Short- and Long-Term Changes in CA1 Network Excitability After Kainate Treatment in Rats

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INTRODUCTION

Kainate treatment in rats causes status epilepticus (i.e., a period of prolonged and repetitive seizures) and leads to the eventual development of a chronic epileptic state characterized by an increasing frequency of spontaneous recurrent seizures weeks after the initial treatment. The kainate-treated rat thus serves as a model of human temporal lobe epilepsy (TLE) (Ben-Ari 1985; Lothman and Collins 1981; Nadler 1981). In both kainate-treated rats and TLE patients, epileptogenesis is associated with loss of neurons in the hippocampal CA3, CA1, and hilar areas. Increased collaterals of granule cell axons (i.e., mossy fiber “sprouting”) and synaptic reorganization in the dentate gyrus also occur over several weeks and months as chronic epilepsy develops with time (Babb et al. 1991; Ben-Ari 1985; Ben-Ari et al. 1981; Buckmaster and Dudek 1997a,b; Cronin and Dudek 1988; Cronin et al. 1992; De Lanerolle et al. 1989; Franck et al. 1995; Hellier et al. 1998; Nadler et al. 1980; Sutula et al. 1989, 1998; Tauck and Nadler 1985).

Several previous studies have provided evidence that the CA1 area of the hippocampus is a region of both substantial neuronal loss and increased seizure susceptibility in the epileptic brain. The combination of axon sprouting of pyramidal cells coupled with selective neuronal loss in the CA1 area has been hypothesized to be an important component of the development of TLE. CA1 pyramidal cell density is often greatly reduced in humans with TLE and in animal models (Mathern et al. 1995a,b, 1997). Selective loss of putative inhibitory neurons has been observed in the CA1 region in the kainate (Best et al. 1993; Morin et al. 1998a) and pilocarpine models of TLE (Hauser and Esclapez 1996). Even so, much of the inhibitory circuitry appears functional weeks after treatment (Esclapez et al. 1997; Franck et al. 1995; Hellier et al. 1998; Nadler et al. 1980). Some studies have suggested that inhibition is transiently decreased immediately after kainate treatment followed by partial recovery after several weeks (Franck and Schwartzkroin 1985; Franck et al. 1988). Electrophysiological analyses in the isolated CA1 indicated that inhibition is reduced several weeks after kainate treatment (Meier et al. 1992). Months after kainate treatment, CA1 pyramidal cells could generate repetitive, all-or-none bursts of action potentials to afferent stimulation in the presence of the GABA$_\text{A}$-receptor antagonist, bicuculline (i.e., similar to normal CA3 pyramidal cells) (Meier and Dudek 1996). Early studies indicated an increased number of excitatory synapses in the CA1 region after kainate treatment (Nadler et al. 1980), and reconstruction of intracellularly labeled CA1 pyramidal cells revealed increased axonal branching in kainate-treated animals versus controls (Esclapez et al. 1999; Perez et al. 1996), suggesting an increase in excitatory connectivity in the CA1 due to local axon reorganization.

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relationship between anatomical alterations and regional changes in excitability is probably a critical feature of temporal lobe epileptogenesis (see Dudek and Spitz 1997). However, the time-dependent changes in CA1 network excitability after kainate treatment have been only briefly discussed (Franck and Schwartzkroin 1985; Franck et al. 1988).

We examined CA1 neuron density, pyramidal cell axonal morphology, and synaptically evoked spike bursts in CA1 pyramidal cells using slices from rats several weeks after kainate treatment, when they had developed spontaneous recurrent motor seizures (i.e., rats with kainate-induced epilepsy), and compared the results to those obtained in slices from rats that received saline injections and from rats studied 3–8 days after kainate treatment. This allowed us to compare immediate anatomical and electrophysiological changes directly associated with kainate-induced status epilepticus to the subsequent alterations that develop over several weeks, when the rats develop spontaneous seizures. We hypothesized that there is a delayed (i.e., time-dependent) increase in synaptic network excitability in the CA1 region after kainate treatment.

METHODS

Kainate treatment

Sprague-Dawley rats (Harlan) were housed under normal 12 h light/12 h dark cycle and had access to food and water ad libitum. All procedures were approved by the Colorado State University Animal Care and Use Committee. Adult male rats (200–250 g) were given procedures were approved by the Colorado State University Animal Care and Use Committee. Adult male rats (200–250 g) were given

METHODS

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Sprague-Dawley rats (Harlan) were housed under normal 12 h light/12 h dark cycle and had access to food and water ad libitum. All procedures were approved by the Colorado State University Animal Care and Use Committee. Adult male rats (200–250 g) were given intraperitoneal injections of kainate (5 mg/kg in 150 mM NaCl; Sigma, St. Louis, MO) every hour for 1–2 h. Seizure intensity was evaluated using the scale described by Racine (1972). Low-intensity tonic-clonic seizures, mostly of the forelimbs (stage 3), usually began by the third injection. Stage 3 seizures generally progressed into rearing and falling (stage 4–5) after subsequent injections. Occasional episodes of circling and jumping were also observed. Stage 4 and 5 seizures were maintained for 4–6 h and then slowly subsided in most rats, even with more kainate injections. A similar phenomenon has been seen with another multiple-treatment kainate-injection protocol (Sarkisian et al. 1997). In rats with very frequent or continuous stage 4–5 seizures, we either eliminated an injection or gave half the dose. The total dose per rat was 30–50 mg/kg. Each animal had ≥4 h of recurring seizure activity before the treatment was stopped. Control rats were injected hourly with the vehicle in parallel with the kainate-treated rats. All the kainate-treated rats received subcutaneous lactated Ringer (3–5 ml) at the end of the period of status epilepticus to replenish fluids. The survival rate was ~80%.

Following the kainate treatment, the behavior of both control and kainate-treated rats was monitored for 1–2 h/day, 3–5 days/wk (minimum 6 h/wk) over a period of 3–13 mo to confirm that the treatment induced spontaneous, recurrent seizures (i.e., chronic epilepsy). An analysis of seizure activity in kainate-treated rats, including those animals used in this study, has been published (Hellier et al. 1998). One group of control and two experimental groups of animals were used in these studies based on the time after kainate-induced status epilepticus and on development of spontaneous motor seizures in the kainate-treated animals. The control group consisted of saline-treated rats, which were not observed to have seizures. One experimental group consisted of animals examined in the first week after treatment (<5 d) and was therefore defined as a “short-term” group of kainate-treated rats. These animals underwent kainate-induced status epilepticus, and no motor seizures were observed on subsequent days. However, a few seizures could have occurred in some rats from this group, based on 24-h video monitoring of other animals in the first week after kainate treatment (Hellier et al. 1999). Another experimen-

tal group of animals was examined 3–13 mo after kainate treatment (mean = 198 ± 17 d) and was therefore defined as a “long-term” group of kainate-treated rats. All animals in this group were observed to have had multiple spontaneous motor seizures in the 30 days prior to recording (mean = 10.6 ± 1.5 seizures). Animals were excluded if seizures were observed the day of the experiment.

Tissue preparation

Hippocampal slices were prepared and maintained in a manner similar to that described previously (e.g., Meier et al. 1992). Briefly, rats were anesthetized with pentobarbital sodium (100 mg/kg ip) and decapitated. Their brains were then rapidly removed and placed in ice-cold, oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF) for ~1 min. The ACSF contained (in mM) 124 NaCl, 3 KCl, 26 NaHCO3, 1.4 NaH2PO4, 11 mM glucose, 1.3 CaCl2, and 1.3 MgSO4, pH = 7.3–7.4, with an osmolality of 290–305 mOsm/kg. Brains were then block and mounted on a vibrisscor (Campden Instrument, Lafayette, IN), and transverse slices of the temporal hippocampus (400–500 μm) were cut. After isolating the hippocampus from surrounding tissue, the slices were transferred to a storage chamber and constantly immersed in perfusion medium warmed to 32–34°C for ≥1 h prior to placement in an interface-type recording chamber for electrophysiological recording. On placement in the recording chamber, the CA1 area was isolated from the CA2/CA3 regions and from the subiculum with knife cuts. The GABAa antagonist, bicuculline methiodide (30 μM; Sigma), or the glutamate AMPA/kainate receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10–50 μM; Sigma) was added to the ACSF for some specific experiments.

Population and whole cell recording

Field-potential and whole cell patch-clamp recordings were obtained from pyramidal cells in the isolated CA1. An extracellular electrode containing 1 M NaCl was placed in the pyramidal cell layer. Field responses were recorded using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Patch pipettes pulled from thin-wall (0.45-mm thickness) borosilicate glass capillaries (Garner Glass, Claremont, CA) were filled with (in mM) 130 K+-glucuronate, 1 NaCl, 5 EGTA, 10 HEPES, 1 MgCl2, 1 CaCl2, 3 KOH, and 2–4 ATP; pH = 7.2. Pipettes had open resistances of 2–5 MΩ; seal resistances were typically 1–4 GΩ and series resistances were 4–19 MΩ, uncompensated. Whole cell signals were recorded using an Axopatch 1D amplifier (Axon Instruments). All signals were low-pass filtered at 2–5 kHz, digitized at 44 kHz (Neuro-corder; Neurodata Instruments, New York, NY), and stored on videotape.

Once in the whole cell configuration, cells were initially held near the resting membrane potential for 5–10 min to allow equilibration of the recording electrode solution. Electrical stimulation ofafferent fibers in stratum radiatum was made with a bipolar electrode constructed from a twisted pair of Teflon-coated, platinum-iridium wires (75-μm diam). An unpaired, two-tailed Student’s t-test was used for comparing data between recordings. Numbers are reported as the means ± SE unless otherwise noted.

Tissue staining

Slices adjacent to those from which recordings were made were reserved for histological analysis. At least one slice from most animals was processed for glutamic acid decarboxylase (GAD) immunohistochemistry to identify putative GABAAergic neurons. These slices were immersion-fixed overnight in 4% paraformaldehyde in 0.15 M NaH2PO4 buffer. Following several rinses in 0.01 M PBS and cut at 20–30 μm on a sliding microtome. After several rinses in PBS, alternate sections were either left untreated for later Nissl-
staining with cresyl violet or reacted for GAD immunohistochemistry. These latter sections were immersed in PBS containing 10% normal goat serum and a polyclonal GAD antibody (AB108; Chemicon, Temecula, CA) at a dilution of 1:2000 overnight. After rinsing in PBS, sections were treated with fluorescein-conjugated secondary antibody (goat anti-rabbit, IgG; 1:400) for 4–12 h. Sections were mounted on slides, air-dried, and cover-slipped in Vectashield (Vector Labs, Burlingame, CA) to reduce oxidation and fading of the reaction product. Coverslips were sealed with fingernail polish at the edges. Immunoreactive neurons were visualized on a Zeiss Axiophot microscope and images of the tissue were captured digitally. A section from near the middle of the original slice with well-preserved morphology was selected for analysis of each label. A box drawn around an area 200 × 200 μm was superimposed on the image over the CA1 region that corresponded to the region from which recordings were made in adjacent slices and the images were printed. Semi-quantitative cell counts were made by counting manually all the labeled neurons within the boxed area of a single image. Relative numbers of GAD neurons were independently assessed blind by two persons. For pyramidal neuron assessment, the same blinded investigators counted all neuronal nuclei in the pyramidal cell layer that fell within the grid outline. Counts from the two assessments were averaged. Cell densities were normalized to counts from control tissue.

Neuron reconstruction

Electrodes contained 0.1–0.2% biocytin to label recorded neurons and verify their location (Horikawa and Armstrong 1988). Following each recording, slices were fixed in 4% paraformaldehyde in 0.15 M NaPO₄ buffer (pH 7.2–7.4) overnight at 4°C. Following fixation, slices were rinsed (3 × 5 min) in PBS (pH 7.4), cryoprotected in PBS containing 30% sucrose, and sectioned at 30–50 μm on a sliding microtome. After rinsing in PBS, endogenous peroxidase was removed (10% methanol/3% H₂O₂ in PBS; 60–70 min). The sections were again rinsed in PBS and incubated overnight in avidin-biotin-horseradish peroxidase complex (ABC elite kit; Vector Labs) in PBS (1:100; pH 7.3) containing 0.1% Triton X-100. The reaction product was visualized with diaminobenzidine at a concentration of 0.06% with 0.003% H₂O₂ in 0.01 M PBS (pH = 7.4) to confirm the location of the recorded neuron, and the tissue was subsequently dehydrated in alcohols and mounted in permount. Axonal morphology was examined by reconstructing serial sections using computer-assisted neuron-drawing software (Neurolucida; MicroBrightfield, Colchester, VT). Axon reconstructions were limited to neurons in slices from which only a single neuron was recorded. In neurons from each experimental group, total axon length and number of branches were quantified by the Neurolucida software. Dendrites, identified by the presence of spines, were not reconstructed for this study.

RESULTS

Data were obtained from 28 rats in the long-term group (i.e., examined >3 mo after kainate treatment), 9 kainate-treated rats in the short-term group (i.e., used ≤8 days posttreatment), and 13 age-matched control animals (i.e., received vehicle injections). Anatomical analyses were performed on a subset of the animals from which electrophysiological results were obtained.

Anatomical studies

PYRAMIDAL CELL LOSS. Nissl staining was performed on slices adjacent to those from which recordings were obtained to determine if the number of pyramidal cells was reduced after kainate treatment (Fig. 1, A–C). Pyramidal cell density was examined in controls (n = 7), short-term animals (n = 7), and long-term (n = 14). In tissue from short-term animals, CA1 pyramidal cell density was 81 ± 7% of that in age-matched control animals (P < 0.05) and was 69 ± 7% of control in long-term rats. Cell counts in both long- and short-term animals differed from controls (P < 0.05) but not from each other (P > 0.05).

INHIBITORY NEURON LOSS. To determine the relative decrease in the number of putative inhibitory neurons in the CA1 region after status epilepticus in kainate-treated rats, we examined sections from adjacent slices for GAD immunoreactivity (Fig. 1, D–F). The density of GAD-immunoreactive neurons in the short-term group was significantly reduced in stratum oriens (n = 5; 54 ± 24% of control; P < 0.03). Similar to previous reports on interneuron density (Best et al. 1993; Hauser and Esclapez 1996; Morin et al. 1998a), the density of GAD-immunoreactive neurons in the long-term group (n = 7) was also significantly reduced in stratum oriens (37 ± 18% of control; P < 0.03) but not in stratum radiatum (93 ± 11% of control; P > 0.05). The numbers of GAD-immunoreactive neurons in stratum oriens was reduced in both experimental groups, but there was no difference between short- and long-term animals (P > 0.05).

FIG. 1. Nissl staining and immunostaining for glutamic acid decarboxylase (GAD) in the CA1 area. A–C: Nissl staining of CA1 neurons in transverse hippocampal slices. A: cell staining in a saline-treated control rat. B: staining 3 days after kainate treatment. Loss of some pyramidal neurons in CA1 is apparent. C: staining in a section from a rat examined >3 mo after kainate treatment. The pyramidal cell layer is dispersed and less dense. D–F: higher power images of GAD-immunopositive neurons in the stratum oriens in sections adjacent to those shown in A–C. Arrowheads indicate examples of GAD immunoreactivity. D: saline-treated control. E: rat examined 3 days after kainate treatment. F: rat examined >3 mo after kainate treatment. Reduction in density of GAD-immunopositive neurons is apparent in E and F. Scale bars are 50 μm for both sets of figures. dg, dentate gyrus; sp, stratum pyramidal.
**Axon Sprouting.** Axon sprouting in the dentate gyrus (i.e., mossy fiber sprouting) has been associated with an increased propensity for seizure activity in previous studies using this animal model (Buckmaster and Dudek 1999; Hellier et al. 1998; Wuarin and Dudek 1996). Axon sprouting has also been demonstrated in CA1 pyramidal neurons from kainate-treated rats (Esclapez et al. 1999; Perez et al. 1996). To corroborate that there were differences in the axon morphology of CA1 pyramidal cells between the three groups we used, we reconstructed digitally the axons of a few of the biocytin-filled CA1 pyramidal cell neurons from which we recorded. We found that axons of CA1 pyramidal cells were more highly branched \((P < 0.05)\) in slices from long-term animals than in either short-term or control groups. Axon length and number of branch points was greater in the long-term animals than in either short-term or control groups. Axon length from four neurons in control rats was 1,105 ± 213 μm, with 8 ± 2 branches in stratum oriens of the isolated CA1 slice. In neurons from short-term animals \((n = 3)\), the mean axon length was 1,346 ± 474 μm and the number of branches was 9 ± 2. In neurons from long-term rats \((n = 4)\), the mean axon length was 2,505 ± 531 μm \((P < 0.05\) vs. both other groups) and branch number was 30 ± 6 \((P < 0.05\) vs. both other groups). Axon length and number of branch points was greater in the long-term animals than in either short-term or control rats with short-term and control animals having comparable axon measurements.

**Electrophysiological Studies.**

**Evoked Responses: Field-Potential Recordings.** Extracellular field-potential recordings were made in the CA1 pyramidal cell layer, which had been isolated from CA3 and the subiculum by knife cuts. Field recordings were obtained in 27 isolated CA1 slices from 16 kainate-treated rats in the long-term group, 9 slices from 5 kainate-treated rats in the short-term group, and 12 slices from 8 saline-treated control rats. A low-intensity stimulus applied to the fibers in stratum radiatum resulted in a single population spike in the CA1 region of control rats (Fig. 3A). The amplitude of the spike increased as the stimulus intensity was increased, and occasionally a second population spike developed at stimulation intensities greater than three times that required for population spike generation. As in slices from control animals, a stimulus at threshold intensity evoked a single population spike in the CA1 pyramidal cell layer in kainate-treated animals from the short-term group. Unlike controls, increasing the stimulus intensity resulted in a graded increase in spike number \((\leq 8\) spikes) in addition to an increase in the amplitude of the first spike in this short-term group (Fig. 3B).

In isolated slices from kainate-treated rats from the long-term group, stimulation at threshold intensity resulted in an “all-or-none” burst of population spikes (i.e., the number of spikes was not graded with stimulus intensity) in 23 of 27 slices (Fig. 3C). The remaining four slices exhibited a graded response to afferent stimulation, with one to seven spikes being generated, the number being larger with increased stimulus intensity. The burst duration from stimulus to last population spike was variable, ranging from 25 to 150 ms, as were the number of spikes in the burst \((range 3–20; mean 10 ± 1.4)\). Increasing stimulus intensity resulted in an increase in the amplitude of the spikes, plus a shortened and more consistent delay to the onset of the burst. A normalized comparison of the number of population spikes as a function of stimulus intensity for each of the three groups is contained in Table 1. Whereas a graded response was observed in control and short-term animals, the response in slices from long-term animals was less dependent on stimulus intensity. A summary of CA1 pyramidal cell population responses to afferent stimulation across the three groups is contained in Table 1.

**Whole Cell Recordings.** Whole cell patch-clamp recordings were made from pyramidal cells in hippocampal slices of the isolated CA1 area from control rats \((n = 17)\) and short-term \((n = 17)\) and long-term \((n = 38)\) groups of kainate-treated rats. Average resting membrane potential and input resistance were similar between the three groups \((control, −65 ± 1 mV, 139 ± 24 MΩ; short-term, −64 ± 2 mV, 140 ± 18 MΩ; long-term, −65 ± 1 mV, 136 ± 13 MΩ; P > 0.05)\). Afferent stimulation \((minimally twice the minimum intensity for spike generation)\) resulted in a single action potential in slices from control animals (Fig. 4A1). In animals from the short-term group, afferent stimulation at twice threshold intensity resulted in a short burst of action potentials (Fig. 4A2). Similar afferent stimulation resulted in a longer burst of spikes \((mean 8 ± 1 spikes; 85 ± 9 ms duration)\) in CA1 pyramidal cells from kainate-treated rats in the long-term group (Fig. 4A3). As in the field recordings, qualitative “all-or-none” burst responses were also seen with low stimulation intensities.
EVOKED RESPONSES: BICUCULLINE. In the presence of the GABA_A-receptor antagonist, bicuculline (30 \( \mu \text{M} \)), the typical response in slices from control rats was a short burst of action potentials (40–170 ms; 10 ± 2 spikes; Fig. 4B1). A brief burst was also generated by similar stimulation in bicuculline-treated slices from rats in the short-term group. This burst was of somewhat longer duration in the presence of bicuculline than in these same slices recorded in normal ACSF (70–220 ms; 12 ± 2 spikes; Fig. 4B2). The duration of evoked bursts was similar for slices from animals in control and short-term groups when tested in the presence of bicuculline (\( P > 0.05 \)).

A brief “all-or-none” burst was often generated in slices from animals in the long-term group, regardless of stimulus intensity, but addition of bicuculline increased burst duration to as much as 2,500 ms (mean, 920 ± 405 ms) in these animals (Fig. 4B3). The response morphology was characterized by an initial burst of action potentials followed by a period of inactivation prior to repolarization of the membrane. As in normal ACSF, similar responses were seen at threshold stimulus intensities, with the principal difference being the variable latency to burst onset at lower stimulus intensity. The bursts observed in slices from the long-term group in the presence of bicuculline were significantly longer than those in either of the other groups under the same recording conditions (\( P < 0.05 \)).

SPONTANEOUS ACTIVITY. In an earlier study in kainate-treated rats, 1 of 53 isolated CA1 slices (i.e., CA3 input was cut) exhibited spontaneous bursting activity in the presence of bicuculline (Meier and Dudek 1996). To determine if spontaneous, synchronized bursting in the CA1 region developed

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**TABLE 1. Electrophysiological response characteristics of CA1 pyramidal cells in isolated slices from control, short- and long-term groups**

<table>
<thead>
<tr>
<th>Evoked Responses, No. of Spikes</th>
<th>Spontaneous Bursting</th>
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<tr>
<td><strong>Group</strong></td>
<td><strong>Control ACSF</strong></td>
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<td>( T )</td>
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<tr>
<td></td>
<td><strong>Percentage with bursts</strong></td>
</tr>
<tr>
<td>Control</td>
<td>1.0 ± 0.01</td>
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<tr>
<td>Short term</td>
<td>1.4 ± 0.3</td>
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<tr>
<td>Long term</td>
<td>10 ± 1.4*†</td>
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Values are means ± SE. * \( P < 0.05 \) versus control; † \( P < 0.05 \) versus short term; \( T \), threshold stimulus intensity.
after kainate treatment, we examined population and cellular activity in the isolated CA1 region in the presence of bicuculline (30 μM). Spontaneous bursts were not observed when bicuculline was added to normal ACSF in isolated slices from controls (0 of 10 slices from 10 animals) or in slices from the short-term group (0 of 7 slices from 7 animals). Under identical conditions, four of six slices from six animals in the long-term group developed spontaneous bursts of activity after 10–30 min. The mean interburst interval was 57 ± 28 s in these slices. Spontaneous bursting was observed in disinhibited slices from the long-term group but not short-term or control groups.

To determine whether a larger population of the long-term slices could generate spontaneous bursts if excitatory network interactions were enhanced, some experiments were conducted in nominally Mg2+-free ACSF containing bicuculline. This solution was used to remove the Mg2+-dependent blockade of N-methyl-D-aspartate (NMDA) receptors at membrane potentials near resting membrane potential (Mayer et al. 1984), allowing any excitatory synaptic connections to be fully functional while simultaneously suppressing inhibitory connections. In no case did spontaneous bursts develop in slices from control rats that were bathed in nominally Mg2+-free ACSF containing bicuculline (0 of 7 slices from 6 animals) under these conditions. However, spontaneous bursts of 200–800 ms were observed after 20–30 min in the CA1 in two of seven slices (29%) from seven animals from the short-term group under these conditions. The average interburst interval in these two slices was 23 ± 8 s. In addition, spontaneous bursts of 350- to 2,000-ms duration (Fig. 5) developed after 10–20 min in the CA1 region from rats in the long-term group in four of five slices (80%). The average interburst interval for these four slices was 9.9 ± 2.4 s (vs. ~1/min in bicuculline alone). The probability of bursting under these conditions was not significantly different between these two groups (χ2 test; P > 0.05), although the frequency of the bursts was greater in the long-term group (P < 0.05). In all cases tested (n = 8), application of DNQX (50 μM) eliminated spontaneous burst activity (Fig. 5). Spontaneous bursting was therefore observed in animals from the long-term group in the presence of bicuculline and could occasionally also be observed with lower frequency in animals from the short-term group when Mg2+-concentration was reduced in addition to GABA A receptor blockade. A summary of spontaneous activity in CA1 pyramidal cells across the three groups is contained in Table 1.

**DISCUSSION**

Shortly after kainate-induced status epilepticus, the number of inhibitory neurons and pyramidal cells was reduced in the CA1 region. Graded bursts to afferent stimulation were present...
in these same animals. Network bursts in the isolated CA1 region became apparent after a period of months, a time when increased axon branching and spontaneous seizures were also noted. These results are consistent with the hypothesis that axon sprouting is a reactive event that occurs over time in the CA1 region after neuronal injury and leads to an increased propensity for network bursts.

Compared with single injections, the multiple-kainate-injection protocol we used results in a prolonged period of status epilepticus and consistently robust mesial temporal sclerosis and to the development of a chronic state of recurrent seizures (Patrylo and Dudek 1998; Wuarin and Dudek 1996). The robust nature of the lesion may also account for the consistency of some measurements of slice excitability we employed. A previous study showed that practically all of the long-term kainate-treated rats used in the present experiments developed epilepsy (Hellier et al. 1998). By examining the CA1 area at two time points (i.e., 3–8 days and 3–13 mo) after treatment, we aimed to differentiate between immediate effects of kainate-induced status epilepticus in animals that likely would have developed spontaneous seizures (i.e., short-term group) versus the effects that developed over the next several weeks and were observed in animals that had spontaneous recurrent seizures (i.e., long-term group). We performed multiple anatomical and physiological analyses on tissue from the same animals in which the behavior had been determined. Our results suggest that the transition from kainate-induced status epilepticus to development of chronic epilepsy involves neuron loss in the first week posttreatment, probably including an immediate decrease in inhibition, followed by increased axon collateralization and accompanying enhanced network excitability in the rat CA1 area.

Reduced synaptic inhibition

Both anatomical and electrophysiological studies have suggested that kainate-induced status epilepticus leads to a short-latency and long-lasting (months) depression—but not elimination—of synaptic inhibition in the CA1 area, and the present experiments further support this hypothesis. Although some examinations of GAD immunoreactivity have suggested that intraventricular kainate injections do not reduce the number of GABAergic “basket” neurons (Franck et al. 1988), other studies using GAD (Morin et al. 1998a) or parvalbumin (Best et al. 1993) have shown that inhibitory neurons are lost in stratum oriens after kainate treatment. As in the latter studies (Best et al. 1993; Morin et al. 1998a), we also found that the number of putative inhibitory neurons was reduced in stratum oriens but not stratum radiatum. Furthermore our results indicate that a significant loss occurs shortly after systemic kainate treatment.

The observation that orthodromically evoked bursts were graded with stimulus intensity in animals from the short-term group further suggested that inhibition was functionally decreased (Crepel et al. 1997). Several studies have indicated that stimulation of afferent fibers in stratum radiatum results in brief bursts of spikes in the CA1 area in slices from kainate-treated rats (Ashwood et al. 1986; Esclapez et al. 1999; Franck and Schwartzkroin 1985; Franck et al. 1988; Meier et al. 1992; Nakajima et al. 1991; Perez et al. 1996; Williams et al. 1993). The observation that blocking the remaining GABA_A-receptor mediated inhibition with bicuculline led to more robust bursts indicates that the kainate-induced depression of inhibition was only partial. The immediate kainate-induced loss of inhibitory interneurons in stratum oriens and the concomitant depression of synaptic inhibition were not sufficient to reveal the all-or-none, network-mediated bursts, even after bath application of bicuculline. Months after kainate treatment, when the animals were clearly epileptic by virtue of displaying spontaneous recurrent seizures, such network bursts were observed in this and a previous study (Meier and Dudek 1996). This finding is consistent with the hypothesis suggested previously that inhibition is diminished yet sufficient to suppress epileptiform activity in CA1 pyramidal cells (Esclapez et al. 1997; Franck and Schwartzkroin 1985; Franck et al. 1988). However, the partial loss of inhibition may contribute to locally enhanced excitability in the CA1 area. In the short-term, kainate-induced status epilepticus therefore results in reduced inhibition but not a complete breakdown of CA1 inhibitory circuitry.

Increased excitation

Intracellular staining has revealed that CA1 pyramidal cells show an increase in axon collaterals a few weeks after kainate treatment (Esclapez et al. 1999; Perez et al. 1996). In the present study we also observed an increase in axon collaterals weeks after kainate treatment, but this increase was not apparent days afterward (i.e., in the short-term group). Simultaneous intracellular and extracellular recordings revealed that bicuculline-treated slices containing only the CA1 area could generate robust, all-or-none burst discharges that were driven by EPSPs many weeks after kainate treatment (Meier and Dudek 1996), which is widely considered to be a property of interconnected excitatory networks (Gutnick et al. 1982; Miles and Wong 1983; Miles et al. 1988; Smith et al. 1999; Traub and Wong 1982). We have corroborated this result in the isolated CA1 of animals examined months after kainate treatment and found that all-or-none brief network bursts can be initiated even when residual inhibition is not blocked with bicuculline. The present data extend previous research suggesting a time-dependent increase in propensity for seizure-like events, possibly mediated by new recurrent excitatory circuits. These new circuits probably increase in number over time. By inference, the number of previous seizures may influence synaptic excitability if successive seizures contribute to the severity of the lesion.

The bursts in CA1 pyramidal cells from control and short-term groups were similar in the presence of bicuculline. That is, little evidence for increased excitatory connectivity was observed shortly after kainate treatment when changes in inhibition were equalized by receptor blockade. However, some slices from animals in the short-term group, but not controls, could generate network bursts of low frequency in nominally Mg^2+-free ACSF that contained bicuculline. There may be several explanations for this activity. One possibility is a rapid alteration of NMDA receptors (Turner and Wheal 1991). In this scenario, the relatively few excitatory connections that normally exist between CA1 pyramidal cells (Christian and Dudek 1988; Deuchars and Thomson 1996) might be functionally augmented by postsynaptic receptor modifications. Activity in the isolated CA1 used in this and previous studies (Meier and Dudek 1996; Meier et al. 1992) indicates that activation of CA3 neurons are not necessary to generate network bursts in
epileptic rats. Postsynaptic NMDA receptor sensitivity would be expected to increase continuously if this single mechanism explains the enhancement of network excitability we observed over time.

An additional possibility is that a few axons begin to sprout soon after the initial kainate treatment (i.e., days), allowing expression of the bursting activity in a minority of slices. Axon sprouting of gradual but immediate onset might contribute to the increasing propensity for network activity over time and also might contribute to the variability in the latent period to observed seizure onset seen in these animals (Hellier et al. 1998). In a recent examination of identically treated animals, seizures were observed with continuous 24-h video monitoring in some animals within a week after kainate treatment and after a short (i.e., 1–2 d) latent period (Hellier et al. 1999). The increased propensity for network bursting in the long-term group could be derived from increases, over time, in axon collateralization and new excitatory synapse formation, which could begin relatively soon after kainate treatment. However, it should be noted that neither this nor previous studies have provided direct evidence that newly sprouted recurrent CA1 axon collaterals increase connectivity between CA1 pyramidal cells and contribute to the increased excitability. In fact, electrophysiological studies using dual recordings found little or no evidence for increased excitatory synaptic connectivity between pairs of CA1 pyramidal cells in kainate-treated rats (Esclapez et al. 1999; Nakajima et al. 1991). Although anatomical studies have suggested that the new axon collaterals might contact pyramidal neurons, they also suggested that the new terminals could increase excitatory input to specific subtypes of surviving inhibitory interneurons (Morin et al. 1998b). Regardless of remaining inhibition, enhanced excitatory connectivity could allow generation of all-or-none network bursts. This would be especially apparent when the surviving inhibition is suppressed as can occur with rises in extracellular K⁺ concentration. Probably the oldest view of alterations in seizure susceptibility during the process of chronic epileptogenesis is based on the balance of synaptic inhibition and excitation. The studies described here, and many others, lead to the hypothesis that status epilepticus and other forms of neuronal injury cause a rapid but variable decrease in synaptic inhibition in several cortical areas. The consequent axonal sprouting and formation of new recurrent excitatory circuits, probably also in multiple neuronal systems, provides the substrate for local recruitment of network bursts among numerous populations of cortical neurons. The probability of network burst generation would be expected to depend on the density of recurrent excitatory circuits, which would increase over the days, weeks, months, and even years following an injury, and might be further increased by the seizures themselves. Finally, the “masking” effect of persistent inhibition in some cortical areas could hypothetically explain why the prolonged interictal periods are seizure-free. The evaluation of these interrelated hypotheses will require further quantitative and multidisciplinary analyses, plus the merging of molecular, cellular, and network analyses of local synaptic circuits in many cortical areas throughout the development of epilepsy.

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