Recurent mossy fiber pathway in rat dentate gyrus: Synaptic currents evoked in presence and absence of seizure-induced growth

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INTRODUCTION

Temporal lobe epilepsy is the most common form of epilepsy in the adult population, afflicting ≥800,000 Americans. Despite years of study, the etiology of this condition is poorly understood. Roughly two-thirds of persons with temporal lobe epilepsy have lesions in one or both hippocampi (Meldrum and Bruton 1992). The most consistent pathological finding in these brains is a dramatic loss of interneurons from the hilus of the dentate gyrus, a condition referred to as “endfolium sclerosis” (Margerison and Corsellis 1966). This lesion appears to precede the seizures in many cases and is postulated to facilitate seizure generation and propagation (Sloviter 1994). There are a number of ways in which endfolium sclerosis could lead to seizures. One of these is the lesion-induced growth of excitatory axon collaterals, with subsequent formation of novel feedback circuitry.

The mossy fibers are axons of the hippocampal dentate granule cells. The mossy fiber pathway normally projects to the pyramidal cells of the hippocampal area CA3 and to interneurons of the CA3 area and dentate hilus; it is thought to make few, if any, recurrent synapses onto granule cells. A common feature of temporal lobe epilepsy (Babb et al. 1991; Francik et al. 1995; Sutula et al. 1989) and of animal models of temporal lobe epilepsy (Okazaki et al. 1995; Represa et al. 1993; Sutula et al. 1988) is the development of numerous mossy fiber-granule cell synapses. These synapses mediate monosynaptic recurrent excitation that is normally weak or absent (Cronin et al. 1992; Golarai and Sutula 1996; Masakawa et al. 1992; Patrylo and Dudek 1998; Tauck and Nadler 1985; Wuarin and Dudek 1996). Novel innervation of this type is expected to have profound consequences for hippocampal function. Dentate granule cells have been shown to resist the propagation of seizures through the limbic circuit (Collins et al. 1983; Lothman et al. 1992). Several properties of granule cells and their connectivity appear to contribute to this high resistance, including the lack of intrinsic capacity for burst discharge, strong tonic inhibition from GABA interneurons, and absence of a synaptic mechanism for synchronization of burst discharge. Granule cell discharge can be synchronized by nonsynaptic mechanisms (Schweitzer et al. 1992), but this occurs only during very strong afferent bombardment that is likely to compromise synaptic inhibition (Lothman et al. 1992). Synaptic interconnections serve as the anatomic substrate for synchronization of CA3 pyramidal cell discharge (Miles et al. 1984). Area CA3 is one of the major sites of epileptiform activity in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The data suggest that the mossy fiber pathway is altered in seizure-prone rats. Mossy fiber pathways in rat dentate gyrus: Synaptic currents evoked in presence and absence of seizure-induced growth. J. Neurophysiol. 81: 1645–1660, 1999. A common feature of temporal lobe epilepsy and of animal models of epilepsy is the growth of hippocampal mossy fibers into the dentate molecular layer, where at least some of them innervate granule cells. Because the mossy fibers are axons of granule cells, the recurrent mossy fiber pathway provides monosynaptic excitatory feedback to these neurons that could facilitate seizure discharge. We used the pilocarpine model of temporal lobe epilepsy to study the synaptic responses evoked by activating this pathway. Whole cell patch-clamp recording demonstrated that antidromic stimulation of the mossy fibers evoked an excitatory postsynaptic current (EPSC) in ∼74% of granule cells from rats that had survived >10 wk after pilocarpine-induced status epilepticus. Recurrent mossy fiber growth was demonstrated with the Timm stain in all instances. In contrast, antidromic stimulation of the mossy fibers evoked an excitatory postsynaptic current (EPSC) in only 5% of granule cells studied 4–6 days after status epilepticus, before recurrent mossy fiber growth became detectable. Notably, antidromic mossy fiber stimulation also evoked an EPSC in many granule cells from control rats. Clusters of mossy fiber-like Timm staining normally were present in the inner third of the dentate molecular layer at the level of the hippocampal formation from which slices were prepared, and several considerations suggested that the recorded EPSCs depended mainly on activation of recurrent mossy fibers rather than associational fibers. In both status epilepticus and control groups, the antidromically evoked EPSC was glutamatergic and involved the activation of both AMPA/kainate and N-methyl-D-aspartate (NMDA) receptors. EPSCs recorded in granule cells from rats with recurrent mossy fiber growth differed in three respects from those recorded in control granule cells: they were much more frequently evoked, a number of them were unusually large, and the NMDA component of the response was generally much more prominent. In contrast to the antidromically evoked EPSC, the EPSC evoked by stimulation of the perforant path appeared to be unaffected by a prior episode of status epilepticus. These results support the hypothesis that recurrent mossy fiber growth and synapse formation increases the excitatory drive to dentate granule cells and thus facilitates repetitive synchronous discharge. Activation of NMDA receptors in the recurrent pathway may contribute to seizure propagation under depolarizing conditions. Mossy fiber-granule cell synapses also are present in normal rats, where they may contribute to repetitive granule cell discharge in regions of the dentate gyrus where their numbers are significant.
bursting and seizure initiation in the limbic system. By analogy, the recurrent mossy fiber pathway, if sufficiently powerful, could facilitate the synchronized firing of granule cells, thereby enhancing their participation in seizures.

However, the recurrent mossy fibers also may drive GABA inhibition. Some of these fibers appear to contact GABA interneurons (Kotti et al. 1997; Sloviter 1992). They have been suggested to provide a critical excitatory drive needed to activate these neurons that normally is provided by the associational-commissural pathway (which degenerates in the lesioned hippocampal formation) (Sloviter 1991). It also has been suggested that mossy fiber terminals in the epileptic brain release GABA in addition to glutamate, thus becoming at once both excitatory and inhibitory (Sloviter et al. 1996). Furthermore, GABA interneurons may form additional synapses on granule cells (Davenport et al. 1990; Mathern et al. 1995). Under the right conditions, strong recurrent inhibition mediated by the granule cell-GABA interneuron-granule cell circuit could facilitate seizures by assuring that granule cells fire synchronously (Freund and Buzsáki 1996). At other times, enhanced recurrent inhibition may suppress the emergence of seizures.

This study was undertaken to examine the cellular electrophysiological effects of activating the recurrent mossy fiber pathway. We recorded the excitatory postsynaptic current (EPSC) and inhibitory postsynaptic current (IPSC) evoked in granule cells by antidromic stimulation of the mossy fibers at two survival times after pilocarpine-induced status epilepticus: $\geq 10$ wk, a time when mossy fiber growth is essentially complete, and 4–6 days, a time when Timm histochemistry reveals no evidence of mossy fiber growth (Okazaki et al. 1995). For comparison, similar studies were performed with stimulation of the perforant path.

**METHODS**

**Pilocarpine-induced status epilepticus**

Male Sprague-Dawley rats (150–200 g; Zivic-Miller Laboratories, Allison Park, PA) received a single injection of pilocarpine hydrochloride (330–360 mg/kg ip). The animals were pretreated 30 min earlier with scopolamine methyl bromide and terbutaline hemisulfate (2 mg/kg ip, each) to block peripheral side effects and maintain respiration. Not all pilocarpine-treated rats developed status epilepticus. For this study, we accepted for study. The liquid junction potential was determined to be $0.1 \text{ M sodium phosphate buffer, pH 7.3, and set aside for Timm and Nissl staining.}$

**Stimulation and recording**

Slices used for electrophysiological recording corresponded to horizontal plates 98–100 of Paxinos and Watson (1986). Beginning 1.5 h after preparation, individual slices were transferred to a small experimental chamber, placed on a nylon net and barely submerged in the superfusion medium. The superfusion rate was 1.5 ml/min. The monopolar stimulating electrode was a 25-µm-diam nichrome wire implanted into the tip with a polymerized polyvinyl resin (Formvar). It was placed in stratum lucidum of area CA3b (200–250 µm from the opening of the dentate hilus. Then an extracellular electrode fashioned from borosilicate glass (filled with 1 M NaCl; resistance of 2–6 MΩ) was used to probe for location in the granule cell body layer where the antidromic population spike was of maximal amplitude. With the extracellular recording electrode positioned at this location, the stimulating electrode was moved perpendicular to the pyramidal cell body layer until stimulation evoked an antidromic population spike of the greatest possible amplitude. The antidromically evoked response declined dramatically when the stimulating electrode was moved even slightly away from stratum lucidum. The final optimization of the antidromic population spike amplitude was achieved by adjusting the depth of both the stimulating and recording electrodes. The stimulus current was set to a near-maximal value (490 µA) and rectangular pulses of 100-µs duration were applied every 30 s.

Whole cell patch-clamp recordings were made from dentate granule cells located close to the extracellular recording electrode. The patch electrode was fashioned from Sutter (Novato, CA) borosilicate glass pulled to a narrowly tapered tip with a resistance of 5–8 MΩ. The tip was filled by vacuum with a solution that contained (in mM) 140 cesium gluconate, 15.5 HEPES, and 3.1 MgCl$_2$, pH 7.2 and 276–277 mosm. The electrode then was backfilled with the internal solution, which consisted of (in mM) 120 cesium gluconate, 10 HEPES, 2 MgATP, and 10 QX-314 (N-ethyl lidocaine) chloride, pH 7.2 and 276–277 mosm. Seals were formed by the “blind” approach (Blanton et al. 1989), and whole cell access was obtained in current clamp mode. Only cells with $V_m$ more than $–60$ mV on break-in were accepted for study. The liquid junction potential was determined to be 10 mV with use of the method described by Neher (1992). This value was subtracted from all membrane potentials. Whole cell recordings were made with an Axon Instruments (Foster City, CA) Axopatch 200A patch clamp amplifier beginning 15–20 min after break-in. Membrane resistance was determined in current clamp mode by injecting 50-pA hyperpolarizing rectangular pulses of 200-ms duration just before the start of recording. Series resistances ranged from 8 to 20 MΩ and were compensated $\geq 75\%$. The series resistance did not change by $\geq 20\%$ during the experiment. Signals were filtered $<2$ kHz, digitized at 10 kHz, and stored to disk with use of a Digidata board and PClamp 6 running on a Micron P-90 or P-100 microcomputer. Normally, 10 sequentially obtained traces were averaged, and the averaged waveforms were analyzed off-line with functions incorporated in PClamp 6. Unless stated otherwise, all traces presented in the figures are averaged records.

The antidromically evoked EPSC was studied at a holding potential of $–80$ mV in the presence of 30–100 µM bicuculline methiodide or 50 µM picrotoxin and was defined as the inward current abolished by 50 µM d-2-amino-5-phosphonopentanoate (d-AP5) and either 20 µM 6,7-dinitroquininaline-2,3-dione (DNQX) or 5 µM 2,3-dihydroxy-6-nitro-7-sulfamyl-benzof(2)quininaline (NBQX). The antidromically evoked feedback (or mossy fiber-driven) GABA$_A$ IPSC was studied at a holding potential of 0 mV and was defined as the outward current abolished by bicuculline methiodide or picrotoxin. Activation of post synaptic GABA$_A$ receptors was prevented by the use of a cesium-based internal solution that included QX-314 but not GTP.

Responses to perforant path stimulation were studied in a similar...
were cut into 40-μm-thick frozen coronal or horizontal sections. Alternate sections were mounted on slides coated with chrome alum-gelatin, stained for the presence of heavy metals as described by Danscher (1981), and lightly counterstained with cresyl violet. The remaining sections were stained with cresyl violet. Sections used for histological analysis were those that corresponded to horizontal plates 101–103 of Paxinos and Watson (1986), the region of the hippocampal formation immediately rostral to the slices used for electrophysiological recording. Histological analyses were performed without knowledge of the experimental treatment or electrophysiological data. Hilar neurons were counted in two cresyl violet-stained sections, and the results were averaged. The hilar area related to the dentate gyrus (corresponding to area CA4 of Lorente de Nó 1934) was outlined with the aid of a camera lucida. Only cells within this area with a diameter that exceeded 10 μm were included in the count, thus excluding glia and displaced granule cells. Neurons at the lower edge of the granule cell body layer (e.g., basket cells) also were excluded. The cross-sectional area in which neurons were counted was measured with use of NIH Image software. Hilar neuron density was calculated by dividing the number of neurons counted by the cross-sectional area of the counting region.

A separate group of 23 rats that had experienced 3–4 h of pilocarpine-induced status epilepticus and 9 pilocarpine-treated control rats was used to determine the extent of neuronal degeneration in the hippocampal formation. The animals were anesthetized deeply with pentobarbital sodium 1 day after pilocarpine treatment and perfused transcardially with phosphate-buffered 0.9% (wt/vol) saline for 1 min followed by 4% (wt/vol) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. After 3–20 days of postfixation at 4°C, the brains were cut into 40-μm-thick frozen coronal or horizontal sections. Alternate sections were stained with cresyl violet or impregnated with silver to visualize degenerating neuronal somata and terminals (Nadel and Evenson 1989).

Materials

D-Gluconic acid lactone, HEPES, phenobarbital sodium, pilocarpine hydrochloride, (–)-scopolamine methyl bromide, and terbutaline hemisulfate were purchased from Sigma Chemical (St. Louis, MO). D-AP5 and DNQX were purchased from Tocris Cookson (Bristol, UK), bicuculline methiodide from Research Biochemicals (Natick, MA), and cesium hydroxide (99.9%; 50 wt%) from Aldrich (Milwaukee, WI). QX-314 chloride was obtained from Astra USA (Westborough, MA) and Alomone Labs (Jerusalem, Israel). NBQX was a gift from Novo Nordisk (Måløv, Denmark).

RESULTS

Antidromically evoked EPSCs were recorded more frequently and were sometimes larger in slices from rats that had developed status epilepticus

The antidromically evoked inward current recorded at a holding potential of −80 mV consisted of bicuculline-sensitive and -insensitive components (Fig. 1, top). In each of 49 cells, the bicuculline-insensitive component was abolished or nearly abolished by addition of 20 μM DNQX and 50 μM D-AP5 to the superfusion medium (Fig. 1, bottom). It thus was taken to represent the antidromically evoked EPSC. The EPSC typically exhibited slow response kinetics that could not be described adequately by a monoeponential rise and decay. In some instances, multiple components clearly were present. The bicuculline-sensitive inward current represented the GABA<sub>A</sub>-receptor-mediated feedback IPSC. The IPSC was directed inwardly at a holding potential of −80 mV because E<sub>Cl</sub> (calculated as described by Staley and Mody 1992) was about −54 mV under the conditions of our experiments.

A small, brief biphasic current recorded just before the EPSC coincided with the simultaneously recorded antidromic population spike, and its amplitude varied with the antidromic spike amplitude (Fig. 1). This “inflection” of the antidromic spike overlapped the onset of the EPSC (and sometimes the IPSC), and it could not be adequately removed by electronic subtraction of traces in the presence and absence of bicuculline or in the presence and absence of DNQX/D-AP5. Thus the latency to onset of the antidromically evoked EPSC could not be accurately determined.

Antidromically evoked EPSCs were observed most frequently in dentate granule cells from long-term survivors of pilocarpine-induced status epilepticus. The success rate in these animals was ~74% (Fig. 2, Table 1). However, antidromic stimulation at this survival time also evoked an EPSC in ~38% of granule cells from pilocarpine-treated control rats and in ~26% of granule cells from age-matched untreated or saline-treated control rats. The peak amplitudes of 9 of the 25 antidromically evoked EPSCs from the status epilepticus group were greater than the peak amplitude of the largest EPSC recorded from any control granule cell; 4 were >100 pA. Because of this cohort of unusually large responses, the mean peak amplitude of the antidromically evoked EPSC was about three times as large as control in granule cells from the status epilepticus group. The largest EPSCs were not always associated with especially robust mossy fiber growth, as indicated by Timm histochemistry. Among the long-term survivors, there were no significant between-group differences in either the membrane potential on break-in, input resistance or amplitude of the antidromic population spike (Table 1). There was also no correlation between any of these measures and the peak amplitude of the EPSC (P > 0.1, Spearman-rho rank correlation).

No such effects of pilocarpine-induced status epilepticus on
the antidromically evoked EPSC were found 4–6 days after treatment (Table 1). Antidromic stimulation of the mossy fibers evoked an EPSC in only 1 of 19 granule cells from rats that had developed status epilepticus. In contrast, antidromic stimulation evoked an EPSC in roughly the same percentages of control granule cells in short-term survivors (40% of the treated control group and 36% of the untreated control group) as in long-term survivors. Again there were no significant between-group differences in either the membrane potential on break-in or the amplitude of the antidromic population spike. However, input resistance was higher in granule cells from rats that had developed status epilepticus.

Delayed inward currents sometimes were observed in granule cells from both status epilepticus and control groups. Although antidromic stimulation normally evoked at most a single short-latency inward current, in some granule cells it also evoked a delayed inward current during superfusion with bicuculline or picrotoxin (Fig. 3). Delayed currents ranged from large (as large as 700 pA) and complex to small and unimodal. They appeared at varying latencies and were present after some stimulus pulses and not others. In some instances, the delayed inward current appeared during wash-in of the GABAA antagonist and then disappeared after the antagonist had equilibrated. Persistent delayed inward currents most commonly were observed in the long-term status epilepticus group (6 cells) but also appeared in four cells from the long-term treated control group and in three cells each from the short-term treated control and short-term untreated control groups. Each granule cell in which this response was recorded came from a different rat. In each treatment group, the peak amplitude of at least one of these responses was ≥100 pA. Delayed inward currents were abolished by superfusion with DNQX/D-AP5, suggesting that they were polysynaptic EPSCs.

NMDA receptors contributed to the antidromically evoked EPSC

To determine whether antidromic stimulation activated NMDA receptors, we studied the pharmacology of the postsynaptic response recorded at holding potentials of −80 and −30 mV (Fig. 4). Only rats that had survived ≥10 wk after pilocarpine administration were used in this study. At a holding potential of −80 mV, addition of the selective AMPA/kainate receptor antagonist NBQX (5 μM) to the superfusion medium left only a small antidromically evoked inward current. The small residual current disappeared on the addition of 50 μM D-AP5. However, NBQX unmasked a much larger component
when the holding potential was changed to −30 mV. The bicuculline- and NBQX-insensitive inward current exhibited slow response kinetics and was abolished by addition of 50 μM D-AP5 to the superfusion medium. It thus represented the NMDA component of the antidromically evoked EPSC. Because the antidromically evoked EPSC essentially was eliminated with a combination of NBQX and D-AP5 (or DNQX and D-AP5), we conclude that this response is glutamatergic and depends predominantly on activation of AMPA/kainate and NMDA receptors. Similar results were obtained in each of four granule cells from pilocarpine-treated control rats and in each of six granule cells from rats that had developed status epilepticus.

I-V curves were generated in three experiments each from the status epilepticus and pilocarpine-treated control groups. Each of these curves exhibited a region of negative slope conductance with the peak NMDA current between −20 and −40 mV (Fig. 5). However, the NMDA component of the response from granule cells in the status epilepticus group was generally larger. Table 2 compares the total charge transfer through NMDA receptors at −30 mV with the total charge transfer through AMPA/kainate receptors at −80 mV. By this measure, the NMDA component was a small fraction of the AMPA/kainate component in three of the four granule cells from control rats. In contrast, the NMDA component was 1.4–2.2 times as large as the AMPA/kainate component in five of the six granule cells from rats that had developed status epilepticus.

**Amplitude of antidromically evoked IPSCs depended on age and the prior development of status epilepticus**

At a holding potential of 0 mV, antidromic stimulation of the mossy fibers evoked a GABA<sub>δ</sub>-receptor-mediated feedback IPSC (Fig. 6) in all granule cells studied (Fig. 7). The peak amplitude of this response differed among treatment groups in two respects. First, the GABA<sub>δ</sub> feedback IPSC was only about one-third as large in the older control rats compared with the younger controls (Table 3). Second, the amplitude of the feedback GABA<sub>δ</sub> IPSC was depressed significantly 4–6 days after pilocarpine-induced status epilepticus when compared with the control groups. There were no significant between-group differences at the longer survival time and the results from the status epilepticus group did not differ significantly with survival time (P > 0.05).

**Antidromic stimulation evoked synaptically mediated field responses and delayed bursts in the dentate gyrus of some slices from both status epilepticus and control groups**

We and others have recorded complex antidromically evoked field responses from the granule cell body layer in some hippocampal slices from epileptic brain (Cronin et al. 1992; Masukawa et al. 1992; Patrylo and Dudek 1998; Tauck and Nadler 1985). These responses, consisting of repetitive population spikes, were not observed in the present study whether or not a GABA antagonist was present. In many instances, the field response consisted simply of an antidromic
population spike, and GABA and glutamate antagonists were without effect (Fig. 8, right). In other instances, most commonly in slices from the long-term status epilepticus group, a small negative deflection followed the antidromic spike (Fig. 8, left). The peak of this negative wave corresponded in time to the peak of the EPSC when an antidromically evoked EPSC was present in the recorded cell. GABA antagonists had variable effects, if any, on this component of the response, but DNQX/D-AP5 or NBQX either abolished it or markedly reduced its amplitude. In addition, when antidromic stimulation evoked a delayed inward current in the recorded cell, it usually also evoked a delayed burst in the extracellular record. The burst consisted of a biphasic shift in extracellular potential (negative-positive) on which small negative deflections were superimposed. The peak negativity of the burst occurred approximately 100 ms after the stimulus. A delayed field burst was observed in all six slices from the long-term status epilepticus group in which a delayed inward current was recorded and in all but one such slice from each of the long-term treated control, short-term treated control and short-term untreated control groups. When examined 10 wk after pilocarpine-induced status epilepticus, all sections cut from slices adjacent to those used for electrophysiological recording exhibited relatively normal histology. Preservation of the normal lamination in area CA3 facilitated accurate placement of the antidromic stimulating electrode. The only obvious histological abnormality was a substantial loss of hilar neurons. Cell counts revealed a 50% reduction in their density both 4–6 days and 10 wk after treatment (Fig. 13). There was also a significant age-related reduction of hilar neuron density irrespective of treatment. The latter effect could be accounted for by a corresponding increase in the cross-sectional area of the hilus with age.

Responses to perforant path stimulation were unaffected by the prior development of status epilepticus

Electrical stimulation of the perforant path where it crosses the subiculum consistently evoked an EPSC with a latency to onset of ~4–5 ms and a feed forward GABA_A IPSC with a latency to onset of ~7–12 ms (Fig. 9). Pilocarpine-induced status epilepticus did not significantly change the mean peak amplitude, half-width, or latency to onset of the AMPA-kainate component of the EPSC, the NMDA component of the EPSC, or the feed forward GABA_A IPSC (Fig. 10). However, the peak amplitude of the feed forward IPSC, like that of the feedback IPSC, declined significantly with age.

Pilocarpine-induced status epilepticus killed about half the hilar neurons in the caudal hippocampal formation

Silver impregnation performed 1 day after pilocarpine-induced status epilepticus revealed widespread neuronal degeneration in many brain regions, including layers 2 and 3 of the somatosensory neocortex, layers 5 and 6 of the cingulate cortex, the entire pyriform cortex, the lateral septum, the claustrum-insula region, and several nuclei of the amygdala and thalamus. This distribution of damage is consistent with previous reports (Clifford et al. 1987; Turski et al. 1983). Within the hippocampal formation, neuronal degeneration was less extensive than in most other vulnerable brain regions. Argyrophilic (degenerating) granule and pyramidal cells were found scattered and in small clusters throughout the cell body layers (Fig. 11). Degenerating granule cells were most numerous at the apex of the granule cell arch. Numerous argyrophilic neurons were present in the dentate hilus. Silver granules, indicative of terminal degeneration, densely filled the inner third of the dentate molecular layer, but there was little evidence of terminal degeneration in the perforant path zone. We found no evidence of somatic or terminal argyrophilia in pilocarpine-treated control rats.
Pilocarpine-induced status epilepticus increased the density of supragranular mossy fiber-like Timm stain

As reported previously (Okazaki et al. 1995), pilocarpine-induced status epilepticus led to robust recurrent mossy fiber growth as demonstrated by Timm histochemistry. Except in some animals with the most extensive growth (e.g., Fig. 14, D–F), Timm staining of the supragranular zone appeared denser in the infrapyramidal blade of the dentate gyrus than in the suprapyramidal blade. Supragranular mossy fiber-like Timm stain was greatest at the caudal pole of the dentate gyrus and was virtual absent from sections more than \(\sim 400 \mu m\) rostral to the level at which electrophysiological recordings were made. These observations are consistent with previous descriptions of Timm histochemistry in normal rats (Gaarskjaer 1978; Haug 1974). Timm staining of the supragranular zone in treated control rats was indistinguishable from Timm staining in untreated controls.

**DISCUSSION**

Whole cell patch-clamp recordings in pilocarpine-treated rats revealed the following. 1) Antidromic stimulation of the mossy fibers evoked an EPSC in many dentate granule cells from control rats. 2) The same stimulus evoked an EPSC in a much higher percentage of granule cells from rats that exhibited recurrent mossy fiber growth and, in these instances, the peak amplitude of the response tended to be greater. 3) The antidromically evoked EPSC was glutamatergic and had both AMPA-kainate and NMDA components. The NMDA component of the response tended to be considerably greater in rats with recurrent mossy fiber growth. 4) The peak amplitude of the antidromically evoked IPSC was 40% less than control 4–6 days after status epilepticus and not significantly different from control in long-term survivors. However, control values declined with age. 5) In contrast to the antidromically evoked EPSC, neither the AMPA/kainate nor the NMDA component of the perforant path EPSC appeared to be affected by pilocarpine-induced status epilepticus. However, the size of the feed forward IPSC, like that of the feedback IPSC, declined with age.

Antidromically evoked EPSCs in rats with recurrent mossy fiber growth

These responses differed from controls in three ways: they were detected more frequently, the peak amplitude of 36% of them was greater than the peak amplitude of the largest EPSC recorded from any control granule cell, and the NMDA component was generally much larger. A key finding was that neither the probability of evoking an EPSC nor the peak amplitude of the EPSC tended to be enhanced 4–6 days after pilocarpine administration. At this survival time, Timm histochemistry revealed no evidence of recurrent mossy fiber growth. Another important finding was that the effects were specific to the antidromically evoked EPSC; the perforant path EPSC remained unchanged.

Our results are consistent with previous reports on the functional consequences of recurrent mossy fiber growth. Dentate granule cells become hyperexcitable in response to afferent stimulation both in vivo (Buckmaster and Dudek 1997a) and in slice preparations (Cronin et al. 1992; Franck et al. 1995; Masukawa et al. 1992; Patrylo and Dudek 1998; Tauck and Nadler 1985; Williamson et al. 1995). Field recordings in vivo revealed development of a novel current sink in the inner
portion of the molecular layer, the major site of recurrent mossy fiber synapses, in kindled rats with mossy fiber growth (Golarai and Sutula 1996). A $\kappa$-opioid receptor agonist, which inhibits the release of glutamate from mossy fiber terminals, reduced the amplitude of granule cell population spikes in hippocampal slices with supragranular mossy fibers (Simmons et al. 1997). Wuarin and Dudek (1996) reported that microdrop application of glutamate to the granule cell body layer increased the frequency of spontaneous EPSPs in granule cells. Similarly, we reported that laser-evoked photolysis of caged glutamate in the granule cell body layer evoked an apparently unitary EPSC in granule cells (Molnár and Nadler 1997). The basal frequency of spontaneous EPSCs was reported to be substantially greater in granule cells from hippocampal slices with supragranular mossy fibers (Simmons et al. 1997). In the presence of bicuculline, EPSCs of unusually high-amplitude and long duration ("giant" EPSCs) were observed both spontaneously and during stimulation of the perforant path. It was suggested that the giant perforant path-evoked EPSPs resulted from disynaptic activation of mossy fiber-granule cell synapses.

Thus present and past findings can be explained by formation of mossy fiber-granule cell synapses after prolonged or repeated seizures. Lack of correlation between the size of the antidromically evoked EPSC and the extent of recurrent mossy fiber growth, as indicated by Timm histochemistry, does not argue against this conclusion. Timm stain density should correlate with measures of population activity, but not necessarily with responses of an individual granule cell. The only other excitatory input to the dentate granule cells reported to be activated by stimulation of the CA3 area is the dentate associational pathway (Scharfman 1994a, 1996). Activation of granule cell EPSCs through this disynaptic pathway requires stimulus-evoked action potentials in hilar mossy cells. Thus one reason to discount the possibility that an expanded associational projection contributed to the antidromically evoked EPSC is that limbic status epilepticus is believed to kill the majority of hilar mossy cells (Scharfman and Schwartzkroin 1990; Sloviter 1987). Consistent with this idea, degeneration of hilar neurons in the present study was associated with dense terminal argyrophilia in the inner third of the dentate molecular layer, the region to which the associational pathway projects.

The wide variation in peak amplitudes of the antidromically evoked EPSC may arise at least partly from similar variability in mossy fiber terminal morphology. The largest of these terminals in the inner third of the dentate molecular layer is $\sim2 \mu m$ in diameter (Okazaki et al. 1995). Three or four of these large terminals form a collar around the granule cell dendrite and each terminal forms multiple synaptic contacts. Activation of such synaptic complexes may have generated the large amplitude antidromically evoked EPSCs observed in this study, as well as the giant spontaneous EPSCs observed by

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<td>2.2</td>
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<tr>
<td>31.2</td>
<td>7.1</td>
<td>1,167</td>
<td>636</td>
<td>0.5</td>
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<tr>
<td>20.9</td>
<td>19.7</td>
<td>1,251</td>
<td>3,285</td>
<td>2.6</td>
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<tr>
<td>41.7</td>
<td>4.8</td>
<td>2,597</td>
<td>566</td>
<td>0.2</td>
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</tr>
<tr>
<td>39.0</td>
<td>9.3</td>
<td>1,883</td>
<td>872</td>
<td>0.5</td>
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</table>

Each row of values was obtained from a single dentate granule cell, each cell from a different rat. KA, kainate; NMDA, N-methyl-D-aspartate.
Two-way analysis of variance yielded

<table>
<thead>
<tr>
<th>Survival Time</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>4–6 Days</td>
<td></td>
</tr>
<tr>
<td>Status epileptic</td>
<td>198 ± 29* (19)</td>
</tr>
<tr>
<td>Treated controls</td>
<td>394 ± 38 (17)</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>361 ± 62 (10)</td>
</tr>
<tr>
<td>10+ Weeks</td>
<td></td>
</tr>
<tr>
<td>Status epileptic</td>
<td>213 ± 82 (13)</td>
</tr>
<tr>
<td>Treated controls</td>
<td>138 ± 22 (14)</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>113 ± 20† (15)</td>
</tr>
</tbody>
</table>

Values (in pA) are means ± SE for the number of responses in parentheses. Two-way analysis of variance yielded $P < 0.001$ for the effect of survival time and $P < 0.002$ for the interaction between survival time and treatment. *$P < 0.01$ compared with treated controls and $P < 0.05$ compared with untreated controls at the same survival time (Newman-Keuls test). †$P < 0.01$ compared with the same treatment at a survival time of 4–6 days (Newman-Keuls test).

UNITED EPSCS generated distally will be distorted by filtering through the membrane time constant to a greater extent than those generated proximally. The greater the contribution of distal synapses, the slower the time course and the smaller the peak amplitude. A final consideration is variation in the percentage of stimulated mossy fibers that remain within the plane of the slice until they reach the fascia dentata. One might predict that the higher the percentage of these fibers activated, as reflected by the size of the antidromic population spike, the larger the antidromically evoked EPSC would be. However, we found no correlation between the peak amplitudes of the antidromic population spike and antidromically evoked EPSC. Hence differences in mossy fiber activation appear to be weakly contributory at most.

Our findings that NMDA receptors contribute to the antidromically evoked EPSC and that pilocarpine-induced status epilepticus augments the size of the NMDA component are of particular interest considering the well-established role of NMDA receptors in epileptic phenomena (Dingledine et al. 1990; Traub and Jeffreys 1994). Because NMDA-receptor-mediated EPSCs could be evoked in the presence of an AMPA/kainate receptor antagonist, these responses were presumably monosynaptic and resulted from the activation of recurrent mossy fibers. We have confirmed that the unitary mossy fiber-granule cell EPSC has an NMDA component (Molnár and Nadler 1997). NMDA receptors also contribute to the mossy fiber EPSC recorded in CA3 pyramidal cells (Weisskopf and Nicoll 1995) and dentate basket cells (Kneisler and Dingledine 1995). The synaptic NMDA current measured at a holding potential of $-30$ mV was ordinarily a small fraction of the synaptic AMPA/kainate current measured at $-80$ mV. However, the relative size of the NMDA current increased several-fold in most instances after pilocarpine-induced status epilepticus. This change probably did not reflect upregulation of NMDA receptors throughout the granule cell because the NMDA and AMPA/kainate components of the perforant path EPSC appeared to be unaffected. In fact, in five of six cells, NMDA receptors came to play as prominent a role in antidromically evoked excitatory transmission as they do in perforant path transmission. Interestingly, Patrylo and Dudek (1998) reported that d-AP5 either blocked antidromically evoked bursting in slices from rats with kainate-induced recurrent mossy fiber growth or reduced its probability of occurrence. Recurrent NMDA receptor activation may play a significant role in seizure propagation under the depolarizing conditions that others (Simmons et al. 1997). However, many recurrent mossy fiber boutons are much smaller than 2 μm and appear to form synaptic contact with only a single spine. Unitary synaptic currents are expected to vary with the number of release sites. The often complex morphology of the antidromically evoked EPSC suggests that polysynaptic activity in the recurrent mossy fiber pathway also contributes. Antidromic activation of granule cells would be expected to provoke reverberating excitation. Thus many of the EPSCs we recorded probably included both a monosynaptic component from direct activation of granule cells presynaptic to the recorded cell and polysynaptic components from granule cells that were activated synaptically by the stimulus. The variable amplitude, slow time course and complex morphology of the antidromically evoked EPSC also may reflect the asynchronous discharge and broad dispersion of the mossy fiber-granule cell synapses. Three pieces of evidence support this possibility. First, granule cells appear to become innervated by other granule cells located both nearby and some distance away (Okazaki et al. 1995; Sutula et al. 1998). Furthermore, the recurrent mossy fibers often branch from the parent axon deep within the hilus, several hundred micrometers from the target cells. Thus conduction time would be expected to differ considerably for the different mossy fibers activated. Second, Langdon et al. (1993) suggested that action potentials typically invade mossy fiber boutons after a variable delay due to the variable impedance mismatch between the thin mossy fiber axon and the morphologically diverse boutons. Even if action potentials arrive at two recurrent mossy fiber boutons simultaneously, they may release transmitter asynchronously. Third, mossy fiber-granule cell synapses can be located on a rather large percentage of the dendritic tree particularly in cases of robust recurrent growth.

TABLE 3. Peak amplitudes of the antidromically evoked GABA-mediated IPSC

<table>
<thead>
<tr>
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FIG. 7. Cumulative response distributions of feedback GABA A-receptor-mediated IPSCs for the short- and long-term survival groups. Refer to Fig. 2 for details. Note that feedback IPSCs were smaller than control 4–6 days after pilocarpine-induced status epilepticus and that these responses in granule cells from control rats declined in amplitude with age.
exist when granule cells are bombarded by high-frequency activity of the perforant path.

Pilocarpine-induced status epilepticus did not alter the current-voltage relationship of the NMDA component, suggesting that there was no marked change in the ability of Mg\(^{2+}\) to block the NMDA channel. In this respect, the pilocarpine model differs from the kindling model, in which Mg\(^{2+}\) has reduced ability to block NMDA channels in dentate granule cells (Köhr et al. 1993).

**FIG. 8.** Antidromic stimulation evoked synaptically mediated field responses in some slices. Recordings are from 2 rats that had survived >10 wk after administration of pilocarpine. Left: in a granule cell from a rat that had developed status epilepticus, antidromic stimulation in the presence of bicuculline evoked a short-latency negative deflection followed by a small delayed burst. Negative deflection was attenuated and the delayed burst was eliminated by addition of NBQX to the superfusion medium, indicating that these responses were mediated by activation of glutamate synapses. Right: in a granule cell from a pilocarpine-treated control rat studied under the same conditions, no synaptic response can be seen in the field recording. *, antidromic population spike.

**Antidromically evoked EPSCs in control rats**

There is currently no published anatomic evidence for the presence of mossy fiber-granule cell synapses in normal rat hippocampal formation. Thus we were surprised to record an antidromically evoked EPSC in many granule cells from control rats. In a few instances, the EPSC was followed by a

**FIG. 9.** Postsynaptic currents evoked by perforant path stimulation in representative granule cells from a rat that survived >10 wk after pilocarpine-induced status epilepticus (top), from a rat treated with pilocarine that did not develop status epilepticus (middle), and from an age-matched, untreated rat (bottom). Components of the response were isolated as described in METHODS. KA, kainate. No consistent treatment-related differences were evident in these experiments.
delayed inward current and field burst. When using antidromic stimulation to study the recurrent mossy fiber pathway, one is concerned about the possibility of activating other afferent pathways. As stated before, the only other excitatory input to the dentate granule cells reported to be activated in this manner is the associational pathway. Electrically evoked firing of CA3 pyramidal cells drives hilar mossy cells (Scharfman 1994b), which innervate dentate granule cells (Scharfman 1995). Antidromic stimulation of the mossy fiber pathway in area CA3b inevitably activates some CA3 pyramidal cells, and for technical reasons, we could not differentiate a monosynaptic recurrent mossy fiber EPSC from a polysynaptic (CA3 pyramidal cell-hilar mossy cell-dentate granule cell) associational EPSC on the basis of onset latency. For several reasons, however, we do not think that the associational input contributed in a major way to the antidromically evoked EPSC. First, every antidromically evoked EPSC tested had an NMDA component. Again, because these responses were evoked in the presence of an AMPA/kainate receptor antagonist, they must have been activated monosynaptically and thus could not have originated from the polysynaptic associational pathway. Scharfman (1994a) confirmed that blockade of AMPA/kainate receptors prevents the activation of mossy cells by input from area CA3. Second, we could evoke apparently unitary EPSCs in some granule cells from both pilocarpine-treated control rats (Molnár and Nadler 1997) and age-matched untreated controls (Molnár and Nadler, unpublished observations) by laser-evoked photolysis of caged glutamate in the granule cell body layer. Finally, we have not been able to evoke an EPSC in granule cells from control rats by uncaging glutamate within the dentate hilus in the presence of bicuculline. At least half the hilar interneurons are mossy cells (Amaral 1978), and these neurons are highly excitable due to their low spike threshold (7–8 mV from resting $V_m$), small IPSPs, and high-input resistance (Scharfman and Schwartzkroin 1988). Nevertheless, we did not activate a mossy cell-granule cell connection in ~600 attempts; the probability of evoking an EPSC by photolysis in the granule cell body layer with use of the same laser power and pulse duration was much higher. Although these considerations do not eliminate the possibility that the associational pathway contributed to the antidromically evoked EPSC in some instances, they suggest that recurrent mossy fibers played the major role.

Previous electrophysiological tests for mossy fiber-granule cell synapses in hippocampal slices from control rats have produced negative results. In contrast to our work, Wuarin and Dudek (1996) reported that neither antidromic stimulation of the mossy fibers nor glutamate microstimulation in the granule cell body layer evoked an excitatory synaptic response in dentate granule cells. There is no obvious explanation for these discrepant findings. However, Timm histochemistry suggests
that the location in the hippocampal formation from which the slice was taken may be a crucial variable. In our study, slices were prepared from a portion of the caudal hippocampal formation in which clusters of mossy fiber-like Timm staining normally were present in the supragranular zone. These clusters were most numerous at the caudal pole of the dentate gyrus and virtually disappeared at a level just rostral to that at which our recordings were made. They presumably indicate the presence of closely spaced recurrent mossy fiber boutons at those locations. Although we do not know whether the postsynaptic targets of these presumptive recurrent mossy fibers are, in fact, granule cells, our electrophysiological data support the hypothesis that at least some of them are. Previous studies may have utilized hippocampal slices from more rostral levels of the hippocampal formation, where supragranular mossy fibers appear to be scarce.

Thus we suggest that the recurrent mossy fiber pathway, previously thought unique to the epileptic brain, represents expansion of a minimal pathway already present in the caudal dentate gyrus. The putative existence of mossy fiber-granule cell synapses in normal rats requires confirmation by electron microscopy.

**Antidromically evoked field responses**

Previous reports have associated recurrent mossy fiber growth with antidromically evoked repetitive population spikes, delayed field bursts, and spontaneous bursting (Cronin et al. 1992; Masukawa et al. 1992; Patrylo and Dudek 1998; Tauck and Nadler 1985; Wuarin and Dudek 1996). These responses were postulated to have resulted in large part from the establishment of reverberating excitation in the dentate gyrus. Field recordings provided evidence of enhanced population activity in the present study as well. These responses consisted of a glutamate-mediated field EPSP with or without a delayed field burst. Both components of the field response coincided in time with an antidromically evoked glutamatergic synaptic current. The delayed burst, like the delayed inward current, appeared at variable latencies and after some stimulus pulses but not others. These long-latency responses have been attributed to the unmasking by GABA_A receptor antagonists of a polysynaptic recurrent excitatory circuit (Cronin et al. 1992).

**FIG. 13.** Counts of hilar neurons in sections stained with cresyl violet revealed loss of about half these cells after pilocarpine-induced status epilepticus. Values are means ± SE for the number of animals given in Table 1. Two-way ANOVA revealed significant effects of both treatment and survival time (P < 0.001) with no significant interaction between these variables (P > 0.2). Effect of survival time on hilar neuron density was accounted for by expansion of the dentate hilus with age. See METHODS for explanation of the cell counting procedure.

**FIG. 14.** Localization of mossy fiber boutons with the Timm stain >10 wk after pilocarpine administration in 1 rat that did (D–F) and in another rat that did not (A–C) develop status epilepticus. A: Timm histochemistry in a section from the treated control rat shows little mossy fiber-like staining in the inner third of the dentate molecular layer (↓). Clusters of mossy fiber-like Timm staining are present in this layer and are more numerous in the infrapyramidal blade (C) than in the suprapyramidal blade (B). D: Timm histochemistry in a section from the rat that had developed status epilepticus demonstrates mossy fiber growth into the inner third of the molecular layer (↓). Because mossy fiber growth was quite robust in this animal, there is not an obvious difference in Timm stain density between the infrapyramidal (F) and suprapyramidal (E) blades. Scale bars, 0.5 mm (A and D) and 0.1 mm (B, C, E, and F).
Antidromically evoked synaptic field responses were observed in rats from both status epilepticus and control groups, but more commonly from the long-term status epilepticus group. This observation supports the view that the extracellularly recorded antidromically evoked synaptic responses depended on the presence of mossy fiber-granule cell synapses. However, synaptically evoked field responses were observed in only a minority of slices, even in those with robust mossy fiber growth. We attribute this relatively meager evidence of reverberating excitation to recording at room temperature, which would be expected to reduce neuronal excitability. When recordings were made at a temperature of 32°C, we often recorded complex antidromically evoked field responses in slices from pilocarpine-treated rats (Okazaki and Nadler 1994). Previously published recordings of similar antidromically driven waveforms from slices of epileptic hippocampus also were made at 32–35°C.

Feedback and feed forward IPSCs

The GABA_\textsubscript{A}-mediated feedback IPSC was depressed substantially 4–6 days after pilocarpine-induced status epilepticus. This result was expected because 40% of hilar GABA neurons were reported to degenerate under these conditions (Obenaus et al. 1993). Silver impregnation and cell counts also indicated degeneration of about half the hilar neurons, although we do not know what proportion of these were GABA neurons as opposed to mossy cells. Conversely, the GABA_\textsubscript{A}-mediated feed forward (perforant path-activated) IPSC was unaffected. These results are consistent with the limited information we have about the vulnerability of different GABA neuronal subtypes to prolonged seizures. The “HIPP” cells (cell body located in the hilus, axonal projection to the perforant path terminal zone), a type of GABA interneuron that also contains somatostatin and neuropeptide Y, are highly vulnerable to seizures (Buckmaster and Dudek 1997b; Lurton and Cavalezhiro 1997; Mathern et al. 1995; Sloviter 1987). Because the dendrites of the HIPP cell remain confined predominantly to the hilus (Freund and Buzsáki 1996), these cells are thought to mediate feedback, but not feed forward, inhibition. The dentate basket cells and “MOPP” cells (cell body located in the molecular layer, axonal projection to the perforant path terminal zone) remain intact after status epilepticus (Buckmaster and Dudek 1997b; Obenaus et al. 1993; Sloviter 1987). The dendrites of both cell types receive direct innervation from the perforant path and participate in feed forward inhibition (although basket cells also mediate feedback inhibition).

The between-group difference in peak amplitude of the feedback IPSC had disappeared within 10 wk after pilocarpine administration. However, this change with survival time resulted not from restoration of feedback inhibition in the status epilepticus group but rather from a marked reduction of peak IPSC amplitude in the control groups. The size of the antidromic population spike tended to be smaller in the slices from older rats. Thus the antidromic stimulus may have provided a weaker drive for activation of GABA neurons in the feedback circuit. However, for the treated controls, the threefold reduction in peak IPSC amplitude with survival time appears disproportionate to the 20% reduction in peak antidromic population spike amplitude. Furthermore the peak amplitude of the feed forward IPSC also was reduced substantially. This loss of GABA synaptic function in granule cells may have either a biological or technical explanation. There is some evidence for a loss of GABA neurons with aging in the rat hippocampal formation (Shetty and Turner 1998). Some of the GABA neurons that die off during the aging process may be of the same types that are killed by pilocarpine-induced status epilepticus. Alternatively, GABA neurons of the dentate gyrus may survive slice preparation less well as animals grow older.

Several lines of evidence suggest that some form of granule cell inhibition is enhanced in rats after pilocarpine-induced or kainic acid-induced status epilepticus. Buckmaster and Dudek (1997a,b) found enhanced paired-pulse inhibition and an increased threshold for maximal dentate activation with stimulation of the perforant path in vivo. In addition, GABA_\textsubscript{A} receptor current density in acutely dissociated granule cells was 78% greater than control, indicating enhanced expression of those receptors (Gibbs et al. 1997). Conversely, Isokawa (1996) found no difference from control in the peak amplitude of the feed forward IPSC during low-frequency stimulation of the perforant path in hippocampal slices. We confirm the latter result and further report no lasting between-group difference in the feedback IPSC either. These findings do not necessarily contradict reports of enhanced inhibition in vivo, however. Peak amplitude of the IPSC evoked at one standard stimulus intensity serves only as a crude measure of synaptic inhibition. Subtle changes easily could have been missed. For example, high-frequency stimulation of the perforant path at a holding potential of \(-30\) mV (to maximize NMDA current) was reported to reduce the size of the GABA_\textsubscript{A}-mediated IPSC specifically in granule cells from rats that had been made epileptic with pilocarpine (Isokawa 1996). Furthermore, slice preparation removes much of the normal inhibitory circuitry of the dentate gyrus. Thus our stimuli activated only a portion of the granule cell inhibition that is present in the intact animal. Finally the additional GABA_\textsubscript{A} receptors reported to be expressed by dentate granule cells from epileptic brain may not have been activated by the stimulation protocols we used. One possibility is that they are present mainly at extrasynaptic sites and are activated only by stimulus trains.

Although our results support the hypothesis that seizure-induced mossy fiber growth mediates recurrent excitation, they reveal little about its effect on GABA inhibition. Relative to the controls, feedback inhibition increased with survival time after status epilepticus. At first glance, this finding might be regarded as evidence of compensation for the seizure-induced loss of hilar GABA neurons, perhaps through the formation of new mossy fiber-GABA neuron and/or GABA neuron-granule cell synapses. This interpretation would be unjustified, however, because the change resulted not from enhancement of the IPSC in the status epilepticus group but rather from a diminished IPSC in the control groups. Our results neither support nor refute the possibility that seizure-induced mossy fiber growth drives GABA inhibition, as others have suggested (Kotti et al. 1997; Sloviter 1992). Further studies of this issue need to consider the possibility of time-dependent changes in the controls.

Short-term effects of pilocarpine-induced status epilepticus

Antidromic stimulation evoked only a single small EPSC in 19 granule cells tested 4–6 days after pilocarpine-induced
status epilepticus. This frequency of success was significantly less than in either control group, suggesting a reversible loss of connectivity. Mossy fiber synapses may have become anatomically or functionally disconnected from postsynaptic granule cells during the period of mossy fiber growth. There is ample precedence for a reversible loss of excitatory afferent synapses on peripheral neurons during regrowth of their axons after axotomy (e.g., Mendell et al. 1976; Purves 1975). In addition, 30% of perforant path synapses were lost transiently after status epilepticus induced by intracerebroventricular administration of kainic acid (Nadler et al. 1980). The apparent disconnection of synapses occurred in the absence of overt terminal degeneration. A similar process may account for transient loss of the antidromically evoked EPSC in the present study. However, our results do not suggest any disconnection of perforant path synapses in this instance. Silver impregnation revealed degeneration of some dentate granule cells within a day after status epilepticus. Therefore loss of the antidromically evoked EPSC may be explained to some degree by degeneration of presynaptic granule cells. To the extent that the associational pathway contributes to the antidromically evoked EPSC, seizure-induced degeneration of hilar mossy cells also may play a role.

The input resistance of granule cells increased reversibly after pilocarpine-induced status epilepticus. The molecular basis of this change requires further investigation. It probably cannot be explained by reduced resting $K^+$ conductance, because $K^+$ channels were blocked with intracellular Cs$^+$. Along with degeneration of presynaptic GABA neurons, increased membrane resistance may contribute to hyperexcitability of the dentate gyrus during the first few days after status epilepticus. However, this result requires confirmation under recording conditions that do not alter the intracellular milieu.

Relation to epileptic phenomena

Rats that develop status epilepticus after administration of pilocarpine invariably become epileptic, that is, they exhibit unremitting spontaneous seizures (Lemos and Cavalheiro 1996; Mello et al. 1993). The density of supragranular mossy fiber-like Timm staining has been correlated with the development of spontaneous seizures although not with seizure frequency. Our results support the hypothesis that expansion of the recurrent mossy fiber pathway increases the excitatory drive to dentate granule cells and thus facilitates repetitive synchronous discharge. Given that dentate granule cells are normally difficult to recruit into epileptiform activity and thus present a barrier to seizure propagation, recurrent mossy fiber growth may contribute to epileptogenesis in the pilocarpine-treated rat. Although the pilocarpine model differs from human temporal lobe epilepsy in some respects (e.g., extent of cell death in area CA1 and in neocortical layer 2), both are characterized by extensive loss of hilar neurons and recurrent mossy fiber growth. Some studies have reported hyperexcitable electrophysiological responses in the dentate gyrus of hippocampi resected for pharmacologically intractable temporal lobe epilepsy that could be explained by expanded recurrent excitatory circuitry (Franck et al. 1995; Masukawa et al. 1992). However, recurrent mossy fiber growth is probably neither necessary nor sufficient for epileptogenesis. Timm-positive supragranular mossy fibers are present in aged, nonepileptic human hippocampi (Cassell and Brown 1984), but not in all persons with temporal lobe epilepsy (Franck et al. 1995). In addition, administration of cycloheximide before pilocarpine appears to suppress mossy fiber growth without effect on the development of recurrent spontaneous seizures (Longo and Mello 1997). The circumstances under which recurrent mossy fiber growth contributes to epileptogenesis remain to be defined.

The existence of mossy fiber-granule cell synapses in the normal brain, if confirmed anatomically, also must be considered with regard to seizure propagation. By analogy with research on area CA3, these connections, though sparse, would be expected to promote the synchronization of granule cell discharge when inhibition is compromised. Thus mossy fiber-granule cell synapses may play some role in epileptic events even in the absence of mossy fiber growth, for example when seizures originate from a mass lesion or vascular malformation outside the hippocampal formation. It may not be coincidental that supragranular mossy fibers are most numerous at the caudal end of the hippocampal formation (homologous to the human anterior hippocampus), which also has a particularly low threshold for epileptiform discharge (Bragdon et al. 1986; Gilbert et al. 1985; Racine et al. 1977).

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