Exp Brain Res (1987) 67: 363–372

Experimental Brain Research

Control of spontaneous epileptiform discharges by extracellular potassium: an "in vitro" study in the CA1 subfield of the hippocampal slice*

V. Tancredi** and M. Avoli

Montreal Neurological Institute, Department of Neurology and Neurosurgery, McGill University, Montréal, Québec H3A 2B4, Canada

Summary. 1. The effects evoked by changing $[K^+]_o$ upon the synchronous epileptiform discharges (SEDs) generated in the presence of GABA antagonists were studied in the "in vitro" hippocampal slice with extra- and intracellular recordings. $[K^+]$ in the artificial cerebrospinal fluid (ACSF) was varied in steps of 1 or 2 mM between 3.25 and 10.25 mM. 2. Spontaneous SEDs occurred rarely at $[K^+]_o$ lower than 5.25 mM. Augmenting $[K^+]_o$ from 5.25 to 10.25 mM caused a four to five fold increase in the frequency of occurrence of SEDs while the duration of each SED was inversely related to the rate of occurrence. 3. Similar findings were observed when the CA1 subfield had been surgically disconnected from the CA2–CA3 subfields. In these experiments SEDs occurred independently in the two regions, but at any given $[K^+]_o$ SEDs in the CA3 subfield displayed a frequency two to three times higher than that of SEDs generated in the CA1 area. 4. The intracellular correlate of the SEDs in the CA1 subfield either intact or isolated from the CA2–CA3 ones was a large amplitude depolarization (duration 100–600 ms) associated with a burst of action potentials. This intracellular event, which was similar to the paroxysmal depolarizing shift (PDS) recorded in focal models of epilepsy "in vivo", behaved largely like a synaptic phenomenon when the resting membrane potential (Vm) was changed with intracellularly injected current. A long lasting (half-width: 0.3–2 s in 6.25 mM $[K^+]_o$) hyperpolarizing potential usually followed the PDS and could be inverted by hyperpolarizing the Vm by 15–25 mV. When $[K^+]$ in the ACSF was raised from 7.25 to 10.25 mM, pyramidal cells depolarized in a dose related fashion. At the same time the post-PDS hyperpolarization decreased in duration and peaked earlier, thus curtailing the depolarizing envelope of the PDS. Consequently, the effect of increasing $[K^+]_o$ was that of evoking more frequent, but shorter PDSs. 6. These findings demonstrate that the appearance of spontaneous SEDs in the presence of GABA antagonists is dependent upon $[K^+]_o$. The effects of evoked by increasing $[K^+]_o$ are presumably mediated through: (i) a decreased strength of K^+ repolarizing conductances; (ii) an increased efficacy of synaptic potentials; (iii) a steady depolarization of the neuronal membrane. The modulation of the frequency of occurrence of SEDs appears to be related to a decreased duration of the hyperpolarization which follows the PDS, a potential which is largely mediated by a K^+ conductance.

Key words: Epileptic discharges – Extracellular K^+ – GABA antagonists – Hippocampal slice

Introduction

A feature common to several of the drugs used for evoking epileptiform activity (e.g. penicillin, bicuculline, picrotoxine) is the ability to antagonize GABAergic potentials (Curtis and Felix 1971; Curtis et al. 1972; MacDonald and Barker 1977; Dingledine and Gjerstad 1980; Schwartzkroin and Prince 1980a; Hablitz 1984). Therefore, an important factor in the genesis of focal interictal discharges appears to reside in a diminished efficiency of GABAergic Cl^- conductance (for review see Krnjevic 1983). However, it has been observed in our laboratory (Avoli 1984; see also Schwartzkroin and Prince 1978) that synchronous epileptiform discharges (SEDs) do not occur

* Supported by MRC of Canada (grant MA-8109)

** Present address: Dept. of Experimental Medicine (Physiology), Università degli Studi Tor Vergata, I–00100 Rome, Italy
Offprint requests to: M. Avoli (address see above)

spontaneously in hippocampal slices bathed in artificial cerebrospinal fluid (ACSF) containing penicillin (3.4 mM) when the $[K^+]$ in the ACSF is kept at 3.25 mM. This is a value close to that normally detected in the extraneuronal space (cf. Lux et al. 1986). Since the absence of spontaneous SEDs in these experiments is accompanied by a block of the recurrent IPSP and the ability of orthodromic stimuli to induce large amplitude depolarizations with burst of action potential, we interpreted the lack of spontaneous SEDs as being related to the $[K^+]_o$.

Several studies have indeed demonstrated a role played by K^+ in the modulation of epileptiform discharges (Zuckerman and Glaser 1968; Ogata et al. 1976; Oliver et al. 1978; Prince and Schwartzkroin 1978) and in the transition from the interictal to the ictal state (Zuckerman and Glaser 1968; Fertziger and Rank 1970; Dichter et al. 1972; Sypert and Ward 1974). Furthermore, studies employing ion-sensitive electrodes have shown that $[K^+]_o$ increases during enhanced neuronal activity or seizures evoked by iontophoresis of excitatory transmitters, electrical stimulation or chemical convulsants (for review see: Lux et al. 1986; Heinemann et al. 1986). Although this represents probably a consequence rather than a cause of seizure initiation, still the elevation of $[K^+]$ might play a role in sustaining epileptiform activity. In the light of these findings we analyzed in the CA1 subfield of the "in vitro" hippocampal slice the relationship between spotaneous SEDs evoked by GABA antagonists and $[K^+]_o$.

Methods

Preparations and incubation of the slices

Male rats (150–300 g) were decapitated under ether anaesthesia and the hippocampus quickly removed. Slices, 400–500 μm thick, were cut at an angle 35–45° from a plane normal to its longitudinal axis using a McIlwain tissue chopper. They were then transferred into a tissue chamber where they lay in an interface between ACSF and humidified gas (95% O_2, 5% CO_2) at a temperature of 35° C ± 1° C and a pH of 7.4. The composition of the ACSF was (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26 and glucose 10. Bicuculline methiodide (20 μM) or penicillin (3.4 mM) were applied into the ACSF to block GABAergic conductances. $[K^+]$ in the ACSF and therefore $[K^+]_o$ was increased by adding KCl. Changes in osmolarity associated with these procedures were not corrected. In some experiments CsCl (1 mM) was added to the ACSF in order to abolish the time dependent inward rectification which occurs when the resting membrane potential (Vm) is brought at levels larger than –80 mV (Brown and Griffith 1983). In a series of experiments the CA1 subfield was separated from the CA2–CA3 regions by a cut which extended from the alveus, up to the inferior edge of the dentate area. This cut was performed with a knife immediately after slicing.

Recording and stimulation

Extracellular recordings were performed with glass micropipettes filled with 4 M NaCl (resistance 5–20 mΩ) which were positioned in the CA3 and CA1 subfields. Conventional intracellular recordings were made from the soma of CA1 pyramidal cells in the subregion b (Masukawa et al. 1982). These neurons could be electrophysiologically identified by antidromic activation from the alveus. Glass micropipettes were filled with 4 M K-acetate (resistance 80–150 MΩ). The intracellular signals were fed to a high impedance negative capacitance DC amplifier with bridge circuit which allowed square wave current pulses (20–500 ms) and/or steady DC current to be passed through the intracellular microelectrode. The bridge balance was carefully checked throughout the experiment and adjusted if necessary.

Orthodromic and antidromic anodal stimuli (0.05–0.3 mA, 10–90 μs) were delivered through sharpened and insulated tungsten electrodes placed in stratum (s.) radiatum and alveus, respectively. In some slices a lesion was made with a knife in the s. oriens in order to avoid that current spread from the stimulating electrode in the alveus would activate afferent fibers running in the s. oriens.

Experimental protocols and data analysis

The frequency of occurrence of SEDs was measured from either extra- or intracellular data. In the extracellular experiments $[K^+]$ in the ACSF was varied between 3.25 and 10.25 mM in steps of 1 mM in a cumulative fashion. The frequency of occurrence was obtained at any given $[K^+]$ over periods of at least 20 min after having waited 30 min for equilibration of the new $[K^+]$ level in the tissue chamber. When recording intracellularly the changes in $[K^+]$ were usually performed in 2 mM steps.

Extracellular data were collected from over 80 slices. Intracellular observations were gathered from 47 pyramidal cells which displayed overshooting action potentials and the ability to generate repetitive firing with adaptation during intracellular injection of depolarizing square wave pulses (0.2–0.5 nA; 20–500 ms). In $[K^+]_o$ at 3.25 mM these cells were characterized by action potentials larger than 80 mV resting membrane potential of at least 60 mV and input resistance higher than 20 MΩ. Data were displayed on an oscilloscope or on a Gould pen recorder.

Results

Changes evoked by bicuculline and penicillin in the CA1 subfield

In keeping with previous experiments in the "in vitro" slice (Schwartzkroin and Prince 1978, 1980a; Alger and Nicoll 1980; Dingledine and Gjerstad 1980) bath application of either bicuculline or penicillin modified the orthodromic response of CA1 pyramidal cells to s. radiatum stimulation. Intracellularly, the s. radiatum induced EPSP displayed an increased amplitude and decreased repolarization (Fig. 1Bb) while stimuli at higher strength elicited a burst of fast action potentials (Fig. 1Aa, b; Bc). Extracellularly the EPSP-single population spike recorded in control ACSF ($[K^+]$: 3.25 mM) was

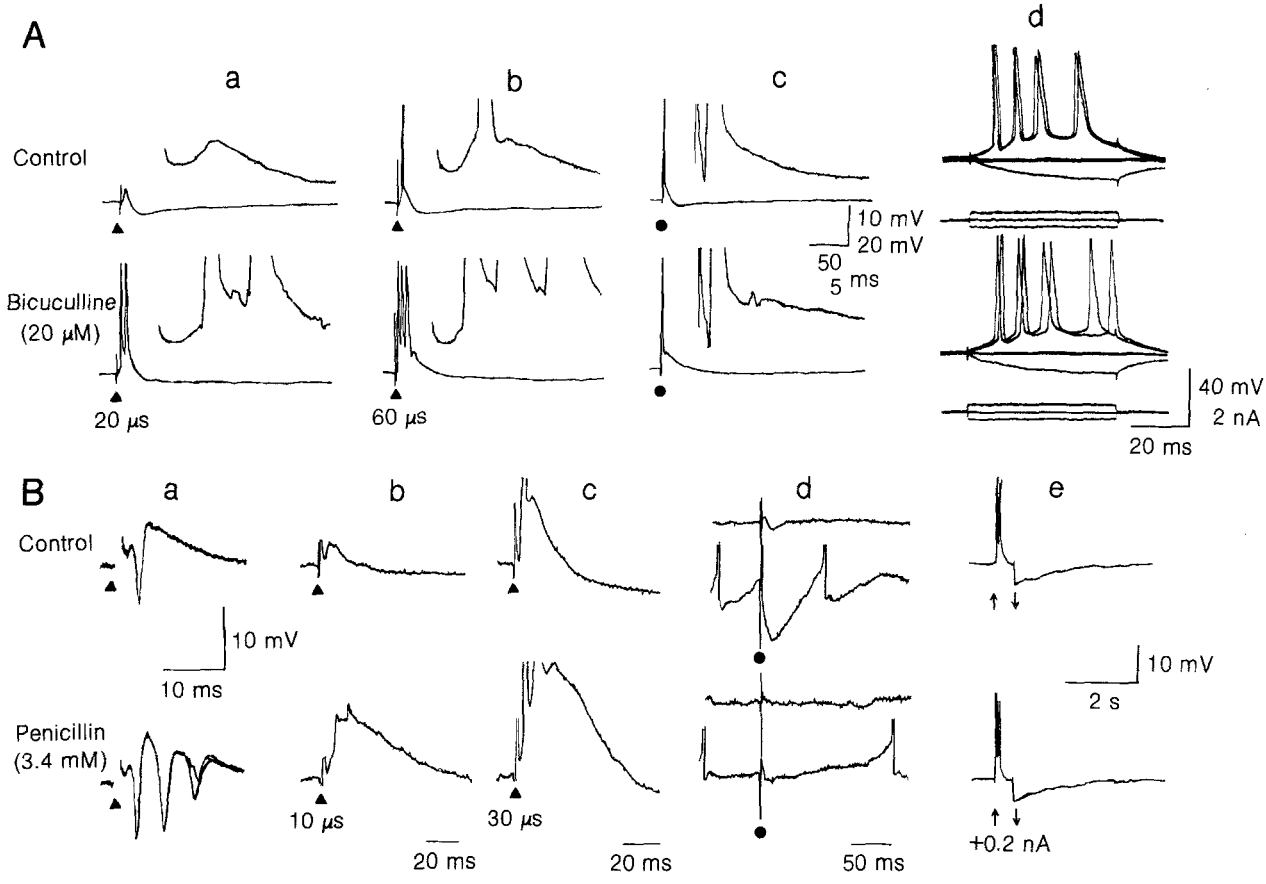

Fig. 1. **A** Effects evoked by bicuculline methiodide (20 μM) on the synaptic and non-synaptic responses of CA1 pyramidal cells bathed in ACSF containing 3.25 mM [K^+]. In **a–c,** intracellular potentials evoked by orthodromic (**a** and **b**) and antidromic (**c**) stimulation of s. radiatum (▲) and alveus (●), respectively; in **d** responses to intracellular injections of depolarizing and hyperpolarizing square pulses. Two different strengths of stimulation were employed in **a, b.** In the presence of bicuculline (45′) orthodromic stimuli were capable of triggering two (**a**) and three (**b**) action potentials. This effect is paralleled by a block of the orthodromic and antidromic (**c**) IPSP while no change can be observed in the input resistance and the repetitive firing properties (**d**). In **a–c,** each stimulus induced response is shown at two different time bases and amplitudes. Resting Vm: –65 mV. Action potential amplitude: 80 mV. **B** Changes induced by penicillin (3.4 mM) on the synaptic and non-synaptic responses of CA1 pyramidal cells bathed in ACSF containing 3.25 mM [K^+]. In **a,** superimposed field potentials (n: 5) evokked by s. radiatum stimulation (▲) in control and 1 hour after penicillin (recording microelectrode in s. pyramidale). In **b–e,** intracellular potentials evoked by orthodromic (**b, c**)and antidromic (**d**) stimulation of s. radiatum (▲) and alveus (●), respectively; in **e,** responses to intracellular injection of depolarizing square pulses. Two different strength of orthodromic stimulation were employed in **b, c.** In **d,** alveus stimuli of equal strength were delivered at resting (upper trace) and depolarized Vm (15 mV positive to rest, lower trace). Note that penicillin modifies the orthodromic response into a large depolarization capable of triggering a burst of action potentials while blocking the recurrent IPSP. These effects are accompanied by no change in the repetitive firing and afterhyperpolarization. Resting Vm: –60 mV. Action potential amplitude: 82 mV

transformed into a burst of 3–5 population spikes (Fig. 1Ba). The orthodromic response modified by either of the two convulsant drugs was consistently accompanied by a blockade of the recurrent IPSP evoked by alvear stimulation (Fig. 1Ac; Bd). Bicuculline or penicillin at the concentration used in the present experiments did not modify the passive properties of the neuronal membrane (i.e. input resistance or time constant), the repetitive firing evoked by intracellular depolarizing pulses or the afterhyperpolarizing potentials (Figs. 1Ad; Be).

[K^+] and spontaneous synchronous epileptiform discharges

In spite of the dramatic changes exerted by both bicuculline and penicillin upon the postsynaptic responses of CA1 pyramidal cells, slices bathed in ACSF containing 3.25 mM [K^+] did not generate spontaneous SEDs. In some slices spontaneous epileptiform events appeared by raising [K^+] by 1 mM and were observed in almost every case when [K^+] was further increased to 5.25–6.25 mM. At

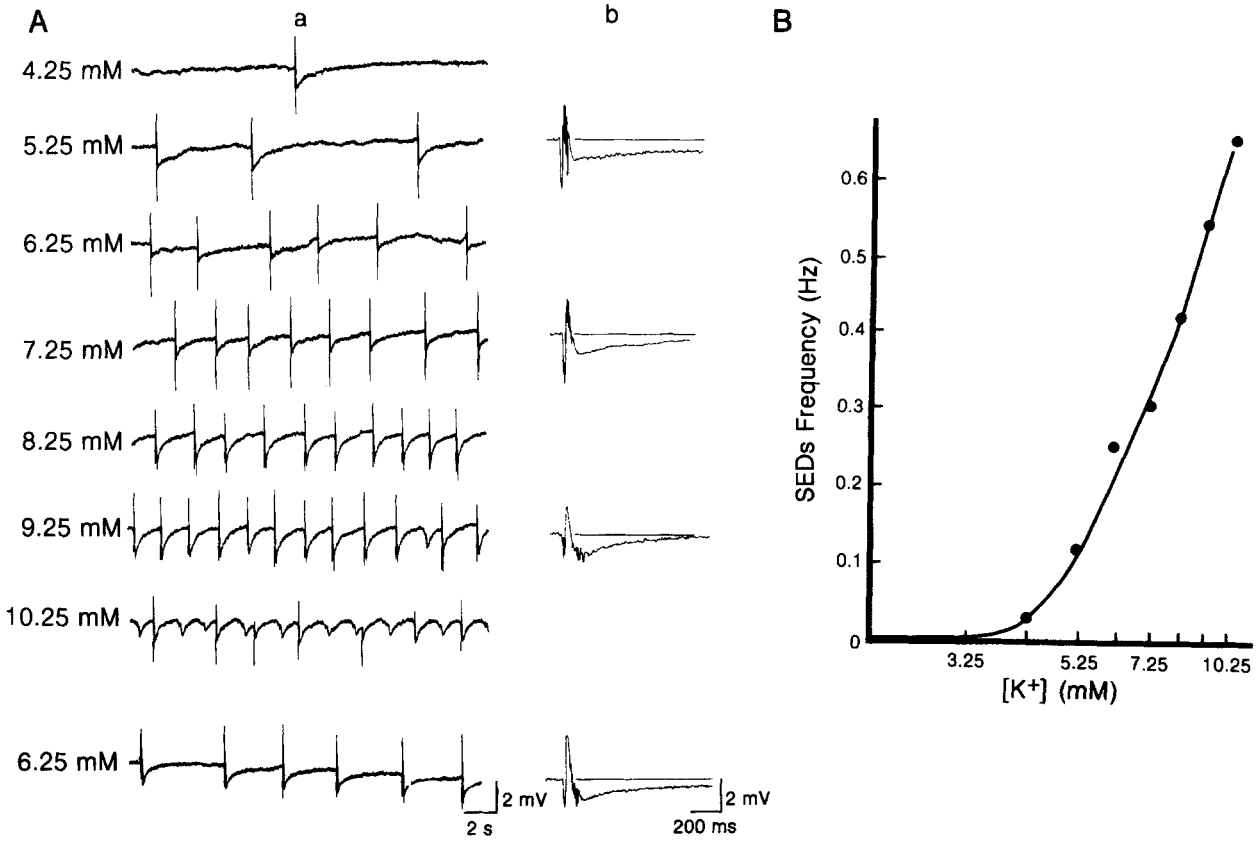

Fig. 2A, B. Effects evoked by raising the $[K^+]$ in the ACSF (artificial cerebro-spinal fluid) upon SEDs (synchronous epileptiform discharge) evoked by bicuculline (20 μM). In **A**, raw data displayed at two different time bases (**a, b**). Note that at 10.25 mM $[K^+]_o$ the SEDs are mainly characterized by a relatively slow, negative field potential which often lacks the fast deflections due to the occurrence of population spikes. In **B**, plot of the frequency of occurrence of the SEDs versus $[K^+]$

these values SEDs recurred in the CA1 subfield rhythmically at a frequency of 0.06–0.3 Hz and displayed a stereotyped shape (Fig. 2Aa). As shown in Fig. 2Ab, SEDs recorded at the border between s. radiatum and s. pyramidale were characterized by an early field potential (duration: 50–400 ms) associated with burst of population spikes followed by a long lasting (up to 2 s) negative, slowly decaying potential.

By varying the $[K^+]$ in the ACSF between 5.25 and 10.25 mM it was possible to demonstrate a close relationship between $[K^+]$ and the frequency of occurrence of SEDs (Fig. 2). When the frequency of occurrence of SEDs at the $[K^+]$ capable of evoking a regular pattern (e.g. 5.25 mM in the experiment illustrated in Fig. 2) was compared with the frequency evoked by 10.25 mM $[K^+]$, the increase observed was four- to five-fold. Also, at $[K^+]$ values higher than 7.25 the increase in frequency was inversely related to the amplitude and the duration of each SED. Similar results have been recently reported for the CA3 subfield by Rutecki et al. (1985). Furthermore, it is known (and could be confirmed in the present experiments) that SEDs evoked by antagonists of GABA are generated in the CA2–CA3 subfields and then synaptically propagated to the CA1 subfield (Schwartzkroin and Prince 1978; Gjerstad et al. 1981; Wong and Traub 1983). Thus, the effects of $[K^+]$ upon SEDs in the CA1 subfield could possibly be explained as resulting from changes occurring in the CA3 subfield.

We therefore repeated the same type of experiments in slices in which the CA1 subfield had been disconnected from the CA2–CA3 regions. In these cases spontaneous SEDs were seen at $[K^+]$ above 6.25 mM, i.e. at values larger than those sufficient for evoking spontaneous SEDs in the intact hippocampal slice (Fig. 3B). As observed when CA1 neurons were connected with the CA3 subfield there existed a close relation between the frequency of occurrence of SEDs and $[K^+]$ over a 6.25–10.25 mM range (Fig. 3C), while the amplitude and the duration of each SED (mainly of the late negative field) was inversely related to the frequency of occurrence (Fig. 3Bb, D). Simultaneous extracellular recordings from the iso-

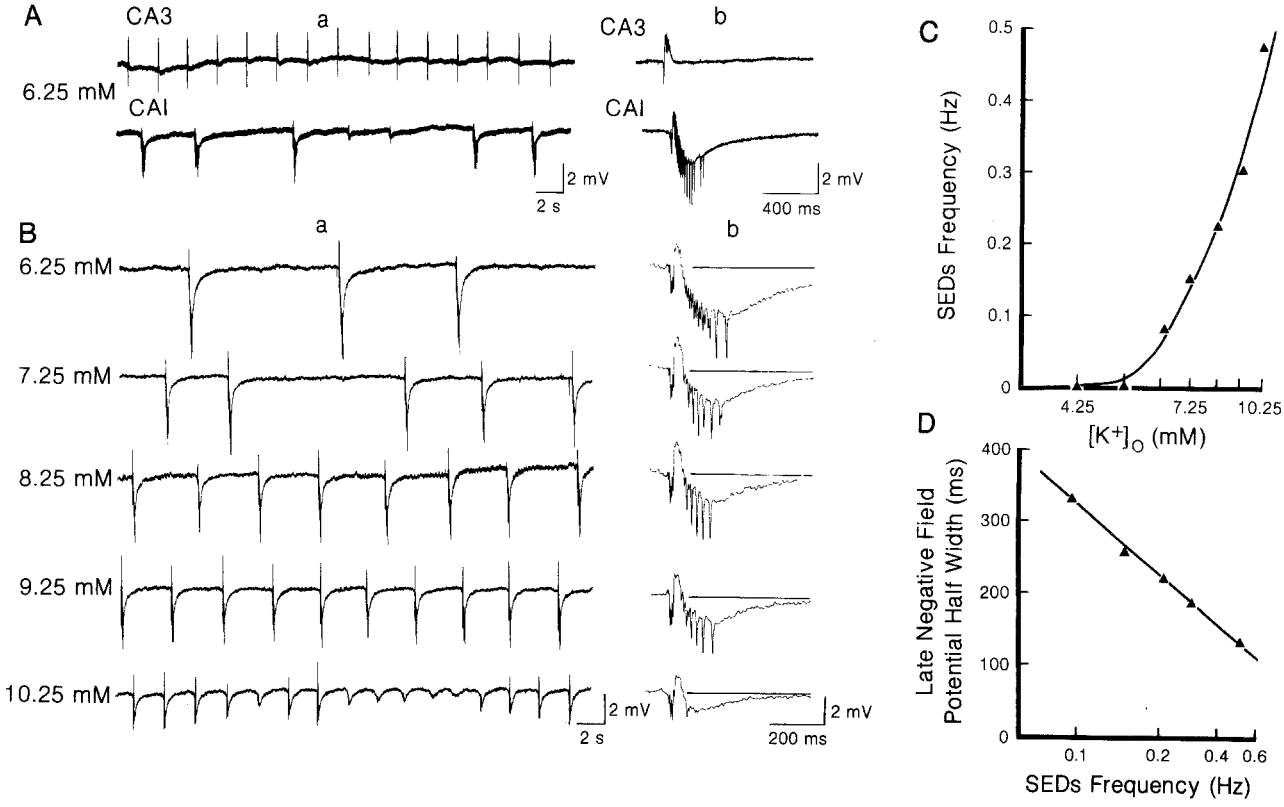

Fig. 3. A Occurrence of spontaneous SEDs (synchronous epileptiform discharge) (bicuculline, 20 μM) in CA1 and CA3 subfields which were disconnected by a cut. Note the higher frequency of occurrence as well as the shorter duration of SEDs in the CA3 subfield. **B** Effects of increasing $[K^+]$ upon the frequency of occurrence and the morphology of SEDs recorded from an isolated CA1 subfield in the presence of bicuculline. In both **A, B,** data shown in **b** were similar to those in **a** but at higher time base for better appreciation of the changes in shape. **C** Plot of the frequency of occurrence of the SEDs versus $[K^+]$ from the experiment shown in **B. D** Plot of the duration of the late negative field versus the frequency of occurrence (data from the experiment shown in **B**)

lated CA1 and CA3 subfields disclosed that SEDs in CA3 occurred at higher frequency and were shorter than those in CA1 (Fig. 3Aa, b).

Intracellular recordings during spontaneous synchronous epileptiform discharges

As shown in Fig. 4(A–C) the cellular correlate of the "in vitro" SED generated by CA1 hippocampal cells was a large amplitude depolarization associated with a burst of fast action potentials (duration: 50–600 ms). This phenomenon, termed paroxysmal depolarizing shift (PDS) because of its resemblance with the cellular event underlying the focal interictal spike "in vivo" (Matsumoto and Ajmone Marsan 1964), was similar in both "intact" and "isolated" CA1 subfields. The PDS was usually followed by a hyperpolarization (hereafter called post-PDS hyperpolarization) which displayed in $[K^+]_o$ 6.25 mM a halfwidth of 0.3–2 s and peak latency (as calculated by considering as zero the onset of the PDS) of 200–600 ms. Thus, it corresponded in time with the late negative extracellular field potential of the SED as recorded in s. pyramidale.

The increase in conductance occuring during the post-PDS hyperpolarization was capable of shunting action potentials induced by intracellular depolarizing pulses (Fig. 4C). However, in some instances action potentials could be observed during the post-PDS hyperpolarization. As shown in Fig. 4D, these action potentials appeared to arise from small amplitude, fast negative transients which represented the extracellular population spikes recorded intracellularly with reference to bath. The action potentials recorded intracellularly during the post-PDS hyperpolarization were presumably generated through field (ephaptic) interactions. They could be aborted or enhanced by hyperpolarizing or depolarizing the Vm with intracellular steady current injection.

The frequency of occurrence of the PDS at a given $[K^+]_o$ was not affected by changing the Vm in the hyperpolarizing direction with intracellular injection of steady current (Fig. 4B). Also, during this

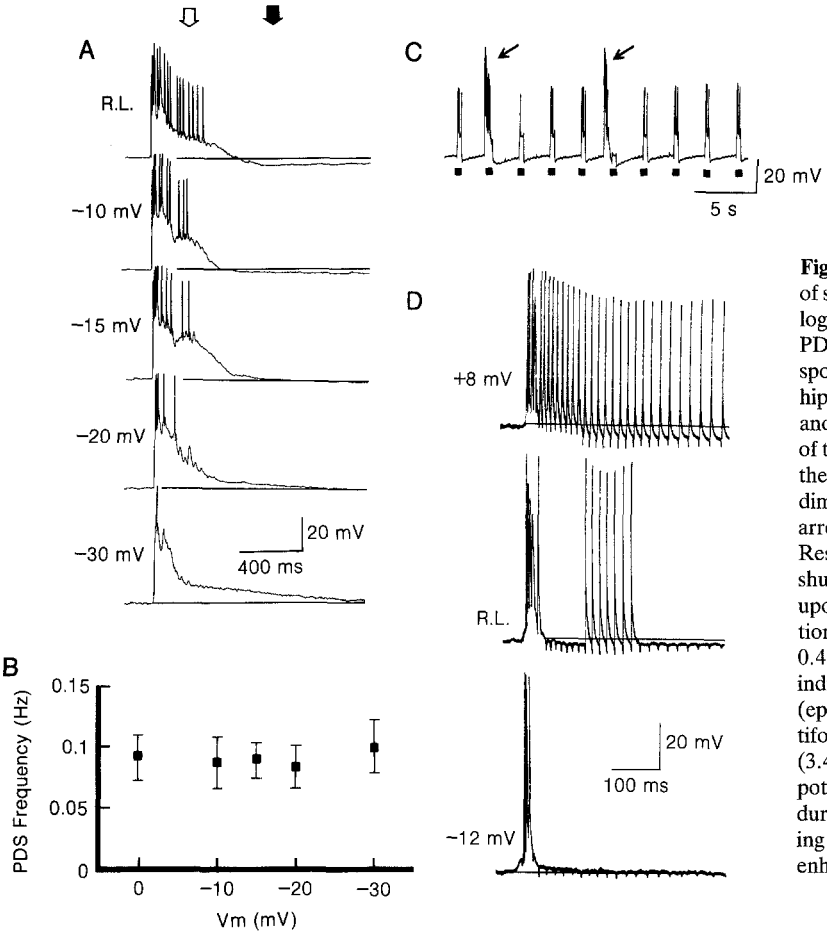

Fig. 4A–D. Effects evoked by intracellular injection of steady hyperpolarizing current upon the morphology (**A**) and the frequency of occurrence (**B**) of the PDS (paroxysmal depolarizing shift) occurring spontaneously in the isolated CA1 subfield of a hippocampal slice bathed in bicuculline (20 μM) and 7.25 mM $[K^+]$. Note that the early component of the PDS increases in amplitude upon increase of the Vm, while the late part (open arrow) is clearly diminished at values larger than –20 mV. The filled arrow points at the post-PDS hyperpolarization. Resting Vm (RL) in this neuron was –65 mV. In **C** shunting ability of the post PDS hyperpolarization upon action potential evoked by intracellular injection of depolarizing pulses (filled squares, 400 ms, 0.4 nA). The spontaneously occurring PDS are indicated by arrows. In **D.** evidence for field (ephaptic) effects during SED (synchronous epileptiform discharge) in the presence of penicillin (3.4 mM) and 7.25 mM $[K^+]$. Note that each action potential rises from a small negative transient during the post PDS hyperpolarization. By changing the Vm, the amount of action potentials can be enhanced (+8 mV) or aborted (–12 mV)

procedure the PDS appeared to change in amplitude. Although the occurrence of action potentials during the depolarizing event did not allow us to quantify this phenomenon, it was quite clear from the raw data that the early part (first 50–100 ms) of the depolarizing envelope increased as a continuum during the full range of hyperpolarized levels studied. Conversely, the late component of the PDS, after an increase over levels of 0–20 mV negative to rest, decreased at more negative Vm values (Fig. 4A, open arrow). By hyperpolarizing the neuron it was also possible to decrease the amplitude and eventually to invert the post-PDS hyperpolarization (Fig. 4A, filled arrow; 4D). The equilibrium potental of this event in 6.25 mM $[K^+]_o$ was 10–20 mV negative to rest (i.e. –85 to –100 mV), a value which was suggestive for a K^+ conductance. CA1 pyramidal cells (either still connected to or isolated from the CA2–CA3 regions) depolarized steadily of 5–10 mV during elevation of $[K^+]_o$ from 7.25 up to 10.25 mM. This change in Vm was accompanied by an increase in the frequency of occurrence of the PDSs which became smaller in amplitude as a result of the decreased electrical gradient across the neuronal membrane (Fig. 5A). In addition, although a relatively larger number of action potentials could be seen at the peak of the PDS at the new Vm reached during elevation of $[K^+]_o$, the duration of each PDS decreased. This phenomenon appeared as if it were caused by the post-PDS hyperpolarization which, at depolarized Vm, peaked earlier thus curtailing the depolarizing envelope of the PDS. At the same time the duration of the post-PDS hyperpolarization potential decreased.

The relationship between duration of the post-PDS hyperpolarization on one hand, and $[K^+]_o$ on the other is clear in Fig. 5B. Both half-width and total duration of the post-PDS hyperpolarization were inversely related to the $[K^+]_o$, a relation which was also observed with the afterhyperpolarization following action potentials elicited by depolarizing pulse of current. In none of our intracellular experiments (n: 3) the duration of the spontaneous PDS increased upon changes in $[K^+]_o$ between 7.25 and 10.25 mM, a finding which is in agreement with the extracellular data reported above.

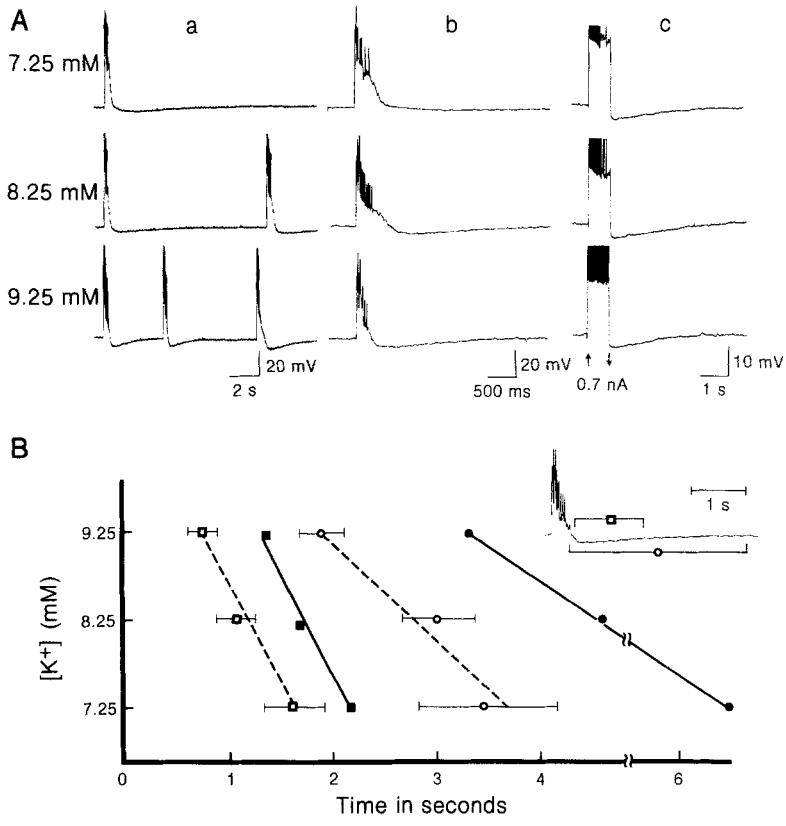

Fig. 5. A Changes evoked by increasing $[K^+]$ upon the PDS (paroxysmal depolarizing shift), the post-PDS hyperpolarization (**a, b**) and the afterhyperpolarization which follows a discharge of action potentials evoked by an intracellular current pulse (**c**). **B** Plot of the duration of the post-PDS hyperpolarizations (duration ○ and halfwidth □) and of the hyperpolarizations following action potentials (duration ● and halfwidth ■) evoked by the intracellular pulses. Vm of this neuron at 7.25, 8.25 and 9.25 mM was −65, −59 and −52 mV, respectively

Discussion

Spontaneous synchronous epileptiform discharges in the CA1 subfield of the "in vitro" slice

These experiments demonstrate that the appearance of spontaneous SEDs generated by hippocampal pyramidal cells in the presence of GABA antagonists is dependent upon the $[K^+]_o$. Accordingly, spontaneous SEDs in the intact CA1 subfield could only be observed when $[K^+]$ in the ACSF was brought to levels larger than 4.25 mM, a value more than 1 mM higher than the one found in the extraneuronal space (cf. Lux et al. 1986). We could also show that the absence of spontaneous SEDs in 3.25 mM $[K^+]$ was not caused by a lack of action of bicuculline or penicillin since they were both able to block the IPSP and to change the orthodromic response to s. radiatum stimulation into an epileptiform burst (Schwartzkroin and Prince 1980a; Dingledine and Gjerstad 1980).

The relation between $[K^+]$ and excitability of hippocampal slices maintained "in vitro" has been described by several investigators. Orthodromic responses are facilitated by increasing $[K^+]$ in the ACSF (Hablitz and Lundervold 1981) and a PDS like response appears at $[K^+]$ of 10 mM (Ogata et al. 1976; Prince and Schwartzkroin 1978). Also a quantitative analysis of the relation between frequency of occurrence of SEDs and $[K^+]$ has recently been reported for the CA3 subfield (Rutecki et al. 1985). Here, in the absence of chemical convulsant, the sole increase in $[K^+]_o$ was capable of evoking spontaneous SEDs.

Our data confirm these previous findings as well as that the CA2–CA3 regions are the sites from which SEDs are triggered in the intact slice bathed in GABA antagonists (Schwartzkroin and Prince 1978; Gjerstad et al. 1981; Wong and Traub 1983). However, we have also demonstrated that hippocampal pyramidal cells in the isolated CA1 subfield can generate spontaneous SEDs under adequate conditions. One of these conditions has been identified as a certain minimal $[K^+]_o$ value. Although the $[K^+]_o$ required for the appearance of spontaneous SEDs in the isolated CA1 subfield was higher than that capable of eliciting them in the intact slice, the morphology of the SEDs and of the intracellular PDS were similar in the "intact" and "isolated" CA1 subfield.

Spontaneous SEDs in the isolated CA1 subfield have been reported by Hablitz (1984) in the presence of picrotoxine. Furthermore, slices bathed in ACSF containing low Ca^{++} (a condition where synaptic

transmission is largely blocked) generate spontaneous synchronous bursts which start usually in the CA1 subfield (Yaari et al. 1983; Haas and Jefferys 1984; Avoli and Agopyan 1986). Finally, spontaneous SEDs have been recorded "in situ" in the presence of penicillin from hippocampal islands consisting mainly of the CA1 region (Dichter et al. 1973).

Synaptic and non-synaptic origins of the synchronous epileptiform discharges

The cellular correlate of the spontaneous SED generated by hippocampal pyramidal cells in either "isolated" or "intact" CA1 subfield was a large amplitude depolarizing potential associated with a burst of action potentials. Thus, from a morphological standpoint the PDS recorded in the present experiments was similar to that described in both "in vivo" and "in vitro" models of focal epilepsy (Matsumoto and Ajmone Marsan 1964; Prince 1968; Ayala et al. 1973; Schwartzkroin and Prince 1978, 1980a; Gjerstad et al. 1981; Hablitz 1984).

As expected for a network generated potential (Johnston and Brown 1981), intracellular injections of hyperpolarizing current did not change the PDS frequency of occurrence at any given $[K^+]_o$. In addition, this procedure caused an increase of the amplitude of the depolarizing potential as expected for a phenomenon which reflects synaptic conductances. However, by increasing the Vm we could observe a late component of the depolarizing envelope which was clearly dependent upon the Vm. High threshold Ca^{++} inward currents in the dendrites (Wong and Prince 1979) and/or voltage dependent conductances related to N-methyl-D-aspartate receptors (Dingledine et al. 1986) might in fact be responsible for this late component of the PDS.

It should be emphasized that during SEDs we could also observe action potentials which appeared to be generated through field (ephaptic) effects. Synchronization of action potentials can occur when evoked neurotransmitter release is blocked in low $[Ca^{++}]$ ACSF (Yaari et al. 1983; Jefferys and Haas 1984; Avoli and Agopyan 1986). Also, Snow and Dudek (1984) have shown that electrical field effects contribute to the synchronization of action potentials generated on top of the PDS evoked by picrotoxine. The evidence gathered in the present experiments is in keeping with these data and in fact we could show that electrical field interactions are capable of making neurons fire action potentials even during the powerful "inhibitory" post-PDS hyperpolarization.

Modulation of synchronous epileptiform discharges by $[K^+]_o$

Several mechanisms underlie the appearance of spontaneous SEDs evoked by the increase $[K^+]_o$. It is well known that high $[K^+]_o$ increases the efficacy of synaptic transmission and indeed this has been a frequently used tool for studying synaptic transmitter release in brain tissue maintained "in vitro". On the other hand, increasing $[K^+]_o$ evokes a dose related steady depolarization of the Vm. Hippocampal neurons are consequently brought to a Vm which is closer to the threshold for the generation of action potentials and within the range of activation of endogenous bursts which have been observed in the CA1 subfield (Masukawa et al. 1984). Also, in this situation, field (ephaptic) effects will be greatly facilitated.

The reduction of the chemical gradient for K^+ across the neuronal membrane will also affect several K^+ repolarizing conductances. Accordingly, K^+ channel blockers such as tetraethylammonium or 4-aminopyridine are able to evoke epileptiform activity (Schwartzkroin and Prince 1980b; Voskyul and Albus 1985). Finally, the increase in $[K^+]_o$ will also induce a shift of the Cl^- equilibrium potential (Martin 1979). Although such a mechanism should play a minor role in our experiments, where GABA conductances have been blocked by bicuculline or penicillin, it might still be capable of affecting non-synaptic Cl^- conductances (Owens et al. 1985).

The experiments reported here demonstrate that the increase in frequency of occurrence of SEDs during elevation of $[K^+]_o$ is related at the intracellular level to a decreased duration of the post PDS hyperpolarization. In keeping with this observation experimental evidence indicates that this potential is mainly underlied by two different K^+ conductances, namely: (i) a Ca^{++} dependent K^+ current (Hotson and Prince 1980; Brown and Griffith 1983) which is powerfully turned on by the depolarization associated with the PDS and (ii) a synaptic hyperpolarizing K^+ conductance which is enhanced in the presence of GABA antagonists (Alger and Nicoll 1980; Hablitz 1981; Thallman and Ayala 1982; Alger 1984; Lancaster and Wheal 1984; Newberry and Nicoll 1984). Accordingly, reduction or blockage of the Ca^{++} dependent K^+ current by cyclic AMP results in an increase of the frequency of SEDs evoked by bicuculline (Newberry and Nicoll 1984). Also, modeling studies have attributed to the post-PDS hyperpolarization a pivotal role in determining the rate of occurrence of spontaneous PDSs (Traub and Wong 1983). However, our data do not suggest the existence of a simple relationship between post-PDS

hyperpolarization and frequency of occurring of SEDs since the interval between spontaneous SEDs appears to be longer than the duration of the post-PDS hyperpolarization. A similar conclusion has been drawn in hippocampal slices bathed in ACSF containing picrotoxine and 5 mM $[K^+]$ (Hablitz 1984). Consequently, in addition to the K^+ mediated post-PDS hyperpolarization, other mechanisms do contribute to determining the frequency of occurrence of spontaneous SEDs.

Acknowledgements. We thank Drs. M. Dichter, P. Gloor, and R. Pumain for their helpful comments on an earlier draft.

References

Alger BE (1984) Characteristics of a slow hyperpolarizing synaptic potential in rat hippocampal pyramidal cells in vitro. J Neurophysiol 52: 892–910

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

Avoli M (1984) Penicillin induced hyperexcitablity in the in vitro hippocampal slice can be unrelated to impairment of somatic inhibition. Brain Res 323: 154–158

Avoli M, Agopyan N (1986) Mechanisms for low calcium, high magnesium synchronous bursts in the in vitro hippocampal slice. In: Speckman EJ et al. (eds) Epilepsy and calcium. Urban and Schwarzenberg, München Wien Baltimore, pp 63–83

Ayala GF, Dichter M, Gumnit RJ, Matsumoto H, Spencer WA (1973) Genesis of epileptic interictal spikes: new knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms. Brain Res 52: 1–17

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

Curtis DR, Felix D (1971) The effect of bicuculline upon synaptic inhibition in cerebral and cerebellar cortices of the cat. Brain Res 34: 301–321

Curtis DR, Game CJA, Johnston GAR, McCulloch RM, MacLachlan RM (1972) Convulsive action of penicillin. Brain Res 42: 242–245

Dichter M, Herman CJ, Selzer M (1972) Silent cells during interictal discharges and seizures in hippocampal penicillin foci. Evidence for the role of extracellular K^+ in the transition from the interictal state to seizures. Brain Res 48: 173–183

Dichter M, Herman CJ, Selzer M (1983) Penicillin epilepsy in isolated islands of hippocampus. Electroenceph Clin Neurophysiol 34: 631–638

Dingledine R, Gjerstad L (1980) Reduced inhibition during epileptiform activity in the in vitro hippocampal slice. J Physiol (Lond) 305: 297–313

Dingledine R, Hynes MA, King G (1986) Involvement of N-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice. J Physiol (Lond) 380: 175–189

Fertziger AP, Rank JB (1970) Potassium accumulation in interstitial space during epileptiform seizures. Exp Neurol 26: 571–585

Gjerstad L, Andersen P, Langmoen IA, Lundervold A, Hablitz J (1981) Synaptic triggering of epileptiform discharges in CA1 pyramidal cells in vitro. Acta Physiol Scand 113: 245–252

Haas HL, Jefferys JGR (1984) Low calcium field burst discharges of CA1 pyramidal neurons in rat hippocampal slices. J Physiol (Lond) 354: L 185–201

Hablitz JJ (1981) Effects of intracellular injections of chloride and EGTA on postepileptiform burst hyperpolarizations in hippocampal neurons. Neurosci Lett 22: 159–163

Hablitz JJ (1984) Picrotoxin-induced epileptiform activity in hippocampus: role of endogenous versus synaptic factors. J Neurophysiol 51: 1011–1027

Hablitz JJ, Lundervold A (1981) Hippocampal excitability and changes in extracellular potassium. Exp Neurol 71: 410–420

Heinemann U, Konnerth A, Pumain R, Wadman WJ (1986) Extracellular calcium and potassium concentration changes in chronic brain tissue. In: Delgado Escueta AV et al. (eds) Advances in neurology, Vol 44. Raven Press, New York, pp 641–661

Hotson JR, Prince DA (1980) A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. J Neurophysiol 43: 409–419

Johnston D, Brown TH (1981) Giant synaptic potential hypothesis for epileptiform activity. Science 211: 294–297

Krnjevic K (1983) GABA-mediated inhibitory mechanisms in relation to epileptic discharges. In: Jasper HH, van Gelder N (eds) Basic mechanisms of neuronal hyperexcitability. Alan R Liss Inc, New York, pp 249–280

Lancaster B, Wheal HV (1984) The synaptically evoked hyperpolarization in hippocampal CA1 pyramidal cells is resistant to intracellular EGTA. Neuroscience 12: 267–275

Lux HD, Heinemann U, Dietzel I (1986) Ionic changes and alteration in the size of the extracellular space during epileptic activity. In: Delgado Escueta AV et al. (eds) Advances in neurology, Vol 44. Raven Press, New York, pp 619–639

MacDonald RL, Barker JL (1977) Pentylenetetrazol and penicillin selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurons. Nature (Lond) 267: 720–721

Martin AR (1979) Appendix. In: Mathews G, Wickelgren WO (eds) Glycine GABA and synaptic inhibition of reticulospinal neurons of lamprey. J Physiol (Lond) 293: 394–415

Masukawa L, Benardo LS, Prince DA (1982) Variation in electrophysiological properties of hippocampal neurons in different subfields. Brain Res 242: 341–344

Matsumoto M, Ajmone C (1964) Cortical cellular phenomena in experimental epilepsy: interictal manifestations. Exp Neurol 9: 286–304

Newberry NR, Nicoll RA (1984) A bicuculline resistant inhibitory postsynaptic potential in rat hippocampal pyramidal cells in vitro. J Physiol (Lond) 348: 239–254

Ogata N, Hori N, Katsuda N (1976) The correlation between extracellular potassium concentration and hippocampal epileptic activity in vitro. Brain Res 110: 371–375

Oliver AP, Hoffer BJ, Wyatt RJ (1978) Interaction of potassium and calcium in penicillin induced interictal spike discharge in the hippocampal slice. Exp Neurol 62: 510–520

Owens DG, Segal M, Barker JL (1984) A Ca^{++}-dependent Cl^- conductance in cultured mouse spinal neurons. Nature (Lond) 311: 567–570

Prince DA (1968) Inhibition in 'epileptic' neurons. Exp Neurol 21: 307–321

Prince DA, Schwartzkroin PA (1978) Non-synaptic mechanisms in epileptogenesis. In: Chalazonitis N, Boisson M (eds) Abnormal neuronal discharges. Raven Press, New York, pp 1–12

Rutecki PA, Lebeda FJ, Johnston D (1985) Epileptiform activity induced by changes in extracellular potassium in hippocampus. J Neurophysiol 54: 1363–1374

Schwartzkroin PA, Prince DA (1978) Cellular and field potential properties of epileptogenic hippocampal slices. Brain Res 147: 117–130

Schwartzkroin PA, Prince DA (1980a) Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. Brain Res 183: 61–73

Schwartzkroin PA, Prince DA (1980b) Effects of TEA on hippocampal neurons. Brain Res 185: 169–181

Snow RW, Dudek FE (1984) Electrical fields directly contribute to action potential synchronization during convulsant-induced epileptiform bursts. Brain Res 323: 114–118

Sypert GW, Ward AA (1974) Changes in extracellular potassium activity during neocortical propagated seizures. Exp Neurol 45: 19–41

Thalmann RH, Ayala GF (1982) A late increase in K conductance follows synaptic stimulation of granule neurons of the dentate gyrus. Neurosci Lett 23: 243–248

Traub RD, Wong RKS (1983) Synchronized burst discharge in disinhibited hippocampal slice. II. Model of cellular mechanism. J Neurophysiol 49: 459–471

Voskyul RA, Albus H (1985) Spontaneous epileptiform discharges in hippocampal slices induced by 4-aminopyridine. Brain Res 342: 54–66

Wong RKS, Prince DA (1979) Dendritic mechanisms underlying penicillin induced epileptiform activity. Science 204: 1228–1231

Wong RKS, Traub RD (1983) Synchronized burst discharge in disinhibited hippocampal slice. I. Initiation in CA2–CA3 region. J Neurophysiol 49: 442–458

Yaari Y, Konnerth A, Heinemann V (1983) Spontaneous epileptiform activity of CA1 hippocampal neurons in low extracellular calcium solutions. Exp Brain Res 51: 153–155

Zuckerman EC, Glaser GH (1968) Hippocampal epileptic activity induced by localized ventricular perfusion with high potassium cerebrospinal fluid. Exp Neurol 20: 87–110

Received August 4, 1986 / Accepted March 20, 1987