Modulation of Burst Frequency, Duration, and Amplitude in the Zero-Ca^{2+} Model of Epileptiform Activity

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Bikson, Marom, Rahul S. Ghai, Scott C. Baraban, and Dominique M. Durand. Modulation of burst frequency, duration, and amplitude in the zero-Ca^{2+} model of epileptiform activity. *J. Neurophysiol.* 82: 2262–2270, 1999. Incubation of hippocampal slices in zero-Ca^{2+} medium blocks synaptic transmission and results in spontaneous burst discharges. This seizure-like activity is characterized by negative shifts (bursts) in the extracellular field potential and a K^+ wave that propagates across the hippocampus. To isolate factors related to seizure initiation, propagation, and termination, a number of pharmacological agents were tested. K^+ influx and efflux mechanisms where blocked with cesium, barium, tetraethylammonium (TEA), and 4-aminopyridine (4-AP). The effect of the gap junction blockers, heptanol and octanol, on zero-Ca^{2+} bursting was evaluated. Neuronal excitability was modulated with tetrodotoxin (TTX), charge screening, and applied electric fields. Glial cell function was examined with a metabolism antagonist (fluroacetate). Neuronal hyperpolarization by cation screening or applied fields decreased burst frequency but did not affect burst amplitude or duration. Heptanol attenuated burst amplitude and duration at low concentration (0.2 mM), and blocked bursting at higher concentration (0.5 mM). $CsCl_2$ (1 mM) had no effect, whereas high concentrations (1 mM) of $BaCl_2$ blocked bursting. TEA (25 mM) and low concentration of $BaCl_2$ (300 μM) resulted in a two- to sixfold increase in burst duration. Fluroacetate also blocked burst activity but only during prolonged application (>3 h). Our results demonstrate that burst frequency, amplitude, and duration can be independently modulated and suggest that neuronal excitability plays a central role in burst initiation, whereas potassium dynamics establish burst amplitude and duration.

INTRODUCTION

The generation and spread of spontaneous epileptiform discharges is generally attributed to synaptic excitatory feedback. However, studies performed "in situ" using ion-selective electrodes (Heinemann et al. 1977; Pumain et al. 1985) have shown that during an epileptic seizure extracellular calcium concentration can decrease to levels where chemical synaptic transmission is abolished. Interestingly, several laboratories have demonstrated the development of synchronized epileptiform activity in hippocampal slices when synaptic transmission is blocked with a calcium-free artificial cerebrospinal fluid solution (Jefferys and Haas 1982; Konnerth et al. 1984). This "nonsynaptic" epileptiform activity is characterized by negative shifts (burst discharges) in the extracellular field potential that propagate slowly across the pyramidal cell layer and are always accompanied by a transient increase in extracellular potassium (Konnerth et al. 1984).

Incubation of an acute hippocampal slice in calcium-free solution results in neuronal hyperexcitability and enhanced synchronization (Taylor and Dudek 1982). This hyperexcitability is generally attributed to reduced cation screening (Frankenhaeuser and Hodgkin 1957; Hahin and Campbell 1983), block of calcium-activated hyperpolarizing currents (Alger and Nicoll 1980; Hotson and Prince 1980), and reduced synaptic GABAergic inhibition (Jones and Heinemann 1987). Enhanced ephaptic and electrotonic interactions also contribute to the observed synchrony (Perez-Velazquez et al. 1994; Taylor and Dudek 1982). These nonsynaptic mechanisms, however, are considered to be too fast to account for the slow propagation (0.5–100 mm/s) of burst discharges observed in the zero-calcium model (Haas and Jefferys 1984; Konnerth et al. 1986). It has been suggested that an individual burst is initiated when intense neuronal firing results in a local increase in extracellular potassium (Hounsgaard and Nicholson 1983). This slow increase in $[K^+]_o$ would in turn depolarize neighboring cells that would be induced to fire. Thus it is generally believed that neurons contribute in a feed-forward manner to the waves of potassium observed in this model. The slow speed of propagation is consistent with the diffusion of potassium passively and through glial spatial buffering mechanisms (Yaari et al. 1986).

Although much is known about the mechanisms of increased excitability and synchronization that occur during zero-calcium bursting, the mechanisms underlying burst initiation, termination, and the source of the extracellular field shifts are largely unknown. We therefore tested the effects of several pharmacological perturbations on burst frequency, duration, and amplitude. To study the role of neuronal excitability in the generation and propagation of zero-calcium burst discharges, we examined the effects of cation screening, sodium channel block, and application of electric fields. To investigate the role of gap junction coupling, we applied heptanol and octanol. Several potassium channel blockers were similarly tested. Our results demonstrate that neuronal excitability plays a central role in establishing burst frequency, whereas burst amplitude and duration are modulated by gap junction coupling and potassium conductances.

METHODS

Preparation of hippocampal slices

All experiments were performed in the CA1 pyramidal cell region of hippocampal brain slices prepared from Sprague-Dawley rats (75–

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250 g). Rats were anesthetized using ethyl ether and decapitated. The brain was then rapidly removed and one hemisphere glued to the stage of a Vibroslicer (Vibroslice, Campden). Slicing was carried out in cold (3–4°C), oxygenated sucrose-based artificial cerebrospinal fluid (ACSF) consisting of (in mM) 220 sucrose, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 dextrose. Sucrose-based slicing medium has been shown to increase cell viability in vitro (Aghajanian and Rasmussen 1989). The resulting 350-μm-thick slices were immediately transferred to a holding chamber filled with "normal" ACSF bubbled with 95% O_2-5% CO_2 (pH 7.4). Normal ACSF consisting of (in mM) 124 NaCl, 3.75 KCl, 1.25 KH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_2$, and 10 dextrose (osmolarity 305 ± 4 mosmol l^{-1}, mean ± SD). Slices were held at room temperature for ≥60 min before being transferred to the recording chamber (Warner Instrument), where they were perfused (3–10 ml/min) with normal ACSF (temperature, 34 ± 2 °c) bubbled with 95% O_2-5% CO_2. Fluid level in the recording chamber was maintained under 1 mm. A total of 123 hippocampal slices were used in this study.

Solutions and drugs

All pharmacological studies were conducted in a submerged recording chamber. Following transfer to the recording chamber, slices were perfused in "zero-calcium" ACSF (zero-Ca^{2+}) bubbled with 95% O_2-5% CO_2 (pH 7.4). Zero-Ca^{2+} ACSF was of the following composition (in mM): 123 NaCl, 4.75 KCl, 1.25 KH_2PO_4, 1.5 $MgSO_4$, 26 $NaHCO_2$, 10 dextrose, and 1 EGTA (osmolarity 300 ± 3 mosmol l^{-1}). For all cation screening experiments (including $BaCl_2$) $MgCl_2$ was used in place of $MgSO_4$. 1-Octanol and 1-heptanol (Aldrich) were diluted in zero-Ca^{2+} ACSF and sonicated immediately before use. Monofluroacetic acid (FAC), 4-aminopyridine (4-AP), tetraethylammonium ion (TEA), and 4,4′-diisothiocyanatostilbebe-s,s′-disulfonic acid (DIDS) were obtained from Sigma. $CdCl_2$, $BaCl_2$, and CeCl were obtained from Fisher. 5-Nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) was obtained from RBI. 4–4′-dinitrostilbene-2,2′disolfonic acid, disodium salt (DNDS) was obtained from Molecular Probes. Tetrodotoxin (TTX) was obtained from Calbiochem. Patch pipettes were filled with internal recording solution consisting of (in mM) 140 KGluconate, 2 $MgCl_2$, 2 $CaCl_2$, 2 Na-ATP, 0.2 Na-GTP, 10 EGTA, and 10 HEPES (pH was adjusted to 7.25 with 10 M KOH and osmolarity adjusted to 285–290 mosmol l^{-1}).

Generation and application of electric fields

For studies involving electric fields, slices were transferred to a standard interface recording chamber (Durand 1986). Electric fields were generated across individual slices by passing current between two parallel AgCl-coated silver wires placed on the surface of the ACSF in the interface chamber. Slices were always aligned such that the dendritic-somatic axis was parallel to the direction of the field. Pulses applied to the wires were generated by a voltage generator (Master-8 Programmable Pulse Generator, A.M.P.I.) and converted to a current pulse by a stimulus isolation unit (Grass Instrument). The electric field (mV/mm) in the chamber was calibrated by measuring the voltage difference (mV) between two recording electrodes of known distance (mm). During field application, two recording electrodes were positioned to allow for differential recording. One electrode was positioned in the CA1 pyramidal layer and the other aligned along an isopotential line (Fig. 3*D*).

Field recording

Extracellular recordings of field potentials were made using glass micropipettes (2–5 MΩ) filled with 150 mM NaCl. Recording electrodes were positioned in the somatic layer of the CA1 region. Signals were amplified and low-passed filtered (1 kHz) with an AxoClamp 2B amplifier (Axon Instruments) and an FLA-01 amplifier (Cygnus Technology) and finally stored on a DAT (MicroData System). Monopolar stimulating electrodes were placed on the surface of the alveus. KCl was ejected by pressure (PV 800, WPI) in the CA1 pyramidal layer via a microelectrode filled with 20 mM KCl. Pressure pulses of 500 ms ranged between 5 and 20 psi. In control experiments, NaCl was similarly injected.

For each slice, control amplitude and duration was calculated by sampling 10 bursts during stable control conditions (at least 25 min after switching to low calcium medium). The amplitude and duration from 10 bursts 15–60 min after pharmacological perturbation were similarly averaged. Results are presented as means ± SD. The effects of all the drugs used in this study were completely reversible after 10–40 min of wash.

Whole cell recording

Tight seal (4–6 GΩ) whole cell voltage- and current-clamp recordings were made with an Axopatch-1D amplifier (Axon Instruments). Patch pipettes were pulled from 1.5-mm borosilicate filament containing glass tubing (Warner Instrument) using a two-stage process, firepolished, and coated with silicone elastomer (Sylgard; Dow Corning). The pipette was positioned under visual control with differential interference contrast optics and infrared light (IR-DIC). During voltage clamp cells were held at a command potential of −60 mV. No holding current was used during current-clamp recordings. Data were transferred directly to a computer (DELL XPS H266) using a Digi-Data 1200 board and pCLAMP software (Axon Instruments).

RESULTS

Zero-Ca^{2+} epileptiform activity in a submerged recording chamber

Ten to 20 min after perfusion with zero-Ca^{2+} ACSF, stable field "bursts" were recorded in the CA1 pyramidal cell layer (Fig. 1*A*). Once fully established, the activity remained stable for up to 8 h. The average burst frequency for submerged chamber experiments was 6.2 ± 2.7 per 30 s ($n = 30$). Within a given slice, interevent intervals varied by as much as 50%, however, the number of bursts in a 30-s period was very stable over time. A saturating concentration of cadmium chloride (100–300 μM), a nonselective blocker of voltage-activated Ca^{2+} channels, had no effect on bursting, as expected ($n = 2$).

CA1 pyramidal cells and interneurons were patched in normal ACSF before perfusion with zero-Ca^{2+} media. Individual pyramidal cells were identified in the pyramidal cell layer using IR-DIC (Fig. 1*B*) and by their response to depolarizing current steps (Fig. 1*C*); pyramidal cells exhibit spike frequency adaptation (Lacaille and Schwartzkroin 1988). Immediately after perfusion with zero-Ca^{2+} solution, pyramidal cells displayed increased spontaneous firing, enhanced afterdepolarizations, and reduced afterhyperpolarization following a spike train (Fig. 1*C*). Whole cell current-clamp recordings ($n = 8$) during bursting showed bursts of action potential superimposed on a slow depolarizing shift (Fig. 1*D*). The depolarizing shift occurred simultaneously with extracellular bursts as expected (Haas and Jefferys 1984). Voltage-clamp recordings ($n = 9$) revealed a slow persistent inward current that underlies this slow depolarization shift (Fig. 1*E*).

Interneurons in stratum lacunosom-moleculare were identified by infrared video microscopy (Fig. 1*F*), by their location in the slice (at least 200 μM from the pyramidal cell layer), and by their response to depolarizing current pulses (Fig. 1*G*); interneurons exhibit little spike frequency adaptation (Lacaille and Schwartzkroin 1988). Immediately after perfusion with

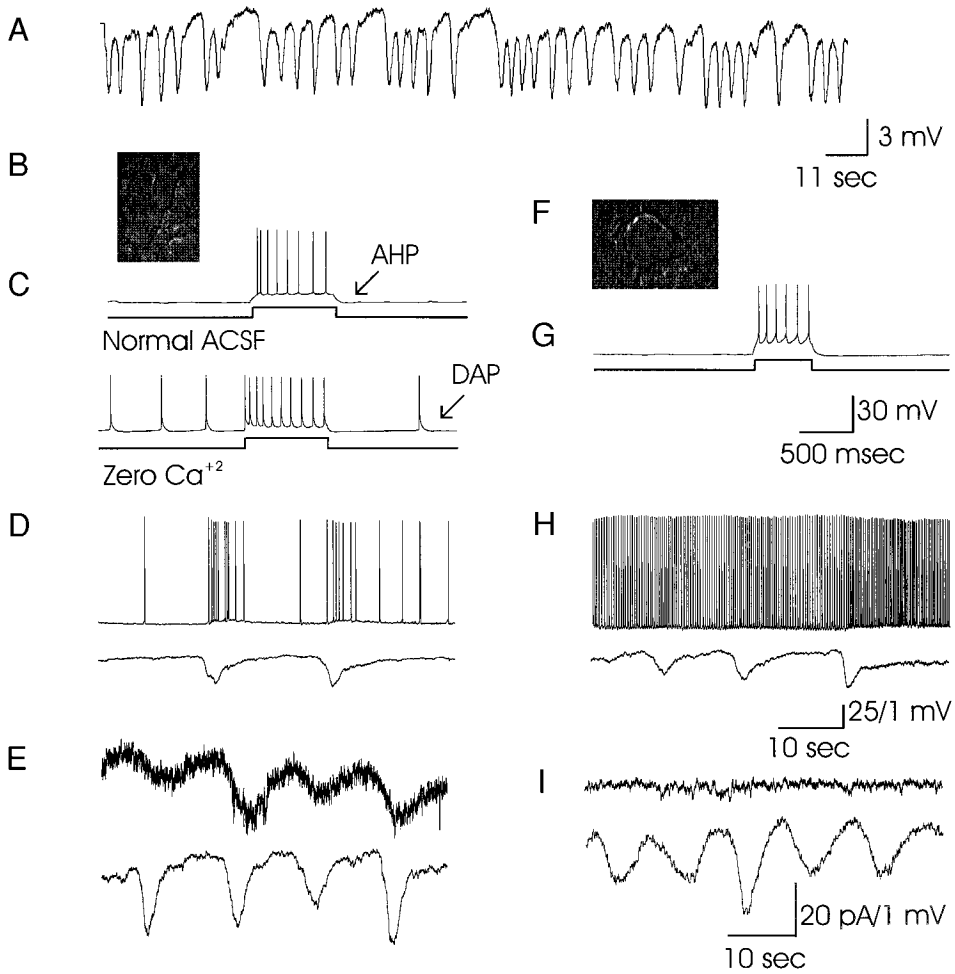

FIG. 1. Effects of incubation in zero-Ca^{2+} medium in a submerged chamber. *A*: field recording in CA1 stratum pyramidale 25 min after switch to zero-Ca^{2+} medium. *B*: frame grabber image of a CA1 pyramidal cell visualized with infrared video microscopy. *C*: response of a patched pyramidal cell to current step before and shortly after perfusion with zero-Ca^{2+} media. Cells displayed increased spontaneous firing, enhanced excitability, enhanced afterdepolarizations (DAP), and reduced slow afterhyperpolarizations following a spike train (AHP). *D*: simultaneous recordings of current-clamped pyramidal cell (*top*) and extracellular field (*bottom*) during zero-Ca^{2+} burst discharges. *E*: simultaneous recordings of voltage-clamped pyramidal cell (*top*) and extracellular field (*bottom*) during zero-Ca^{2+} burst discharges. *F*: frame grabber image of an interneuron visualized with infrared video microscopy. *G*: response of patched interneuron to current step in normal artificial cerebrospinal fluid (ACSF). *H*: simultaneous recordings of current-clamped interneuron cell (*top*) and extracellular field (*bottom*) during zero-Ca^{2+} burst discharges. *I*: simultaneous recordings of voltage-clamped interneuron (*top*) and extracellular field (*bottom*) during zero-Ca^{2+} burst discharges.

zero-Ca^{2+} medium, interneurons, recorded in whole cell current clamp ($n = 3$), began firing tonically and continued to fire at a high rate during zero-Ca^{2+} bursting (Fig. 1*H*). However, no increase in firing rate was observed coincident with field burst activity. Furthermore, voltage-clamp recordings ($n = 4$) showed no change in holding current during bursting (Fig. 1*I*). These results suggest that stratum lacunosom-moleculare interneurons do not play a significant role in the generation and modulation of bursting in the zero-Ca^{2+} model.

Role of neuronal firing during zero-Ca^{2+} bursting

Addition of 1 μM TTX, a sodium channel antagonist at a concentration that blocks action potential generation (Hille 1992), to the perfusate medium, completely blocked spontaneous bursting ($n = 3$; Fig. 2*A*). Localized pressure injection of 2 mM KCl in the stratum pyramidale has been shown to be sufficient to initiate a zero-Ca^{2+} burst (Yaari et al. 1986). Following the block of bursting with 1 μM TTX, subsequent injection of KCl resulted in a local negative shift in the field potential (Fig. 2, *B* and *C*). Multiple field electrodes positioned along the CA1 layer, however, showed that this shift decayed quickly over distance (i.e., never measurable >0.5 mm away from injection site) and always followed the injection with no delay (Fig. 2*C*). Varying the amplitude of the KCl injection varied the size of the induced potential shift. Pressure injection of 2 M NaCl did not produce a negative shift in the field potential ($n = 2$). Thus, although neuronal firing does not appear to be essential for the generation of a burstlike shift in the field potential, neuronal firing is required for burst propagation.

Effects of neuronal excitability on zero-Ca^{2+} burst frequency

Because neuronal firing is required for the propagation of zero-Ca^{2+} bursts, we next studied how burst activity is affected by changes in neuronal excitability. To modulate neuronal excitability we used cation screening and electric fields to directly polarize cells. Consistent with earlier findings (Haas and Jefferys 1984), increasing extracellular magnesium concentration ($[Mg^{2+}]_o$) from 1.5 to 3.2 mM decreased burst frequency but did not affect burst amplitude or duration (Fig. 3, *A* and *B*) in 10 slices. In two other slices in which high burst frequency was observed (defined as >1 per 3 s), increasing ($[Mg^{2+}]_o$) increased burst amplitude. This could be due to each burst no longer arriving during the relative refractory period of the previous one (Konnerth et al. 1986). Increasing $[Mg^{2+}]_o$ above 3.7 mM arrested bursting, reversibly, in all slices tested ($n = 3$). Figure 3*C* shows the changes in burst frequency observed during incremental increases in $[Mg^{2+}]_o$ from a representative slice. A similar linear relationship was seen with all slices tested (average slope, -3.8 ± 2.0; $n = 3$).

Application of uniform electric fields has also been shown to modulate neuronal excitability (Durand 1986). In the zero

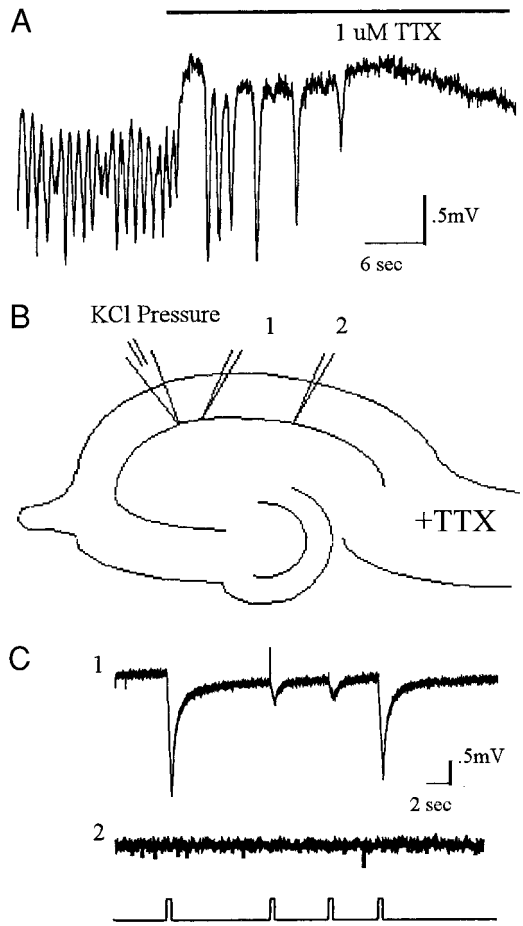

FIG. 2. Induction of bursts by KCl injection. *A*: time course of bursting block with TTX. *B*: schematic of the electrode placement of field and KCl injection electrodes. *C*: induced "bursts" measured at *electrode 1*. Notice no spontaneous or induced activity was measured at *electrode 2*. *Bottom trace*: times of injection. First and last injections: 20 psi. Middle two injections: 10 psi.

calcium model, exogenous electric fields caused changes in burst frequency but not burst amplitude or duration ($n = 10$) except at high burst frequencys (>1 per 3 s; Fig. 3, *D* and *E*). Electric fields generating a depolarization of the somatic membrane (anode near tip of apical dendrites) enhanced burst frequency, whereas hyperpolarizing fields (anode near basal dendrites) reduced burst frequency. Incremental steps in field strength resulted in a linear modulation of burst frequency (Fig. 3*F*) with an average slope of 1.5 ± 0.2 ($n = 3$).

Factors affecting zero-Ca^{2+} burst amplitude

Because amplitude was not effected by neuronal excitability, we tested the hypothesis that burst amplitude could be modulated by network properties. We therefore added heptanol and octanol to the perfusate media to study the role of intracellular gap junction coupling on zero calcium bursting. Heptanol (0.5–3 mM; $n = 8$) and octanol (0.3 mM; $n = 3$) reversibly blocked bursting in all slices tested. Lower concentrations of heptanol (0.2 mM) attenuated bursting in eight slices and blocked bursting in two (Fig. 4, *A* and *B*). Both burst amplitude and duration were significantly reduced at lower concentrations of heptanol (0.2 mM). Burst frequency, however, was not consistently altered at this concentration.

Because heptanol can antagonize sodium currents (Largo et al. 1997; Nelson and Makielski 1991), we tested the effect of heptanol perfusion on the intrinsic firing properties of patched CA1 pyramidal neurons (Fig. 4*C*). We found that resting membrane potential (97 ± 5.1% control), input resistance (96 ± 10% control), action potential amplitude (97 ± 1.5% control), threshold (106 ± 13% control), and width (94 ± 9.7% control) were not significantly effected by heptanol at a concentration (0.2 mM) shown to modulate burst activity ($n = 3$). Taken together, these results suggest that the movement of ions through either glial or neuronal gap junctions contributes to the generation of the extracellular field shift.

Factors affecting burst duration

Because potassium concentration is known to rise during a burst (Yaari et al. 1986), we investigated the effect of potassium channel blockers on zero-Ca^{2+} bursting. Cesium and barium have been shown to reduce K_{ir} (Ransom and Sontheimer 1995) and reduce potassium uptake by astrocytes (Ballanyi et al. 1987; Janigro et al. 1997; Walz and Hinks 1985). To investigate the role of the K_{ir} channel and glial potassium uptake, we perfused slices with CeCl (0.1–1 mM) and $BaCl_2$ (0.3–1 mM). Zero-Ca^{2+} bursting was not affected at any concentration of cesium tested ($n = 7$). Similarly, 300 μM Ba^{2+} had no effect on bursting ($n = 2$), whereas 700 μM Ba^{2+} increased burst duration two- to sixfold ($n = 4$; Fig. 5*A*). In experiments where individual bursts became so wide that they followed one another with no delay, a concomitant decrease in burst frequency was observed. Higher concentrations of barium (1 mM) blocked bursting in all slices tested ($n = 3$).

Interestingly, Agopyan and Avoli (1988) observed a similar dramatic increase in burst duration after removal of extracellular Cl^-. Because Ba^{2+} has been shown to inhibit KCl uptake by glia (Ballanyi et al. 1987), we tested the effect of DNDS, a glial Cl^- channel blocker (Muller and Schlue 1998), on burst duration. We found that low concentrations of DNDS (20–200 μM; $n = 6$) had no effect of bursting. A higher concentration of DNDS (2 mM; $n = 2$) resulted in a 6- to 10-fold increase in burst duration (Fig. 5*C*). Two other chloride channel blockers (Muller and Schlue 1998), NPPB (0.1–0.2 mM, $n = 2$) and DIDS (10–200 μM, $n = 9$), blocked bursting at all concentrations tested.

To determine the role of outward potassium currents, we tested the effects of TEA, an antagonist of the voltage-activated delayed rectifier channel (I_k) (Hille 1992). TEA (25 mM, $n = 4$) attenuated burst amplitude and increased burst duration (Fig. 6*A*). 4-AP (5 mM, $n = 3$), which blocks the fast transient voltage-activated potassium current (I_A) (Storm 1990) did not affect bursting ($n = 3$; Fig. 6*B*). Preliminary data indicate that the above effects were not due to changes in solution osmolarity.

DISCUSSION

The main finding of this study is that burst frequency, amplitude, and duration can be independently modulated in the zero-Ca^{2+} model of nonsynaptic bursting. The following discussion will address each aspect of this finding in greater detail.

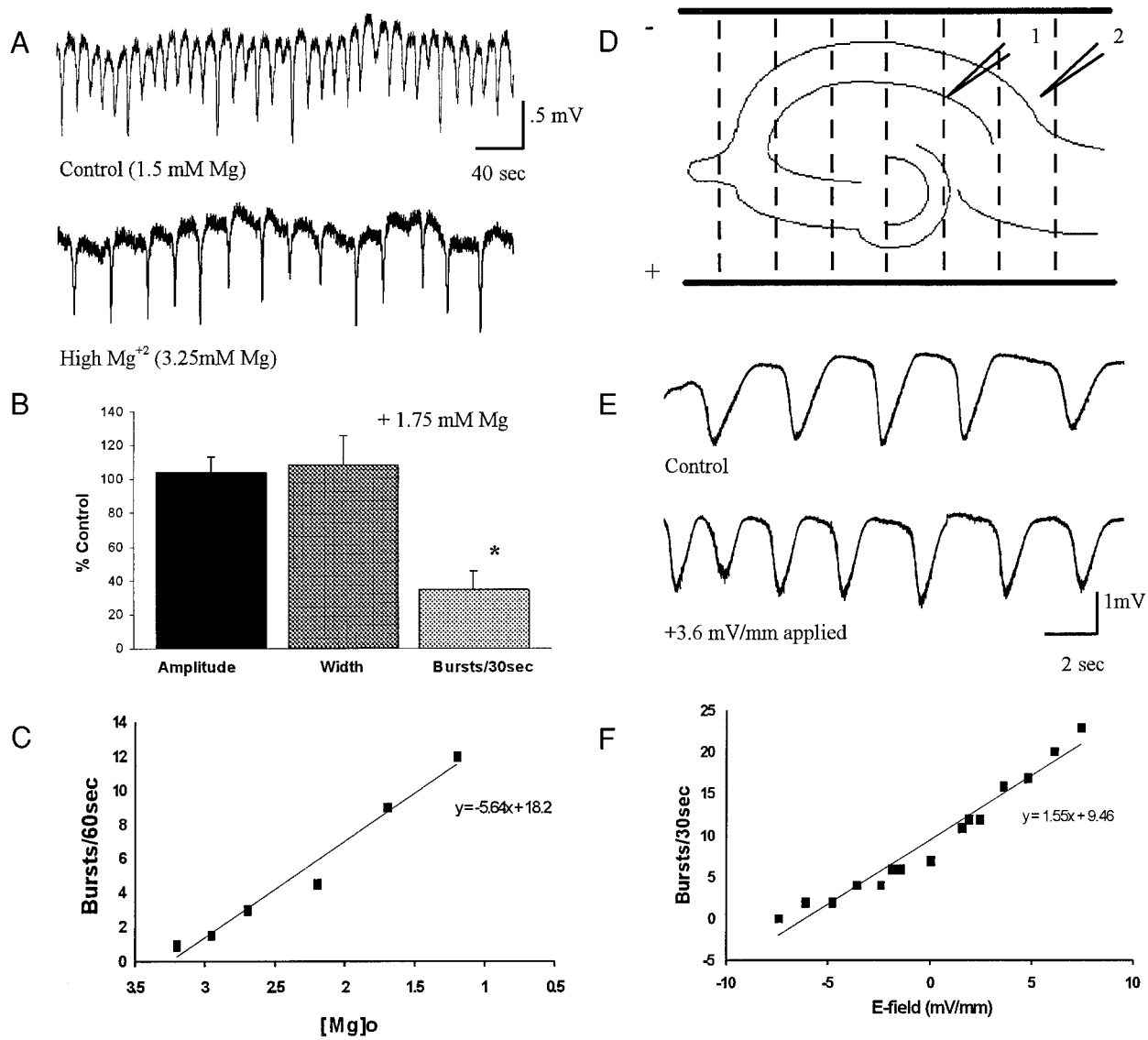

FIG. 3. Neuronal excitability was altered by both cation screening and application of electric fields. *A*: effect of increasing $[Mg^+]_o$ from 1.25 to 3.25 mM during zero-Ca^{2+} bursting. *B*: cumulative results of cation screening experiments ($n = 8$). *C*: effect of incremental increases in $[Mg^+]_o$ on burst frequency. *D*: experimental setup for electric field experiments. Fields were generated across individual slices by passing current between 2 parallel AgCl-coated silver wires. Slices were always aligned such that the dendritic-somatic axis was parallel to the direction of the field (dotted lines) with bias as indicated in the diagram. The potential at *electrode 2* was subtracted from *electrode 1* to eliminate the applied field artifact. *E*: effects of application of electric fields during zero-Ca^{2+} bursting. *F*: effect of electric field polarity and magnitude on burst frequency.

Factors affecting burst frequency or initiation

Previous studies have shown that several drugs and transmitter candidates, which presumably interfere with neuronal excitability, modified burst frequency but not burst amplitude or duration (Jefferys and Haas 1982). Consistent with these studies, using both cation screening and electric fields to polarize CA1 cells, we were able to vary the frequency of zero-calcium field bursts without affecting burst size or duration. The effect of $[Mg^{2+}]_o$ on action potential threshold changes has been shown to be linear over the concentration range used in this study (Hahin and Campbell 1983). Similarly, the amplitude of the membrane polarization varies linearly with the strength of the applied electric field (Nakagawa and Durand 1991). Interestingly, for both electric fields and cation screening, the relationship between degree of polarization and burst frequency was found to be linear. One explanation for this finding is that cation screening and electric fields polarize a pacemaker cell or group of cells, modulating their intracellular burst rate in a linear fashion, and thus the rate of field burst initiation.

Linear modulation of action potential frequency, by neuron polarization, has been demonstrated for maximal dentate activation-like bursting (Pan and Stringer 1996) and for endogenously bursting CA3 cells (Johnston and Brown 1984). Polarization of cells with electric fields has been shown to specifically modulate the population burst frequency of high-K^+–induced epileptiform activity (Gluckman et al. 1996). Elevation of extracellular potassium, which has the effect of depolarizing cell membranes, has been shown to increase burst frequency linearly in the zero-Ca^{2+} (Yaari et al. 1986), 4-AP,

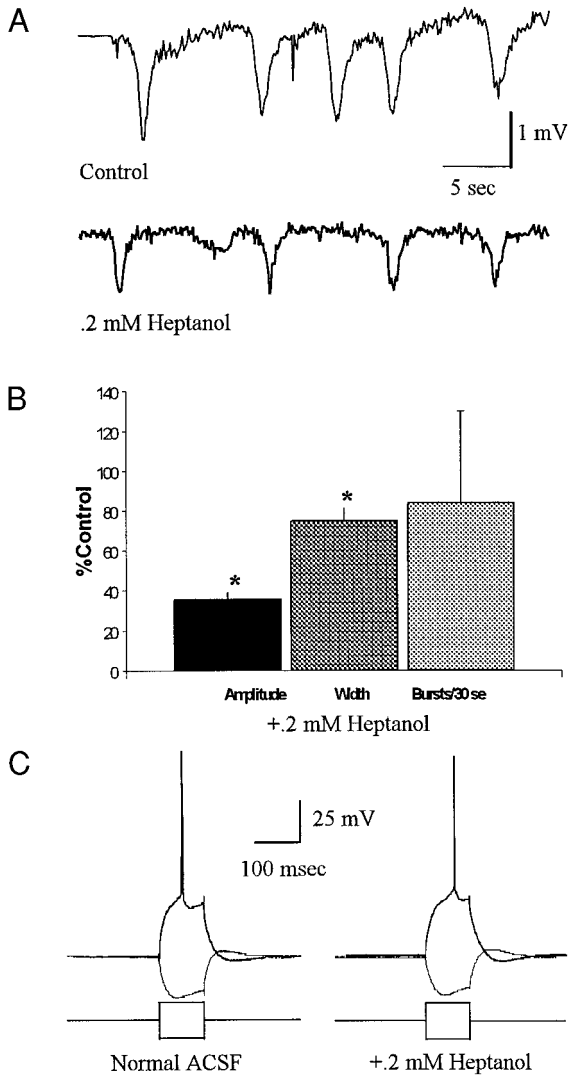

FIG. 4. Modulation of zero-Ca^{2+} field burst activity in CA1 stratum pyramidale by gap junction blockers. *A*: effect of 0.2 mM heptanol on spontaneous epileptiform bursts. *B*: cumulative results after heptanol perfusion ($n = 5$). *C*: response of pyramidal cell, recorded in whole cell current clamp, to 100-ms depolarizing and hyperpolarizing pulses before and after addition of 0.2 mM heptanol.

high-K^+, and bicuculline (Rutecki et al. 1985, 1987) models of epilepsy. Taken together these results suggest that membrane polarization plays a central role in establishing the frequency of epileptiform bursting.

Factors effecting burst amplitude

Electric fields, cation screening, and drugs that effect neuronal excitability (Haas and Jefferys 1984; Jefferys and Haas 1982) have been shown to alter the interburst interval but not burst size or duration. Similarly, Watson and Andrew (1995) found no correlation between neuronal excitability and burst amplitude. Thus once a nonsynaptic burst is generated, its amplitude and duration appear to be independent of the level of neuronal excitability. This suggests that neuronal action potentials are not directly responsible for the generation of the slow field shift. Treatments that enhanced neuronal excitability increased the frequency of the population spikes that are sometimes observed superimposed on the slow zero-Ca^{2+} bursts induced in an interface chamber (Hass and Jefferys 1984). There is no doubt that these fast spikes are summations of synchronized neuronal action potentials. We propose, however, that the slow extracellular potential shift observed in the zero-calcium model is due, at least in part, to an influx of potassium into glia (Dietzel et al. 1989). Recently, it was shown that block of a field burst is always coincident with block of the accompanying potassium wave (Ghai et al. 1998). Moreover, local KCl injection, in the presence of TTX, could still elicit a local negative field shift. Several groups have correlated a slow shift in the extracellular field potential with the influx of potassium into glia (Gabriel et al. 1998; Heinemann and Walz 1998). These results suggest that the field shift observed during zero-calcium bursting is due in part to glial spatial buffering of extracellular potassium (Yaari et al. 1986). Another likely contributor to the extracellular field shift is a persistent inward sodium current into neurons (Fig. 1*E*).

Consistent with earlier reports, we found that heptanol

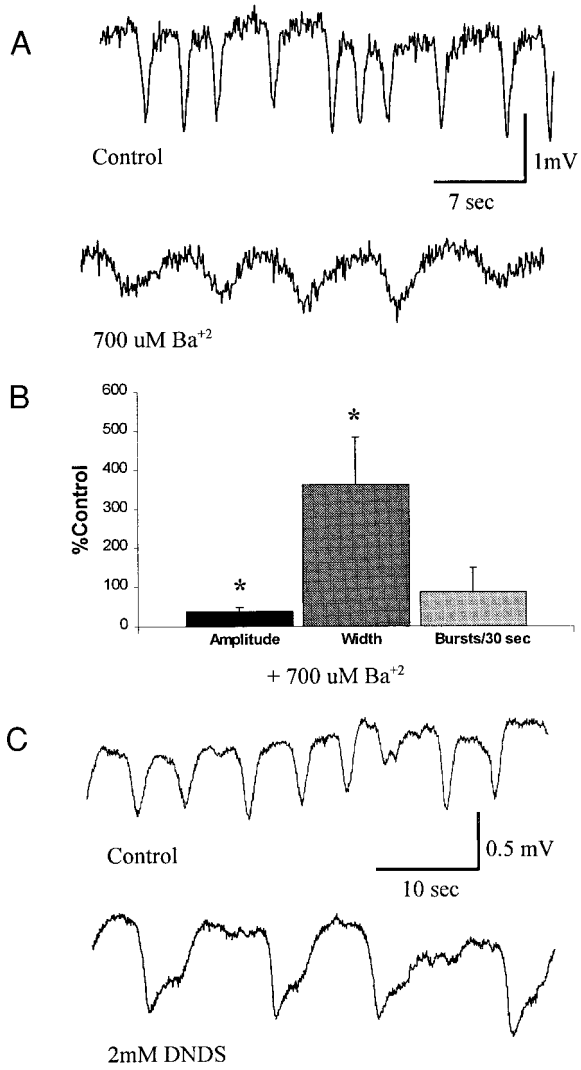

FIG. 5. Modulation of zero-Ca^{2+} field burst activity in CA1 stratum pyramidale by Ba^{2+} and 4–4′-dinitrostilbene-2,2′disolfonic acid, disodium salt (DNDS). *A*: effect of 700 μM Ba^{2+} on spontaneous epileptiform bursts. *B*: cumulative results after Ba^{2+} perfusion ($n = 4$). *C*: effect of 2 mM DNDS on spontaneous epileptiform bursts.

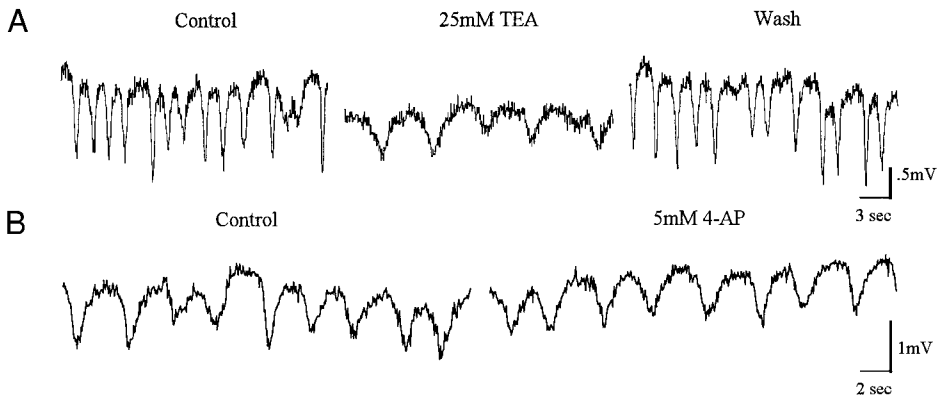

FIG. 6. Effects of K^+ channel blockers on zero-Ca^{2+} bursting. *A*: bursting before, during, and after wash of 25 mM TEA. *B*: effect of 5 mM 4-aminopyridine (4-AP) on bursting.

(Perez-Velazquez et al. 1994) and octanol, two gap junction blockers, annihilated bursting. Several lines of evidence would suggest that gap junction blockers modulate nonsynaptic bursting via a glial specific mechanism. First, CA1 pyramidal cells exhibit very restricted coupling (Haas and Jefferys 1984) and exhibit little connexin expression (a gap junction protein) (Dermietzel and Spray 1993; Shiosaka et al. 1989). Second, although it has been shown that perfusion with low calcium media can enhance coupling between a small number of nearby CA1 pyramidal cells (Perez-Velazquez et al. 1984), CA1 astrocytes are characterized by an extremely high degree of cell-to-cell coupling among hundreds of cells (D'Ambrosio et al. 1998) and express high levels of connexins (Dermietzel and Spray 1993; Yamamoto et al. 1990). Third, each burst is accompanied by a transient increase in extracellular K^+. Glial cell membranes exhibit a selective permeability to K^+ and have been postulated to play a central role in the buffering of extracellular K^+ changes (Nilius and Reichenbach 1988). Taken together, these results are consistent with our hypothesis that direct block of glial gap junctions inhibits zero-calcium bursting and suggests that movement of K^+ through glial gap junctions directly contributes to the generation of extracellular field shifts.

Proliferating glial tissue is found in nearly all epileptogenic lesions (Somjen 1980; Vital et al. 1994). Naus et al. (1991) found that Connexin43, the principle gap junction protein of astrocytes, is elevated in surgical samples removed during epilepsy resection. Lee et al. (1995) found that gap-junction coupling was more pronounced in cells isolated from epileptic tissue than from normal tissue. Glial proliferation and enhanced coupling have been proposed to be factors in the generation of seizures, however, by what mechanisms remains unclear (Lee et al. 1995; Somjen 1980). Our results suggests that electrotonic coupling between glia may be critical for the buffering of extracellular potassium waves associated with seizure activity.

Factors effecting burst duration or termination

In astrocyte cultures, inward-rectifier potassium (K_{ir}) currents were blocked by both cesium (K_d = 189 μM) and barium (K_d = 3.5 μM) (Ransom and Sontheimer 1995). Extracellular application of 1 mM cesium or 300 μM barium, however, had no effect on bursting in our studies, suggesting that the K_{ir} current does not contribute to zero-calcium bursting. Furthermore, it was recently shown that potassium channels on most CA1 astrocytes are not affected by extracellular cesium (D'Ambrosio et al. 1998), suggesting that K_{ir} channels are not expressed in this region. Because barium had no effect on burst duration at concentrations below 700 μM (a concentration that far exceeds the K_d to block K_{ir} channels), the elongation and blockage of bursts at higher concentrations is not likely to be mediated by an action at K_{ir} channels.

As shown above, application of TEA resulted in an elongation of zero-Ca^{2+} burst duration similar to that observed with 700 μM barium. The increase in burst integral observed during these two treatments may reflect an increase in net potassium uptake by glia. This paradoxical result is supported by work showing an increase in stimulation-induced K^+ efflux after addition of barium or TEA (Ballanyi et al. 1987; Gabriel et al. 1998; Jones and Heinemann 1987). The failure of 25 mM TEA and 5 mM 4-AP to block bursting suggests that the annihilation of bursting by 1 mM barium is not due to inhibition of outward potassium channels.

Several researchers have found that in addition to glial spatial buffering (i.e., Cl^--independant K^+ uptake), KCl cotransport and accumulation by glia accompanies neuronal discharges (Dietzel et al. 1989; Walz and Hinks 1985). During zero-Ca^{2+} bursting, large decreases in $[Cl^-]_o$ are observed (Heinemann et al. 1992). Removal of extracellular Cl^- (Agopyan and Avoli 1988), addition of the Cl^- channel blocker DNDS (2 mM), or addition of 700 μM Ba^{2+} results in a 6- to 10-fold increase in burst duration. As such, the increase in burst duration by 700 μM Ba^{2+} and block by 1 mM Ba^{2+} may be explained by the inhibition of KCl uptake (Ballanyi et al. 1987). The difference between the actions of heptanol and Ba^{2+} could be due to the former affecting spatial buffering (which requires coupling via gap junctions) and the latter affecting KCl accumulation by glia. If this were the case, however, it is unclear why TEA, which should not interfere with glial KCl accumulation, would cause an increase burst duration. The nonspecific effects of high concentrations of TEA on glial potassium channels have not been investigated.

It has been suggested that monofluroacetic acid (FAC) interferes with glial cell metabolism (Largo et al. 1997) and acts as a "suicide" agent for glial cells (Clarke 1991). To directly test the role of glia on zero-Ca^{2+} bursting, slices were perfused in 100 μM FAC. Prolonged incubation in FAC resulted in some attenuation of burst amplitude and block of bursting in two slices (n = 5). However, this attenuation was always followed by a decrease in the antidromic response size, suggesting overall tissue damage. Therefore until glial specific

agents are developed that do not produce overall tissue damage, we will be unable to determine the precise role of glia in the modulation of nonsynaptic bursting.

Summary: relevance to epileptogenisis in vivo

From our results we conclude the following. *1*) Neuronal firing plays a central role in zero-Ca^{2+} burst initiation and is required for propagation, but the level of neuronal excitability does not effect the generation of the slow shift in the field potential. *2*) Movement of ions through gap junctions (presumably glial) contributes to the amplitude of the extracellular field shift. *3*) Zero-Ca^{2+} burst termination is mediated by neuronal potassium efflux or glial KCl accumulation. Most current treatments of epilepsy target seizure activity by attempting to calm neurons. Our results suggest that these agents interfere with seizure initiation. Furthermore, we have shown that agents that interfere with burst termination are effective at blocking epileptiform activity. Thus rational therapies for epilepsy could be aimed at interfering with burst termination and propagation mechanisms, or glial cell function (Armand et al. 1997; Somjen 1980).

This work was supported by The Whitaker Foundation and National Science Foundation Grant IBN93-19591.

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Received 18 March 1999; accepted in final form 12 July 1999.

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