 2004; Sailer et al. 2002; Stocker 2004; Stocker et al. 1999; Villalobos et al. 2004; however, see Discussion). In addition, the Kv7/KCNQ M-current and the hyperpolarization-activated I\(_h\) were previously shown to contribute to the mI\(_{AHP}\)/mAHP (Gu et al. 2005; Storm 1987; Young et al. 2004). In contrast, the channels mediating the sI\(_{AHP}\) are different and their nature has not been clarified (Bond et al. 2004; Sah and Faber 2002; Villalobos et al. 2004). There is no known specific blocking agent for the sI\(_{AHP}\) that is insensitive to tetraethylammonium (TEA) and micromolar concentrations of 4-AP (Alger and Williamson 1988; Martín et al. 2001; reviewed in Sah and Faber 2002; Stocker 2004). The clotrimazole analogue UCL2027-2 (PZ233) has been shown to induce a relatively selective inhibition of the sAHP in cultured hippocampal neurons (Shah et al. 2001), but bath application of PZ233 (5–10 \(\mu M\)) had no significant effects on the sAHP in CA3 pyramidal neurons in our experimental conditions (data not shown, \(n = 2\)), even when GABAergic inhibition was blocked with PTX (40 \(\mu M\)) and saclofen (100 \(\mu M\); \(n = 3\)). However, the sI\(_{AHP}\) is strongly inhibited in a nonspecific manner by muscarinic, adrenergic, and mGluR agonists (e.g., Borde et al. 2000; Madison and Nicoll 1986; Martín et al. 2001; Melyn et al. 2002; Pedarzani and Storm 1993; reviewed in Sah and Faber 2002; Stocker 2004). We investigated the action of t-ACPD (20 \(\mu M\); \(n = 6\)) added to the 4-AP solution (t-ACPD + 4-AP, Fig. 3C) and in a few cases (\(n = 5\)) to the control ACSF (+t-ACPD, Fig. 3C).

The t-ACPD challenge (Fig. 3C), CCh (10 \(\mu M\); \(n = 6\); Fig. 3D), and isoproterenol (10 \(\mu M\); \(n = 5\); see following text) inhibited the slow outward component, consistent with this current being the sI\(_{AHP}\). Therefore the I\(_{AHP}\)/AHPs that follow epileptiform burst are most likely the faster initial mI\(_{AHP}\) that
FIG. 3. Two components of the AHP that follow epileptiform bursts are mediated by K⁺ conductances. A: representative voltage-clamp trace obtained with a Cs⁺-glucamate-filled electrode 15 min after the onset of 4-AP superfusion. Note epileptiform burst of inward unclamped action currents (truncated) followed by prolonged inward current (IC) and total absence of outward currents. B: representative voltage-clamp trace showing the mAHP and sAHP evoked by depolarizing command pulses (shown above) in control ACSF (top) and after block of the mAHP with apamin (bottom). C: representative voltage-clamp traces of outward “tail currents” evoked by brief depolarizing command pulses (shown above). D: summary data showing amplitude modifications of the mAHP and sAHP (% of control values) when apamin (100 nM; n = 9) and carbocoll (CCh, 10 μM; n = 6) were added to the control solution and 20 min after the onset of the epileptiform activity when the 4-AP + Mg²⁺-free (n = 10) and the 4-AP + Mg²⁺-free + CCh solutions (n = 6) were superfused. All recordings were at a −50 mV Vₗ.

A decrease of the mAHP/sAHP parallels the induction of epileptiform bursts

When the mAHP was isolated after blocking the sAHP with t-ACPD the mAHP was not modified, but after the induction of epileptiform activity there was a gradual and marked reduction of the mAHP (to 40.8 ± 4.3% of control values; P < 0.001; n = 6) (t-ACPD + 4-AP, Fig. 3C).

To further analyze the possible modifications of the mAHP/sAHPs that follow epileptiform bursts we compared them with the mAHP/sAHPs induced by identical depolarizing current pulses both in control conditions and during interictal-like activity (n = 11). With this methodology the changes of the mAHP/sAHPs during the epileptiform activity could be compared with the mAHP/sAHPs in control conditions in the same cells. In addition, the contributions of variations in the burst characteristics to the mAHP/sAHPs during the interictal-like activity were minimized. Moreover, manipulations that modified the mAHP/sAHPs that followed interictal-like bursts induced parallel changes of the pulse-evoked mAHP/sAHPs, suggesting that they were mediated by the same conductances.

In current-clamp conditions the pulse-evoked mAHP was reduced in amplitude (40.1 ± 6.2% of control values; P < 0.001; n = 6) or even disappeared altogether (n = 5) when measured 10–20 min after the establishment of the abnormal activity (Fig. 4, A–D). The mAHP did not recover after a prolonged nearly 45-min washout. In contrast the pulse-evoked sAHP did not change during the abnormal bursting activity (96.9 ± 4.3% of control; P > 0.05; n = 11) (Fig. 4, A–D). Under voltage-clamp conditions the control pulse-evoked mAHP was reduced in amplitude (by 45.9 ± 5.7% from control values; P < 0.001; 8/11 cells or roughly 73%) and could even disappear (3/11 cells or roughly 27%) during the epileptiform activity (Fig. 4, E–G), whereas the sAHP did not change (98.2 ± 5.9% of control; P > 0.05; n = 9).

The above results are consistent with the observed reduction of the mAHP/mAHP being a key factor in the induction of the interictal-like activity. The results suggest that a downregulation of the negative feedback supplied by the mAHP/mAHP may contribute to the induction of the epileptiform activity. We tested the above assumption both by inhibiting and enhancing the mAHP/mAHP with apamin and EBIO, respectively, during the epileptiform activity. Apamin (100 nM), a selective blocker of SK Ca²⁺-activated K⁺-mediated channels, added to the 4-AP solution increased the epileptiform burst duration (188.0 ± 17.7%; P < 0.01, n = 5), the amplitude of the PDS (233.1 ± 18.2%, P < 0.001, n = 5), the number of spikes in the bursts (8.8 ± 1.1 spikes, P < 0.01, same cells), totally blocked the mAHP/mAHP, and could disclose an ADP (Fig. 5A). In contrast, adding EBIO (400 nM) to the 4-AP solution increased the mAHP (143.3 ± 82%, P < 0.001, n = 10), decreased the amplitude of the PDS (66.3 ± 84%, P < 0.001, n = 10), the burst frequency (0.20 ± 0.06 Hz, P < 0.02; 0.09 ± 0.06 Hz, P < 0.01, n = 10), the burst duration (143.3 ± 64 ms, 6 min, P < 0.001; n = 10), and the number of spikes in the bursts (1.4 ± 0.7 spikes, P < 0.005, same cells). Eventually, EBIO could suppress the abnormal bursting activity (Fig. 5C; cf. Garduño et al. 2005). Interestingly, we previously showed that EBIO enhances the mAHP/mAHP without...
activity as occurs in CA1 pyramidal cells (Martín et al. 2001). In addition, activation of mGluRs may also induce a reduction of the background conductance and the activation of a voltage-gated inward current that contributes to the ADP (Chuang et al. 2002; Young et al. 2004). However, neither MCPG (0.5–1.0 mM) nor LY341495 (20 µM), which block type I and type II mGluRs, prevented the $m_{AHP}$/mAHP reduction that paralleled the epileptiform activity ($n = 4$; data not shown), suggesting that other mechanisms were active to depress the conductance. This view is consistent with the action of the mGluR agonist t-ACPD that inhibited the $s_{AHP}$ but not the $m_{AHP}$ (Fig. 3C).

The $s_{AHP}$/sAHP regulates the interburst interval and rhythmicity

Another important issue that remains to be clarified is what factors determine the timing of the periodic network interictal-like activity. Therefore we tested the possible contribution of the $s_{AHP}$/sAHP to the timing of the bursting activity. The constancy of epileptiform bursts characteristics in different cells suggests that a uniform influx of Ca$^{2+}$ was induced in all cells in the bursting network. Therefore we hypothesized that: 1) bursts were supported by the increased excitability caused by the reduction of the $m_{AHP}$/mAHP and 2) the synchronized bursting and the resulting massive influx of Ca$^{2+}$ activated the $s_{AHP}$/sAHP that had similar characteristics and tended to terminate at fixed intervals after a network burst. This view is in accordance with the absence of epileptiform bursts and the reduction of the synaptic activity during the $s_{AHP}$/sAHP. It also agrees with the occurrence of the subsequent synchronized bursts in the network when $s_{AHP}$/sAHP had terminated, as observed with paired recordings (see following text). This notion is consistent with the synchronized bursting being caused by a recovery of the $V_m$ and excitability that brought cells in the network to fire in close synchrony to a level of population firing that depolarized neurons and triggered the burst (Menéndez de la Prada et al. 2006).

We further tested the above assumptions in two ways. First, by estimating the probability of occurrence of epileptiform bursts before and after pulse-evoked $s_{AHP}$/sAHPs, we found that there was a dramatic reduction of bursting both in current-clamp (by 77.9 ± 0.8% from control values; $P < 0.01$; $n = 10$) and voltage-clamp conditions (by 60.2 ± 0.3% from control values; $P < 0.01$; $n = 10$) during the pulse-evoked $s_{AHP}$/sAHP (Fig. 6A–C). In addition, under current-clamp mode the pulse-evoked burst–sAHP sequence induced a reset of the abnormal bursting interictal-like activity that was characterized by repeated bursts that tended to occur at specified times after the pulse. The successive bursts were synchronized by the consecutive pulse-evoked burst–sAHP sequences (Fig. 6A). Second, we tested the effects of blocking the $s_{AHP}$/sAHP during the epileptiform activity with t-ACPD (20 µM) that disrupted rhythmicity, reduced the silent interval that followed pulse-evoked bursts, and increased the frequency of epileptiform bursts by 175.5 ± 22.3% ($P < 0.005$, $n = 6$). Therefore under block of the $s_{AHP}$ with t-ACPD epileptiform bursts occurred at irregular intervals and with similar probability through the record (Fig. 6D), suggesting that synchronization was not a direct consequence of the bursts but resulted from the combined pulse-evoked burst–sAHP sequence.
clamp traces showing pulse-evoked sAHPs (n = 6), as in the rest of the traces) after 20 min of superfusion with 4-AP. Note the synchronization of bursts after the pulse-evoked sAHP; i.e., the sAHP “resets” the ongoing interictal-like activity. B, top: superimposed current-clamp traces showing pulse-evoked sAHPs 20 min after superfusion with 4-AP. Note the absence of bursts during the sAHP (double-headed arrow). B, bottom: histogram showing sAHP burst occurrences (% before and after spike bursts and sAHPs obtained from the same cell as in B, top. C, top and bottom: same as B, top and bottom, but in voltage-clamp conditions in another cell. D, top and bottom: same as C, top and bottom, but 30 min after onset of superfusion with 4-AP and added r-ACPD (20 μM) in another neuron. Note the absence of sAHP and of burst synchronization. Histograms in each experimental group (n = 10 cells) were constructed with responses evoked by 20 successive stimuli in each case. E: current-clamp recordings, showing examples of the increased synaptic activity (dotted squares) immediately preceding (about 1.5 s) epileptiform bursts (i.e., “follower cell”). F: same as E, but the increased synaptic activity (dotted squares) occurred after (about 1.0 s) bursts in another neuron (i.e., “leader cell”). G: same as E, showing complete bursting cycle and interburst interval (IBI). E and G: same cell. H: histogram showing postsynaptic potential occurrences % after (n = 6 cells) and before (n = 5 neurons) bursts. Data were averaged from synaptic potentials paralleling 10 successive bursts in each cell. IBI was normalized by scaling to the briefest one.

We also found that epileptiform bursts were in some cells preceded (6/35 or roughly 17%), whereas in other neurons bursts were followed (8/35 or roughly 23%) by increases in synaptic activity (Fig. 6, E and F). In the cells in which the increased synaptic activity preceded bursts it lasted 1–2 s and terminated by rapid depolarization that initiated the PDS. The above results suggest that the synaptic activity that depolarized the recorded neuron and drove it to the bursting threshold was generated by presynaptic neurons forming part of the bursting network that tended to fire in synchrony when the sAHP had terminated. This view is consistent with the recorded neuron being a “follower cell” in the network. The other cells in which the increased synaptic activity occurred immediately after bursts (1–2 s) behaved as “leader cells” in the network. In addition, synaptic activity was considerably reduced during the sAHP, probably indicating a modulation of network excitability and a reduction of synaptic interactions by the sAHP. To estimate the variations in synaptic activity during the sAHP we constructed histograms of the proportion of postsynaptic potential occurrences in both leader (n = 8) and follower (n = 5) cells (see methodology in the caption of Fig. 6). The histogram closely paralleled the profile of the sAHP and revealed few

FIG. 6. Epileptiform bursts are inhibited during the sAHP and preceded by increased synaptic activity. A: superimposed representative current-clamp recordings showing pulse-evoked sAHPs (n = 6, as in the rest of the traces) after 20 min of superfusion with 4-AP. Note the absence of bursts after the pulse-evoked sAHP; i.e., the sAHP “resets” the ongoing interictal-like activity. B, top: superimposed current-clamp traces showing pulse-evoked sAHPs 20 min after superfusion with 4-AP. Note the absence of bursts during the sAHP (double-headed arrow). B, bottom: histogram showing sAHP burst occurrences (% before and after spike bursts and sAHPs obtained from the same cell as in B, top. C, top and bottom: same as B, top and bottom, but in voltage-clamp conditions in another cell. D, top and bottom: same as C, top and bottom, but 30 min after onset of superfusion with 4-AP and added r-ACPD (20 μM) in another neuron. Note the absence of sAHP and of burst synchronization. Histograms in each experimental group (n = 10 cells) were constructed with responses evoked by 20 successive stimuli in each case. E: current-clamp recordings, showing examples of the increased synaptic activity (dotted squares) immediately preceding (about 1.5 s) epileptiform bursts (i.e., “follower cell”). F: same as E, but the increased synaptic activity (dotted squares) occurred after (about 1.0 s) bursts in another neuron (i.e., “leader cell”). G: same as E, showing complete bursting cycle and interburst interval (IBI). E and G: same cell. H: histogram showing postsynaptic potential occurrences % after (n = 6 cells) and before (n = 5 neurons) bursts. Data were averaged from synaptic potentials paralleling 10 successive bursts in each cell. IBI was normalized by scaling to the briefest one.

We also found that epileptiform bursts were in some cells preceded (6/35 or roughly 17%), whereas in other neurons bursts were followed (8/35 or roughly 23%) by increases in synaptic activity (Fig. 6, E and F). In the cells in which the increased synaptic activity preceded bursts it lasted 1–2 s and terminated by rapid depolarization that initiated the PDS. The above results suggest that the synaptic activity that depolarized the recorded neuron and drove it to the bursting threshold was generated by presynaptic neurons forming part of the bursting network that tended to fire in synchrony when the sAHP had terminated. This view is consistent with the recorded neuron being a “follower cell” in the network. The other cells in which the increased synaptic activity occurred immediately after bursts (1–2 s) behaved as “leader cells” in the network. In addition, synaptic activity was considerably reduced during the sAHP, probably indicating a modulation of network excitability and a reduction of synaptic interactions by the sAHP. To estimate the variations in synaptic activity during the sAHP we constructed histograms of the proportion of postsynaptic potential occurrences in both leader (n = 8) and follower (n = 5) cells (see methodology in the caption of Fig. 6). The histogram closely paralleled the profile of the sAHP and revealed few synaptic potentials occurrences at the peak hyperpolarization of the sAHP, whereas the proportion of synaptic potentials increased gradually toward briefer and longer delays from the peak sAHP (Fig. 6, G and H).

These results are consistent with the regulation of network interactions by the sAHP that probably acts by decreasing population activity by reducing excitability in the cells that compose the bursting network. Many neurons showed synaptic potentials during bursts (21/35 or 60.0%), suggesting that they fired in close synchrony with other cells in the network (see following text). The above determinations were made in the first 20 min after the onset of the interictal-like activity. However, synaptic activity preceding and following burst was scarce (4/35 or 11.4%) later during the evolution of epileptiform activity, suggesting that burst synchronization improved in the network with the evolution of the abnormal activity (see following text). The above results are consistent with the burst–sAHP sequence being a key factor in the regulation of the frequency and synchronization of the bursting rhythm, whereas the burst per se was not because desynchronization was observed in conditions where the burst persisted and the sAHP was absent. The abnormal activity was inhibited by 20 μM CNQX (n = 3; data not shown), indicating that excitatory synaptic interactions were of major importance in the genesis of the interictal-like activity.

We verified the above assumptions by recording pairs of CA3 pyramidal neurons (about 50–100 μm apart; n = 22 pairs). We could not detect synaptic interactions between pairs, but their absence is not surprising in view of the low probability of functional excitatory interconnections in acute slices (Miles and Wong 1986).

First, during epileptiform activity we blocked the sAHP with 10 μM CCh (n = 5 pairs) that desynchronized bursting between neurons and increased the bursting rate (by 148.3 ± 18.3%, 3/10 cells or 30%). The ACh challenge also usually increased synaptic activity (4/10 cells or 40%). Both effects were probably caused by the depolarization and the increased excitability induced by CCh in the cells composing the network. Indeed, CCh inhibits the sAHP/sAHP and also increases excitability by blocking several K+–mediated conductances (reviewed in Storm 1987). However, it was previously reported that at higher temperature (about 32°C) and under GABA A blockade with bicuculline, instead of desynchronization, CCh per se induces synchronized population activity in the CA3 region in vitro (Psarropoulou and Dallaire 1998). The synchronized activity is blocked by muscarinic antagonists and is thought to be mediated by local excitatory circuits enhanced by muscarinic activity in the absence of inhibition. We tested the effects of CCh applied at 32–34°C on the behavior of pyramidal neuron pairs (separated by about 100 μm; n = 5 pairs) in experiments in which GABA A inhibition was blocked with FTX (40 μM) in replacement of the bicuculline used by Psarropoulou and Dallaire (1998) because this drug also blocks the mAHp (Debarbieux et al. 1998; Stocker et al. 1999), thus favoring epileptogenesis. Synchronized bursts (5.1 ± 1.1 spikes, 340 ± 20.8 ms duration, at 0.38 ± 0.1 s−1, n = 5) were induced by CCh (10 μM) (CCh, Fig. 7A), thus confirming the results of Psarropoulou and Dallaire (1998). In addition, synchronized bursts at a higher more irregular rate (8.3 ± 1.8 spikes, 450 ± 30.2 ms duration, at 0.75 ± 0.1 s−1, n = 5 pairs) continued when 4-AP (100 μM) was added to the CCh solution
AFTERHYPERPOLARIZATIONS REGULATE CA3 EPILEPTOGENESIS

Third, we recorded pairs with one cell loaded with Cs-
gluconate to test the effects blocking all K\(^+\) conductances in
the recorded cell (\(n = 5\) pairs) without affecting other neurons
in the bursting network or the cell recorded with the normal
intracellular solution. With intracellular Cs\(^+\) the AHPs were
completely blocked and the Cs\(^+\)-loaded cells initially (<20
min after the onset of bursts) tended to burst irregularly at a
higher rate (by 192.3 \(\pm\) 7.2\%, \(P < 0.001, n = 5\) pairs) and
were not synchronized. However, later (>20 min) both cells
tended to burst in synchrony, although bursts were much
longer and were followed by ADPs in the neuron dialyzed with
Cs\(^+\) (\(n = 6\)) (Fig. 8A). Pulse-evoked bursts were followed by
ADPs in control conditions but ADPs that could generate
plateaulike depolarization topped by prolonged spike bursts
were evoked under Cs\(^+\) (ACSF, Fig. 8A).

Finally, we tested the effects of a intracellular pipette solu-
tion containing 40 mM BAPTA (\(n = 6\) pairs) that blocks both
the mAHP and sAHP in the recorded cell by chelating intra-
cellular free Ca\(^{2+}\) without affecting other neurons in the
bursting network (Fig. 8B). The effects of intracellular BAPTA
measured <20 min after the induction of the first interictal-like
bursts were statistically comparable to those of Cs\(^+\). As in all
other cases, later (>20 min) both cells tended to burst in
synchrony (4-AP, Fig. 8B). We then superfused isoproterenol
(10 \(\mu M; n = 3\)) in pairs loaded with BAPTA in one cell and
control solution in the other neuron (as above) and there was
a marked increase in firing rate (by 210.3 \(\pm\) 12.3, \(n = 3\)) and
spontaneous synaptic activity and a complete desynchronized

(CCh + 4-AP, Fig. 7A). In contrast, the synchronized interict-

tal-like bursting activity induced by 100 \(\mu M\) 4-AP (4-AP, Fig.
7B) was totally desynchronized by adding CCh (10 \(\mu M\),
confirming our results obtained at room temperature (4
pairs). The above differences between the effects of superfu-
sion with CCh before and after the induction of the interictal-
like activity by 4-AP at high temperatures might be caused by
divergence in the sequences of the blockade of different po-
tassium conductances by both agents, especially because the
muscarnic metabotropic activity may be long lived, may
induce potentiation (Fernández de Sevilla et al. 2005), and may
disclose an ADP (McQuiston and Madison 1999). In any case
the differences are highly interesting and should be analyzed in
detail in future studies.

It is noteworthy that essentially identical desynchronizing
effects were observed in paired recordings under superfusion
with t-ACPD (20 \(\mu M; n = 2\) or isoproterenol (5–10 \(\mu M; n = 3\))
added to the 4-AP solution (see following text).

Second, we tested the contribution of the sAHP to the
bursting rhythm by enhancing the sAHP in one cell with an
intracellular KMeSO\(_4\) solution (Zhang et al. 1994), whereas the
other cell was recorded with normal K-glucosiate pipette solu-
tion (\(n = 4\) pairs). The cells initially (<20 min after the start
of the abnormal bursts) did not burst in synchrony. The neuron
diallyzed with KMeSO\(_4\) showed larger sAHPs of longer dura-
tion and tended to burst at a lower frequency than the other
(30.9 \(\pm\) 5.2\%, \(P < 0.005, n = 4\) pairs) (Interictal,
Fig. 7C). However, both cells fired in synchrony during the
ictal-like episodes when the sAHPs were substantially reduced
in both cells and a prolonged depolarization was evoked (Ictal,
Fig. 7C). In addition, both cells also tended to burst in syn-
chrony later (>20 min) with the evolution of the epileptiform
activity (data not shown).

Fig. 7. Paired recordings: effects of blocking or enhancing the sAHP in one of the cells. A, left: representative current-clamp recordings in a pair of pyramidal neurons (1 and 2; about 50-\mu m separation) showing rhythmic bursting activity induced by CCh at 32–24°C (10 \(\mu M\)). A, right: after adding 4-AP (50 \(\mu M\)) the bursting frequency increased and bursts remained synchronised in both neurons. B, left: records showing synchronized bursting interictal-like activity induced by 4-AP (50 \(\mu M\)). B, right: when 50 \(\mu M\) CCh was added bursts were at a higher frequency in both cells and totally desynchronous. C1: representative traces obtained with an electrode filled with the KMeSO\(_4\) solution that enhances the sAHP. D1: simultaneous recording with the normal K-glucosiate electrode solution. Note the absence of burst synchronisation and the more prolonged and larger sAHP in D1 during the interictal-like activity. Cells synchronized at the onset of the ictal episode. E: digitized differential interference contrast (DIC) image of the pyramidal neuron pair corresponding to the recordings shown in D.

Fig. 8. Paired recordings: abnormal bursting in cells dialyzed with Cs\(^+\) and 2-bis(2-aminophenox)-ethane-N,N,N',N'-tetracetic acid (BAPTA) and with control intracellular solution. A, left: representative current-clamp records obtained in control conditions showing pulse evoked responses (the 200-ms pulse shown below) of a neuron with control intracellular solution (1) and another dialyzed with Cs\(^+\) (2). Note sAHP in (1) is absent in (2) and a ADP (McQuiston and Madison 1999). In any case the differences are highly interesting and should be analyzed in detail in future studies.

In contrast, the synchronized interictal-like bursting activity induced by 100 \(\mu M\) 4-AP (4-AP, Fig. 7B) was totally desynchronized by adding CCh (10 \(\mu M\), confirming our results obtained at room temperature (4 pairs). The above differences between the effects of superfusion with CCh before and after the induction of the interictal-like activity by 4-AP at high temperatures might be caused by divergence in the sequences of the blockade of different potassium conductances by both agents, especially because the muscarinic metabotropic activity may be long lived, may induce potentiation (Fernández de Sevilla et al. 2005), and may disclose an ADP (McQuiston and Madison 1999). In any case the differences are highly interesting and should be analyzed in detail in future studies.

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chrony later (>20 min) with the evolution of the epileptiform
activity (data not shown).
bursting throughout the recording $>\text{30 min}$ $(n = 4)$ (4-AP + isoproterenol, Fig. 8B).

The above results offer additional support to the notion that the $s_{\text{AHP}}/s_{\text{AHP}}$ plays a key role in determining the interburst cycle and regulating the frequency and synchronization of the bursting network. The results also explain why, when the $s_{\text{AHP}}$ has different kinetics in different cells, bursts may not be entirely synchronized. However, bursts became synchronized later even if the AHPs and other $K^+\text{-mediated currents}$ were inhibited in one of the recorded cells, indicating that other neurons in the network with active AHPs and $K^+\text{-mediated conductances}$ were driving the recorded neurons.

**Synaptic excitation synchronizes CA3 pyramidal neuron ensembles**

As described above, in many cells there was an increase in synaptic activity preceding or following the interictal burst in the recorded cell (Fig. 10A). The synaptic activity increased in amplitude with imposed hyperpolarization and decreased with depolarization, consistent with excitatory postsynaptic potentials (EPSPs, not shown). Therefore the bursting network was driven by mutual excitatory interconnections that depolarized and drove the recorded cell to the bursting threshold (Menéndez de la Prida et al. 2006).

We further analyzed this possibility with paired recordings that showed that the epileptiform activity typically started in one neuron and rapidly (5–10 min) spread to the other cell. A period of incompletely synchronized irregular bursting at a low frequency (4-AP 10 min; Fig. 9, A and B) was followed by the establishment of well-synchronized rhythmic bursting at a higher frequency in the two neurons (4-AP 20 min; Fig. 9, C and D). The stabilized interictal-like activity was typified by synchronous bursts, usually of similar characteristics, terminated by AHPs of analogous amplitude and duration in both cells (Fig. 9, C and D). Cross-correlations revealed that PDSs and bursts in one cell tended to precede those in the other neuron, initially by about 20 ms (4-AP 10 min; Fig. 9) and later when the bursting activity was fully organized the delay stabilized at a much shorter interval of about 2 ms, suggesting an improvement of burst synchronization between cells (4-AP 20 min; Fig. 9). These small differences in the timing of bursts and the improvement of synchronization during epileptiform activity were observed in all pairs analyzed $(n = 22)$ pairs, suggesting that excitatory synaptic network interactions were gradually enhanced. Synchronization increased, on average, from 16.2 $\pm$ 2.1 to 3.1 $\pm$ 2.2 ms $(P < 0.01; \text{same cells})$, as measured from cross-correlations. The augmented interactions could be caused by the increased temporal and spatial summation of EPSPs attributable to the rise in network excitability that paralleled the decreased $m_{\text{AHPs}}/m_{\text{AHPs}}$.

Additional support to the increased excitatory synaptic interactions was provided by paired recordings, which revealed that initially during the interictal-like activity (5–10 min) EPSPs could occur simultaneously in both cells (18/22 pairs or 86%), consistent with a common excitatory input from other neurons in the network (arrows, left records, Fig. 10A). Later with the stabilization of the abnormal activity (20–30 min) simultaneous EPSPs disappeared and coincident spikes and EPSPs could occur during bursts (20/22 or 90%) (arrows, right records, Fig. 10A). These changes are also consistent with an increase in excitability and excitatory synaptic interactions within the network.

The above results suggest that the activation of a single cell could eventually, as a consequence of the increase synaptic interactions, control the interictal-like network activity. Therefore we used paired recordings and direct stimulation of one of the recorded cells with a brief high-intensity current pulse that evoked the burst–sAHP sequence to test the possible evolution of network interactions. Activation of either of the neurons of the pair never revealed direct excitatory connections in control conditions in our sample $(n = 22)$. However, when the interictal-like activity had stabilized (>20 min) a brief current pulse in one cell, which induced the usual spike burst followed by a silent period, was paralleled by a brief period of increased bursting activity followed by a silent interval in the other.
neuron (Fig. 10B). This synchronization of activity suggests that the pulse-evoked burst and subsequent sAHP in the recorded cell induced changes in the excitability of the other neuron (4/22 pairs or roughly 18%), implying that activation of a single neuron could control bursting activity in the network (e.g., Menéndez de la Prida et al. 2006; Miles and Wong 1983), probably by indirect excitatory interconnections that increased or synapses that were silent and became active with the evolution of the epileptiform activity.

We also analyzed the effects of stimulating an excitatory synaptic input on burst synchronization and timing between simultaneously recorded CA3 pyramidal neurons (n = 7 pairs) under block of GABA_A inhibition with 40 μM PTX. At the onset of the abnormal activity the burst–sAHP sequence evoked by MF stimulation inhibited the epileptiform bursts during a somewhat variable interval in both cells that terminated by bursts that were not well synchronized (15 min + Stimulation, Fig. 11A). Later, when the epileptiform activity had stabilized, the burst–sAHP sequences evoked by MF stimulation were of similar duration and inhibited epileptiform bursts during a similar interval (4.5 ± 0.5 s, n = 6) that terminated by well-synchronized bursts when the sAHP ended in both neurons (30 min + Stimulation, Fig. 11A). The synchronization, estimated by measuring the reduction of the temporal dispersion between bursts in successive responses, increased by 208 ± 18.3% (P < 0.001, n = 6). Therefore the sAHP evoked by synaptic stimulation silenced the cells during a relatively fixed interval that synchronized and timed the subsequent rhythmic bursting activity. In addition, the response evoked by the MF stimulation was modified during the evolution of the epileptiform activity because in control conditions EPSPs were smaller, evoked bursts with fewer spikes, and were followed by a mAHP (Control, Fig. 11B), whereas later when the interictal-like activity had stabilized EPSPs were larger and evoked longer bursts with more spikes (the number of spikes increased by 167.3 ± 17.3%, P < 0.005, n = 7) and were followed by an ADP in all seven cells (30 min, Fig. 11B).

DISCUSSION

We present evidence demonstrating that in the CA3 region, where epileptiform activity is initiated and from there spreads to other hippocampal regions (Colom and Saggau 1994; Luhmann et al. 2000; MacVicar and Dudek 1982; Miles and Wong 1983; Schwartzkroin and Prince 1978), the interictal-like activity in pyramidal neurons is precisely regulated by two Ca^{2+}-activated K^+–mediated currents with different kinetics. A downregulation of the mAHP/mAHP and the resulting increased excitability are central mechanisms contributing to the generation and regulation of the network bursts that characterize CA3 interictal-like activity. The results also point to a key role of the decreased excitability induced by the activation of the postsynaptic sAHP/mAHP in controlling both the frequency and synchronization of the interictal-like network activity.

We show that there is a close relationship between the decline of the mAHP amplitude, the increased excitability, and the induction of interictal-like activity. Pharmacological manipulations that reduce the mAHP, as bath-applied apamin, that specifically inhibits SK Ca^{2+}-activated K^+–mediated currents, caused a marked increase of the bursting activity (McCown and Breese 1990). In contrast, EBIO that enhances Ca^{2+}-activated K^+ currents, and in CA3 pyramidal cells specifically augments the mAHP/mAHP, reduced bursting or even blocked the epileptiform activity (Garduño et al. 2005). The above-discussed results are consistent with a key involvement of the mAHP/mAHP acting as a negative feedback in the regulation of excitability and in the genesis of CA3 epileptogenesis.

Participation of SK channels in the genesis of the mAHP has recently been questioned (Gu et al. 2005) because, although a clear SK-mediated apamin-sensitive component was evoked by brief depolarization under voltage-clamp mode, these authors could not evoke the Ca^{2+}-activated K^+–mediated current with similar depolarizations under current-clamp mode. Several ionic conductances may contribute to the mAHP/mAHP besides the apamin–EBIO-sensitive Ca^{2+}-activated K^+–mediated SK component. The Kv7/KCNQ M-current at depolarized (> −60 mV) and the H-current at hyperpolarized V_m (< −70 mV) may add to the mAHP/mAHP (Bond et al. 2004; Sah and Faber 2002; Sailer et al. 2002; Stocker 2004; Stocker et al. 1999; Villalobos et al. 2004). Nevertheless, an apamin–EBIO-sensitive component without an important contribution of the M-current was found in our experimental
conditions because both t-ACPD and CCh, which are potent inhibitors of the M-current, did not induce important reductions of the mI_{AHP}/mAHP. Activation of the H-current was not expected in our experiments because V_m values of > −75 mV at which the H-current is activated were never reached. The divergence between our results and those mentioned above may either reside in differences in the channels expressed in CA1 and CA3 pyramidal cells (Chuang et al. 2002; Sailer et al. 2002; Schiller 2004; Schwartzkroin and Prince 1980; Young et al. 2004) or as a result of the masking of the muscarinic M-type component by the ADP (McQuiston and Madison 1999).

Present results could indicate the mAHP may be masked by the enhancement of an inward current that underlies the ADP that effectively contributes to increase excitability (Chuang et al. 2002; Sailer et al. 2002; Schiller 2004; Schwartzkroin and Prince 1980; Young et al. 2004). Consistent with this view—when the mI_{AHP}/mAHP was not apparent during epileptiform activity—apamin always enhanced bursts and could disclose an ADP, suggesting that the apamin-sensitive SK Ca^{2+}-activated K^-mediated component of the mAHP was nevertheless present and masked the ADP (McCown and Breese 1990). The mechanisms that induce the downregulation of the mI_{AHP}/mAHP and the enhancement of the ADP during epileptiform activity could be of key importance but remain to be investigated.

Several studies established the crucial role of the sI_{AHP}/sAHP as negative feedback controlling excitability in normal conditions (reviewed in Sah and Faber 2002; Stocker 2004; Vogalis et al. 2003) and during epileptiform activity (reviewed in de Curtis and Avanzini 2001; McCormick and Contreras 2001). Present results suggest that after each synchronized epileptiform burst the resulting massive Ca^{2+} influx activates the sI_{AHP}/sAHP that sets the interburst interval of the interictal-like activity by reducing the excitability of the network, thus decreasing synaptic interactions and burst generation during a relatively fixed time interval after each burst. The intracellular Ca^{2+} concentration is then gradually reduced, inducing the deactivation of the sI_{AHP}/sAHP that leads to the return of the V_m, the membrane conductance, and the excitability to previous values, favoring the generation of a subsequent burst. The increased excitability would bring cells in the network to firing threshold, thus increasing synaptic interactions to a level of population firing that depolarizes neurons and triggers the burst. Paired recordings show that when the epileptiform activity has stabilized, the activation—deactivation of the sI_{AHP}/sAHP tend to occur in parallel with practically identical duration in the pyramidal cells that form part of the bursting network. Therefore the burst–sAHP sequence is unceasingly repeated at relatively fixed intervals, determined by the duration of the sAHP, thus setting the bursting cycle and timing the rhythmic interictal-like network activity.

The above conclusions were sustained by the effects of inhibiting the sI_{AHP}/sAHP with CCh, isoproterenol, or t-ACPD that increase the bursting frequency and disrupt burst regularity and synchronization between pairs of pyramidal neurons. Although those drugs have other actions besides a potent inhibition of the sI_{AHP}/sAHP they share essentially identical effects on the abnormal interictal-like activity, suggesting an action most likely exclusively caused by the inhibition of the sI_{AHP}/sAHP (Borde et al. 2000; Buño et al. 2004; Martín et al. 2001). In addition, enhancing the sI_{AHP}/sAHP in one of the recorded neurons by filling the electrode with a solution containing KMeSO_4 (Zhang et al. 1994) induced burst desynchronization because it increased the interburst interval, whereas the other cell (recorded with the normal K-glucuronate intracellular solution) showed a briefer sAHP and a higher bursting rate (present results). Burst desynchronization was also observed when, in one of the neurons of the pair, K^+-mediated currents were blocked with intracellular Cs^+ or when the AHPs were inhibited by chelating intracellular Ca^{2+} with BAPTA. All the above effects are consistent with the sI_{AHP}/sAHP being central in determining the interburst interval, the frequency, and the synchronization of the bursting network.

Changes of the sAHP induced by extracellular drug applications caused stable modifications of the interictal-like activity because they act on all the cells of the network. In contrast, the changes occurred only in the dialyzed cell and exclusively early on during the evolution of epileptiform activity (<20 min) when the sAHP was modified by intracellular drug applications (i.e., KMeSO_4, Cs^+, or BAPTA). However, when epileptiform activity stabilized the dialyzed neuron showed partial burst synchronization in the three different experimental conditions (i.e., intracellular KMeSO_4, Cs^+, or BAPTA), suggesting that the dialyzed cell was “driven” by the convergent synaptic activity generated by bursting cells in the network. The switch from desynchronization to partial synchronization is consistent with an improvement in excitatory interconnections with the progression of the interictal-like activity, although the mechanisms that mediate these effects remain to be investigated. A factor that could contribute is the necessary recovery of synaptic function after a burst before another burst can be initiated (Staley et al. 1998).

In harmony with a key influence of the sI_{AHP}/sAHP in controlling the bursting frequency, we show that the ongoing rhythm can be effectively “reset” by introducing a pulse-evoked or a synaptically evoked burst followed by a sI_{AHP}/sAHP. Interestingly, a similar reset of the hippocampal theta rhythm was induced by synaptic inputs in vivo (Buño et al. 1978), suggesting that similar mechanisms may be functional in normal conditions.

The unlike behaviors of the sAHP and mAHP suggest that both conductances may be regulated by different mechanisms. It was recently shown that activation of the kainate (KA) receptor subunit GluR6 mediates a metabotropic inhibition of the mI_{AHP}/mAHP that increases excitability and firing frequency in CA3 pyramidal cells (Fisahn et al. 2005). Therefore a likely explanation for the decreased mI_{AHP}/mAHP is that it is mediated by the activation of KA receptors caused by the abnormally enhanced synaptic activity and glutamate release that characterize epileptiform activity—an interesting possibility that remains to be investigated. We previously showed that glutamate released during epileptogenesis inhibited the sI_{AHP}/sAHP in CA1 pyramidal neurons and that this effect was mediated by group I and group II mGluRs because it was prevented by superfusion with MCPG, an action that is mediated downstream of the increased intracellular Ca^{2+} signal (Martín et al. 2001). However, the sAHP was not decreased and the reduction of the mAHP was not prevented by MCPG or LY341495 (present results), suggesting that the mGluR-mediated inhibition was not functional. In addition, the consistency of the sAHP during epileptiform activity also suggests
that the KA-mediated inhibition of the sAHP (Fisahn et al. 2005) was absent in our experiments.

With paired recordings activation of a single cell never revealed direct excitatory connections in control conditions but when interictal-like activity stabilized a similar brief current pulse in one cell it could induce a burst followed by a sAHP that was occasionally paralleled by a brief period of increased activity followed by a silent interval and bursting in the other neuron. Therefore activation of a single neuron might control network activity probably by indirect excitatory interconnections that eventually increase or become functional with the evolution of the epileptiform activity, suggesting dynamic modifications in excitability and synaptic efficacy during the abnormal activity.

Other differences between the activation of the mAHP and sAHP may also play a role in the dissimilar behaviors of both conductances during epileptiform activity. Indeed, the sAHP is inhibited by nifedipine but unaffected by omega-conotoxin GVIA. In contrast, the mAHP is insensitive to nifedipine and inhibited by omega-conotoxin GVIA, suggesting that different voltage-gated Ca\(^{2+}\) channels mediate the major calcium influx pathway for activation of both conductances (Borde et al. 2000; Empson and Jefferys 2001; Viana et al. 1993). These Ca\(^{2+}\) channels may be regulated in different ways by epileptiform activity, thus determining the different behaviors of both conductances (Empson and Jefferys 2001). In addition, sAHP channels require an elevation of Ca\(^{2+}\) in the cytoplasm, rather than at the membrane, consistent with a role for a cytoplasmic intermediate between Ca\(^{2+}\) and the sAHP channels (Abel et al. 2004), where Ca\(^{2+}\)-induced Ca\(^{2+}\) release may play a role (Borde et al. 2000), suggesting that the sAHP and mAHP are regulated by different mechanisms during epileptiform activity and that those that inhibit the mAHP are active, whereas those that influence the mAHP are inoperative in CA3 pyramidal cells.

GABAergic inhibition does not contribute to the AHPs that follow bursts in our experimental conditions. Controversy exists regarding the contribution of GABA receptors to the hyperpolarization that follows epileptiform bursts because both a significant involvement (Perez-Velazquez and Carlen 1999; reviewed in Delgado-Escueta et al. 1986; Wong and Miles 1980) and the mAHP is insensitive to nifedipine and inhibited by omega-conotoxin GVIA, suggesting that different voltage-gated Ca\(^{2+}\) channels mediate the major calcium influx pathway for activation of both conductances (Borde et al. 2000; Empson and Jefferys 2001; Viana et al. 1993). These Ca\(^{2+}\) channels may be regulated in different ways by epileptiform activity, thus determining the different behaviors of both conductances (Empson and Jefferys 2001). In addition, sAHP channels require an elevation of Ca\(^{2+}\) in the cytoplasm, rather than at the membrane, consistent with a role for a cytoplasmic intermediate between Ca\(^{2+}\) and the sAHP channels (Abel et al. 2004), where Ca\(^{2+}\)-induced Ca\(^{2+}\) release may play a role (Borde et al. 2000), suggesting that the sAHP and mAHP are regulated by different mechanisms during epileptiform activity and that those that inhibit the mAHP are active, whereas those that influence the mAHP are inoperative in CA3 pyramidal cells.

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The reduced GABA release would diminish both GABAA and GABAB-mediated presynaptic inhibition of glutamate release may be substantially reduced after seizures in the hippocampus (Poon et al. 2006), an action that would increase glutamate release and seizure susceptibility. Finally, prolonged epileptiform activity may reduce GABAergic inhibition in vitro by rapid internalization of GABA receptors (Goodkin et al. 2005).

The existence of different mechanisms for the decline of inhibitory feedback during epileptiform activity strongly suggests a major contribution of the reduction of inhibition to the abnormal activity. Therefore the above effects could increase network excitability and induce the abnormal activity by the cooperative action of downregulating both synaptic inhibition and the mAHP/mAHP. The reduction of inhibition in some types of epilepsies but not in others may explain why barbiturates and benzodiazepines that act on GABA receptors to inhibit some types of epilepsies are totally ineffective in controlling other forms (Treiman et al. 1998).

Interesting are the differences between the epileptiform activities induced by the identical procedures in the CA1 (Martin et al. 2001) and the CA3 pyramidal cells (present results) because they indicate different network dynamics that could determine the higher susceptibility of the CA3 region to develop and command the induction of epileptic activity. In both regions there is an increased synaptic activity during epileptogenesis (Martin et al. 2001; Rutecki et al. 1987; present results) that activate mGluRs that inhibit the sAHP/sAHP in CA1 pyramidal neurons (Martin et al. 2001). In contrast, reduction of the mAHP/mAHP is present and instead the mAHP/mAHP is reduced by yet unknown mechanisms during epileptiform activity in CA3 pyramidal cells (present results). The CA1–CA3 differences could also depend on the distinct characteristics of the mAHP/sAHP is strongly activated during the interburst interval because the Ca\(^{2+}\)-dependent K\(^{+}\) currents in CA1 and CA3 pyramidal neurons, especially because inhibition of the mAHP/mAHP in CA3 pyramidal neurons may disclose an inward current that could greatly enhance the excitability and contribute to bursting discharges by mediating a postburst ADP (Chuang et al. 2002; Sailer et al. 2002; Schiller 2004; Schwartzkroin and Prince 1980; Young et al. 2004), whereas this inward current and ADP are not important in CA1 pyramidal neurons (Sailer et al. 2002; Schwartzkroin and Prince 1980). Network properties may also contribute to the differences because the CA3 region is characterized by recurrent excitatory connections made by axon collaterals of pyramidal cells, whereas recurrent connections are minor in the CA1 region (reviewed in Knowles 1992).

In conclusion present results suggest that the interictal-like bursting is sustained by active processes where the mAHP/mAHP and the mAHP/sAHP play crucial roles that are initiated by the massive Ca\(^{2+}\) influx during a burst. After the burst network excitability first decreases and then increases as the intracellular Ca\(^{2+}\) concentration rises by the calcium influx and then declines with the intracellular Ca\(^{2+}\) buffering and extrusion. During this period the Ca\(^{2+}\)-activated mAHP/mAHP and sAHP/sAHP act cooperatively and in sequence. First, the reduction of the mAHP/mAHP increases neuronal excitability and facilitates bursting, resulting in a massive Ca\(^{2+}\) influx. Finally, the mAHP/mAHP is strongly activated during the interburst interval because the Ca\(^{2+}\) concentration is high as a result of the substantial Ca\(^{2+}\) influx. The increased Ca\(^{2+}\) concentration activates the sAHP that reduces excitability, network interactions, and bursting until the Ca\(^{2+}\) concentration is lowered by Ca\(^{2+}\)-buffering extrusion, ultimately reducing the mAHP/mAHP and thus recovering the excitability that increases population activity to generate a subsequent burst.
Finally, we emphasize that acute animal models may not provide unambiguous information on the pathophysiology of chronic and human epileptogenesis. Even with these intrinsic limitations acute animal models are particularly fruitful in revealing the cellular pathophysiology of epileptic disorders, including synaptic and ionic channel alterations that cannot be completely answered with human investigation. Moreover, the development of novel models and of innovative analysis techniques may broaden our knowledge of the processes underlying epilepsy, may be useful in the understanding of human epilepsy, and inspire the development of new treatment strategies.

ACKNOWLEDGMENTS

Many thanks to Dr. A. Araque for valuable suggestions.

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GRANTS

This work was supported by Ministerio de Ciencia y Tecnología Grants BFI2002-01107 and BFU2005-07486) and Comunidad Autónoma de Madrid (Spain) Grant GR/SAL/0877/2004 to W. Buño. D. Fernández de Sevilla was previously awarded a postdoctoral contract funded by Ministerio de Ciencia y Tecnología Grant BFI2002-01107 and Comunidad Autónoma de Madrid Grant GR/SAL/0877/2004, and at present by a Ministerio de Ciencia y Tecnología Grant BFI2005-07486. J. Garduño was a postdoctoral fellow supported by a Fundación Carolina (Spain) fellowship. E. Galván was funded by a Dirección General de Estudios de Postgrado–Universidad Nacional Autónoma de México (Mexico) fellowship.

REFERENCES


Miles R and Wong RKS. Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. *J Physiol* 373: 397–418, 1986.


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


