Seizures Induce Simultaneous GABAergic and Glutamatergic Transmission in the Dentate Gyrus-CA3 System

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INTRODUCTION

Glutamic acid decarboxylase (GAD) and GABA are normally present in the excitatory granule cells of the dentate gyrus (DG) (Sandler and Smith 1991; Sloviter et al. 1996). They transiently overexpress GAD mRNA and GAD after seizures (Lehmann et al. 1996; Schwarzer and Sperk 1995), suggesting that they can synthesize GABA de novo, enabling them to utilize it for fast neurotransmission. Supporting this, glutamate receptors antagonists (GluRAs) uncover a fast bicuculline-sensitive inhibitory postsynaptic potential (IPSP) in CA3 pyramidal cells (PC) on DG stimulation in kindled animals (Gutiérrez and Heinemann 1997). However, a permanent state of epilepsy in humans and animal models of chronic epilepsy induces sprouting of mossy fibers (MF) (Babb et al. 1991; Cavazos et al. 1991; Qiao and Noebels 1993) and of inhibitory interneurons (Davenport et al. 1990) that can innervate PC in CA3, and which can underlie aberrant inhibitory responses. Since these changes may obscure the correct identification of the origin of the synaptic responses of PC to DG stimulation, it was important to test whether the fast IPSP in CA3 was provoked by stimulation of rearranged neuronal processes that accompany kindling epilepsy, or to the sole occurrence of a seizure, which is unlikely to induce sprouting (Cavazos et al. 1991). Therefore, this work analyzes the synaptic responses of CA3 PC to DG stimulation, in kindled epileptic animals with and without seizures, and in nonepileptic animals, immediately after an acutely pentylentetrazol (PTZ)-induced seizure.

METHODS

Adult Wistar rats were implanted in the amygdala (Paxinos and Watson 1997) for daily kindling stimulation (1-s train at 60 Hz; 0.1-ms pulse duration; ≈500 μA) until five seizures were evoked. Acute seizures were induced with a single administration of PTZ (70 mg/kg ip). Intracellular recordings of CA3 PC were obtained in vitro, from the following groups of animals: kindled animals (n = 19), 1) 24 h and 2) 1 mo after the last seizure; 3) 24 h after one seizure (n = 8), kindled after 1 mo, during which no seizures were provoked and 4) 2 h after a single PTZ-induced seizure (n = 10). Combined entorhinal cortex-hippocampus slices (400 μm) were cut and transferred to an interface recording chamber and constantly perfused with oxygenated artificial cerebrospinal fluid (ACSF) at 35°C containing (in mM) 124 NaCl, 3 KCl, 1.25 NaH2PO4, 1.8 or 2.4 MgSO4, 1.6 or 0.8 CaCl2, 26 NaHCO3, and 10 glucose, pH 7.35. The drugs used were diluted in the ACSF; namely (DL)-2-amino-5-phosphonovaleric acid (5APV; 30 μM; Tocris); 6-nitro-7-sulfamoylbenzo(f)-quinoxaline-2,3-dione (NBQX; 10 μM; Tocris), and bicuculline methiodide (20 μM; Sigma); halothane (10 μM); (1+)2-amino-4-phosphonobutyric acid (AP4; 10 μM; Tocris). Intracellular activity of PC was recorded with microelectrodes filled with 2 M potassium acetate (70–90 MΩ) using an AxoClamp 2B amplifier. Acquisition and offline analysis were done with the program pClamp6 (Axon Instruments). Stimulation (pulse of 0.1 ms) was delivered over the intersection of the blades of the granular layer of the DG, with a bipolar glass-insulated platinum wire (50 μm) electrode, at an intensity that evoked 70% of the excitatory postsynaptic potential (EPSP) amplitude needed to reach threshold for evoking action potentials.

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RESULTS

DG stimulation evoked EPSP/IPSP sequences in PC (Fig. 1A), which were blocked by perfusion of the GluRAs, NBQX, and 5APV, in 50 of 50 PC, confirming that IPSPs were disynaptically mediated (Fig. 1, A and B1, and C, top; Fig. 2A). However, in PC from rats in which seizures were induced, GluRAs blocked the EPSP and isolated a fast IPSP (Fig. 1, B2 and B3, and C, bottom; Fig. 2B) as follows: 1) in freshly kindled rats, 30 of 30 cells; 2) in preparations recorded a month after completion of the kindling process, 4 of 20 cells; 3) after a kindled seizure, 1 mo after completion of the initial kindling process, in 16 of 19 cells; and 4) 1 h after a single PTZ-induced seizure, in 14 of 20 cells. Neither the resting membrane potential nor the input resistance of the cells (−64 ± 3 mV; 33.8 ± 1.7 MΩ; mean ± SE; n = 50) was altered by the presence of seizures (Fig. 1C). The reversal potential for the seizure-induced IPSP was −67.5 ± 0.3 mV (n = 35; Fig. 1C). The onset latency of the DG-evoked EPSP, measured from the beginning of the stimulus artifact to the beginning of the rising phase, was 4.2 ± 0.7 ms (n = 33) and of the pharmacologically isolated IPSP was 4.8 ± 0.5 ms (n = 27; mean ± SD; Fig. 2C). They were not statistically different. Perfusion of the high Mg²⁺–low Ca²⁺ medium in control preparations blocked the IPSP but not the EPSP, whose latency was not affected (Fig. 2D, top). This also confirms that the control IPSP is disynaptically mediated and, as expected, its apparent kinetics are different from those of the postseizure fast IPSP (Fig. 1, A, B2, and B3), since it was preceded by the monosynaptic EPSP. However, in postseizure preparations, the low Ca²⁺ medium did not block the pharmacologically isolated IPSP nor alter its

FIG. 1. A: control synaptic responses of a pyramidal cell (PC) to dentate gyrus (DG) stimulation. B1: synaptic potentials were blocked by glutamate receptor antagonists (GluRAs) in control preparations and in the majority of cells from kindled nonseizing rats. After an acute pentyleneetetrazol (PTZ)-induced seizure (B2) or a day after a rekindled seizure (B3), a fast inhibitory postsynaptic potential (IPSP) was evident under GluRAs. C: current-voltage (I-V) curves of a control PC (top) and a PC recorded after seizures, under GluRAs (bottom). The traces in the bottom panel show the reversal potential of the postseizure IPSP. Calibration bars in B also apply to A; those in C apply to both panels. Dots signal the stimulus artifact and arrows the afferent volley. Traces in A and B are averages of 10 responses. NBQX, 6-nitro-7-sulfamoylbenzo(f)-quinolaxine-2,3-dione; 5APV, (R)-2-amino-5-phosphonovaleric acid.

FIG. 2. A: control responses to DG stimulation were blocked by GluRAs. B: after seizures, GluRAs block the excitatory postsynaptic potential (EPSP) and isolate a fast IPSP. C: histogram showing the onset latencies of the control DG-evoked EPSP (n = 33) and the postseizure pharmacologically isolated IPSP (n = 27). D: perfusion of a low Ca²⁺–high Mg²⁺ medium blocks the polysynaptic IPSP in control preparations (top), but not the postseizure DG-evoked fast IPSP (bottom), without affecting the onset latencies. Calibration bars in B also apply to A (10 mV for the EPSP; 5 mV for the IPSP). Dots signal the stimulus artifact and the arrow the afferent volley. All traces are averages of 10 responses.
The induction of fast inhibition from DG to CA3 after a seizure, besides the existing feed-forward inhibition (Buzsáki 1984) and the inhibition from adjacent synapses (Vogt and Nicoll 1999), can further modulate MF-CA3 neurotransmission after enhanced excitability. Interestingly, it coincides with postictal depression and inhibition (Hong et al. 1979; Post et al. 1984) that follows seizures, and it can be relevant in the anterograde amnesia that appears in this period. Finally, the present results provide electrophysiological evidence that supports the hypothesis that the MF are able to release GABA, as previously suggested (Gutiérrez and Heinemann 1997; Lehman et al. 1996; Sandler and Smith 1991; Sloviter et al. 1996).

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REFERENCES


