Network Properties of the Dentate Gyrus in Epileptic Rats With Hilar Neuron Loss and Granule Cell Axon Reorganization

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Buckmaster, Paul S. and F. Edward Dudek. Network properties of the dentate gyrus in epileptic rats with hilar neuron loss and granule cell axon reorganization. J. Neurophysiol. 77: 2685–2696, 1997. Neuron loss in the hilus of the dentate gyrus and granule cell axon reorganization have been proposed as etiologic factors in human temporal lobe epilepsy. To explore these possible epileptogenic mechanisms, electrophysiological and anatomical methods were used to examine the dentate gyrus network in adult rats that had been treated systemically with kainic acid. All kainate-treated rats, but no age-matched vehicle-treated controls, were observed to have spontaneous recurrent motor seizures beginning weeks to months after exposure to kainate. Epileptic kainate-treated rats and control animals were anesthetized for field potential recording from the dentate gyrus in vivo. Epileptic kainate-treated rats displayed spontaneous positivities (“dentate electroencephalographic spikes”) with larger amplitude and higher frequency than those in control animals. After electrophysiological recording, rats were perfused and their hippocampi were processed for Nissl and Timm staining. Epileptic kainate-treated rats displayed significant hilar neuron loss and granule cell axon reorganization. It has been hypothesized that hilar neuron loss reduces lateral inhibition in the dentate gyrus, thereby decreasing seizure threshold. To assess lateral inhibition, simultaneous recordings were obtained from the dentate gyrus in different hippocampal lamellae, separated by 1 mm. The perforant path was stimulated with paired-pulse paradigms, and population spike amplitudes were measured. Responses were obtained from one lamella while a recording electrode in a distant lamella leaked saline or the γ-aminobutyric acid-A receptor antagonist bicuculline. Epileptic kainate-treated and control rats both showed significantly more paired-pulse inhibition when a lateral lamella was hyperexcitable. To assess seizure threshold in the dentate gyrus, two techniques were used. Measurement of stimulus threshold for evoking maximal dentate activation revealed significantly higher thresholds in epileptic kainate-treated rats compared with controls. In contrast, epileptic kainate-treated rats were more likely than controls to discharge spontaneous bursts of population spikes and to display stimulus-triggered afterdischarges when a focal region of the dentate gyrus was disinhibited with bicuculline. These spontaneous bursts and afterdischarges were confined to the disinhibited region and did not spread to other septo-temporal levels of the dentate gyrus. Epileptic kainate-treated rats that displayed spontaneous bursts and/or afterdischarges had significantly larger percentages of Timm staining in the granule cell and molecular layers than epileptic kainate-treated rats that failed to show spontaneous bursts or afterdischarges. In summary, this study reveals functional abnormalities in the dentate gyri of epileptic kainate-treated rats; however, lateral inhibition persists, suggesting that vulnerable hilar neurons are not necessary for generating lateral inhibition in the dentate gyrus.

INTRODUCTION

The hippocampus is thought to be an epileptogenic focus in human temporal lobe epilepsy because its removal eliminates or reduces the frequency of seizures in most patients (Falconer et al. 1964; Ojemann 1987) and because it displays neuropathological abnormalities. The most consistently and severely affected region of the hippocampus is the dentate gyrus (Margerison and Correllis 1966). Hilar neurons die (Babb et al. 1984; Mouritzen Dam 1980) and granule cell axons sprout into areas of the dentate gyrus where they normally are not found (Babb et al. 1991; de Lanerolle et al. 1989; Franck et al. 1995; Houzer et al. 1990; Isokawa et al. 1993; Sutula et al. 1989). Both hilar neuron loss and axon reorganization could affect functional characteristics of the dentate gyrus network. In this study we examine electrophysiological properties of the dentate gyrus circuit in kainate-treated rats, which display hilar neuron loss and granule cell axon reorganization, to better understand how those changes might contribute to epileptogenesis.

The vulnerable hilar region of the dentate gyrus consists of a variety of different neuron classes, but “mossy cells” are the predominant type (Amaral 1978), and they are extremely vulnerable to excitotoxic damage (for reviews see Buckmaster and Schwartzkroin 1994; Sloviter 1994). Sloviter (1987, 1991b, 1994) proposed that mossy cells drive lateral inhibition in the dentate gyrus, and that mossy cell death thus reduces lateral inhibition, permitting hypersynchronous discharge of granule cells across distant hippocampal lamellae. Others have suggested that mossy cell death itself does not increase granule cell excitability and synchrony (Buckmaster and Schwartzkroin 1994; Buckmaster et al. 1996b). To help distinguish between these alternatives, lateral inhibition in the dentate gyrus was assessed with paired-pulse stimulation by recording simultaneously at two locations (i.e., between hippocampal lamellae) of the dentate gyrus. Responses obtained from control and kainate-treated rats, which were epileptic and displayed hilar neuron loss (including, presumably, loss of mossy cells), were compared.

It has been proposed that hilar neuron loss vacates postsynaptic sites on granule cell dendrites, thereby triggering (or permitting) the formation of excitatory recurrent collaterals (Babb et al. 1991; Buckmaster et al. 1996b; Cavazos and Sutula 1990; Nadler et al. 1980; Okazaki et al. 1995; Represa et al. 1993). Excitatory recurrent collaterals could produce positive feedback, having an epileptogenic effect, and electrophysiological evidence is consistent with a recurrent excitatory mechanism among granule cells after axon reorganization caused by kainate treatment or kindling (Cronin et al. 1992; Golarai and Sutula 1996; Tauck and Nadler 1985; Wuarin and Dudek 1996). In contrast, others have suggested...
that granule cell axon reorganization restores inhibition after hilar neuron loss, thereby having an antiepileptic effect (Sloviter 1992). To explore the effects of granule cell axon reorganization (and hilar neuron loss) on dentate gyrus excitability, we measured and compared spontaneous burst activity, maximal dentate activation (MDA) threshold, and afterdischarge occurrence in the presence of bicuculline in control and epileptic kainate-treated rats with granule cell axon reorganization. Some of these data have been presented in abstract form (Buckmaster and Dudek 1996).

METHODS

Animals

Forty-eight rats were used in these experiments; 29 were treated with kainic acid and 19 were vehicle-treated age-matched controls. The kainate-treated rat, an experimental model of temporal lobe epilepsy, displays hilar neuron loss and granule cell axon reorganization like that seen in human temporal lobe epilepsy (Nadler et al. 1978, 1980; Okazaki et al. 1995; Represa et al. 1993). Male Sprague-Dawley rats (Harlan) weighed ~225 g when treated with kainic acid (dissolved in 0.9% sodium chloride, 5 mg/kg, administered intraperitoneally; Sigma Chemical) at intervals of ~1 h, until they experienced ~3.5 h of recurrent motor seizures. The average cumulative dose of kainic acid was 29 mg/kg. Age-matched control rats received a similar volume of vehicle.

After recovering from acute seizures caused by kainate treatment, rats were observed ~6 h/wk for chronic spontaneous seizures. Only motor seizures of grade 3 or greater on the Racine scale (i.e., forelimb clonus ± rearing ± falling; Racine 1972) were scored. Spontaneous recurrent seizures began after a latent period lasting weeks to months. Seizure frequency was calculated by dividing the number of observed seizures by the cumulative observation time in the period between the first observed seizure and euthanasia. The average period between kainate treatment and electrophysiological recording and perfusion was 179 days (range 66–335 days).

Electrophysiology

To explore the functional effects of hilar neuron loss and granule cell axon reorganization, experiments were performed in vivo, thereby preserving dentate gyrus circuitry and synaptic inputs in their entirety. Rats were anesthetized with urethane (1.2 g/kg ip) and placed in a stereotaxic apparatus with the nose bar set at ~3.0 mm. Body temperature was maintained with a heating pad. Holes were drilled through the skull and electrodes were directed toward the dentate gyrus and the angular bundle at the following stereotaxic coordinates: ~4.6 posterior and 2.2 lateral to bregma for recording, and ~8.3 posterior and 3.6 lateral to bregma for stimulating. Electrode depths were determined by optimizing responses to stimulation. Recording electrodes were glass micropipettes broken to an inner diameter of ~15 μm and filled with 0.9% sodium chloride. In some experiments, recording electrodes were filled with 10 mM bicuculline methiodide [a γ-aminobutyric acid-A (GABA_A) receptor antagonist, Sigma Chemical] dissolved in 0.9% sodium chloride or with 10 mM bicuculline methiodide dissolved in 4 M potassium chloride. For simultaneous recording of dentate gyrus field potentials at different septotemporal levels, electrodes were glued together with tips separated by 1 or 2 mm. A bipolar, concentric electrode (SNEX-100, Rhodes Medical Instruments) activated perforant path fibers. Dentate gyrus field potentials were amplified (Axoprobe-1A, Axon Instruments), observed on-line, and stored on video tape (Neuro-corder model DR-484, Neuro Data Instruments) and computer (pClamp, Axon Instruments).

A normal dentate gyrus field potential response to perforant path stimulation, recorded at the granule cell layer/hilar border, consists of a field excitatory postsynaptic potential (EPSP) and a population spike (Lomo 1971). Field EPSP amplitude was measured from prestimulus baseline voltage to EPSP peak. Population spike amplitude was measured from peak negativity of the spike to a line at the base of the spike where it arose from the field EPSP. Stimulus intensity was standardized by the stimulus threshold for a population spike (T), which was determined by delivering stimuli (frequency 0.1 Hz, duration 150 μs, intensity 4 × T). The frequency of spontaneous field potential positivities was determined for both hippocampi, and values from both hippocampi per rat were averaged.

SPONTANEOUS FIELD POTENTIAL POSITIVITIES. At least 5 min of spontaneous dentate gyrus field potential activity was obtained and analyzed per hippocampus. Spontaneous positivities were defined as brief (<30 ms), positive deflections in the field potential whose amplitude, measured from baseline to peak, exceeded a threshold level determined for each hippocampus and defined as 10% or 25% of the field EPSP amplitude evoked by perforant path stimulation (frequency 0.1 Hz, duration 150 μs, intensity 4 × T). The frequency of spontaneous field potential positivities was determined for both hippocampi, and values from both hippocampi per rat were averaged.

LATERAL INHIBITION. Lateral inhibition in the dentate gyrus was measured with the use of a modification of the technique of Sloviter and Brisman (1995). Pairs of perforant path stimuli were delivered at interstimulus intervals of 30, 70, and 250 ms, which generate fast inhibition, facilitation, and slow inhibition, respectively, of the second population spike of the pair (Buckmaster et al. 1996a). Pairs of stimuli were delivered at frequencies reported to evoke different pathways of inhibition. Slower frequencies (0.1 Hz) are thought to emphasize feedback inhibition, whereas faster frequencies (1.0 Hz) emphasize a combination of feedback and feedforward inhibition (Sloviter 1991a). Simultaneous recordings were obtained with a pair of saline-filled electrodes from different hippocampal lamellae, separated along the septotemporal axis by 1 mm. Then the saline-filled pair of electrodes was replaced with a saline-filled and a bicuculline-filled electrode, and another set of responses to paired-pulse stimulation was obtained. In both cases, population spikes recorded with the saline-filled electrodes in the more septal position (Fig. 3A, electrode position labeled 1) were measured and used to calculate population spike amplitude ratios. At least 10 responses were evoked and averaged at each combination of interstimulus interval (30, 70, or 250 ms) and stimulus frequency (0.1 or 1.0 Hz). Custom software controlled stimulation, acquired data, and measured population spike amplitudes, which were normalized by the maximum amplitude of the first spike in each set of responses. Population spike amplitude ratios were calculated by dividing the amplitude of the second response of the pair by that of the first. Amplitude ratios <1 indicate that the second population spike of the pair was inhibited; ratios >1 indicate that the amplitude of the second population spike was facilitated.

MDA STIMULUS THRESHOLD. MDA stimulus threshold was determined with the use of a modified protocol of Stringer and Lothman (1989). The perforant path was stimulated with trains of 300-μs-duration pulses at 20 Hz. Stimulus trains lasting 30 s were delivered at intensities beginning at 0.3 × T and increasing in 0.3 × T increments. At least 5 min elapsed between trains. MDA was recognized by the onset of large-amplitude dentate gyrus population spike (>10 mV) and a negative DC shift in the field potential (Stringer and Lothman 1989). MDA stimulus thresholds were determined for both hippocampi and values from both hippocampi per rat were averaged.
DENTATE GYRUS AFTERDISCHARGES. To test for afterdischarges, field potentials were recorded with an electrode containing bicuculline (± potassium chloride) that leaked into a focal region of the dentate gyrus near the electrode tip (Sloviter and Brismann 1995). Bicuculline blocked GABA_A-receptor-mediated inhibition that might mask recurrent excitatory circuits (Cronin et al. 1992). Potassium chloride depolarized dentate neurons, including granule cells whose normal resting membrane potential is quite negative, thereby increasing the probability of detecting recurrent excitatory effects. The perforant path was stimulated (pulse duration 150 μs, 2.0 Hz, intensity 5 × T, 40 pulses), and responses were examined for afterdischarges, i.e., bursts of population spikes following the initial burst.

Anatomy

Immediately after electrophysiological recordings were obtained, rats were killed (100 mg/kg ip pentobarbital sodium) and perfused through the ascending aorta with 10 ml of heparinized saline, ~400 ml of 0.37% sodium sulfide, and ~500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was placed in fixative overnight, then hippocampi were isolated and placed in 30% sucrose in 0.1 M phosphate buffer until equilibrated. Hippocampi were extended slightly along the septotemporal axis, frozen, and mounted on the freezing stage of a sliding microtome where they were sectioned nominally at 30 μm. Serially ordered sections were placed in 0.1 M phosphate buffer and chosen for staining with the use of an unbiased, systematic method (West et al. 1991). Starting from a random position near the septal pole, every 15th section was sampled, yielding ~10 samples per hippocampus. Each sample consisted of two sections, one for Nissl staining and the other for Timm staining.

STAINING. Cresyl violet was used to stain Nissl substance. Timm staining was used to label the zinc-rich axon terminals of granule cells according to a modified protocol of Babb et al. (1991). Slide-mounted sections were placed in a solution consisting of 180 ml gum arabic (500 g/l water), 30 ml citrate buffer (7.65 g citric acid, 7.05 g sodium citrate), 2.5 g hydroquinone in 45 ml water, and 365 mg silver lactate in 50 ml water (all chemicals from Sigma Chemical, except citrate buffer ingredients, which were from Fisher Scientific). Staining developed for ~70 min in darkness at room temperature, then sections were rinsed in water, counterstained lightly with cresyl violet, dehydrated, cleared, and overslipped with Permount.

QUANTITATIVE ANALYSIS. Quantitative analysis was performed by an investigator who was blind to the experimental subjects’ treatment. The number of hilar neurons per dentate gyrus was estimated by the optical fractionator method (West et al. 1991). Total section thickness was used for dissector height, and ‘caps’ (defined as neuronal nuclei that came into focus while focusing down through the dissector height) were counted. An average of ~10% of the total hilar area per section was sampled systematically and randomly, as described for the optical fractionator method (West et al. 1991). The hilus was defined by its border with the granule cell layer and by straight lines drawn from the ends of the granule cell layer to the proximal end of the CA3 pyramidal cell layer. In rats with CA3 pyramidal cell loss, the proximal end of the CA3 pyramidal cell layer was estimated by observing remaining neurons and patterns of gliosis that were different in CA3 versus the hilus.

Extent of Timm staining was estimated by the Cavalieri method (Gundersen and Jensen 1987). With the use of a low-power (×4) objective, total area and the Timm-positive region of the granule cell and molecular layers were measured, and the percent of the granule cell layer plus molecular layer volume infiltrated by black reaction product in each dentate gyrus was calculated. As with the neuron counting procedure, a camera-lucida-like microscope-computer interface (Lucivid, MicroBrightField) and image analysis software (Neurolucida, MicroBrightField) were used for quantitative morphological analysis.

RESULTS

Seizure frequency

Seizure frequency was determined to establish that kainate treatment was effective in producing chronically epileptic rats. All kainate-treated rats and no controls displayed chronic spontaneous recurrent motor seizures. The mean latency between kainate treatment and the first observed spontaneous seizure was 78 days (range 19–195 days). The mean seizure frequency of kainate-treated rats was 0.25 seizures per hour (range 0.01–0.76 seizures/h).

Anatomy

Hilar neuron numbers and extent of Timm staining were estimated to establish that kainate-treated rats displayed hilar neuron loss and granule cell axon reorganization (Fig. 1). Control rats had 42,792 ± 1,508 (SE) hilar neurons per dentate gyrus (n = 16). Kainate-treated rats had 16,981 ± 1,923 hilar neurons per dentate gyrus (n = 28), i.e., they lost an average of 60% of their hilar neurons. CA3 pyramidal cells also were lost in kainate-treated rats, but this was not analyzed quantitatively. Control rats showed Timm staining in 3 ± 1% of the granule cell and molecular layers (n = 10). Kainate-treated rats displayed Timm staining in 30 ± 2% of the granule cell and molecular layers (n = 27). Abnormal Timm staining concentrated in the inner third of the molecular layer. Differences between kainate-treated and control rats in hilar neuron numbers and extent of Timm staining were significant (P < 0.001, t-test).

Spontaneous positivities

Spontaneous positivities generated in the dentate gyrus are a type of interictal spike, and they might reflect changes in epileptiform activity. Spontaneous positivities with amplitudes >10% of field EPSP amplitude occurred in all control and kainate-treated rats examined. The frequency of these events was 1.58 ± 0.56 per second (mean ± SE, n = 7) in kainate-treated rats and 0.10 ± 0.05 per second (n = 5) in controls; the difference was significant (P < 0.04, t-test). In addition to the frequency difference, traces obtained from kainate-treated rats appeared to contain larger-amplitude spontaneous positivities than those from controls. Therefore the traces from the same group of animals plus four more rats were analyzed for larger-amplitude events. Large-amplitude (>25% of field EPSP amplitude) spontaneous positivities were observed in most kainate-treated rats and no controls (7 of 9 vs. 0 of 7 rats, respectively; P < 0.005, χ² test; Fig. 2). The frequency of these large-amplitude spontaneous positivities in kainate-treated rats was 0.20 ± 0.10 per second (n = 9 rats). Synchronous spontaneous positivities were observed in 12 of 12 kainate-treated rats examined with electrodes separated by 1 mm. A subset of these kainate-treated rats was also recorded with electrodes separated by...
FIG. 1. Epileptic kainate-treated rats display hilar neuron loss and granule cell axon reorganization. Nissl-stained sections (A, C, and E) reveal many neurons in the hilus (h) of a control rat (A) and fewer hilar neurons in epileptic kainate-treated rats (C and E). Estimated number of hilar neurons per hippocampus: 46,170 (control rat, A), 23,433 (kainate-treated rat in C), 15,741 (kainate-treated rat in E). Timm-stained sections (B, D, and F) reveal a normal pattern of staining in a control rat (B) and aberrant staining in the granule cell layer (g) and in the inner third of the molecular layer (m) of epileptic kainate-treated rats (D and F; →). Estimated proportion of granule cell layer plus molecular layer that was Timm positive: 3% (control rat, B), 26% (kainate-treated rat in D), 39% (kainate-treated rat in F). The kainate-treated rat with more severe hilar neuron loss and granule cell axon reorganization (E and F) displayed spontaneous bursts of dentate gyrus population spikes and afterdischarges; the other kainate-treated rat and the control animal did not. Scale bar = 500 μm.

2 mm along the septotemporal axis, and all of these animals (6 of 6) displayed synchronous spontaneous positivities (Fig. 2C).

**Lateral inhibition**

It has been proposed that the hilar neurons vulnerable to excitotoxic damage generate lateral inhibition in the dentate gyrus (Sloviter 1994; Sloviter and Brisman 1995). To test this hypothesis, we used paired-pulse stimulation while recording at two locations (i.e., between hippocampal lamellae) and compared lateral inhibition in control rats (n = 6) and in epileptic kainate-treated rats (n = 8) with hilar neuron loss.

The technique used to measure lateral inhibition required collection of baseline paired-pulse responses obtained with a pair of saline-filled recording electrodes. Dentate gyrus field potential responses were recorded simultaneously at two sites, but only the waveforms obtained from the more septally positioned electrode (Fig. 3A, electrode labeled 1) were quantitatively analyzed. In control rats, recorded with a pair of saline-filled electrodes, paired-pulse stimulation at intervals of 30, 70, and 250 ms evoked inhibition, facilitation, and inhibition, respectively, of the second population spike of the pair (Fig. 4). At the 30-ms interstimulus interval...
DENTATE GYRUS NETWORK PROPERTIES IN EPILEPTIC RATS

FIG. 2. Dentate gyrus field potential recordings reveal large-amplitude spontaneous positivities in epileptic kainate-treated rats (B, X) but not in controls (A). C: simultaneous dentate gyrus field potential recordings reveal synchronous large-amplitude spontaneous positivities (X) in an epileptic kainate-treated rat at septotemporal levels separated by 2 mm.

there was marked inhibition with 1.0-Hz frequency stimulation and slight inhibition with 0.1-Hz stimulation. At the 70-ms interstimulus interval there was marked facilitation at both stimulus frequencies. At the 250-ms interstimulus interval there was slight inhibition at both stimulus frequencies. Paired-pulse responses of kainate-treated rats, recorded with a pair of saline-filled electrodes, were qualitatively similar to those of controls (Figs. 3B and 4), except they tended to be more inhibited, as reported previously (Buckmaster and Dudek 1995a; Milgram et al. 1991).

Immediately after or before responses were obtained with saline-filled electrodes, another set of responses was obtained with an electrode pair consisting of a saline-filled electrode and a bicuculline-filled electrode. The saline-filled member of the pair was directed toward the more septal position in the dentate gyrus (Fig. 3A, electrode labeled 1), at the same location as the electrode whose population spike amplitudes were measured with the pair of saline-filled electrodes. The responses recorded with the bicuculline-filled member of the electrode pair always consisted of multiple population spikes (e.g., Fig. 3C2), indicating that bicuculline leaked from the electrode and disinhibited a region of the dentate gyrus. Multiple population spikes never were observed in responses obtained by the saline-filled electrode in the more septal position 1 mm away (Fig. 3C1), indicating that the disinhibited region was focally confined. Population spike amplitudes were measured and amplitude ratios were calculated only from responses obtained with the saline-filled, septally positioned electrode (Fig. 3A, electrode labeled 1).

Control rats showed significantly enhanced paired-pulse inhibition (i.e., smaller amplitude ratios, Fig. 4) at the 30- and 70-ms interstimulus intervals when the distant region of the dentate gyrus was hyperexcitable, indicating that burst discharges in the surrounding (lateral) region inhibited responses in the analyzed region. There was little effect on amplitude ratios at the 250-ms interstimulus interval. Like control animals, kainate-treated rats displayed significantly more paired-pulse inhibition at the 30- and 70-ms interstimulus intervals when the distant region of the dentate gyrus was hyperexcitable, despite having more paired-pulse inhibition than controls at their baseline level (i.e., when the distant electrode contained saline instead of bicuculline). Control and kainate-treated rats both showed enhanced paired-pulse inhibition with stimulation at 1.0 and 0.1 Hz when the distant region of the dentate gyrus was hyperexcitable, suggesting that the lateral inhibitory effect occurred at stimulation frequencies that evoke both feedback and feedforward inhibition (Sloviter 1991a).

Comparison of mean population spike amplitude ratios, obtained when burst discharges occurred in a lateral hippocampal lamella (Fig. 4, gray bars), revealed no significant difference (P > 0.50, t-test) between control and epileptic kainate-treated rats at the 30-, 70-, and 250-ms interstimulus intervals with 1.0-Hz stimulation and at the 30- and 70-ms interstimulus intervals with 0.1-Hz stimulation. At the 250-ms interstimulus interval with 0.1-Hz stimulation, the mean population spike amplitude ratio of kainate-treated rats was significantly smaller (P < 0.02, t-test) than that of controls. The similarities between control and epileptic kainate-treated rats in population spike amplitude ratios, measured when a lateral region of the dentate gyrus was discharging bursts of population spikes, suggests persistence of lateral inhibitory control in kainate-treated animals.

**MDA stimulus threshold**

MDA thresholds were measured and compared in control versus kainate-treated rats to assess excitability changes in the dentate gyrus after hilar neuron loss and granule cell axon reorganization. Stimulus threshold for
FIG. 3. Measurement of lateral inhibition in the dentate gyrus. A: schematic of electrode configuration for measurement of lateral inhibition. Stimulating electrode (stim.) was positioned to activate perforant pathway fibers. A pair of recording electrodes (labeled 1 and 2), with 1-mm tip separation along the septotemporal axis of the hippocampus, was placed to obtain simultaneous dentate gyrus field potentials from different hippocampal lamellae. B and C: dentate gyrus field potential responses of an epileptic kainate-treated rat to perforant path stimulation [interstimulus interval 30 ms, stimulus frequency 0.1 Hz, stimulus intensity 5 \times \text{stimulus threshold for a population spike (T)}] obtained with a pair of saline-filled recording electrodes (B) and with a saline-filled (C1) and a bicuculline-filled electrode (C2). Traces in B were obtained first. Then the pair of saline-filled electrodes was replaced with a saline-filled electrode in position 1 and a bicuculline-filled electrode in position 2, and the traces shown in C were obtained. Note the multiple population spikes recorded by the bicuculline-filled electrode (C2). Also note that the amplitude of the 2nd population spike was reduced (compare B1 and C1; \(Z\)) when the 1-mm distant region of dentate gyrus was hyperexcitable (C2).

evoking MDA in control rats was \(0.38 \pm 0.03 \times T\) (mean ± SE, \(n = 6\)). In kainate-treated rats it was \(1.07 \pm 0.16 \times T\) (\(n = 13\)). The average MDA threshold of kainate-treated rats was 2.8 times higher than that of controls, and the difference was significant (\(P < 0.005\), \(t\)-test; Fig. 5). The highest MDA thresholds tended to occur in kainate-treated rats with the most hilar neuron loss (Fig. 5B) and with the largest proportions of Timm staining in the granule cell and molecular layers (\(R^2 = 0.54, P < 0.005\), analysis of variance).

Afterdischarges and spontaneous bursts of dentate gyrus population spikes

Spontaneous dentate gyrus field potentials and responses to tetanic stimulation in the presence of focally applied bicuculline were examined to determine whether local inhibition might be masking the epileptogenic effects of granule cell axon reorganization in kainate-treated rats. Afterdischarges were evoked by perforant path stimulation (frequency 2 Hz, 40 pulses, intensity \(5 \times T\)) at the site of a bicuculline-containing electrode in 3 of
FIG. 4. Mean population spike amplitude ratios (2nd population spike amplitude divided by 1st population spike amplitude) in control (n = 6) and kainate (KA)-treated rats (n = 8). Ratios were calculated for 3 interstimulus intervals (30, 70, and 250 ms) and 2 stimulus frequencies (1.0 and 0.1 Hz). White bars: amplitude ratios when the region distant from the recording electrode used to measure population spike amplitudes had normal excitability (i.e., the electrode in the distant lamella contained saline). Gray bars: amplitude ratios when the region of the dentate gyrus distant from the recording electrode used to measure population spike amplitudes was hyperexcitable (i.e., the electrode in the distant lamella contained bicuculline). Note that the amplitude ratios at the 30- and 70-ms interstimulus intervals were significantly smaller (i.e., there was more paired-pulse inhibition) when bicuculline was present in the distant electrode. There was little effect at the 250-ms interstimulus interval. Epileptic kainate-treated rats tended to have smaller amplitude ratios than controls under normal conditions (i.e., when a pair of saline-filled electrodes was used, white bars). Like controls, epileptic kainate-treated rats showed significantly smaller amplitude ratios at the 30- and 70-ms interstimulus intervals when the distant electrode contained bicuculline (gray bars) than when it contained saline. Note that the population spike amplitude ratios of control and epileptic kainate-treated rats were similar when burst discharges occurred in a lateral hippocampal lamella (see text for results of statistical comparison). Error bars: SE. Single asterisk: \( P < 0.025 \). Double asterisk: \( P < 0.01 \), t-test.

9 kainate-treated rats and 0 of 11 controls (Fig. 6A). Afterdischarges were observed with an electrode containing bicuculline plus potassium chloride in 4 of 11 epileptic kainate-treated rats and in 0 of 9 controls. Spontaneous bursts of population spikes were observed with a bicuculline-containing electrode in 4 of 14 kainate-treated rats and in 0 of 11 controls, and these bursts tended to be immediately preceded by individual or compound spontaneous positivities (Fig. 6C). In summary, afterdischarges and/or spontaneous bursts of population spikes were observed in 6 of 14 epileptic kainate-treated rats and in 0 of 11 controls; the difference was significant (\( P < 0.025 \), \( \chi^2 \) test). Epileptic kainate-treated rats that displayed spontaneous bursts of population spikes and/or afterdischarges (n = 6) had significantly larger proportions of their granule cell and molecular layers infiltrated with Timm-positive granule cell axon terminals (35 ± 3%, mean ± SE) than did epileptic kainate-treated rats (n = 8) that failed to show these epileptiform events (22 ± 4%; \( P < 0.03 \), t-test). When spontaneous bursts of population spikes or afterdischarges occurred, they always were confined to the electrode containing bicuculline (± potassium chloride); they never were observed to spread to the saline-containing electrode, 1 mm away (Fig. 6, B and C).

DISCUSSION

The main results of this study are: 1) lateral inhibition in the dentate gyrus persists despite hilar neuron loss, and 2) rats with the greatest extent of granule cell axon reorganization were most likely to display spontaneous bursts and afterdischarges in the dentate gyrus. In addition, epileptic kainate-treated rats displayed higher-frequency and larger-amplitude spontaneous positivities in their dentate gyrus field potentials, and they had higher stimulus thresholds for MDA than did controls.

Lateral inhibition persists despite hilar neuron loss

Lateral inhibition is a common feature of neuronal circuits (Shepherd and Koch 1990). Sloviter and Brisman (1995) demonstrated lateral inhibition in the rat dentate gyrus with the use of paired-pulse stimulation and recording electrodes that leaked pharmacological agents. They showed that paired-pulse inhibition increased after a region of the dentate gyrus, 800 μm away from the recording site, was made hyperexcitable with the GABA<sub>A</sub> receptor antagonist bicuculline. Bicuculline disinhibited a focal group of neurons that responded to perforant path stimulation with a burst instead of a single action potential. Presumably, these burst discharges resulted in increased release of inhibitory neurotransmitter in surrounding (lateral) regions of the dentate gyrus, so that when the second afferent pulse of the pair arrived at the recording site, it was more inhibited than when bursting in the distant region did not occur. The results of
interneurons project their axon collaterals long distances (Buckmaster and Schwartzkroin 1995a,b; Struble et al. 1978), and many GABAergic interneurons survive treatments that kill mossy cells (Buckmaster and Dudek 1995b; Davenport et al. 1990; Obenaus et al. 1993; Sloviter 1987, 1991b). We propose that surviving inhibitory interneurons include cells that generate lateral inhibition. This would account for evidence from kainate-treated rats of increased paired-pulse inhibition when a distant region was hyperexcitable and for the confinement of the hyperexcitability to a focally disinhibited region.

There are some caveats with our conclusion. First, counting mossy cells is hindered by the absence of a specific marker. Mossy cell loss, therefore, is based on indirect evidence. Our data indicate that an average of 60% of the total hilar neuron population was killed by kainate treatment. It is likely that mossy cells were among the killed neurons, because 1) they are the predominant hilar neuron type (Amaral 1978); 2) they display electrophysiological evidence of degeneration in response to prolonged stimulation (Scharfman and Schwartzkroin 1990); 3) similar treatments that kill hilar neurons produce a band of degenerating axons in the inner molecular layer where mossy cell axons concentrate (Buckmaster et al. 1996b; Nadler et al. 1980; Obenaus et al. 1993; Sloviter 1987, 1991b); and 4) hilar neurons remaining after similar treatments are likely to be GABAergic (Davenport et al. 1990; Obenaus et al. 1993; Sloviter et al. 1987, 1991b), but mossy cells are glutamatergic (Soriano and Frotscher 1994). However, the possibility that some mossy cells survived kainate treatment and drove lateral inhibition in our epileptic rats cannot be ruled out. Second, it might be argued that mossy cell death results in acute loss of lateral inhibition in the days following kainate treatment, but over subsequent weeks, granule cell axon reorganization restores excitatory synaptic input to inhibitory interneurons (Sloviter 1992). This mechanism might restore local inhibitory circuits, but its relevance to lateral inhibition is doubtful. Reorganized granule cell axon collaterals largely remain close to the parent cell body (Buckmaster and Dudek 1995a); they do not extend to distant septotemporal regions like the axons of mossy cells (Buckmaster et al. 1996b), and therefore they could not restore the proposed lateral inhibitory circuits driven by long mossy cell axons (Sloviter 1994). Despite these caveats, we believe that the most parsimonious explanation for the persistence of lateral inhibition in epileptic kainate-treated rats is that it is not generated by mossy cells but instead by inhibitory interneurons that survive kainate treatment and whose axons extend far along the septotemporal axis of the hippocampus (Fig. 7).

**Spontaneous bursts and afterdischarges occur in the dentate gyrus after granule cell axon reorganization**

It has been proposed that granule cell axon reorganization, induced by hilar neuron loss, has an antiepileptic effect by restoring excitatory synaptic input to inhibitory interneurons (Sloviter 1992). However, anatomic and electrophysiological evidence suggests that granule cell axon reorganization results in the formation of excitatory recurrent collaterals.
Labeled granule cell axons have been observed at the ultrastructural level making synaptic contacts with nonlabeled complex dendritic spines of presumed granule cells in the inner third of the molecular layer of kindled and kainate-treated rats (Represa et al. 1993) and in tissue from patients with temporal lobe epilepsy (Babb et al. 1991; Franck et al. 1995). Synaptic contacts have been observed between biocytin-labeled granule cell axons and dendrites in pilocarpine- and kainate-treated rats (Okazaki et al. 1995). Antidromic stimulation of granule cell axons in tissue from epileptic humans and kainate-treated rats (but not controls) evokes multiple discharges, consistent with a recurrent excitatory circuit (Cronin et al. 1992; Masukawa et al. 1992; Tauck and Nadler 1985; Wuarin and Dudek 1996). Current-source-density analysis of perforant-path-evoked responses reveals a current sink in the inner molecular layer of kindled rats with granule cell axon reorganization (Golarai and Sutula 1996), and glutamate microdrops applied to the granule cell layer evoke excitatory synaptic potentials in neighboring granule cells of kainate-treated rats (Wuarin and Dudek 1996).

Most of the results of the present study are consistent with the view that granule axon reorganization produces recurrent excitatory circuits that underlie positive feedback and generate epileptiform activity. Focal application of bicuculline fails to cause spontaneous bursts of dentate gyrus population spikes in control animals (Sloviter and Brisman 1995; this study); however, such bursts occurred in kainate-treated rats with granule cell axon reorganization. Responses to perforant path stimulation obtained with electrodes filled with bicuculline (± potassium chloride) never revealed afterdischarges in control animals but did in a significant proportion of kainate-treated rats with granule cell axon reorganization. Furthermore, kainate-treated rats that displayed these epileptiform events had significantly more granule cell axon reorganization than kainate-treated rats that failed to show spontaneous bursts and afterdischarges. These findings demonstrate a possible link between granule cell axon reorganization and epileptiform activity; however, it is unclear whether there is a cause-effect relationship. Other contributing factors might include nonsynaptic properties (e.g., intrinsic physiology of constituent neurons) and synaptic inputs from extrinsic structures to the dentate gyrus. We favor the view that granule cell axon reorganization underlies these epileptiform events, because the largely locally restricted nature of the reorganized granule cell axons (Buckmaster and Dudek 1995a) (and persistence of lateral inhibition) is consistent with the focal, nonspreading characteristic of the discharges. Bicuculline’s ability to reveal epileptiform discharges suggests that recurrent excitation usually is masked.

**Fig. 6.** Epileptiform discharges in the dentate gyri of epileptic kainate-treated rats. A: stimulus-triggered (frequency 2 Hz, intensity 5 × T) afterdischarges (✓) occurred in epileptic kainate-treated rats (A2) but not in controls (A1) when bicuculline leaked from a recording electrode. B: stimulus-triggered (frequency 2 Hz, intensity 5 × T) afterdischarges in epileptic kainate-treated rats remained confined to the region of the dentate gyrus near the bicuculline-containing electrode (B2, ✓); they did not spread to the region of the dentate gyrus recorded simultaneously with a saline-containing electrode, 1 mm away (B1). C: spontaneous bursts of dentate gyrus population spikes were observed in epileptic kainate-treated rats but not in controls. These spontaneous bursts occurred only in the region recorded by the bicuculline-filled electrode (C2, ✓) and not in the region recorded with a saline-filled electrode 1 mm away (C1), and they frequently were preceded by dentate electroencephalographic spikes like the compound dentate spike shown in these traces.
FIG. 7. Schematic diagram of proposed dentate gyrus circuitry in control and epileptic kainate-treated rats after mossy cell (mc) loss and granule cell (gc) axon reorganization. In epileptic kainate-treated rats, hilar neurons die (including, presumably, mossy cells), but inhibitory neurons (in) persist. We propose that 1) via axon collaterals that extend along the septotemporal axis of the dentate gyrus, inhibitory neurons (not mossy cells) mediate lateral inhibition; 2) granule cell axon reorganization in epileptic kainate-treated rats establishes local recurrent excitatory collaterals that underlie epileptiform discharges when inhibition is blocked; and 3) persistent lateral inhibition prevents the spread of epileptiform discharges from a focally disinhibited region to distant septotemporal levels.

by GABA_\(_A\)_receptor-mediated inhibition (Cronin et al. 1992).

In contrast, MDA thresholds were higher in epileptic kainate-treated rats and tended to correlate with hilar neuron loss and granule cell axon reorganization. The cause-effect relationship between these neuropathological changes and MDA threshold is questionable, because MDA thresholds tend to increase in other models of temporal lobe epilepsy that do not show hilar neuron loss or granule cell axon reorganization (Buckmaster et al. 1996a; Stringer and Lothman 1989). Repeated seizures are a common factor in models of epilepsy that show increased MDA thresholds; therefore it might be a compensatory response to recurrent seizures.

Other evidence for functional abnormalities in the dentate gyrus network of epileptic kainate-treated rats

Spontaneous positivities in dentate gyrus field potentials observed in these experiments closely resemble “dentate electroencephalographic (EEG) spikes” described by Bragin et al. (1995), with similarities in polarity, duration, amplitude, frequency, and synchrony along the septotemporal axis of the hippocampus. On the basis of the divergent axon projections from the entorhinal cortex to the dentate gyrus (Amaral and Witter 1989) and of data from experiments involving surgical lesions of the entorhinal cortex and colchicine injections into the dentate gyrus, dentate spikes appear to be triggered by the entorhinal cortex and generated by inward currents to granule cell dendrites (Bragin et al. 1995). In normal rats, dentate spikes appear to have an inhibitory effect on hippocampal activity, because they are associated with synchronized bursts of inhibitory hilar interneurons and reduced probability for occurrence of sharp waves in the CA3 and CA1 fields of the hippocampus (Bragin et al. 1995).

In the present study, spontaneous positivities occurred at higher frequency and larger amplitude in epileptic kainate-treated rats than in controls. The factors underlying this change are unknown but might include alterations intrinsic to the entorhinal cortex (Du et al. 1995), changes in pharmacological sensitivity of granule cells to perforant path input, and/or alterations in dentate gyrus circuitry (e.g., granule cell axon reorganization). If spontaneous positivities in the dentate gyrus inhibit activity in Ammon’s horn (Bragin et al. 1995), then their increased frequency and amplitude in epileptic animals might be compensatory. However, in epileptic rats recorded with bicuculline-filled electrodes, dentate spikes immediately preceded spontaneous bursts of population spikes, suggesting that dentate EEG spikes trigger epileptiform discharges after granule cell axon reorganization.

Conclusions

Kainate-treated rats with hilar neuron loss and granule cell axon reorganization clearly demonstrate functional abnormalities in the dentate gyrus. However, lateral inhibition persists, suggesting that vulnerable hilar neurons are not necessary for generating lateral inhibition. In the presence of focal bicuculline, epileptiform electrographic events were observed in kainate-treated but not control animals. These events occurred only after granule cell axon reorganization, consistent with the view that recurrent excitatory circuits underlie seizure activity. Concurrent changes in dentate EEG spikes and MDA threshold suggest that compensatory mechanisms might react to the epileptic state of the dentate gyrus network.
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