Network Interactions Mediated by New Excitatory Connections Between CA1 Pyramidal Cells in Rats With Kainate-Induced Epilepsy

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Smith, Bret N. and F. Edward Dudek. Network interactions mediated by new excitatory connections between CA1 pyramidal cells in rats with kainate-induced epilepsy. J Neurophysiol 87: 1655–1658, 2002; 10.1152/jn.00581.2001. Axon sprouting and synaptic reorganization in the hippocampus are associated with the development of seizures in temporal lobe epilepsy. Synaptic interactions among CA1 pyramidal cells were examined in fragments of hippocampal slices containing only the CA1 area from saline- and kainate-treated rats. Glutamate microapplication to the pyramidal cell layer increased excitatory postsynaptic current (EPSC) frequency, but only in rats with kainate-induced epilepsy. In bicuculline, action potentials evoked in single pyramidal cells increased the frequency of network bursts only in slices from rats with kainate-induced epilepsy. These data further support the hypothesis that excitatory connections between CA1 pyramidal cells increase after kainate-induced status epilepticus.

INTRODUCTION

Kainate treatment causes prolonged seizure activity (i.e., status epilepticus) and leads to the development of spontaneous recurrent seizures (e.g., Ben-Ari 1985; Hellier et al. 1998; Nadler et al. 1981; Stafstrom et al. 1992). Neuronal loss and synaptic reorganization have been studied in the dentate gyrus, where mossy fibers appear to form new synapses (Lynch and Sutula 2000; Wuarin and Dudek 1996, 2001). The hippocampal CA1 region may also undergo synaptic reorganization during chronic epileptogenesis (Esclapez et al. 1999; Meier and Dudek 1996; Smith and Dudek 2001). After kainate-induced neuronal loss, asymmetrical synapses on CA1 pyramidal cells increased in number (Nadler et al. 1980). Intracellular staining of CA1 pyramidal cells has shown that their axons become more highly branched after status epilepticus (Esclapez et al. 1999; Perez et al. 1996; Smith and Dudek 2001). The isolated CA1 area from rats with kainate-induced epilepsy could generate afterdischarges of repetitive, all-or-none bursts of action potentials in bicuculline, while graded bursts were observed in slices from control rats and shortly after kainate treatment (Meier and Dudek 1996; Smith and Dudek 2001). However, the new axon collaterals of CA1 pyramidal cells have not been shown to be connected to other pyramidal cells, and paired recordings have failed to detect increased excitatory synapses between CA1 pyramidal cells after intraventricular kainate (Nakajima et al. 1991) or systemic kainate or pilocarpine treatment (Esclapez et al. 1999). Using slices from kainate-treated rats, we examined changes in network interactions in the CA1 area. Our results support the hypothesis that functional connectivity between CA1 pyramidal cells is increased in rats with kainate-induced epilepsy.

METHODS

Kainate treatment

Animals were housed in a vivarium under normal 12-h light/12-h dark cycle. All procedures used in the study adhered to guidelines approved by the Colorado State University Animal Care and Use Committee. The treatment of rats has been described previously, and the animals used herein are a subset of those for which seizure behavior and some aspects of structural and functional changes in the CA1 area and the dentate gyrus have been reported (Hellier et al. 1998; Smith and Dudek 2001; Wuarin and Dudek 2001). Briefly, adult male Sprague-Dawley rats (Harlan; 200–250 g) were given hourly intraperitoneal (ip) injections of kainate (5 mg/kg in 150 mM NaCl; 30–35 mg/kg total; Sigma, St. Louis, MO). Each animal had ≥4 h of recurring seizure activity before the treatment was stopped. Control rats were injected hourly with the vehicle in parallel with the kainate-treated rats. Following the kainate treatment, the behavior of both control and kainate-treated rats was monitored for 1–2 h/day, 3–5 days/wk (minimum of 6 h/wk) over a period of 8–13 mo to determine if the treatment induced spontaneous, recurrent seizures (i.e., chronic epilepsy). After a period of several weeks, all kainate-treated rats in this study experienced spontaneous seizures and had histological and electrophysiological characteristics associated with temporal lobe epilepsy (TLE) (Hellier et al. 1998; Smith and Dudek 2001).

Tissue preparation and recordings

Transverse slices of temporal hippocampus (400–500 μM) were prepared, and field-potential and whole-cell recordings were obtained from pyramidal neurons in fragments containing the CA1 area, which was isolated by knife cuts. An extracellular electrode containing 1 M NaCl was placed in the pyramidal cell layer. Field responses were recorded using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Whole-cell patch-clamp recordings were made from CA1 pyramidal cells using pipettes filled with the following (in mM): 130 K+-gluconate, 1 NaCl, 5 EGTA, 10 HEPES, 1 MgCl₂, 1 CaCl₂, 3 KOH, 2–4 ATP, pH 7.2. Whole-cell signals were recorded using an Axopatch 1D amplifier (Axon Instruments). Signals were low-pass...
filtered at 2-5 kHz, digitized at 44 kHz (Neuro-corder; Neurodata Instruments, New York, NY), and stored on videotape.

Chemical stimulation of neurons in the pyramidal cell layer was made by pressure-applying glutamate (20 nM; Sigma; 20–50 μM diam drops) through a patch pipette positioned at the surface of the slice. The effectiveness of the glutamate in evoking action potentials was verified by applying the glutamate directly at the tip of the recording pipette to evoke unclamped, rapid voltage-dependent inward currents in the recorded neuron (i.e., the fast Na⁺ currents underlying action potential generation). An unpaired, two-tailed t-test was used to compare data between recordings. A χ² test was used for comparing the occurrence of spontaneous bursts between groups. Regression analysis was made to determine if seizure frequency or number were related to survival time or to the degree of burst potentiation. The mean ± SE are reported unless otherwise noted.

RESULTS

Whole-cell recordings were made in isolated CA1 slices from 8 saline-treated rats, 12 rats used <8 days after kainate treatment, and 14 rats with kainate-induced epilepsy examined 60-233 days after kainate injection. The total number of seizures observed in rats with kainate-induced epilepsy ranged from 10 to 52 (mean 25 ± 5 seizures) and occurred at a rate of 0.50 ± 0.07 seizures/h observed after the first observed spontaneous seizure. The total seizure number was related to the number of days the animals survived after kainate treatment (P < 0.05), but was not related to the frequency of observed spontaneous seizures (seizures/h observed; P > 0.6).

Glutamate microstimulation

Glutamate was applied at three to eight positions 300–1000 μM from the recorded neuron (5 applications per position) in the pyramidal cell layer while recording excitatory postsynaptic currents (EPSCs) at resting potential (i.e., near −65 mV). In normal artificial cerebrospinal fluid (ACSF), glutamate microapplication failed to elicit EPSCs in any of five neurons from saline-treated rats or eight neurons from rats studied <8 days after kainate treatment (Fig. 1). When examined in bicusculine (30 μM), no increases in EPSC frequency were observed for any of 20 application sites (9 neurons, 6 slices) in slices from either saline-treated control rats or from rats examined <8 days after kainate treatment. In contrast, glutamate microapplication to the pyramidal cell layer in ACSF evoked EPSCs from at least one stimulation site in most slices (7 of 9) from each of six rats with kainate-induced epilepsy (Fig. 1). Addition of bicusculine revealed excitatory connections from at least one previously unresponsive site in five slices. Therefore glutamate microstimulation in the CA1 pyramidal cell layer evoked EPSCs in pyramidal cells in slices from rats observed to have spontaneous seizures and studied several weeks after kainate injection, but not in slices from saline-treated control rats or from rats examined <8 days after kainate treatment.

Potentiation of spontaneous network bursts by single-cell stimulation

Spontaneous, synchronous bursts were observed in the CA1 area of kainate-treated rats in bicusculine, and burst frequency increased in nominally Mg²⁺-free ACSF (Smith and Dudek 2001). Trains of action potentials were evoked in recorded neurons with one rectangular depolarizing current pulse (330 ms) or a series of pulses (3-ms duration at 30 Hz for 330 ms). Intracellular stimulations were 0.5–1 Hz for 60–120 s. Spontaneous activity was examined for ≥2 min before and after single-cell stimulation. In five of nine slices from eight rats with kainate-induced epilepsy, the current-evoked spikes significantly increased burst frequency (Fig. 2). Interburst intervals were decreased to a similar extent in both Mg²⁺-free (42 ± 8%) and Mg²⁺-containing ACSF (40 ± 2%). In one slice, synchronous bursts were observed only after single-cell stimulation. In two slices with long-term recordings, the effect partially recovered after 20–35 min. Regression analysis indicated that there was no quantitative relationship between number of observed spontaneous seizures (R = 0.52) or spontaneous seizure frequency (R = 0.03) and changes in burst frequency or increases in EPSC frequency (P > 0.2 for both measures).

Network potentiation could not be obtained in seven slices examined <8 days after kainate treatment (including 2 slices with synchronous bursts) or eight saline-treated controls. Likewise, network potentiation could not be induced (i.e., bursting was not initiated) in saline-treated controls (0/8 slices). In an additional three slices from control animals, spontaneous bursting of individual neurons could be induced by elevating extracellular [K⁺] to 6 mM in the presence of bicusculine and reduced extracellular [Mg²⁺]. Concomitant field potential bursts were not consistently observed in these cells, and the bursts were not sensitive to DNQX, indicating they were not synaptically driven network bursts. The single-cell stimulation paradigm had no effect on spontaneous burst frequency in these cells. Therefore intracellular stimulation of single pyramidal cells potentiated synchronous bursts in slices from rats.
with kainate-induced epilepsy, but not in slices from control animals or in animals examined in the first week after kainate treatment.

**DISCUSSION**

Microstimulation of CA1 pyramidal cells with glutamate evoked EPSCs in other CA1 pyramidal cells in most slices from rats with kainate-induced epilepsy, but not in slices from other rats. Based on previous findings (e.g., Christian and Dudek 1988a), glutamate activated the somatodendritic region of CA1 pyramidal cells but not axons of passage. Previous studies in slices from normal rats (Christian and Dudek 1988b) have indicated that connections between CA1 pyramidal cells are sparse (particularly when compared with the CA3 area), with only about 1% of pyramidal cell pairs being connected monosynaptically (Deuchars and Thomson 1996). Evidence for excitatory connections was rarely observed in control rats or rats examined in the first week after kainate treatment, even in the presence of bicuculline, but interactions were frequently observed months after kainate treatment.

Intracellular stimulation of single CA1 pyramidal cells could increase the frequency of spontaneous network bursts, which also strongly suggests that excitatory connections had formed. One characteristic of a network of interconnected excitatory neurons is that increased activity of one neuron or a small group of neurons in the network can enhance network bursting, particularly when inhibition is depressed (Miles and Wong 1983, 1986, 1987; Traub and Wong 1982). Only modest mono-synaptic connectivity (2–4%) is required (Traub and Wong 1982). Since CA1 pyramid pairs normally have about 1% connectivity (Deuchars and Thomson 1996), single-cell stimulation would not be expected to elicit burst changes in the absence of enhanced connections. Two previous studies found no evidence from dual recordings for increased excitatory connections between CA1 pyramidal cells in epileptic rats (Esclapez et al. 1999; Nakajima et al. 1991), but these negative results may reflect the small number of connections between individual pyramidal cells. Our results suggest that connections between CA1 pyramidal cells are more common in epileptic rats, but they do not indicate the number of connections present or required to activate the population. In fact, only a subtle increase in excitatory connectivity appears necessary to allow the network effects of new recurrent excitatory circuits to be expressed. We previously reported that spontaneous bursting was observed in a minority of slices from kainate-treated rats that had not developed epilepsy or undergone significant CA1 pyramidal cell axon reorganization (Smith and Dudek 2001). Bursts in those animals could not be modified by single-cell stimulation. By inference, burst modification, and perhaps other aspects of the epileptiform activity observed here and elsewhere (Meier and Dudek 1996; Smith and Dudek 2001), appears to be an emergent property of reorganized excitatory circuits among CA1 pyramidal cells.

TLE in humans and animal models is often marked by cell loss in several temporal lobe regions, including the CA1 area (Mathern et al. 1995; Smith and Dudek 2001). Axon sprouting of granule cells and consequent synaptic reorganization in the dentate gyrus is a hallmark of TLE in humans and animal models (Lynch and Sutula 2000; Nadler et al. 1980; Wuarin and Dudek 1996). Enhanced synaptic excitability and pyramidal cell axon sprouting in the CA1 region have been demonstrated in the kainate-treated rat and other models of TLE (Esclapez et al. 1999; Perez et al. 1996; Smith and Dudek 2001). The present evidence that excitatory connections between CA1 pyramidal cells are increased in rats with kainate-induced epilepsy is consistent with the hypothesis that synaptic reorganization occurs throughout the temporal lobe, including areas with substantial loss of pyramidal cells.

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