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Epilepsy Research 21 (1995) 195–204

EPILEPSY RESEARCH

An in vitro model of persistent epileptiform activity in neocortex

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Received 26 October 1994; accepted 20 March 1995

Abstract

An in vitro model of persistent epileptiform activity was developed to study the mechanisms involved in epileptogenesis. Extracellular recordings were obtained from rat neocortical slices exposed to magnesium-free solution for 2 h. During exposure to magnesium-free solution spontaneous epileptiform activity consisting of interictal bursting and ictal-like discharges were observed. Interestingly, this activity persisted for hours after the slices were returned to magnesium-containing control solution. The *N*-methyl-D-aspartate (NMDA) receptor antagonist CPP prevented the development of the epileptiform activity, while the non-NMDA receptor antagonist CNQX abolished the epileptiform discharge that persisted after slices were returned to control solution. These findings suggest there are two distinct phases in the development of epileptic activity in this model, namely, induction (mediated by NMDA receptor activity) and maintenance (supported largely by non-NMDA receptor activity). The similarities and possible parallels between the mechanisms underlying this epileptogenesis and other forms of use-dependent modification of synaptic excitation, such as long-term potentiation, are discussed. This in vitro model of neocortical epileptogenesis may provide insights into the events underlying the development of clinical partial epilepsy.

Keywords: AMPA/kainate; Experimental epilepsy; Glutamate; *N*-Methyl-D-aspartate; Neocortex

1. Introduction

Partial epilepsy, which is largely acquired [49], first requires an induction period following some imposed focal pathology (e.g., trauma or ischemia). For complete epileptogenesis to occur, there must be progression beyond this induction stage to one characterized by recurrent seizure discharges. The most useful models to study epilepsy are those in which these two separate phases can be recognized, since different therapies may be indicated at each stage.

Most models of epilepsy studied in vitro examine the activities which ensue following acute drug exposure [13,16,40] or those which develop after some experimental maneuver has been performed (e.g., alumina gel exposure [41] or undercutting the cortex [19]). Of the several acute models of epileptiform activity, the best known include treatments which block fast GABAergic inhibition [17,39,40], or expose slices to high concentrations of extracellular potassium [7,17,48]. The drawbacks to acute models like these are that they focus on generating interictal

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SSDI 0920-1211(95)00024-0

activity. Furthermore, the induced paroxysmal activity does not appear to persist upon removal of the convulsant agent. On the other hand, chronic epilepsy models produce a maintained epileptic state [19,41], but unfortunately in these models the mechanisms giving rise to this condition were not examined.

Recent progress in understanding epileptogenesis and seizure expression has come from examining the role glutamatergic transmission plays in these processes. One major finding is that *N*-methyl-D-aspartate (NMDA) receptor activation may be fundamental in the initiation and propagation of epileptiform discharge, since NMDA antagonists inhibit seizures produced by a variety of treatments [2,18,21,37,48]. Of interest, in one kindling-like model of epilepsy utilizing hippocampal slices [1], further pharmacological differentiation of the epileptogenesis into separate induction and maintenance phases was suggested. Here, NMDA antagonists prevented the development of the kindled state, yet failed to suppress previously kindled seizures [1].

The present study was undertaken to identify a model of epilepsy in neocortical slices in which epileptiform discharges persisted following a period of seizure induction. Such a model would prove valuable to the study of epilepsy and its treatment. Exposure of slices to magnesium-free solutions is known to enhance responses to NMDA [28,33], leading to increases in polysynaptic transmission in neuronal aggregates [45], and sustained seizure discharge [4,5,47,52]. We now report that following such exposure, epileptiform discharges persist for hours after slices have been returned to control solution containing magnesium. Here we characterize this seizure activity and analyze the pharmacology of its induction and maintenance. A brief report describing these results has appeared [50].

2. Methods

Procedures for preparing and maintaining unilateral and bihemispheric slices of rat somatosensory cortex have been published [5]. Slices 400 μm thick were obtained from Sprague–Dawley rats (P16-48) and placed in an interface-type recording chamber maintained at 35.5 ± 1°C. Slices were superfused continuously (1 ml/min) with Ringer's solution, while resting on a nylon net. The composition of control physiological saline was in mM: NaCl, 124; KCl, 5; $MgCl_2$, 1.6; $CaCl_2$, 2; $NaHCO_3$, 26; D-glucose, 10. The pH was 7.4.

Solutions designated magnesium-free had no added magnesium. Some magnesium-free solutions also contained the $GABA_A$ antagonist picrotoxin (50 μM) to further enhance excitability. Our analysis did not reveal any specific differences in the activities generated by slices exposed to magnesium-free solutions with or without picrotoxin. Moreover, picrotoxin alone did not provide the kinds of activity seen in magnesium-free solutions. Accordingly, all data for slices exposed to magnesium-free solutions were pooled. Some solutions also contained 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), or 3-[(−)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP). Given the solution flow rates used and the volume of the experimental chamber, (one-time) turnover of the chamber volume occurs within 3 to 5 min. In practice full drug action (complete mixing) occurs within 10 to 15 min [4,5].

All slices were allowed to adapt within the recording chamber for at least an hour prior to recording. Extracellular recordings were obtained with microelectrodes pulled from thin-walled, filament-containing borosilicate glass tubing (1.0 mm O.D.) on a Brown–Flaming micropipette puller. Electrodes were filled with 2 M NaCl and were broken back to a resistance of 2–5 MΩ. Standard recording techniques were employed using an Axoclamp 2A amplifier. Data were digitized and stored on a microcomputer or on VHS tape for subsequent analysis.

Synaptic field potentials were elicited by means of a coated monopolar tungsten electrode positioned usually in the deep white matter or in layers V or VI. Single cathodal shocks (200 μA) of 100–200 μs duration were delivered through a digitally controlled stimulus isolation unit.

Data are expressed as mean $\pm$ standard deviation. Statistical significance was determined by Student's *t*-test.

3. Results

The data presented here are based on extracellular recordings obtained from 92 neocortical slices, rou-

tinely recorded in layer V. Under control conditions, spontaneous field potential activity was never seen, and evoked activity was limited to single, brief negative potentials. During exposure to magnesium-free solutions epileptiform activity developed. We have empirically defined the period of exposure of slices to convulsant solutions as the induction phase, and the period when slices were returned to control media (during which seizure discharges persisted) as the maintenance phase.

3.1. Induction of epileptiform activity

Slices were exposed to magnesium-free solutions for at least one hour, though two-hour exposure was routine. Within 20 to 35 min following exposure to magnesium-free solution all slices developed spontaneous epileptiform activity ($n = 67$). Three different types of epileptiform activities were recognized, as described below.

Interictal epileptiform discharges

Interictal discharges were those spontaneous or evoked activities consisting of single bursts, lasting seconds, composed of multiple spikes (arrow, Fig. 1A). The amplitude of interictal potentials remained constant in individual slices, but had a range of 0.8 to 4 mV. These epileptiform discharges occurred in near synchrony, but appeared to be led by discharges occurring in the middle layers, as previously reported [43]. Spikes tended to occur at higher frequencies at the beginning of the discharge, and slowed down somewhat prior to their termination (range, 7 to 36 Hz). Interictal events could also be evoked by single orthodromic stimuli applied to the deep gray or white matter (Fig. 1A2). The individual spikes within bursts (see detail Fig. 1A1–2) are the recognized field potential equivalent of interictal spikes seen in the electroencephalogram [6,34,35].

Relevant parameters defining this interictal activity for a representative experiment are presented in Fig. 2. As shown, burst duration, the number of spikes per burst and the interval between bursts were all quite variable, but burst frequency remained relatively constant. Among slices, burst duration ranged from 1 to 14 s, the number of spikes per burst varied from 2 to 63, the interval between bursts ranged from 1 to 96 s, and the frequency of burst firing was between 1 to 13 per minute.

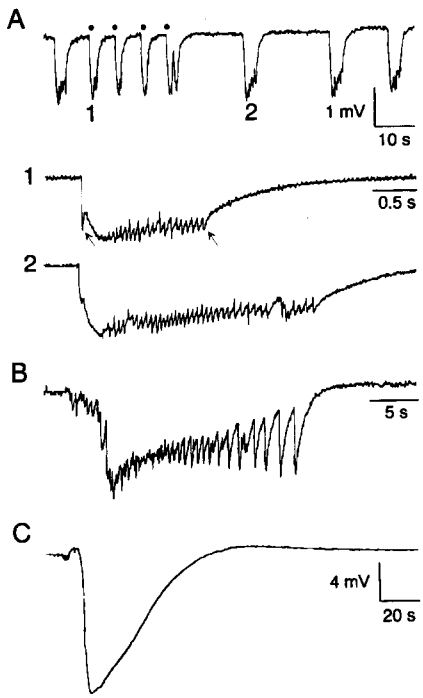

Fig. 1. Extracellular recordings of spontaneous and evoked epileptiform activity in neocortical slices exposed to magnesium-free solution. (A) Upper trace: continuous recording showing spontaneous and evoked (indicated by dots) interictal epileptiform discharges. Traces marked 1 (evoked) and 2 (spontaneous) are shown on an expanded time scale below. Arrows in 1 indicate first and last 'spikes' (see text). (B) Ictal-like event. (C) Spreading depression-like event. Repetitive spiking preceded these long-lasting events which were often followed by an overshoot of the baseline.

Ictal-like discharges

In addition, we observed discharges which have previously been described as ictal like ($n = 40$ slices) [26,39,51]. They were differentiated from interictal activity by their long duration (15 to 45 s), their multiple bursts riding a sustained negativity, and their evolving pattern, resembling the electrographic tonic-clonic seizure discharge seen in vivo epilepsy models [22,36], and in human epilepsy [14]. Fig. 1B illustrates an ictal-like discharge which initially had a series of repetitive spikes superimposed on a prolonged negative potential, which were then followed by recurring bursts. Ictal events occurred on average about 7.5 times per hour (range 1–50).

Spreading depression-like events

About 60% of slices developed longer-lasting negative potentials, indicative of spreading depression-like events [12,30,39,44]. This activity was always preceded by interictal or ictal-like discharge (Fig. 1C). These potentials lasted 0.5 to 2.6 min in duration and ranged from 12 to 21 mV in amplitude. Immediately following the spreading depression-like potential an overshoot of the baseline (0.5 to 1 mV) was often observed, which lasted 0.5 to 7 min. Throughout the duration of the spreading depression, epileptiform bursting could be superimposed, though it was less frequent. Spreading depression-like events were very infrequent, occurring only 2–3 times per hour.

3.2. Maintenance of epileptiform discharges

After exposure to magnesium-free solution for up to two hours, slices were returned to control solution. Though the experimental chamber requires approximately 10 min for complete exchange of solution, all sixty-seven slices continued to show epileptiform activity for prolonged periods of time (mean, 101.0 $\pm$ 57.5 min, range 30–240 min). This activity, consisting of interictal bursting and ictal-like discharges (in 25 slices), continued to be widespread, occurring spontaneously and in response to single orthodromic stimuli. Spreading depression-like potentials were also present during this period in about 40% of slices. Fig. 3 shows examples of the persistent

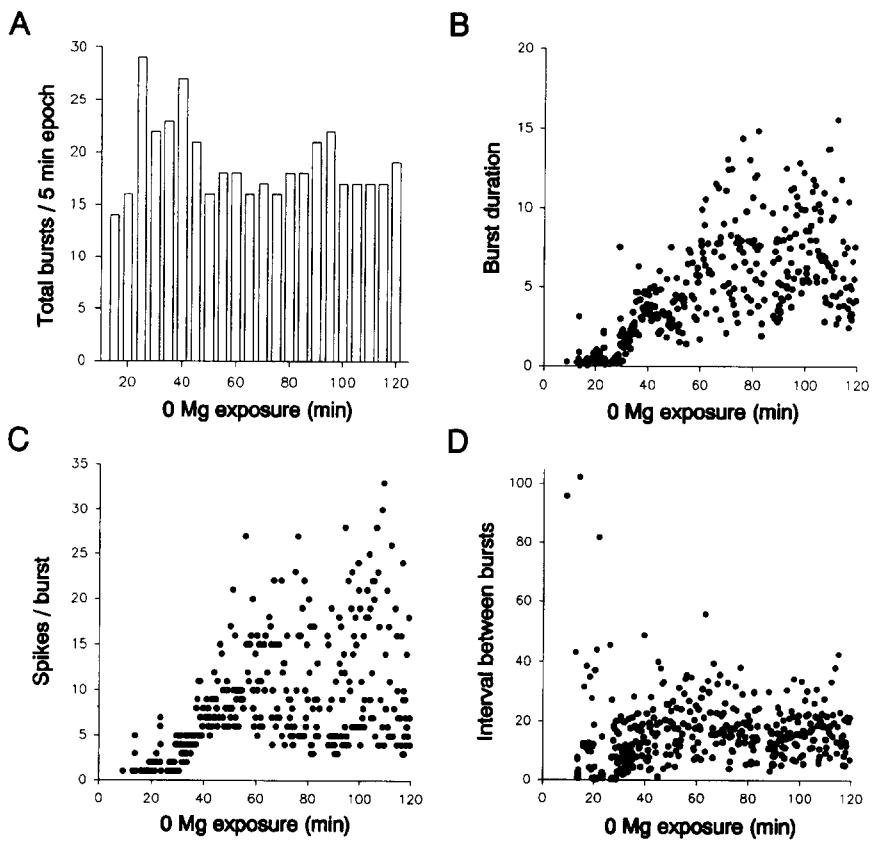

Fig. 2. Indices of epileptiform bursting during induction (exposure to magnesium-free solution). Burst frequency expressed as the total number of bursts during successive 5-min periods (A), duration of the epileptiform bursts (B), total number of spikes per burst (C), and the interval between bursts (D), all plotted against time of exposure to magnesium-free (0 Mg) solution. All data obtained from the same slice.

epileptiform activity at different times throughout the maintenance phase. For the first hour, activity closely resembled that seen during induction. After this, discharges were of shorter duration (e.g. at 74 and 165 min), but continued to occur spontaneously for a total of 4 h.

Fig. 4 displays representative data describing this persistent burst activity in another slice. As shown in the figure, after 40–45 min burst duration, the number of spikes per burst and burst frequency decreased, while the interval between bursts increased. The last several spontaneous events usually lasted 1 to 4 s and consisted of one or two negative events. Even after the spontaneous epileptiform activities had ceased, evoked field potentials remained enhanced for at least an additional 30 min.

3.3. Pharmacology of induction and maintenance

The next set of experiments determined whether induction and maintenance were separable on the basis of their pharmacology. This was accomplished by exposing slices in each condition to glutamate ionotropic receptor antagonists while observing the ongoing spontaneous activity.

Effect of NMDA receptor antagonist

No spontaneous epileptiform activity developed in 12 slices simultaneously exposed to magnesium-free solution and the competitive NMDA blocker CPP (10 μM) for two hours (Fig. 5A). In two slices a total of two or three spontaneous, slow (2.0–6.5 s), low amplitude (1.0–1.6 mV) potentials were noted, but these potentials had no superimposed spikes. Additional slices ($n = 4$) were exposed to magnesium-free solution for one hour, allowing them to develop epileptiform activity. When CPP was subsequently added to the solution, this activity ceased within 3 to 4 min. Orthodromic stimulation in the subcortical white matter failed to trigger epileptiform activity, and no spontaneous epileptiform activity developed upon switching to control solution. These findings supported the notion that NMDA receptors were involved in the induction of epileptiform activity.

Next, the involvement of the NMDA receptor activity during the maintenance phase was determined (Fig. 5B). Slices ($n = 12$) were exposed to magnesium-free solutions for two hours, then returned to normal solution for 15 min. This allowed for complete washing of the preparation and allowed

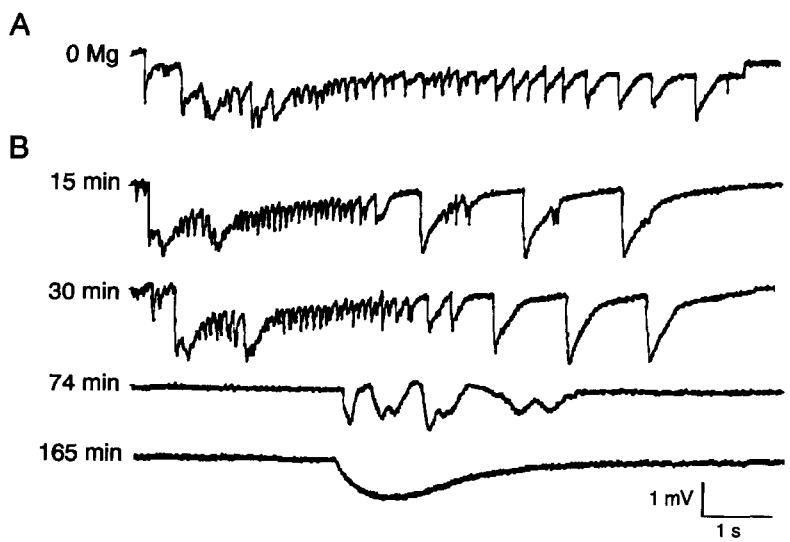

Fig. 3. Persistent spontaneous epileptiform discharges seen after a slice was returned to control solution. (A) Spontaneous epileptiform discharge during exposure to magnesium-free (0 Mg) solution (induction). (B) Spontaneous epileptiform discharges at 15, 30, 74 and 165 min after the slice was returned to control solution (maintenance). The epileptiform discharges at 15 and at 30 min after return to control solution are unchanged from those observed during exposure to magnesium-free solution. Later occurring events were of shorter duration but occurred up to 4 h after return to control solution.

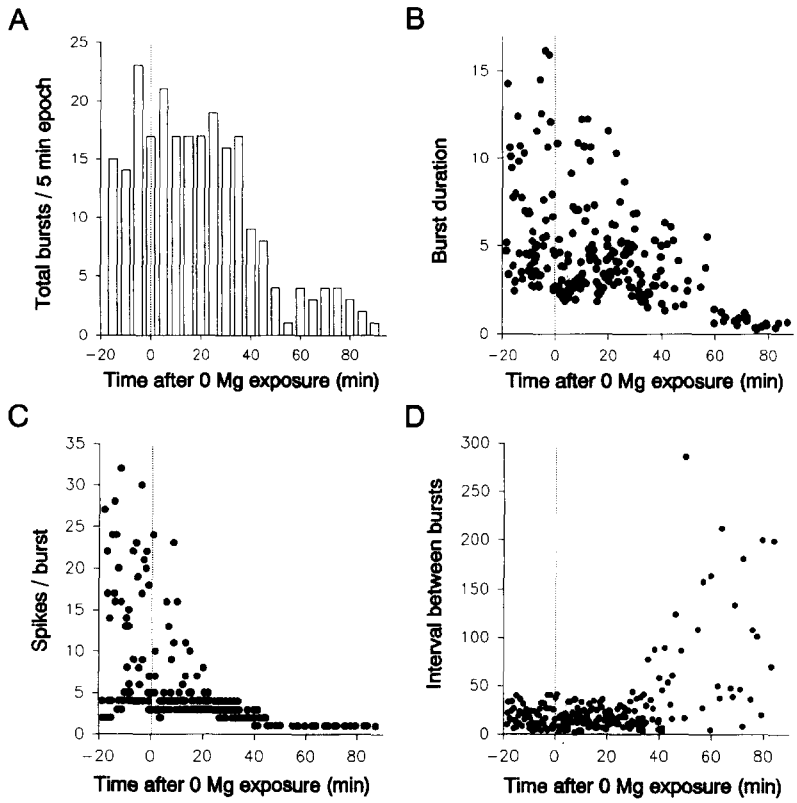

Fig. 4. Indices of persistent epileptiform bursting from a single representative slice returned to solution containing magnesium. Data for epileptiform bursts occurring during the last 20 min of exposure to 0 Mg are shown (negative values). Time 0 (dotted line) corresponds to the time when magnesium-containing solution was introduced. Burst frequency expressed as total number of bursts in 5-min epochs (A), burst duration (B), number of spikes per burst (C), and the interval between bursts (D), were plotted against time before and following exposure to 0 Mg solution.

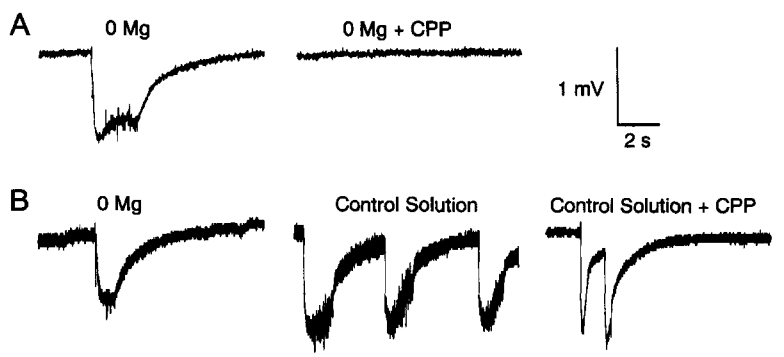

Fig. 5. Effect of the NMDA receptor antagonist CPP on the induction and maintenance of persistent epileptiform activity. When CPP was added to magnesium-free solution (induction) spontaneous epileptiform discharges ceased (A). However, spontaneous epileptiform discharges persisted when CPP was added 15 min after slices were returned to magnesium-containing solution (i.e., during maintenance; B).

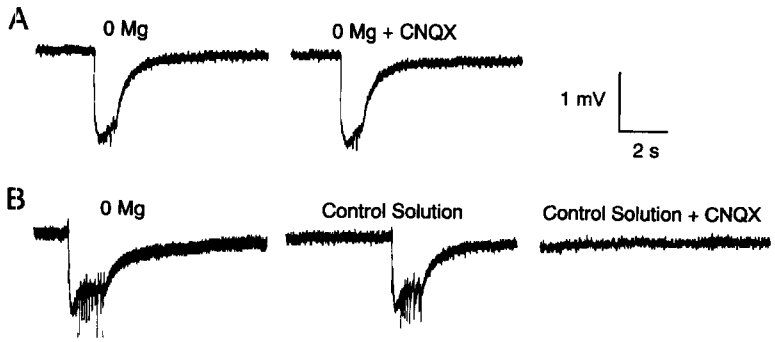

Fig. 6. Effect of the non-NMDA receptor antagonist CNQX on the induction and maintenance of epileptiform discharges. Spontaneous epileptiform activity persisted in the presence of magnesium-free solution and CNQX (induction; A). However, the epileptic discharges which persisted when slices were returned to magnesium-containing control solution, were abolished by the addition of CNQX (maintenance; B).

us to verify the persistence of epileptiform activity. CPP was then bath applied, and in all slices treated, spontaneous epileptiform field potentials continued to occur for an additional period of up to 75 min (i.e. 90 min total; mean, 71.6 ± 10.7 min). This was not significantly different from control values ($P > 0.05$), nor were the duration or frequency of individual discharges affected significantly, although there was a trend toward slightly briefer events. These findings suggested that NMDA receptor activation was not required for the expression of the epileptiform events during the maintenance phase.

Effect of non-NMDA receptor antagonist

To determine whether the epileptiform activity seen during induction relied upon non-NMDA receptor activation, the competitive non-NMDA antagonist CNQX (5 μM) was added to the bath during exposure to magnesium-free solution. Such treatment did not prevent the development of spontaneous epileptiform activity ($n = 12$; Fig. 6A), which was not significantly different from that seen in magnesium-free solution alone [4]. Next, we investigated the role of non-NMDA transmission in supporting the epileptiform activity present during the maintenance phase. When CNQX was added to the bath 15 min after spontaneously discharging slices had been returned to control solution ($n = 13$), the epileptiform activity abated within 5 to 17 min (Fig. 6B). This strongly suggested that the persistent epileptiform discharge was dependent upon activation of non-NMDA receptors.

4. Discussion

This study examined the persistent epileptogenic state that follows a limited exposure to magnesium-free solution in rat neocortical slices. This prolonged seizure-prone state was characterized by the presence of spontaneous, epileptiform extracellular events and constitutes a unique in vitro model for studying the mechanisms involved in epileptogenesis. Pharmacological studies showed that the induction of this activity was dependent upon NMDA receptors (i.e., during exposure to magnesium-free solution), and that the epileptiform potentials which persisted once slices had been returned to normal magnesium-containing solution were supported predominantly by non-NMDA receptors.

Epileptiform discharges are thought to appear whenever the balance between excitation and inhibition exceeds some crucial level [5]. Thus, either enhanced excitation or decreased inhibition produce increased excitatory drive among cortical neuronal aggregates, leading to spontaneous, synchronous epileptiform field potentials [5,47]. In the model presented here, as in other studies in which magnesium-free solutions have been used to create an epileptogenic state, the development of epileptiform

discharge is due to activation of NMDA receptors subsequent to the removal of extracellular magnesium, which normally blocks NMDA channels [28,33]. But numerous in vitro and in vivo studies have implicated the NMDA subtype of glutamate receptors in the generation of epileptiform activity induced by a variety of agents causing epileptogenesis [2,18,21,37,48]. Our results showing that the appearance of the epileptiform discharges was prevented by the specific NMDA receptor antagonist CPP, corroborates the notion that the induction of epileptiform bursting requires the participation of NMDA receptors.

The mechanisms providing the persistent epileptogenic state have been difficult to elucidate, in part because there are very few models of recurrent epilepsy. The best known such model is the in vivo kindling model. While some studies demonstrated enhancement of NMDA receptor activity in the maintenance phase of kindling [15,25,29,31], others noted the additional involvement of non-NMDA transmission [38]. The participation of non-NMDA receptors has been suggested to underlie the persistent seizure-prone state that results from kindling-like stimulation of the hippocampus in vitro, since NMDA receptor antagonists do not suppress the epileptic discharges once the process of epileptogenesis has been established [1]. The involvement of non-NMDA receptors in maintaining epileptic discharges was also suggested in an in vitro model of neocortical traumatic brain injury, because of the variable response of the resulting epileptiform activity to NMDA receptor antagonism [19]. Moreover, in slices which include hippocampal, entorhinal and perirhinal areas, or those from piriform cortex, the abnormal discharges which followed exposure to magnesium-free solution appeared to be non-NMDA mediated [20,27]. These studies are in agreement with our finding that the maintenance of the epileptogenic state is primarily non-NMDA receptor dependent.

The persistence of spontaneous epileptiform discharges in slices returned to control solution suggests neuronal network properties were altered during the exposure to magnesium-free solution. Long-lasting changes within neuronal networks are a well-known feature of the mammalian central nervous system and often involve use-dependent modification of synaptic strength. Long-term potentiation (LTP), a widely used model for studying the cellular basis of memory, is an important example of this activity-dependent synaptic modification. In LTP, enhanced excitatory synaptic transmission is induced following the repeated electrical stimulation of a synaptic pathway. This increased synaptic efficacy persists for several hours in vitro, and up to several weeks in vivo [9,11,42]. NMDA receptor activation appears to be critical for the induction of LTP [3,10,32], but does not appear to be required during the maintenance period [10,32]. Rather, the expression of synaptic potentiation appears to be supported by non-NMDA receptors. Thus, our model of epilepsy bears many similarities with LTP. First, both processes involve enhanced excitatory transmission. Second, their induction phases are mediated by NMDA receptors. Third, their maintenance phases are supported by non-NMDA receptors. Fourth, the duration of potentiated excitatory transmission for both lasts hours [8,24]. In our experiments the spontaneous repetitive epileptiform discharges which occurred in magnesium-free solutions may have played the same role as that of the repetitive high frequency stimulation used to induce LTP. Though LTP appears to be a physiological phenomenon, and epilepsy a pathological condition, the difference in their generation may be a quantitative one, related to the number of pathways involved and the size of the aggregate of neurons recruited by synaptic potentiation. In LTP the strength of a specific pathway is potentiated [46], whereas in epilepsy, several pathways may well be facilitated. Thus, epilepsy may simply be an aberration of a physiological process related to neuronal plasticity.

The present model of epileptogenesis has clinical relevance to partial epilepsy, in which recurrent seizures develop after an area of the cortex sustains an acute injury (e.g., traumatic brain injury, ischemia). As in our model, such insults result in excessive accumulation of extracellular glutamate which subsequently activates glutamate receptors [23]. Given our results, the activation of glutamate receptors of the NMDA subtype, if sufficiently strong, could initiate a process akin to LTP in the affected glutamatergic pathways. The enhanced excitatory synaptic activity, if sufficiently widespread might give rise to interictal, or even ictal discharges. The presence of interictal and ictal activity may

induce further strengthening of synaptic transmission along excitatory pathways participating in the epileptic discharge, thus leading to a chronic epileptic state. Accordingly, therapeutic interventions aimed at seizure prevention might be more properly directed at precluding the establishment of this persistent epileptogenic state. Furthermore, this model would be useful in testing the potential benefit of conventional as well as newly developed antiepileptic drugs, and could differentiate seizure prophylactic agents from those which treat chronic epilepsy. For example, NMDA antagonists should serve as prophylactic agents when given immediately following a potentially epileptogenic injury, while non-NMDA blockers would be better suited to the treatment of epilepsy once it has become established.

Acknowledgements

This work supported by the National Institutes of Health (NS01386 and MH51677 to L.S.B.). We would like to thank Drs. R.Q. Cracco and D.S.F. Ling for critical comments on the manuscript.

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