Role of Intrinsic Burst Firing, Potassium Accumulation, and Electrical Coupling in the Elevated Potassium Model of Hippocampal Epilepsy

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INTRODUCTION

The hippocampus has a low seizure threshold (Green 1969), which may explain why most complex partial (focal) epileptic seizures either originate in or are elaborated by this structure (Spencer et al. 1987). In experimental animals, the hippocampus can be readily induced to generate recurring seizures by topical applications of convulsant drugs (Dichter and Spencer 1969) or high-K⁺ solutions (Zuckermann and Glaser 1968). During a hippocampal seizure (ictal episode), large neural aggregates are recruited into excessive, highly synchronous discharge that endures several seconds or minutes. In the interseizure (interictal) periods the epileptogenic focus remains active, generating more or less regularly brief (lasting tens or hundreds of ms) and spatially restricted bursts of synchronized neuronal discharge. Before seizure onset the interictal paroxysms may increase progressively in size and duration until they give way to sustained ictal discharge (Dichter and Spencer 1969; Dichter et al. 1972). Procedures to induce hippocampal ictal activity in vitro also have been described, e.g., perfusion of hippocampal slices with saline deficient in Ca²⁺ (Haas and Jefferys 1984; Konnerth et al. 1986) or in Mg²⁺ (Anderson et al. 1986), or enriched with K⁺ (Jensen and Yaari 1988; Leschinger et al. 1993; Traynelis and Dingledine 1988; Yaari and Jensen 1988). The high-K⁺ model is particularly attractive, because it is manifested in many cases by progressive transitions from interictal bursts to maximal seizure activity (Jensen and Yaari 1988), reminiscent of interictal-ictal transitions in animal models of focal epilepsy (Dichter et al. 1972; Matsumoto and Ajmone-Marsan 1964a,b; Ralston 1958). Moreover, ictal episodes in this model are focal in nature and consist of a sequence of sustained and intermittent synchronous discharges (Jensen and Yaari 1988; Traynelis and Dingledine 1988), resembling the paroxysmal activity during tonic-clonic seizures (Matsumoto and Ajmone-Marsan 1964b). Thus this model may render several features of seizure electrogenesis in vivo to electrophysiological analyses in the advantageous in vitro situation (Jensen and Yaari 1988, 1991, 1992; Traynelis and Dingledine 1988, 1989).

We have used extra- and intracellular recordings to characterize the mechanisms involved in the generation of interictal bursts and ictal episodes in CA1 in the high-K⁺ model of hippocampal epilepsy. We were particularly interested in appraising the role of intrinsic bursters in this model, because it was recently shown that intrinsic burst firing is up-regulated by moderately elevated concentrations of extracellular K⁺ (K⁺ₑ) (Jensen et al. 1994, 1996). Here we present data suggesting that neuronal recruitment during interictal bursts and ictal discharges is mediated by an interplay of synaptic excitation, electrical interactions, and K⁺ₑ accumulation. Because intrinsic bursters are recruited long before other neurons, they may provide a critical amplification for recruitment of the remaining neuronal population into seizure discharge.

METHODS

Preparations and solutions

Experiments were performed on transverse hippocampal slices from 8- to 10-wk-old Wistar and Sabra rats. After ether anesthesia...
and decapitation, the hippocampi were dissected out and kept in cold (0–1°C) saline for 1–2 min. Transverse slices (450 μm) were then cut with a vibratome and placed on a small piece of lens paper supported by a nylon mesh in an interface recording chamber. The slices were kept at 36°C and perfused from below with oxygenated (95% O₂, 5% CO₂) saline. The gas mixture was also bubbled through the water bath in the chamber and directed toward the upper surface of the slices. The slices were equilibrated in the chamber for >1 h before the experiments were started.

The standard saline comprised (in mM) 124 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 1.2 MgSO₄, 1.2 CaCl₂, 26 NaHCO₃, and d-glucose, 10. High-K⁺ saline contained 7.5 mM KCl.

Stimulation and recording

Focal stimulation in stratum radiatum (in CA3 or CA2) was performed with bipolar electrodes made of sharpened glass-insulated platinum wires (50 μm). For stimulation, square pulses of either 1–20 V or 50–1,000 μA amplitude and 50–70 μs duration were delivered by an isolation unit. All recordings were performed in the pyramidal layer of CA1a or CA1b, with the use of the morphological landmarks provided by Masukawa et al. (1982). Extracellular recordings were performed with microelectrodes filled with 150 mM NaCl (5–10 MΩ resistance). Double-barreled K⁺-sensitive microelectrodes were prepared with the use of the method described by Lux and Neher (1973). The K⁺-sensitive barrel contained a K⁺ ionophore (Flucka); the reference barrel was filled with 150 mM NaCl. It was used for recording the extracellular field potential, which was electrically subtracted from the compound response recorded by the K⁺-sensitive barrel. The microelectrodes were calibrated before and after each experiment with solutions of known K⁺ concentrations, thus enabling the conversion of electrode responses into changes in K⁺ concentration ([K⁺]ₑ). The minimum acceptable microelectrode response for a 10-fold change in [K⁺]ₑ was 55 mV. The response time of these microelectrodes to an iontophoretic K⁺ pulse delivered near their tips in a saline-filled dish was <50 ms.

For intracellular recordings glass microelectrodes filled with 4 M K⁺ acetate (50–80 MΩ) were used. An active bridge circuit in the amplifier (Axoprobe, Axon Instruments) allowed simultaneous injection of current and measurement of membrane potential. The bridge balance was carefully monitored and adjusted before each measurement.

In paired extracellular/intracellular recordings care was taken to position the microelectrode tips in close proximity (Taylor and Dudek 1984a). The field potentials recorded extracellularly by the two microelectrodes were compared after cell impalement was lost. Calculation of transmembrane (intracellular minus extracellular) potentials was made only if the two microelectrodes registered similar extracellular signals (difference <15%). Given values of membrane potentials represent the transmembrane potential differences.

Neurons were identified as pyramidal cells (PCs) if they responded with short-latency spikes to stimulation of the alveus and manifested strong spike frequency accommodation during sustained depolarization. The PCs accepted for this study had stable resting potentials of at least −55 mV and overshooting action potentials.

Data analysis

The extra- and intracellular signals were recorded on a digital tape recorder (VT-100, Instrutech), digitized, and stored by a personal computer with the use of a data acquisition system (TL-1 DMA, Axon Instruments). Off-line data analyses were performed with the use of commercial software.

Averaged data are expressed as mean ± SD. The significance of the differences between the measured parameters was evaluated with the use of the paired Student’s t-test, with a significance level of P < 0.05.

Terminology

The terms used here for the different patterns of synchronous neuronal discharge recorded in the in vitro hippocampal slice (interictal and ictal or seizure; tonic and clonic) have been adopted from the terminology used to describe electrophysiologically similar epileptiform phenomena in vivo (Lothman et al. 1991; Matsumoto and Ajmone-Marsan 1964a,b), which, in the unanesthetized animal, may have distinct behavioral correlates (e.g., tonic-clonic convulsions).

RESULTS

Brief population bursts at 0.5–1 Hz appeared in all 121 slices exposed to high-K⁺ saline. These events originate in CA3 and spread from there into CA1 (Korn et al. 1987; Rutecki et al. 1985). A subset of 51 slices (42%) also developed recurring tonic-clonic ictal episodes every 0.5–2 min limited to CA1, as described previously (Jensen and Yaari 1988; Traynelis and Dingledine 1988). The following data were collected from these 51 slices manifesting interictal-ictal transitions.

Slow potential shifts during an interictal-ictal cycle

Prolonged (>1 h) simultaneous intracellular/extracellular recordings of seizure activity were obtained from 25 PCs in 15 different slices. As illustrated in Fig. 1A, the negative-and positivegoing extracellular potential shifts during an interictal-ictal cycle were tightly correlated with slow intracellular depolarizations and repolarizations, respectively, indicating that neighboring PCs undergo similar potential oscillations.

The membrane potential immediately before the beginning of ictal discharge was −61.4 ± 5.6 (SD) mV. The onset of an ictal discharge was signaled by the appearance of sustained synchronous discharge in the form of large popul-
PC properties during an interictal-ictal cycle

The mean input resistance of the PCs, determined from voltage deflections produced by small hyperpolarizing 100-ms current pulses, was steady through most of the interseizure period (Fig. 2A), amounting to 20.1 ± 7.3 MΩ (n = 24). A rather low neuronal input resistance of CA1 PCs was previously noted in high-K⁺ saline (e.g., 20.9 MΩ, Traynelis and Dingledine 1988; 23.6 MΩ, Jensen et al. 1994). During the ictal episode the input resistance decreased (Fig. 2A). This reduction was maximal during the tonic depolarization (14.4 ± 7.4 MΩ, n = 18), but was also detectable throughout the clonic phase.

Injecting 500-ms depolarizing current pulses into PCs evoked a high-frequency discharge that rapidly accommodated (Fig. 2B). This previously described spike frequency accommodation, caused by the activation of slow K⁺ conductances (Madison and Nicoll 1984), persisted unchanged throughout the interictal period (Fig. 2B; n = 7). The slow afterhyperpolarization that followed the depolarizing current pulses (known to be mediated by a slow Ca²⁺-activated K⁺ current; Lancaster and Adams 1986) was smallest in amplitude immediately after an ictal episode and increased toward the next seizure (Fig. 2B; n = 7).

Pattern of population discharge during an interictal-ictal cycle

The characteristic pattern of synchronous population discharges during an interictal-ictal cycle is illustrated in Fig. 3 (bottom traces in A and B). The first component of each interictal, tonic, and clonic discharge was a spindle-shaped cluster of five to seven population spikes, the primary burst. The size of the population spikes within the primary burst immediately after a seizure episode was small, but grew steadily during the interseizure period (compare Fig. 3, a–c). It became maximal during the tonic and clonic events (Fig. 3, d and f). In contrast, the discharge rate within the primary burst during interictal, tonic, and clonic events was the same (165 Hz). Likewise, when averaged over 19 slices, the population spike frequencies during interictal (171 ± 22 Hz), tonic (178 ± 46 Hz), and clonic (182 ± 38 Hz) primary bursts were strikingly similar.

Large primary bursts were followed by an afterdischarge, comprising a variable series of population spikes generated at a lower rate than the primary burst. The afterdischarge concurred with a negative field potential, whose amplitude and duration were proportional to the size and number of the population spikes, respectively (Fig. 3B). However, a clear separation between primary burst and afterdischarge was not always evident. An afterdischarge of up to 10 population spikes usually appeared in the late part of the interseizure period (compare Fig. 3, Ba–Bc). The afterdischarge during the tonic event lasted for several seconds (4.5 ± 3.0 s), gradually decrementing in frequency (Fig. 3, Bd and Be). Each clonic event comprised an afterdischarge of up to 20 population spikes following the primary burst (Fig. 3Bf).

Recruitment of nonbursters to the primary burst

Intracellular impalements of PCs were used to characterize the intrinsic firing pattern of each neuron and its contribution to the various phases of an interictal-ictal cycle. Of 25 PCs, 20 generated a solitary spike in response to 5-ms depolarizing current pulses injected through the intracellular microelectrode. Of these 20 nonbursters, 13 neurons (65%) fired during interictal bursts, whereas the remaining 7 PCs were excited below their threshold during these events. All
20 neurons fired during the tonic and clonic events. Action potentials were always coincident with population spikes, indicating that the neurons fire in tight synchrony with the population, albeit intermittently.

The characteristic behavior of a regular firing PC throughout an interictal-ictal cycle is illustrated in Fig. 3 (top traces in A and B). During the early interseizure period the intracellular correlate of an interictal population burst was a subliminal depolarizing shift of ~5 mV (Fig. 3Ba). This potential presumably is a composite excitatory postsynaptic potential evoked by a CA3 population burst. From the mid- to the late interseizure period the size of the interictal depolarizing shift did not increase substantially, if at all. Yet the neuron fired one to three spikes, the number of spikes increasing with the size of the corresponding population spikes (Fig. 3, Bb and Bc). During the tonic and clonic primary bursts the neuron generated three spikes as well (Fig. 3, Be and Bf).

It was noted that the initial recruitment of all 20 nonbursters into interictal, tonic, or clonic discharges always concurred with one of the larger population spikes in the primary burst. On average it was delayed by 14 ms with respect to the beginning of the primary burst (minimum delay 10 ms) (Fig. 3, Bc, Bd, and Bf; see also below, Fig. 4B). Thus these neurons could not contribute directly to the formation of the early component of the primary burst.

Recruitment of intrinsic bursters to the primary burst

Of the 25 PCs, 5 displayed spontaneous burst firing that was blocked by hyperpolarizing them a few millivolts with steady negative current injection, thus identifying them as intrinsic bursters (Jensen et al. 1994, 1996). The number of spikes within intrinsic bursts ranged from three to five (averaging 3.8 ± 0.4). The average frequency of intraburst spikes was 173 ± 30 Hz (n = 5).

In contrast to the delayed recruitment of nonbursters described above, bursters always were recruited either slightly before or at the start of the interictal, tonic, and clonic primary bursts. On average, these five neurons fired 3.2 ± 1.5 spikes during the midinterictal bursts. This was fourfold higher (significant at $P < 0.05$, unpaired Student’s t-test) than the midinterictal burst discharge of the 20 nonbursters.

The firing of a representative burster throughout an interictal-ictal cycle is illustrated in Fig. 4 (top traces in A and B). Intrinsic bursts consisted of three to four spikes capping a slow depolarizing potential (Fig. 4, Bb–Bd). The neuron was recruited at the very onset of each interictal burst and fired four to five spikes (Fig. 4, Ba, Bc, and Bd). Of these, the first spike was not coincident with an obvious population spike, but latter spikes became synchronized with the population. During tonic and clonic discharges as well, the neuron was recruited before the appearance of obvious population spikes (Fig. 4, Be and Bg). Thus this neuron contributed to the generation of both early and late components of the interictal, tonic, and clonic primary bursts.

In the five bursters, the size of the depolarizing shift underlying the interictal, tonic, and clonic primary bursts markedly varied, depending on the temporal overlap of the intrinsic and population bursts. The depolarizing shift was maximal when the two bursts concurred (~30 mV; Fig. 4g), and smaller when they were separated (~10 mV; Fig. 4, a, c, and d). However, in all cases the depolarization shift underlying the primary burst was larger in bursters than in nonbursters.

Afterdischarge in single PCs

Both nonbursters and bursters participated in the generation of interictal, tonic, and clonic afterdischarges. As illustrated in Figs. 3B and 4B, the neurons fired coincidently with population spikes during the afterdischarge, albeit intermittently. Intracellular spikes were superimposed on a slowly declining afterdepolarizing potential (ADP), which succeeded the initial depolarizing shift. During the tonic event the ADP first increased to 12.8 mV, as described above.
The ADPs were clearly network-driven potentials, because isolated intrinsic bursts were followed by an afterhyperpolarization (compare Fig. 4, a and c, with b). The size and time course of the ADPs correlated with a negativegoing shift in the extracellular potential (Fig. 3, A and B).

Some PCs fired at low frequencies also after cessation of the tonic afterdischarge (Fig. 5Bd; see also Fig. 4Bf). These asynchronous spikes appeared to be triggered by slow (8–20 Hz) oscillations in membrane potential (Fig. 5Bd), similar to those described in artificially depolarized CA1 PCs (Leung and Yim 1991).

Fast transmembrane potentials during an interictal-ictal cycle

It was shown previously that population spikes in CA1 are associated with rapid transmembrane depolarizing transients in single PCs, presumably mediated by extracellular electrical field interactions (Taylor and Dudek 1984b). This finding was corroborated in the present study. Figure 5 describes the transmembrane potential of a PC during an interictal-ictal cycle. Population spikes during interictal (Fig. 5Ba), tonic (Fig. 5Bb and Be), and clonic discharges (Fig. Be) were coincident with fast (duration 4–7 ms) transmembrane depolarizing potentials. The amplitudes of these potentials were proportional to the respective population spikes, ranging from 5 to 10 mV. On summing with the underlying slow interictal, tonic, or clonic DSs, some of these potentials attained spike threshold, thereby recruiting the neuron in synchrony with its nearby population.

Changes in \( [K^+]_o \) during an interictal-ictal cycle

The extracellular potential and \( [K^+]_o \) were simultaneously recorded with \( K^+ \)-sensitive microelectrodes (\( n = 4 \)). As shown in Fig. 6A, \( [K^+]_o \), steadily increased from 6.5 mM, its lowest postictal value, to 7.5 mM, the apparent “threshold” \( [K^+]_o \) for seizure initiation. Tonic discharges were accompanied by a precipitous rise in \( [K^+]_o \) to \( \sim 12 \) mM, the ordinary ceiling level of \( [K^+]_o \) in the CA1 pyramidal layer in adult rodent hippocampal slices (Benninger et al. 1982). This increase appeared biphasic. In the first 1–2 min \( [K^+]_o \) increased rapidly to 10–11 mM. This was followed by a much slower rise to 12 mM, lasting \( \sim 4 \) s.

After cessation of tonic discharge \( [K^+]_o \) declined, but the appearance of clonic bursts at \( [K^+]_o \) of \( \sim 9 \) mM maintained \( [K^+]_o \) elevated throughout the clonic phase. Each clonic burst was accompanied by a \( K^+ \) transient of \( \sim 0.5 \) mM, superimposed on the elevated basal \( [K^+]_o \) (Fig. 6A). Clonic discharge ended when basal \( [K^+]_o \) decreased to \( \sim 8 \) mM. Subsequently, \( [K^+]_o \) declined slowly to 6.5 mM, from which it recycled again in exactly the same way (Fig. 6A).

Whereas the biphasic rise in \( [K^+]_o \), correlated with the changes in the extracellular potential, the subsequent
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FIG. 5. Transmembrane potentials in a PC during an interictal-ictal cycle. A: paired transmembrane (top trace, beginning at the indicated membrane potential) and extracellular (bottom trace) recordings of interictal and ictal discharge. The transmembrane recording was obtained by subtracting the extracellular recording from the ordinary intracellular recording (not shown). The events indicated (a±e) are shown expanded in B. B: rapid transmembrane potentials concurrent with population spikes are seen through all phases of seizure. Expanded traces show an interictal burst (a), early (b), mid- (c), and late (d) components of tonic discharge, and a clonic burst (e). Inset in a shows with high gain the early portion of the interictal depolarizing shift (marked with a bar on the low-gain trace). Three fast transmembrane depolarizations (arrows) can be compared with a conventional excitatory postsynaptic potential (asterisk). The unlabeled up-going deflection is a truncated spike. Note asynchronous discharges of the neuron during the late tonic phase (d), triggered by spontaneous oscillations of the depolarized membrane potential.

Orthodromically evoked \( K^+ \), transients during an interictal-ictal cycle

Maximal orthodromic activation of the CA1 PCs between seizures at 0.05 Hz produced increasingly larger and longer population spike bursts (Fig. 6B). These evoked responses were associated with correspondingly larger and longer transient \( K^+ \) elevations, surmounted on the smoothly rising basal \( [K^+]_o \) (Fig. 6B, stimuli numbered 2–4). Indeed, when a

changes in \( [K^+]_o \), did not (Fig. 6A). The negative extracellular potential shift associated with tonic discharge recovered to baseline while \( [K^+]_o \) was \(-9\) mM. The extracellular positive shift following the clonic phase attained its peak 10 s before \( [K^+]_o \), undershot to 6.5 mM. Because slow changes in extracellular potential are tightly correlated with changes in PC membrane potentials (see above, Fig. 1), these observations suggest that the decline in \( [K^+]_o \), lags several seconds behind PC repolarization.

FIG. 6. Changes in extracellular \( K^+ \) concentration (\([K^+]_o\)) during an interictal-ictal cycle in CA1. Recurring ictal episodes were induced by perfusing the slice with high-\( K^+ \) (7.5 mM) saline. A \( K^+ \)-selective microelectrode was used to record simultaneously the \([K^+]_o\) (top traces) and the extracellular potential (bottom traces) in the pyramidal layer. A: recurring ictal episodes erupted at a \([K^+]_o\) of 7–7.5 mM, attained a maximal ceiling level of 11.5 mM, and subsequently declined to 6–6.5 mM. The segment denoted by the bar is shown expanded at bottom. Note that \([K^+]_o\) reaches a steady ceiling level during the tonic phase, and remains elevated throughout the clonic phase. B: changes in evoked extracellular \( K^+ \) \((K^+_o)\) transients in relation to a seizure cycle. Stimulation of afferent fibers in stratum radiatum at 0.05 Hz evoked field potentials and transient \([K^+]_o\) increases. Some of the stimuli are denoted with numbers. The \( K^+_o \) transients decayed relatively rapidly during the postictal period (e.g., responses numbered 1 and 7). During the interseizure period they became prolonged (e.g., responses numbered 4 and 10).
stimulus was applied shortly before seizure onset, the evoked $K^+$ transient did not decay but seemingly gave way to the tonic rise in $[K^+]_o$, (Fig. 6B; stimulus numbered 10). By contrast, the $[K^+]_o$ transients accompanying giant clonic bursts (Fig. 6B) or orthodromically evoked during the clonic phase (Fig. 6B; stimulus numbered 6) decayed much more rapidly than those evoked before the seizure. These observations suggest that interseizure rise in basal $[K^+]_o$ may be associated with reduced buffering of neuronally evoked $[K^+]_o$ increases.

Discussion

In this study we have expounded the description of an in vitro model of focal electrographic seizures induced in rat hippocampal slices by moderately elevating $[K^+]_o$ (Jensen and Yaari 1988; Traynelis and Dingledine 1988). Paired intracellular/extracellular recordings were used to identify the mechanisms underlying the regenerative recruitment and synchronization of single PCs into interictal and ictal population discharges. Although the number of synaptic and nonsynaptic positive feedback mechanisms putatively operating in CA1 is large (Haglund and Schwartzkroin 1990; Traynelis and Dingledine 1988; Yaari and Jensen 1988), our data indicate those that may be more critical for $K^+$-induced interictal and ictal activity.

Interseizure depolarization

The finding that CA1 PCs depolarized by 2.4 mV between seizures is consistent with a previous report (Traynelis and Dingledine 1988). This slow depolarization is readily accounted for by the modest interictal rise in basal $[K^+]_o$, measured here with $K^+$-selective microelectrodes or inferred from recordings from glial cells (Traynelis and Dingledine 1988). According to the Nernst equation, a $[K^+]_o$ increase from 6.5 to 7.5 mM would depolarize a $K^+$-permeable membrane (at 35°C) by 3.8 mV. A somewhat smaller depolarization would be expected to occur in PCs, because their membrane permeability is not exclusively selective to $K^+$ even at rest (e.g., Jensen et al. 1993).

Generation of the primary population burst

In the high-$K^+$ model of hippocampal epilepsy, brief bursts in CA1 are triggered by rhythmic burst activity in CA3 (Korn et al. 1987; Rutecki et al. 1985). When the connections between the two fields are severed, brief bursts in CA1 disappear (Jensen and Yaari 1988). Thus a composite excitatory postsynaptic potential must contribute to the depolarizing shift in single PCs during a primary burst. However, given that spike threshold in CA1 PCs in high-$K^+$ saline is $\pm 57$ mV (Jensen et al. 1993, 1996), then a 5-mV depolarizing shift would trigger spikes in nonbursters only in the late interseizure period. Thus other factors must play a role in transforming the distributed excitatory postsynaptic potential in CA1 into coherent population discharge.

Most PCs were silent for $\approx 10$ ms after the onset of the primary population burst. The only PCs firing before or during this period appeared to be bursters, suggesting that intrinsic burst firing is crucial for the formation of the early part of the primary interictal burst. Moreover, the similarity in intraburst spike frequency between intrinsic bursts (173 Hz) and primary population bursts (171, 178, and 182 Hz for interictal, tonic and clonic bursts, respectively), and the fact that bursters fired, on average, fourfold more than regular cells during the interictalprimary burst, suggest that bursters may determine the discharge pattern of the population through the entire primary burst.

Assuming that bursters and nonbursters receive the same excitatory synaptic drive from CA3, the earlier recruitment of bursters may result from their lower firing thresholds. Because the distribution of intraburst spike rate is quite narrow (173 $\pm$ 30 Hz in the 5 bursters encountered in this study), simultaneously recruited bursters conceivably may fire in a time-locked fashion even without interacting. The synchronous firing of bursters may then produce effective local synaptic and/or electrical currents, which would facilitate the delayed recruitment of nonbursters. These interactions would be amplified by local increases in $[K^+]_o$ (see below).

The depolarization shift during the primary population burst was larger in bursters (10–30 mV) than in nonbursters (5 mV). This accounts for the greater number of spikes generated by bursters during the primary population burst. The large depolarization shift in bursters is due to the overwhelming contribution of intrinsic currents to the depolarizing drive. A likely candidate is the persistent $Na^+$ current ($I_{Na,p}$; French et al. 1990) that was recently shown to underlie generation of intrinsic bursts in CA1 PCs (Azouz et al. 1996).

Growth of interictal bursts

The size of the population spikes within the primary burst progressively increased in the interseizure period. Because concurrent brief bursts in CA3 do not oscillate in size (Jensen and Yaari 1988; Traynelis and Dingledine 1988), the changes in the latter must reflect local increases in neuronal excitability.

A population spike would grow in amplitude if the number and/or synchronization of conjointly activated PCs would increase (Andersen et al. 1971). As evidenced from the paired intracellular/extracellular recordings, the increase in population spike size was mostly due to recruitment of hitherto subthreshold neurons, rather than to synchronization of preexisting discharge. Several factors may be involved in the steady growth of interictal bursts. First, the interseizure depolarization of the PCs would progressively increase their excitability. Second, the interseizure rise in basal $[K^+]_o$ may augment the propensity of some PCs to generate intrinsic bursts (Jensen and Yaari 1992; Jensen et al. 1994). Third, decrease in extracellular space volume, reported to occur between sequential seizures in this model (Traynelis and Dingledine 1989), would promote nonsynaptic excitatory interactions among PCs, mediated by the electric field and/or by $K^+$ release into the interstitium (see below).

Development of afterdischarge

Large interictal bursts in the late interseizure period were generally followed by an afterdischarge of several population spikes. This prolongation of interictal discharge was not a consequence of reduced spike frequency accommodation in individual PCs, because accommodation was undiminished throughout the interseizure period.
In paired intracellular/extracellular recordings, the development of afterdischarge was correlated with a progressive prolongation of the interictal ADP. Two mechanisms may synergistically contribute to the electrogensis of afterdischarges and their underlying ADPs. First, CA1 PCs were shown to be functionally, albeit scarcely, interconnected by recurrent axon collaterals (Christian and Dudek 1988b). Re- verberation of spike discharge through these connections would cause prolonged excitation of PCs. This mechanism was used successfully to simulate the development of interictal burst afterdischarge in the convulsant-treated CA3 field (Traub et al. 1993), where recurrent excitatory connections are more dense (Christian and Dudek 1988a). Second, the primary burst may evoke a local increase in \([K^+]_o\), which in turn may excite the PCs for a lengthy period (Swann and Brady 1984). The close association of the ADP with a negative extracellular field in the pyramidal layer suggests that it may be generated predominantly by a local rise in \([K^+]_o\) (Yaari et al. 1986).

**Transition to seizure**

A tonic ictal event emanated from a large primary burst in an explosive, all-or-none manner, suggesting that it is underlain by a regenerative process. As suggested previously in other models of hippocampal epilepsy (e.g., Fetziger and Ranck 1970; Yaari et al. 1986), the large increase in \([K^+]_o\), accompanying the afterdischarge may provide the critical positive feedback for supporting and prolonging the tonic discharge. A rise in \([K^+]_o\), would depolarize CA1 PCs and reduce the driving force for inhibitory \(K^+\) and \(Cl^-\) currents (Jensen et al. 1993). Both effects would tend to excite the PCs and promote their sustained discharge. Because of their high packing density (Green and Maxwell 1961) and the associated small extracellular space (McBain et al. 1990), CA1 PCs in situ would be particularly prone to \(K^+_o\)-mediated excitation. The smaller extracellular volume fraction in CA1 compared with CA3 (0.12 vs. 0.18, respectively; McBain et al. 1990) may contribute to the unique propensity of CA1 to generate ictal episodes in high-\(K^+\) saline. However, other interfield differences, such as weaker Na /K\(^+\) pump activity in CA1 compared with CA3, may augment this propensity as well (Haglund et al. 1985).

Ictal episodes were triggered at an apparent threshold \([K^+]_o\) concentration of 7.5 mM. Presumably at this \([K^+]_o\), the neuronal and glial mechanisms that buffer \([K^+]_o\) increase (reviewed by Barres 1991; Walz 1989) fail to do so effectively. Consequently, increases in neuronal discharge and in \([K^+]_o\) become mutually reinforcing (Nelken and Yaari 1987). Within 2 s of tonic discharge \([K^+]_o\), rose to 12 mM, while the PCs depolarized, on average, by 12.8 mV. An increase in \([K^+]_o\), from 7.5 to 12 mM is expected to depolarize a \(K^+\)-permeable membrane (at 35°C) by 12.5 mV. Thus the \(K^+_o\) accumulation hypothesis accounts well for the tonic ADP. An associated increase in membrane conductance would be expected because of the nonlinearity of the PC membrane, the \(K^+\)-mediated release of many hippocampal neurotransmitters, and the putative direct action of elevated \([K^+]_o\), on membrane properties (Jensen et al. 1993).

**Transition from tonic to clonic discharge**

What factors account for the termination of tonic discharge? The sustained tonic discharge and depolarizing shift eventually lead to spike refractoriness (because of inactivation of fast Na current and activation of slow K currents). Additionally, the increases in \([K^+]_o\), and intracellular Na\(^+\) concentration associated with repetitive discharge may enhance \(K^+_o\) clearance and neuronal repolarization by stimulating the activity of Na /K pumps in neurons and glia (e.g., Frank et al. 1983). This notion is supported by several lines of evidence. First, \([K^+]_o\), attained a ceiling level of 12 mM despite the continuation of tonic discharge, suggesting that the rate of \(K^+_o\) uptake increased. Second, \(K^+_o\) transients orthodromically evoked after the tonic phase dissipated much faster than those evoked before seizure onset. Finally, a \([K^+]_o\) undershot to 6.5 mM (1 mM below \([K^+]_o\), in the saline) followed the ictal episode.

The temporal discrepancies between recovery of the extracellular potential and decline in \([K^+]_o\), suggest that the PCs repolarize faster than \([K^+]_o\), decreases. This may reflect the electrogenic nature of the neuronal Na /K\(^+\) pump activated during the tonic phase of seizure. Indeed, previous work has shown that sustained excitation of CA1 PCs enhances electrogenic Na /K\(^+\) pumping (Gustaffson and Wigström 1983; Thompson and Prince 1986).

Because of the development of spike refractoriness in CA1 PCs, the steady burst activity arriving from CA3 evoked minimal burst responses in CA1 during the second part of the tonic phase. However, as the PCs repolarized and regained their excitability, the evoked bursts markedly increased. The reason for the bursts becoming very large at this specific time most likely is due to the sustained elevation of \([K^+]_o\). However, the enhanced activity of Na /K\(^+\) pumps prevents the conversion of the clonic bursts into regenerative tonic events.

**Electrical coupling**

The differential intracellular/extracellular recordings clearly indicated the presence of rapid transmembrane potentials of up to 10 mV coincident with the population spikes. Such potentials are ubiquitous also in other models of hippocampal epilepsy (Snow and Dudek 1984; Taylor and Dudek 1984b) and are generally attributed to ephaptic (electric field) interactions between neighboring neurons (reviewed by Dudek et al. 1986). These interactions may be more prominent in the present model because of the high-\(K^+\)-induced decrease in extracellular volume fraction in CA1 (McBain et al. 1990), which would strengthen ephaptic interactions. The tonic increases in \([K^+]_o\), to 12 mM may be accompanied by additional decrease in extracellular space (Traynelis and Dingledine 1989), reinforcing neuronal recruitment and synchronization even further.

The transmembrane depolarizations also may reflect electrotonic coupling, i.e., electrical excitation mediated via gap junctions (Dudek et al. 1986). Although gap junctions between CA1 PCs in ordinary conditions reportedly are scarce (Knowles et al. 1982; Taylor and Dudek 1982), recent evidence suggests that they may be induced in CA1 by epileptogenic manipulations (e.g., elevating extracellular pH (Church and Baimbridge 1991) or removing extracellular Ca\(^2+\) (Perez-Velazquez et al. 1994)). Increasing \([K^+]_o\), also was shown to enhance gap junction communication, albeit between glia (Enkvist and McCarthy 1994). Thus the contribution of electrotonic interactions to formation of rapid
transmembrane depolarizing potentials and to neuronal hypersynchrony in the present model cannot be excluded.

Model of seizure generation in elevated $K^+$

The scheme in Fig. 7 describes our working hypothesis regarding the sequence of events leading to the initiation, buildup, and termination of ictal episodes in CA1 in the high-$K^+$ model of hippocampal epilepsy. Electrographic seizures are initiated by a chain reaction that begins with the recruitment of a subset of the neuronal population, mostly bursters, by a small excitatory synaptic input. The excitatory signal is then amplified in time and anatomic space by intrinsic burst firing, $K^+$ accumulation, and electrical (ephaptic and/or electrotonic) coupling. Once a critical mass of neurons is recruited into the discharge zone, the process becomes highly regenerative, and excitation spreads rapidly throughout the neuronal population. Concurrently, negative feedback provided by spike inactivation and electrogenic $Na^+ / K^+$ pumping increases slowly, eventually arresting the seizure.

According to this scheme intrinsic bursters are the leading edge in the cascade of events leading to development of electrographic seizures. Whether intrinsic bursters are crucial elements in this cascade has yet to be determined.

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