Glutamatergic Propagation of GABAergic Seizure-Like Afterdischarge in the Hippocampus In Vitro

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1Department of System Neuroscience, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183-8526; 2The Japan Society for the Promotion of Science, Chiyoda-ku, Tokyo 102-8471; and 3Core Research for the Evolutional Science and Technology Program, Japan Science and Technology Corporation, Kawaguchi, Saitama 332-0012, Japan

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Isomura, Yoshikazu, Yoko Fujiwara-Tsukamoto, and Masahiko Takada. Glutamatergic propagation of GABAergic seizure-like afterdischarge in the hippocampus in vitro. J Neurophysiol 90: 2746–2751, 2003; 10.1152/jn.00057.2003. Previous investigations have suggested that GABA may act as an excitatory mediator in the generation of seizure-like (ictal) or interictal epileptiform activity in several experimental models of temporal lobe epilepsy. However, it remains to be known whether or not such GABAergic excitation may participate in seizure propagation into neighboring cortical regions. In our in vitro study using mature rat hippocampal slices, we examined the cellular mechanism underlying synchronous propagation of seizure-like afterdischarge in the CA1 region, which is driven by depolarizing GABAergic transmission, into the adjacent subiculum region. Tetanically induced seizure-like afterdischarge was always preceded by a GABAergic slow posttetanic depolarization in the pyramidal cells of the original seizure-generating region. In contrast, the slow posttetanic depolarization was no longer observed in the subicular pyramidal cells when the afterdischarge was induced in the CA1 region. Surgical cutting of axonal pathways through the stratum oriens and the alveus between the CA1 and the subiculum region abolished the CA1-generated afterdischarge in the subicular pyramidal cells. Intracellular loading of fluoride ions, a GABA<sub>A</sub> receptor blocker, into single subicular pyramidal cells had no inhibitory effect on the CA1-generated afterdischarge in the pyramidal cells. Furthermore, the CA1-generated afterdischarge in the subicular pyramidal cells was largely depressed by local application of glutamate receptor antagonists to the subiculum region during afterdischarge generation. The present results indicate that the excitatory GABAergic generation of seizure-like activity seems to be restricted to epileptogenic foci of origin in the seizure-like epilepsy model in vitro.

INTRODUCTION

For exploring the cellular mechanism of seizure activity in temporal lobe epilepsy, numerous investigations have been conducted toward the establishment of in vitro and in vivo experimental epilepsy models (McNamara 1994). In rat hippocampal slices, intense electrical stimulation (tetanization) readily induces seizure-like (ictal) epileptic afterdischarge in which hippocampal neurons are activated rhythmically and synchronously for more than 20 s even in normal extracellular fluid (Fujiwara-Tsukamoto et al. 2003; Rafiq et al. 1993; Stusheff et al. 1989, 1993a,b). Although epileptic activity was conventionally believed to occur in disinhibited conditions where GABAergic inhibition was diminished, it has increasingly become evident that GABAergic depolarization might play a key role in the generation of ictal or interictal epileptic activity in this experimental model using tetanization (Fujiwara-Tsukamoto et al. 2003; Higashima et al. 1996, 2000; Velazquez and Carlen 1999), as well as in other experimental models (Köhling et al. 2000; Lamsa and Kaila 1997; Perreault and Avoli 1992; Uusisaari et al. 2002), including human epilepsy patients’ tissues (Cohen et al. 2002). In fact, our previous study has revealed that each cycle of seizure-like afterdischarge is directly driven by GABA<sub>A</sub>-mediated excitation in the pyramidal cells of hippocampal CA1-isolated slices (Fujiwara-Tsukamoto et al. 2003). Thus it is most likely that rhythmic synchronization of seizure activity may be generated by excitatory GABAergic transmission per se in an epileptogenic focus of temporal lobe epilepsy patients.

The seizure activity would, in turn, propagate from a focal site of origin into other cortical regions synchronously. In rat hippocampal-entorhinal slices, the seizure-like afterdischarge that was tetanically generated in the CA1 region has been shown to propagate synchronously into the entorhinal, dentate, and CA3 regions (Rafiq et al. 1993). Similarly, synchronous propagation into other cortical regions was observed in vivo: the rat hippocampus (Bragin et al. 1997a,b), the cat neocortex (Timofeev et al. 2002), and the human temporal lobe (Gloor et al. 1982). In the study of Timofeev et al. (2002), an active role of GABAergic depolarization has been implicated in the evoked and spontaneous seizure-like activity. However, little is as yet known about the cellular mechanism of such synchronous seizure propagation throughout the temporal lobe. Given that seizure activity is generated by excitatory GABAergic transmission, it could be hypothesized that the seizure activity propagating into adjacent cortical regions may also be mediated by the “excitatory GABAergic outputs” from interneurons in the original region. Unlike other epilepsy models by convulsant applications or ionic modifications, the tetanus-induced seizure model, in which its GABAergic generation has been well characterized especially in the CA1 region, will be suitable to assay its propagation into adjacent cortical regions with cell excitability kept normal. Using hippocampal CA1-subi-
anesthetized Wistar rats (P20–P27) with a microslicer (DTK-1500, Dosaka EM, Kyoto, Japan), and the CA1 and subiculum regions were routinely isolated from the CA3 and entorhinal regions (CA1-subiculum–isolated slices), unless otherwise mentioned. After recovery for ≥1 h, each slice was transferred to a submerged-type recording chamber continuously circulated with normal artificial cerebrospinal fluid (ACSF; 30–32°C) consisting of (in mM) 124 NaCl, 2.5 KCl, 1.2 KH2PO4, 26 NaHCO3, 1.2 MgSO4, 2.5 CaCl2, and 25 D-glucose and saturated with 95% O2–5% CO2 (Isomura et al. 2002). To induce the afterdischarge, tetanic stimulation (100 Hz for 0.5 s; intensity 50–400 μA, usually 400 μA; duration 400 μs) was delivered by a monopolar glass stimulating electrode (0.5–1 ΜΩ, filled with 2.5 M NaCl) placed in the stratum radiatum of the CA1, subiculum, or CA3 region (Fujisawa-Tsukamoto et al. 2003). Whole cell patch-clamp recordings were obtained from the CA1 or subicular pyramidal cells in hippocampal slices under visual guidance (Isomura and Kato 1999). In current-clamp mode (I = 0), membrane potentials [resting membrane potential (r.m.p.)]: −61.6 ± 3.9 mV in CA1, −60.6 ± 1.9 mV in subiculum] were recorded with a patch-clamp amplifier (Axopatch 1D or Axopatch 200B, Axon Instruments, Union City, CA), through glass patch electrodes filled with a low-Cl− internal solution containing (in mM) 140 K-glucuronate, 2 NaCl, 1 MgCl2, 10 HEPES, 0.2 EGTA, 2 5’-ATP Na2, 0.5 GTP Na2, and 10 biocytin (pH 7.4; 5–10 ΜΩ). The patch electrode solution used for intracellular blockade of GABAA receptors consisted of (in mM) 140 KF, 10 HEPES, 0.2 EGTA, and 10 biocytin (pH 7.4). For simultaneous whole cell and extracellular recordings, field potentials were additionally recorded with one of the amplifiers through glass electrodes (2–5 ΜΩ, filled with 2.5 M NaCl) placed in the CA1 stratum pyramidale. Recorded signals were low-pass-filtered at 3.5 kHz and digitized at 5 kHz with an A/D interface (Digidata 1200, Axon Instruments). After the whole cell recordings, biocytin-loaded neurons were visualized by an avidin–biotin–HRP complex (ABC) method to identify their morphology. Bicuculline methiodide was purchased from Sigma (St. Louis, MO); t-2-amino-5-phosphonopentanoic acid (t-AP-5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) from Tocris Cookson (Ballwin, MO); and d-serine (D-serine; Tama-maki and Nojyo 1990). In our morphological observation, axonal projections of CA1 interneurons also occasionally reach the subiculum region through the s. oriens, pyramidale, or radiatum (Fig. 2A, right). Hence, one can presume that excitatory GABAergic output from these CA1 interneurons may activate the subicular target neurons synchronously during the afterdischarge. To test this possibility, we attempted to record subicular afterdischarge activity propagated from the CA1 region in “orizens/aleves-cut” slices, where both the s. orients and the alveus were knife-cut between the CA1 and the subiculum region (Fig. 2B). In all of these partially cut slices, the CA1-generated afterdischarge was abolished in the subiculum pyramidal cells, while the afterdischarge normally occurred in the CA1 region (Fig. 2C; control, n = 7; orients/alveus cut, n = 5). This suggests that the axonal fibers passing through the s. orients or the alveus may be critical for synchronous propagation of the CA1-generated afterdischarge. As previously shown by Fujisawa-Tsukamoto et al. (2003), oscillatory responses during the CA1-generated afterdischarge were completely blocked in single CA1 pyramidal cells loaded with fluorode (F−) ions [Fig. 3A, left; F−-loaded, afterdischarge amplitude 0.97 ± 0.38 mV (discharge probability 0%), n = 7; control (data not shown), 8.0 ± 1.8 mV (20.4 ± 40.0%), n = 6; P < 0.002], which inactivate GABAA receptors intracellularly (Bormann et al. 1987; Smirnov et al. 1999). In contrast, F−-loaded subiculum pyramidal cells still exhibited prominent oscillatory responses during the CA1-generated afterdischarge [Fig. 3A, right; F−-loaded, 7.0 ± 2.6 mV (62.9 ± 51.1%), n = 5; control (data not shown), 6.6 ± 4.0 mV (23.6 ± 36.7%), n = 6; P > 0.8]. Thus it is quite unlikely that the oscillatory depolarizing responses may be mediated by GABAA receptors activation in the subicular pyramidal cells during the CA1-generated afterdischarge. Moreover, local application of glutamatergic receptor antagonists during the CA1-generated after-
FIG. 1. Generation of GABAergic seizure-like afterdischarge in the hippocampal CA1 and subiculum regions. A: rhythmic depolarization at about 3 Hz (seizure-like afterdischarge) following a slow posttetanic depolarization (arrows) was induced by a strong tetanus (100 Hz, 0.5 s; third trace), but not by a weak (first trace) or a moderate (second trace) tetanus within the CA1 region. The slow posttetanic depolarization and afterdischarge were completely blocked by 25 μM bicuculline, a GABA_A receptor antagonist (fourth trace). Similar results were obtained by the use of another GABA_A receptor antagonist, 50 μM picrotoxin (see Fujiwara-Tsukamoto et al. 2003). Thick underlines indicate the duration of tetanus, and downward artifacts during the tetani are truncated in all figures. B: representative (top) and averaged (bottom) traces of membrane potential changes in CA1 (left, CA1) and subicular (middle and right, Sub) pyramidal cells, after tetanization in the CA1 (left and right, indicated by parentheses) or subiculum (middle) region. The averaged membrane potentials were calculated after all spikes were deleted. Black and gray lines show the mean ± SE (n = 7 in each group). Slow posttetanic depolarization was always accompanied by the afterdischarge generation when tetanization and whole cell recording were performed in the same region (left and middle), whereas no slow posttetanic depolarization was observed in subicular pyramidal cells if tetanization was delivered in the CA1 region (right). C: afterdischarge propagation from the CA3 region into the CA1 region in CA3-CA1—isolated slices. Note that seizure-like afterdischarge, but not posttetanic depolarization, appeared in CA1 pyramidal cells when tetanization was given to the CA3 region (top trace), and that 25 μM bicuculline completely inhibited the afterdischarge generation (bottom trace). Scale bars: 1 s, 10 mV in A–C.
FIG. 2. Propagation of CA1-generated afterdischarge through the stratum oriens or the alveus into the subiculum region. A: dendritic and axonal arborization of a CA1 pyramidal cell (left) and a CA1 interneuron (right). Axonal projections in both types of neurons extend into the subiculum region (dotted lines). s.l.m., stratum lacunosum-moleculare; s.r., stratum radiatum; s.p., stratum pyramidale; s.o., stratum oriens; alv., alveus. Scale bar: 100 μm. B: recorded slice in which both the stratum oriens and the alveus were cut between the CA1 and the subiculum region (arrow). DG, dentate gyrus. C: inter-regional propagation of afterdischarge through the s. oriens and/or the alveus. Afterdischarge generated in the CA1 region was completely abolished in the subicular pyramidal cells of oriens/alveus-cut slices (left, control slice; right, oriens/alveus-cut slice). Note that population spikes in the CA1 pyramidal cell layer [top; field potential (FP)] were time-locked to oscillatory depolarizing responses in subicular pyramidal cells [bottom; membrane potential (MP)] during afterdischarge in control (intact) slices (left). Scale bars: 1 s, 0.2 (FP) or 10 mV (MP).

FIG. 3. Glutamatergic propagation of CA1-generated afterdischarge into the subiculum region. A: intracellular inhibition of GABA<sub>A</sub>-mediated response (Cl<sup>-</sup>-dependent conductance) by loading of fluoride ions (F<sup>−</sup>) into single recorded pyramidal cells. Oscillatory depolarizing responses were completely blocked only in F<sup>−</sup>-loaded CA1 pyramidal cells [left bottom, CA1 (MP, F<sup>−</sup>)] during afterdischarge generation in the CA1 region [left top, CA1 (FP)]. In contrast, oscillatory responses were not depressed, or rather enhanced, in F<sup>−</sup>-loaded subicular pyramidal cells [right bottom, Sub (MP, F<sup>−</sup>)] during the CA1-generated afterdischarge [right top, CA1 (FP)]. Slight membrane depolarization due to intracellular F<sup>−</sup>-loading was compensated by constant current injection (approximately −0.25 nA). Scale bars: 1 s, 0.2 (FP) or 10 mV (MP). B: local blockade of subicular afterdischarge responses by glutamate receptor antagonists during CA1-generated afterdischarge. Pressure application of a mixture of 5 mM D-AP-5 [N-methyl-D-aspartate (NMDA) receptor antagonist] and 1 mM CNQX (AMPA receptor antagonist) to recorded subicular pyramidal cells during the CA1-generated afterdischarge (middle, thick underline) greatly reduced oscillatory depolarizing responses in the recorded neurons. Left and right, control trials at 10 min before and 20 min after the drug-application trial, respectively; afterdischarge responses were below discharge threshold in this case. Scale bars: 1 s, 5 mV. Insets: *single responses. Scale bars: 50 ms, 2 mV.
discharge reversibly depressed the oscillatory depolarizing responses in the subicular pyramidal cells (Fig. 3B; n = 5, amplitude 8.3 ± 8.0% of control, P < 0.02), indicating that glutamatergic, but not GABAergic, output might play a major role in synchronous propagation of the CA1-generated afterdischarge into the neighboring subiculum region.

**DISCUSSION**

On the basis of the present results, we conclude that slow posttetanic depolarization always precedes the appearance of seizure-like afterdischarge that is induced in the same region and that the seizure-like afterdischarge activity propagates into adjacent regions through glutamatergic, but not GABAergic, pathways. The slow posttetanic depolarization is thought to be caused by drastic changes in ionic gradients of chloride, bicarbonate, and/or potassium, which are primarily dependent on GABA_A receptor activation (Smirnov et al. 1999; Staley and Proctor 1999; Staley et al. 1995). Similar slow GABA-mediated depolarization has been reported to occur in other experimental epilepsy models, e.g., 4-aminopyridine (4-AP)-induced giant depolarizing potential. The giant depolarizing potential, which occurs spontaneously in the presence of 4-AP, is mediated through GABA_A receptors (Avoli et al. 1996; Lamsha and Kaila 1997; Perreault and Avoli 1992) and is often followed by the generation of seizure-like activity (Avoli et al. 1996). In view of the fact that the seizure-like afterdischarge following the slow posttetanic depolarization is also dependent on excitatory GABAergic transmission (Fujisawa-Tsukamoto et al. 2003), such a slow GABA-mediated depolarization may be associated with an initializing process of seizure-like activity in the same cortical region.

On the other hand, synchronous propagation of seizure-like activity into adjacent cortical regions is likely to be mediated by glutamatergic output originating probably from the pyramidal cells. Slow depolarizing GABAergic activity was no longer observed outside the initial seizure-generating site. Such an inter-regional propagation may occur in other hippocampal and neocortical regions synchronously (Rafiq et al. 1993). The CA1 region may often be subjected to CA3- or entorhinal cortex-driven seizure-like oscillations in an intact limbic system (Avoli et al. 2002). Moreover, although other types of seizure-like activity are apparently evoked in GABA_A-blocked (dihydrexibited) conditions in the CA3 region, GABAergic generation of seizure-like afterdischarge has been observed not only in the CA1 region but also in other cortical regions such as the CA3, entorhinal and temporal cortices similarly (Kaneda et al. 2002). Therefore the GABAergic generation and the glutamatergic propagation might be a common nature among the limbic cortical regions in some in vitro epilepsy models.

It has been believed that reduced GABAergic inhibition would underlie the neuronal hyperexcitability in human temporal lobe epilepsy. However, GABAergic neurons and their terminals containing glutamate decarboxylase are preserved in the human epileptic hippocampus (Babb et al. 1989), and GABA, as well as glutamate, is released in the hippocampus during spontaneous seizures in temporal lobe epilepsy patients (During and Spencer 1993). Although a loss of GABA transporters could decrease nonsynaptic GABA release by “GABA transporter reversal” (During et al. 1995), hippocampal GABA transporters are also preserved in temporal lobe epilepsy patients (Mathern et al. 1999). Furthermore, Cohen et al. (2002) have recently reported that interictal epileptic activity is mediated by depolarizing GABAergic transmission, in cooperation with glutamatergic transmission, in hippocampal (subicular) slices obtained from temporal lobe epilepsy patients. Taken together, a reduction in GABAergic inhibition might not result in neuronal hyperexcitability, but rather a conversion of GABA action from inhibition to excitation might play an essential role in focal generation of ictal and/or interictal activities in the in vitro epilepsy models and, possibly, in human temporal lobe epilepsy. Once the GABAergic seizure-like activity occurs in the focus, glutamatergic transmission would, in turn, mediate fast propagation into surrounding or even distant cortical regions synchronously.

**DISCLOSURES**

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**REFERENCES**


