Decrease in Synaptic Transmission Can Reverse the Propagation Direction of Epileptiform Activity in Hippocampus In Vivo

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Many types of epileptiform activity, such as partial seizures, are known to initiate from a primary focus and then propagate to other brain regions (McCormick and Contreras 2001). Therefore understanding the propagation pathways of epileptiform activity can be helpful to reveal the mechanisms of epilepsy and develop new treatments. In this paper, the effect of the synaptic transmission suppression on the propagation of epilepsy in vivo was investigated by using multiple-channel recording probes in CA1. Nonsynaptic epileptiform activity was induced by calcium chelator EGTA with varied concentrations of potassium. For comparison, disinhibition synaptic epileptiform activity was induced by picrotoxin (PTX) with or without partial suppression of excitatory synaptic transmission. The propagation velocity was calculated by measuring the time delay between two electrodes separated by a known distance. The results show that in vivo nonsynaptic epileptiform activity propagates with a direction and velocity comparable to those observed in vitro preparations. The direction of propagation for nonsynaptic activity is reversed from the PTX-induced synaptic activity. A reversal in propagation direction and change in velocity were also observed dynamically during the process of synaptic transmission suppression. Even a partial suppression of synaptic transmission was sufficient to significantly change the propagation direction and velocity of epileptiform activity. These results suggest the possibility that the measurement of propagation can provide important information about the synaptic mechanism underlying epileptic activity.

INTRODUCTION

Many types of epileptiform activity, such as partial seizures, are known to initiate from a primary focus and then propagate to other brain regions (McCormick and Contreras 2001). Therefore understanding the propagation pathways of epileptiform activity can be helpful to reveal the mechanisms of epilepsy and develop new treatments. The hippocampus is one of the most epilepsy-prone brain regions. Many types of epileptiform activity involving synaptic transmission have been observed to propagate from CA3 to CA1 in hippocampus, such as the interictal burst induced in high-K solution (Korn et al. 1987), the epileptiform discharge induced by zero-Mg solution (Kohling et al. 2001), the afterdischarge evoked by stimulus trains (Rafiq et al. 1993), and the bursts caused by convulsant drugs such as picrotoxin, 4-aminopyridine, or pilocarpine (Hablitz 1984; Luhmann et al. 2000; Nagao et al. 1996). However, it has been observed in vitro that the nonsynaptic epileptiform activity induced in low-Ca2+ solution with high K+ propagates from the caudal end of the CA1 to the CA3 with a slow velocity (Haas and Jefferys 1984; Lian et al. 2001; Yaaari et al. 1983, 1986).

Since the nonsynaptic epilepsy model was established in hippocampal slices in 1980s (Jefferys and Haas 1982; Konnerth et al. 1984; Taylor and Dudek 1982), nonsynaptic epileptiform activity had not been observed in vivo until recently (Feng and Durand 2003). Yet little is known about the propagation of this type of epileptiform activity in vivo. In this paper, we address the two following questions: does nonsynaptic epileptiform activity induced in vivo propagate in the similar manner as in vitro? How does suppression of synaptic transmission modulate the propagation direction and velocity during already induced epileptiform activity? These questions were investigated by inserting multiple-channel recording probes into an exposed rat hippocampus in vivo. Several different types of nonsynaptic and synaptic epileptiform activity were induced by calcium chelator (EGTA) or by γABAergic blocker picrotoxin (PTX). The relationship between synaptic transmission suppression and changes in both propagation direction and velocity of epileptiform activity was also observed dynamically.

METHODS

Surgical procedures

All procedures used in this study were approved by the Institutional Animal Care and Use Committee, Case Western Reserve University, Cleveland. Adult Sprague Dawley rats (260–360 g, n = 21) were anesthetized with urethan (1.5 g/kg ip) and placed in a stereotaxic apparatus. Body temperature was maintained at 37°C with a heating pad. The skull over the left cortex was opened, and the cortex overlying the left dorsal hippocampus was removed. Artificial cerebrospinal fluid (ACSF) solution was warmed to 37°C and placed over the surface of the exposed dorsal hippocampus. The solution was refreshed every 5 min throughout the experiment by sucking away old solution and dropping in fresh solution using syringes (Feng and Durand 2003).

Solutions and drugs

Normal ACSF consisted of (in mM) 124 NaCl, 5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1.5 MgSO4, 26 NaHCO3, and 2 g/l Ω-glucose. Calcium chelator ethylene glycol-bis (β-aminoethyI ether)N,N,N’,N’-tetraacetic acid (EGTA) 5 mM replaced CaCl2 to lower [Ca2+]o and block synaptic transmission in hippocampus to induce nonsynaptic

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epileptiform activity in vivo with 7.5 or 12 mM KCl. ACSF without CaCl₂ was used to partially block synaptic transmission in the in vivo preparation. Because of the difficulty in washing out [Ca²⁺]₀ from the hippocampal tissue in this in vivo preparation, zero Ca²⁺ solution can only partially block synaptic transmission. Picrotoxin (PTX, 0.1–0.4 mM) was used to induce epileptic bursts by blocking the GABA_A receptor. All chemicals were obtained from Sigma or Fisher.

Recording of spontaneous and evoked potentials

Multi-channel silicon recording probes with two shanks provided by the Center of Neural Communication Technology, University of Michigan, were used for recording. The distance between the two shanks was 600 μm with four electrodes distributed in each shank at a distance of 200 μm. Bipolar stimulating electrodes were made from pairs of insulated nichrome wires (80 μm diam) with a 0.5-mm vertical tip separation. Recording probes were positioned in the exposed left hippocampus (AP –3.0, ML 2.6) to record field potentials in the CA1 pyramidal stratum and in the CA1 stratum radiatum. Stimulation electrodes were inserted into the area (AP –2.0, ML 2.3) for stimulating the Schaffer collaterals. Patterns of the evoked potentials guided vertical positioning of both the recording probes and the stimulation electrodes (Kloosterman et al. 2001). The stimulus was 0.1-ms duration with 0.35-mA constant current. Two stainless steel screws fixed in the bone of the nose served as ground and reference electrodes.

CA1 field potential signals were amplified 1,000 times by two Model 1700 four-channel amplifiers (A-M System) with filter frequency ranges from 0.1 Hz to 5 kHz for both spontaneous and evoked potentials. Signals were then sampled at a rate of 20 kHz by using a ML795 PowerLab/16SP data-acquisition system (ADInstruments) before they were stored into a hard disk for off-line analysis. The amplitude of orthodromic-evoked population spikes (PS) and the PS latency were calculated from the pyramidal layer recordings to evaluate the change in synaptic transmission.

The time delay between signals obtained from two recording shanks was calculated by the latency difference between the most negative points of the spikes or waves recorded from the pyramidal layer. Signals in the pyramidal layer, especially those with spike activity, have more abrupt slopes facilitating the measurement of the delay. The propagation velocity was calculated by dividing the distance (0.6 mm) between two electrodes by the delay. Data are expressed as means ± SD. A Student’s t-test was used for statistical analysis.

RESULTS

Propagation difference between nonsynaptic and synaptic epileptiform activities

Figure 1 shows the placements of a recording probe and a stimulating electrode (S1) in the CA1 of an exposed left hippocampus. The recording probe included two shanks (R1 and R2) each having four electrode contacts. The two shanks were placed parallel with the middle line of the brain in a presumably transverse plane of the hippocampus. R1 is located near the CA3 region and R2 is near the subiculum. Two of the four contacts in each shank were used to record extracellular field potentials from pyramidal layer and from s. radiatum, respectively. About 30 min after the application of ACSF solution with 7.5 mM K⁺ and 5 mM EGTA over the exposed hippocampus, slow-wave activity with or without superimposed spikes appeared when synaptic transmission was depressed as a result of lowering [Ca²⁺]₀ (Fig. 2, A and B). This activity was similar to that previously reported (Feng and Durand 2003). The suppression of synaptic transmission was clearly indicated by the disappearance of orthodromically evoked PS in the pyramidal layer. The slow-waves in the pyramidal layer were out of phase with those in the s. radiatum and propagated from R2 to R1 (Fig. 2A). Slow waves with superimposed spikes usually propagated faster with a shorter delay between R2 and R1 than the slow waves without superimposed spikes (Fig. 2B). Figure 2C shows a histogram of slow-wave delay calculated from 155 slow waves without superimposed spikes collected within a same preparation. The delay was measured from pyramidal layer field potentials recorded by R1 and R2, as illustrated in Fig. 2A. The delays varied significantly (–187 ± 158 ms/0.6 mm). The negative value indicates propagation from R2 to R1. Figure 2D shows a delay histogram of slow-wave calculated from 172 slow-waves with superimposed spikes in another preparation. The mean delay was –33 ± 39 ms/0.6 mm.

However, for epileptiform activity induced by the GABAergic blocker PTX with intact excitatory synaptic transmission, the propagation was different both in direction and velocity (Fig. 3). The application of an ACSF solution containing PTX 0.1–0.4 mM (7 mM K⁺, 2 mM Ca²⁺) over the exposed hippocampus induced interictal activity or periodical bursts (Fig. 3A, middle). The orthodromic-evoked response displayed multiple PSs resulting from a loss of GABA_A inhibitory synaptic transmission (Fig. 3A, left). The spikes in PTX induced bursts propagated from R1 to R2 (expansions in Fig. 3A). Figure 3B shows a histogram of spike delay between R1 and R2 measured from 464 spikes collected in a single preparation. All of the delays were positive indicating propagation from R1 to R2.

FIG. 1. Schematic diagram of the experimental preparation and the locations of electrodes in CA1. The recording probe included 2 shanks (R1 and R2) with a distance of 0.6 mm. Two contacts in each shank were used to collect extracellular field potentials from the pyramidal layer and stratum radiatum, respectively. The stimulating electrode (S1) was placed to orthodromically stimulate the Schaffer collaterals.
The average delay of the PTX induced spikes was 1.4 ± 0.53 ms/0.6 mm $(n = 4)$ equal to an average velocity of 472 ± 150 mm/s, whereas the average delay of nonsynaptic slow-waves induced by EGTA was −177 ± 122 ms/0.6 mm $(n = 4)$ equal to an average velocity of −11 ± 15 mm/s (Table 1, group 1). The delays were significantly different between the two groups $(P < 0.05)$.

These results show that the block of synaptic transmission by lowering $[Ca^{2+}]_o$, can change both the propagation direction and velocity of epileptiform activity. Next, the effect of synaptic transmission suppression on the propagation changes was tested dynamically in a single experimental preparation.

Dynamic propagation changes in epileptiform activity

By perfusing an ASCF solution with high $K^+$ (12 mM), zero $Ca^{2+}$ and 5 mM EGTA over the exposed hippocampus, a novel epileptic model characterized by sustained spike activity was observed. Potassium ($K^+$) 12 mM was used for ~40 min to raise the neuronal excitability without any epileptiform activity. After the application of EGTA, epileptiform activity started several minutes later as synaptic transmission was blocked, indicated by evoked potentials without PSs in pyramidal layer (only potentials in pyramidal layer were shown). They propagated from R2 to R1, as illustrated by three expanded portions of the waves. C: delay histogram of slow waves without superimposed spikes between R1 and R2 measured from 155 waves of an experiment. D: delay histogram of slow-waves with superimposed spikes between R1 and R2 measured from 172 waves of an experiment. Negative value means propagation from R2 to R1.
synaptic transmission being gradually blocked, as indicated by the amplitude decrease of evoked PS until a total disappearance, the spikes in spontaneous potential appeared as sustained 3- to 5-Hz paired-spikes (termed as late spike). Their propagation direction was from R2 to R1 (Fig. 4A, middle and right). Figure 4B shows the changes in spike delay due to suppression of synaptic transmission for an 80-min period of 12 mM K\(^+\) with 5 mM EGTA. For multiple spikes, the delay was always measured from the first spike. During the process of synaptic transmission suppression, the delay changed from small positive values for early spikes to large negative values for late spikes. Statistical analysis (Table 1, group 2) shows that the delay increased of synaptic transmission could be observed directly in a single preparation. A similar change was also observed in the propagation of PTX-induced epileptiform activity during partial suppression of synaptic transmission.

In this PTX experimental group \((n = 4)\), ACSF with 12 mM K\(^+\) and Ca\(^{2+}\) free was first perfused over the exposed hippocampus for >40 min to lower CA1 [Ca\(^{2+}\)]\(_i\), to partially suppress the synaptic transmission, as indicated by a significant increase of PS latency in evoked potentials. The amplitude increase of the PS was due to the increase of neuronal excitability by higher K\(^+\) and lower Ca\(^{2+}\) concentrations. PTX (0.1 mM) was then added to the solution to evoke epileptiform activity. This activity propagated from R2 to R1 (Fig. 5A). After the addition of 2 mM Ca\(^{2+}\) to the perfusion solution, the pattern of spontaneous epileptiform activity changed and it propagated in a reversed direction from R1 to R2 (Fig. 5B).

Statistical analysis (Table 1, group 3) shows that during the Ca\(^{2+}\)-free period the propagation delay of –8.1 ± 6.0 ms/0.6 mm (a velocity of –131 ± 107 mm/s) was significantly different from the propagation delay of 1.9 ± 0.44 ms/0.6 mm (a velocity of 361 ± 87 mm/s) during the 2 mM Ca\(^{2+}\) period \((P < 0.01; n = 4)\). This result shows that even a partial block of synaptic transmission is sufficient to significantly change both the direction and velocity of PTX-induced epileptiform activity.

**DISCUSSION**

The main findings of this study are: 1) nonsynaptic epileptiform activity induced in vivo in the CA1 region propagates from caudal CA1 to rostral CA1, a reverse direction from

### Table 1. Effect of synaptic transmission suppression on propagation direction and velocity

<table>
<thead>
<tr>
<th>Paired Group</th>
<th>Activity Name</th>
<th>Delay, ms/0.6mm</th>
<th>Velocity, mm/s</th>
<th>Activity Name</th>
<th>Delay, ms/0.6mm</th>
<th>Velocity, mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PTX-spike (Ca(^{2+}) 2 mM)</td>
<td>1.4 ± 0.53 (4)</td>
<td>472 ± 150</td>
<td>Slow-wave (EGTA 5 mM)</td>
<td>–177 ± 122* (4)</td>
<td>–11 ± 15</td>
</tr>
<tr>
<td>2</td>
<td>Early-spike (EGTA 5 mM)</td>
<td>0.73 ± 0.96 (9)</td>
<td>742 ± 300</td>
<td>Late-spike (EGTA 5 mM)</td>
<td>–4.4 ± 2.3** (9)</td>
<td>–192 ± 147</td>
</tr>
<tr>
<td>3</td>
<td>PTX-spike (Ca(^{2+}) 2 mM)</td>
<td>1.9 ± 0.44 (4)</td>
<td>361 ± 87</td>
<td>PTX-spike (Ca(^{2+}) 0 mM)</td>
<td>–8.1 ± 6.0** (4)</td>
<td>–131 ± 107</td>
</tr>
</tbody>
</table>

Negative delay or velocity indicate activity propagating from the caudal CA1 to the rostral CA1. The number of experiments is in parentheses under delay. *\(P < 0.05\); **\(P < 0.01\) activity with little suppression of excitatory synaptic transmission vs. activity with significant suppression of excitatory synaptic transmission, student’s t-test.
PTX-induced synaptic epileptiform activity. The propagation velocity of the nonsynaptic epileptiform activity was also significantly lower than PTX-induced epileptiform activity. 2) Suppression of excitatory synaptic transmission can change both the propagating direction and velocity of epileptiform activity.

Propagation of nonsynaptic activity in in vivo and in in vitro preparations

Two types of nonsynaptic epileptiform activity were observed in this study: slow wave with or without superimposed spikes induced by 7.5 mM K\textsuperscript{+}/H\textsubscript{11001} and 5 mM EGTA and sustained 3- to 5-Hz paired spikes (late spike) induced by 12 mM K\textsuperscript{+}/H\textsubscript{11001} and 5 mM EGTA. Both types of activity propagated from caudal end of CA1 to the rostral CA1 in the same hippocampus as the nonsynaptic activity in vitro (Haas and Jefferys 1984; Lian et al. 2001; Yaari et al. 1983, 1986). They are discussed separately in the following text.

The slow wave induced by EGTA is similar to the nonsynaptic depolarization burst induced by low Ca\textsuperscript{2+} in vitro (Jefferys and Haas 1982; Konnerth et al. 1984; Taylor and Dudek 1982). Because it is difficult to wash out [Ca\textsuperscript{2+}]\textsubscript{o} from hippocampal tissue in vivo, adding the calcium chelator EGTA is necessary to lower [Ca\textsuperscript{2+}]\textsubscript{o} and induce nonsynaptic epileptiform activity. The average propagation velocity of slow waves (−11 ± 15 mm/s) in vivo is within the range of propagation velocities observed in vitro, 1–100 mm/s (Haas and Jefferys 1984) or near 0.44–4.2 mm/s (Konnerth et al. 1986). Also, the propagation velocity is as variable in vivo as it is in vitro (Haas and Jefferys 1984; Konnerth et al. 1986).

The late spikes propagate faster (−192 ± 147 mm/s) than the in vitro nonsynaptic bursts (1–100 mm/s). Several reasons might account for this difference. 1) The propagation velocity of the nonsynaptic burst is [K\textsuperscript{+}]\textsubscript{o} dependent and increases markedly with increasing [K\textsuperscript{+}]\textsubscript{o} in vitro (Yaari et al. 1986). We used a solution with a high concentration of 12 mM K\textsuperscript{+} thereby explaining the higher propagation speed. 2) In vitro, nonsynaptic epileptiform activity with more abrupt onsets (spike-like activity) was also observed to propagate faster with a velocity ~100 mm/s in vitro (Haas and Jefferys 1984; Jefferys 1995).

Another type of potential waveform propagating slowly with nonsynaptic mechanisms observed in brain tissue is spreading depression (SD) (Somjen 2001; Somjen et al. 1992). SD is very different from the low-calcium nonsynaptic epileptiform activity reported here in vivo and in vitro. SD is characterized by larger and longer depolarization potential shift preventing neuronal firing and a much lower propagation velocity in the range of a few millimeters per minute. The mechanisms involve the diffusion of either K\textsuperscript{+} or glutamate, (Somjen 2001).

Suppression of synaptic transmission change the propagation of epileptiform activity

The significant differences in the propagation delays among the three paired groups in Table 1 show that the suppression of synaptic transmission changed the propagation direction and
lowered propagation velocity. This result was not only observed in the first group between the fully developed nonsynaptic activity (slow wave) and synaptic activity (PTX-induced spike) but also was shown by the dynamic propagation evolution recorded during EGTA-induced changes in synaptic transmission (group 2). Moreover, it was also observed with partial depression of synaptic transmission in PTX-induced epileptiform activity (group 3). This result is consistent with previous in vitro studies showing that suppression of excitatory synaptic transmission can decrease the propagation velocity of disinhibited activity in the brain slices (Golomb and Amitai 1997; Traub et al. 1993).

However, it is interesting that the suppression of synaptic transmission can also change the propagating direction. Interictal synaptic activity usually initiates in CA3 and propagates to CA1 (McNamara 1994; Luhmann et al. 2000; Kohling et al. 2001). Ictal activity was observed originating in the entorhinal cortex and propagating via dentate gyrus to CA3, then to CA1 (Barbarosie and Avoli 1997; Dzhala and Staley 2003; Nagao et al. 1996). Therefore most types of epileptiform activity propagate in the direction from CA3 to CA1. Nevertheless, in isolated subiculum-CA1 slices, spontaneous activity induced by low magnesium media was also found propagating from subiculum to CA1 and spreading from the caudal to the rostral part of CA1 (Harris and Stewart 2001). One possible mechanism for this phenomenon is that the subiculum generates spontaneous epileptiform activity that then propagates into CA1 through a backward projection from subiculum to CA1 region when the projection from the CA3 to CA1 is eliminated (Behr and Heinemann 1996). However, it seems unlikely that this mechanism could account for the change in propagation direction observed in the present study because the low Ca$^{2+}$ would suppress the synaptic connections in the CA1 region projecting from both the CA3 and subiculum. Moreover, nonsynaptic activity has been shown to spread through potassium wave diffusion, a nonsynaptic mechanism (Konnerth et al. 1986; Lian et al. 2001). An alternative explanation for the change in propagation direction is based on the fact that the low-Ca$^{2+}$ nonsynaptic epileptiform activity is more readily evoked in the CA1 region than in both the CA3 and dentate gyrus (Konnerth et al. 1984; Schweitzer et al. 1992; Snow and Dudek 1984). The more densely packed pyramidal cells in the CA1 region may facilitate nonsynaptic processes such as gap junctions, ephaptic interaction and potassium coupling (Jefferys 1995; Konnerth et al. 1984).

Therefore the nonsynaptic epileptiform activity might tend to invade into CA3 from the original CA1 area resulting in a propagation direction from CA1 to CA3.

Conclusions

The results of this study show, for the first time, that the nonsynaptic epileptiform activity in vivo propagates slowly in a direction from caudal CA1 toward the CA3. It is also clearly shown that the suppression of excitatory synaptic transmission can change both the propagation direction and velocity of an epileptiform activity. These results suggest that the synaptic and nonsynaptic epilepsy propagate in different modes, implying that measuring the propagation of seizure activity in the brain could provide important information about the synaptic mechanisms underlying epilepsy.

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