Altered Dentate Filtering During the Transition to Seizure in the Rat Tetanus Toxin Model of Epilepsy

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Finnerty, G. T., M. A. Whittington, and J.G.R. Jefferys. Altered dentate filtering during the transition to seizure in the rat tetanus toxin model of epilepsy. J Neurophysiol 86: 2748–2753, 2001. The dentate gyrus is thought to be a key area in containing the spread of seizure discharges in temporal lobe epilepsy. We investigated whether it actively contributes to the transition to seizure in vivo using the tetanus toxin chronic experimental epilepsy. Brief epileptic discharges lasted <2 s in freely moving animals and were clearly distinguishable from spontaneous seizures that lasted tens of seconds. This suggested that the changes underlying the transition to seizure started within the first few seconds of seizure onset. During this period, we found that the amplitude of dentate gyrus population spikes decreased initially, but from 1.1 s after seizure onset, they potentiated. The amplitude and number of CA3 population spikes paralleled the pattern found in the dentate gyrus. We used hippocampal slices to study dentate filtering in more detail. The perforant pathway was stimulated repetitively at the frequency of field postsynaptic potentials found during epileptic discharges in vivo. The amplitude of dentate gyrus population spikes decreased to a steady state in naïve hippocampal slices. In hippocampal slices prepared from rats previously injected with tetanus toxin, population spike amplitude decreased transiently and then potentiated. We found that the biphasic profile and rate of potentiation of dentate population spikes in vivo can be reproduced in naïve hippocampal slices by blocking GABAB receptors. We conclude that the filtering properties of the dentate gyrus are altered in the tetanus toxin model of epilepsy and propose how this contributes to the transition to seizure in our animals.

INTRODUCTION

Epilepsy is characterized by recurrent spontaneous seizures. Electroencephalogram (EEG) recording in localization-related (focal) epilepsy syndromes suggests that the brain is not entirely normal between seizures because brief subclinical epileptic discharges such as interictal spikes or polyspikes can frequently be recorded. What causes the transition from brief epileptic discharges to seizures is a poorly understood but important problem.

The dentate gyrus is one area that has been identified as being critical in blocking the propagation of epileptic discharges into the hippocampus (Heinemann et al. 1992). This may be because the dentate gyrus acts as a low-pass filter. Repetitive stimulation ofafferent pathways demonstrates this property clearly. Activation of the perforant pathway at θ (4–8 Hz) frequencies results in potentiation of the granule cell response (Muñoz et al. 1991). In contrast, at higher stimulation rates (>8 Hz) the granule cell response depresses (Alger and Teyler 1976).

The frequency filtering properties of the dentate gyrus are altered in several epilepsy models (Behr et al. 1998; Collins et al. 1983; Heinemann et al. 1992). However, it remains unclear whether the dentate gyrus simply blocks the spread of epileptic discharges once spontaneous seizures in vivo have started or whether it can actively contribute to the generation of seizures. The latter suggestion would be more likely if changes in the dentate gyrus could explain why epileptic brains sometimes generate brief epileptic discharges such as polyspikes, and on other occasions, seizures. Here, we use the tetanus toxin model of epilepsy to investigate whether dentate filtering properties are altered during the early part of spontaneous seizures and how altered dentate filtering may contribute to the transition to seizure.

METHODS

In vivo recordings of seizures

Male Sprague-Dawley rats (280–400 g) were anesthetized with halothane and nitrous oxide. Bipolar recording electrodes (twisted Teflon-coated stainless steel wire, tips 250–350 μm apart) were placed into CA3 and dentate granule cell body layers of both dorsal hippocampi using the evoked response produced by ventral commisural stimulation as a guide (Finnerty and Jefferys 1993). Coordinates were: CA3, 2.7 mm posterior and 3.3 mm lateral to bregma, and dentate gyrus, 3.3 mm lateral and 3.1 mm posterior (Pellegrino et al. 1979).

One microliter phosphate-buffered saline either without (controls) or with 4–5 ng (12 mouse LD50) tetanus toxin (gift from Wellcome Foundation Research Laboratories, Beckenham, Kent, UK) was injected, using a Hamilton 7101N syringe, into the right hippocampus 3.5 mm lateral and 2.7 mm posterior to bregma over 1 min. A dental cement headstage was constructed to protect the recording contact points. Animals were housed separately postoperatively with free access to food and water.

Recording started 3–6 days postoperatively. The headstage contacts were connected, via counterbalanced wires and a slip ring to a preamplifier (D169, input impedance 100 MΩ), gain ×1, Digitimer, Welwyn Garden City, UK) whose output fed into differential amplifiers (Digitimer D160; band-pass, 0.5 Hz–3 kHz). The amplified EEG
signal was stored on FM tape (Racal V Store, Racal, Southampton, UK; band-pass, d.c. –3.25 kHz). Selected recordings were digitized (1401, Cambridge Electronic Design, Cambridge, UK) and analyzed using SPIKE2 (Cambridge Electronic Design). After completion of the recording protocol, each animal was killed with an overdose of halothane. Each brain was dissected out, fixed in formalin, and embedded in wax prior to staining 10-μm parasagittal sections with cresyl fast violet or hematoxylin and eosin to confirm the electrode sites.

Hippocampal slice experiments

Male Sprague Dawley rats (280–400 g) were anesthetized with halothane as described in the preceding text. Tetanus toxin was injected into the right hippocampus at the same coordinates and in the same amount as detailed for the in vivo experiments, and the rats were allowed to recover. Ten to 14 days later, transverse hippocampal slices (400-μm thick) were prepared from the dorsal hippocampi ±1 mm from the injection site or within 1 mm of the point homotopic to the injection site in the uninjected hippocampus. The same part of the dorsal hippocampus was used when we prepared hippocampal slices from naïve rats for pharmacological experiments. The slices were immediately transferred to an interface recording chamber at 34°C (Whittington and Jefferys 1994). The artificial cerebrospinal fluid (ACSF) flowed at 0.5 ml/min and contained (in mM) 135 NaCl, 16 NaHCO3, 3 KCl, 2 CaCl2, 1.25 NaH2PO4, 1 MgCl2, and 10 D-glucose and was equilibrated with 95% O2-5% CO2. A concentric electrode (Clark Electromedical, Reading, UK) was used to stimulate (50-µs, 2- to 80-V square-wave pulses) the perforant path. Extracellular recordings from the suprapyramidal blade of dentate granule layer were made using glass micropipettes filled with 2 M NaCl (resistances, 5–15 MΩ). Stimulation intensities were adjusted to generate a small (2 mV) population spike. In slices prepared from tetanus toxin-injected rats, the presence of afterdischarges after stimulus trains was further monitored with an additional recording electrode in the CA3c pyramidal layer. Stimulus intensities were then reduced to minimize afterdischarges.

Data analysis

We studied dentate filtering during seizures in vivo by measuring the amplitude of the largest dentate gyrus population spike in each burst during the first few seconds of seizures and plotted the results against time after seizure onset. Measurements were restricted to the hippocampus with the larger epileptic field potentials (injected hippocampus, 67% seizures; uninjected hippocampus, 33% seizures). The average time course of population spike amplitude during seizures was calculated by grouping the results from each seizure into 0.5-s bins. The average of each bin for every seizure was then used to calculate a grand average across all seizures (see Fig. 2). The value at time 0 is the average amplitude of the largest population spike on the first burst of each seizure.

Where necessary, we used a logarithmic transform on our raw data to ensure that they fulfilled the assumptions for ANOVA (Berry 1987). Statistics are quoted as means ± SE.

RESULTS

Unilateral intrahippocampal injection of tetanus toxin results in three different types of electrophysiological activity: interictal spikes, polyspikes and seizures. Interictal spikes last <100 ms and comprise an envelope, which we refer to as a field postsynaptic potential, with superimposed population spikes (Fig. 1A). Polyspikes last 0.4–2 s and resembled a series of interictal spikes (Fig. 1B). Seizures last tens of seconds. The clear difference in duration of seizures and brief epileptic discharges suggests that the process or event that underlies the transition to seizure must start within the first few seconds of the seizure.

Dentate gyrus population spike potentiation

The main input to the dentate gyrus originates in the entorhinal cortex (Ruth et al. 1988; Steward and Scoville 1976). We explored whether the dentate gyrus filtered the entorhinal cortex input normally during the early part of spontaneous seizures (n = 93, 4 rats) induced by intrahippocampal tetanus toxin injection. We measured the amplitude of the largest population spike superimposed on the granule cell field postsynaptic potentials, the number of population spikes on each field postsynaptic potential, and the amplitude of dentate granule cell field postsynaptic potentials.

The granule cell population spike depressed initially with population spikes disappearing within 2 s of seizure onset in 83% (10 of 12, 4 rats) seizures (Fig. 1, C and D). In 17% (2 of 12) seizures, depression was followed by a step increase in population spike amplitude and a second phase of depression. All seizures then showed a phase of dentate gyrus population
spike potentiation (Figs. 1, C and D, and 2A). The rate of increase over the period while dentate gyrus population spike amplitude was increasing was estimated by linear regression of the amplitude of the largest population spike on each field excitatory postsynaptic potential (EPSP) against time (Fig. 1D). This allowed us to compare seizures irrespective of the frequency of bursting during the seizure. The mean rate of increase was 1.31 ± 0.48 mV/s (n = 12 seizures from 4 rats). The onset of potentiation, which was estimated by the time when the regression line attained the lowest population spike amplitude from the beginning of the seizure, was 1.13 ± 0.26 s after seizure onset (n = 12 seizures from 4 rats).

Potentiation of dentate population spike amplitude was accompanied by an increase in the number of population spikes on each dentate granule cell field EPSP (Figs. 1, C and F, and 2C). The mean number of spikes at seizure onset was 3.2 ± 1.3. Three seconds later this number had increased to 6.4 ± 1.6 (P = 0.003, paired t-test, n = 12 seizures). The mean amplitude of dentate population spikes on each field EPSP increased from 0.48 ± 0.08 to 1.17 ± 0.10 mV over the same period (P < 0.001, t-test, n = 107). In contrast, there was no change in the mean amplitude of dentate field EPSPs [1-way ANOVA, F(6,76) = 0.196, P = 0.98; Fig. 2E].

Control (buffer-injected) rats were seizure free and showed no evidence of repeated dentate gyrus population spikes. Therefore our results from seizures were categorically different from the controls. We could not test for potentiation of the granule cell population spike amplitude in control rats in vivo because we did not implant chronic stimulation electrodes into the perforant pathway. Therefore we compared the effects of repetitive perforant path stimulation of hippocampal slices in vitro from control and toxin-injected rats (for details, see GABA<sub>B</sub> blockade) with the results from seizures in vivo. We found that the rate of potentiation during seizures in vivo and during repetitive stimulation in vitro were significantly greater than during repetitive stimulation of the perforant pathway of naïve hippocampal slices (Tukey comparison test following 1-way ANOVA, P < 0.05).

Our data showed that there was an initial decrease followed by an increase in the dentate granule population spike amplitude and number of spikes per field postsynaptic potential during the first few seconds of a seizure. Field EPSP amplitudes were unchanged over the same epoch. These results suggested that the normal filtering properties of the dentate gyrus were altered in rats injected with tetanus toxin.

**CA3 population spike potentiation**

The dentate granule cells provide a major input to CA3 (Amaral and Witter 1989). Therefore we explored the effect of altered dentate filtering on ipsilateral CA3 population spike amplitude using the same seizures. We measured the amplitude of the largest population spike superimposed on each CA3 field postsynaptic potential (Fig. 1E) and the number of population spikes on each field postsynaptic potential (Fig. 1G).

The temporal pattern of the amplitude (Fig. 2B) and number (Fig. 2D) of CA3 population spikes mirrored that recorded in the dentate gyrus. The onset of CA3 potentiation was 1.21 ± 0.26 s after seizure onset (n = 12 seizures from 4 rats). This was not different from the onset of dentate gyrus spike potentiation (P = 0.71; paired t-test, n = 12 seizures). The mean rate of potentiation of CA3 population spikes was 1.21 ± 0.32 mV/s (n = 12 seizures, 4 rats). This was not statistically different from the rate of dentate gyrus population spike potentiation (P = 0.55, paired t-test, n = 12 seizures). The mean amplitude of CA3 population spikes on each field EPSP increased from 0.56 ± 0.05 to 1.22 ± 0.10 mV over the same period (P < 0.001, t-test, n = 100).

**Altered dentate filtering**

During spontaneous seizures, we had no control over the strength of synaptic inputs to the dentate gyrus. However, the increase in dentate population spike amplitude with minimal change in dentate granule cell field postsynaptic potential amplitude suggested that there was potentiation of the population spike amplitude.

We prepared hippocampal slices from rats previously injected with tetanus toxin to address this issue in greater detail. Afferent inputs from the entorhinal cortex were mimicked by stimulating the perforant pathway at the same frequencies as dentate gyrus field postsynaptic potentials during spontaneous seizures. Extracellular field potential recordings were made in the dentate granule cell layer of slices prepared from the injected hippocampus (Fig. 3A). Both 10-Hz (n = 5 slices, 3 rats) and 15-Hz (n = 3 slices, 2 rats) stimulus trains showed an early depression followed by potentiation (Fig. 3, B and C).
similar to that recorded during spontaneous seizures in vivo. We pooled the data from 10- and 15-Hz stimulation trains. The mean rate of increase of dentate gyrus population spike amplitude during the phase of potentiation was $2.3 \pm 0.6 \text{ mV/s}$. The onset of potentiation was $0.47 \pm 0.10 \text{ s}$.

The amplitude of the dentate gyrus population spike in control slices ($n = 9$ prepared from naïve rats) depressed to a steady state, without subsequent potentiation (Fig. 3D) as described previously (Alger and Teyler 1976).

**GABA$_B$ blockade**

Our results indicated that prior exposure to tetanus toxin altered the frequency filtering properties of the dentate gyrus. The known effects of tetanus toxin suggested that this could be due to disinhibition caused by tetanus toxin (Empson and Jefferys 1993) or, alternatively, sprouting with the formation of immature synapses (Mitchell et al. 1996). The former hypothesis should be replicated in naïve hippocampal slices by drugs that mimic the reduction in fast and slow IPSPs reported for the tetanus toxin model (Empson and Jefferys 1993).

We found that we could reproduce the temporal pattern of early depression and late potentiation of the dentate gyrus population spike amplitude with 100–200 μM 2-hydroxysaclofen, a GABA$_B$ antagonist. The results were similar at different concentrations. Therefore we pooled the data. The dentate gyrus population spike amplitude in the latter half of the stimulus train was well fitted by linear regression against time (Fig. 3D). The slope of the regression line increased significantly after 2-hydroxysaclofen was added to the bath [presaclofen = $0.12 \pm 0.04 \text{ mV/s}$, postsaclofen = $1.52 \pm 0.42 \text{ mV/s}$; 2-way ANOVA, $F(1,12) = 10.8, P = 0.006, n = 9$ slices]. The onset of the phase of potentiation was $0.55 \pm 0.17 \text{ s}$ ($n = 9$ slices). The GABA$_B$ antagonist, bicuculline methiodide, at a concentration of 3–5 μM did not duplicate the late potentiation (slope after bicuculline = 0.11 mV/s, $n = 3$ slices; Fig. 3).

We compared analysis of our results from hippocampal slices with the results from spontaneous seizures in vivo. We found that the regression slopes calculated during seizures and the regression slopes calculated from repetitive stimulation of hippocampal slices prepared from rats injected with tetanus toxin or hippocampal slices bathed in 2-hydroxysaclofen were not statistically different. However, all of these results were significantly greater than control slice responses [1-way ANOVA, $F(3,34) = 7.8, P < 0.001$; Tukey pairwise, $P < 0.05$]. The time of onset of the potentiation phase did not differ significantly (1-way ANOVA, $P = 0.76$) between hippocampal slices and spontaneous seizures in vivo, although the former did appear shorter (tetanus toxin, $0.47 \pm 0.10 \text{ s}$; 2-hydroxysaclofen, $0.55 \pm 0.17 \text{ s}$, vs. spontaneous seizures, $1.13 \pm 0.26 \text{ s}$). We concluded that a loss of GABA$_B$ receptor-mediated activity was sufficient to reproduce the late potentiation of population spike firing in the dentate gyrus.

**Discussion**

We studied the altered filtering properties of the dentate gyrus in the tetanus toxin model of epilepsy. Our major conclusions were 1) the amplitude and number of dentate gyrus population spikes depress immediately after seizure onset but start to increase approximately one second after seizure onset. 2) The temporal profile of CA3 population spike number and amplitude mirrors that in the dentate gyrus. 3) Repetitive stimulation of the perforant pathway of hippocampal slices prepared from tetanus toxin-injected rats, to mimic the frequency of field postsynaptic potentials in vivo, results in an initial decrease in population spike amplitude followed by a phase of potentiation. The temporal progression is similar to that recorded in vivo. And 4) the same pattern of population
spike response can be reproduced in naïve hippocampal slices by blocking GABA_B receptors to mimic the functional effects of tetanus toxoid.

Population spike amplitude in vivo

The potentiation of dentate gyrus population spike amplitude during the early part of spontaneous seizures suggested that there was increased synchronous neuronal firing. We do not think that the measured change was due to shrinkage of the extracellular space (Jefferys 1995; Traub et al. 1985) because: field EPSP amplitude was unchanged during the period of dentate population spike potentiation; population spike number increased as well as amplitude; and the late potentiation phase was reproduced in naïve slices by GABA_B receptor antagonists.

Altered dentate filtering

The frequency filtering properties of the dentate gyrus are altered in several epilepsy models. A loss of dentate gyrus filtering properties has been reported following kindling (Behr et al. 1996), but the mechanism remains unclear (Behr et al. 1998). In the low magnesium model of epilepsy in vitro, the dentate gyrus blocks the propagation of epileptic discharges from the entorhinal cortex to the hippocampus. Phacolofen, a GABA_B antagonist, reverses the filtering properties of the dentate and promotes the spread of epileptiform activity from the entorhinal cortex to the hippocampus. This is associated with a change in the response of the dentate gyrus EPSP from paired-pulse depression to paired pulse-facilitation (Rausche et al. 1989). We focused on population spike amplitude to assess how transmission of epileptic activity through the dentate gyrus is modified during spontaneous seizures. We found that granule cell population spikes in the chronic focus depress transiently and then potentiate ~1 s into the stimulus train. This pattern was reproduced in control tissue with GABA_B antagonists. It remains unclear whether the origin of this effect is presynaptic, postsynaptic or a combination of the two.

Other mechanisms have been invoked to explain the role of the dentate gyrus in the transition to seizure. Afferent stimulation of the dentate gyrus sufficient to elevate extracellular potassium concentration to 8–9 mM results in “maximal dentate activation” (Stringer and Lothman 1989; Stringer et al. 1989b). Hippocampal slice experiments suggest that maximal dentate activation is a field burst (Schweitzer and Williamson 1995; Schweitzer et al. 1992). Thus the cellular mechanism of maximal dentate activation is fundamentally different from the mechanism underpinning late potentiation of granule cell population spikes in the tetanus toxin model.

Our data suggest that the dentate gyrus contributes to the transition to seizure in our animals. Recordings of spontaneous seizures indicate that ictal onsets are not restricted to the injected hippocampus but occur in the contralateral hippocampus and probably also in the entorhinal cortex (Finnerty and Jefferys, unpublished data). We propose that seizures occur when epileptic discharges in these different regions become coupled together. The coupling occurs because of the oscillatory nature of epileptic discharges and the reciprocal pathways that link the discharging brain regions (Finnerty and Jefferys 2000). Dentate filtering could prevent coupling of discharges in the entorhinal cortex and hippocampus by blocking propagation of epileptic discharges between these two regions. However, dentate filtering fails in tetanus toxin-injected animals if discharges in the entorhinal cortex last longer than a few seconds. The result is that the input to the hippocampus is enhanced thereby promoting seizures. This provides a simple explanation for why there is a clear separation between brief epileptic discharges, which last <2 s, and seizures in vivo in our animals.
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