Limbic Network Interactions Leading to Hyperexcitability in a Model of Temporal Lobe Epilepsy

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1Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec H3A 2B4, Canada; 2Istituto di Ricovero e Cura a Carattere Scientifico Neurmed, 87036 Pozzilli (Isernia); 3Dipartimento di Scienze Biomediche, Università degli Studi di Modena e Reggio Emilia, 41100 Modena; and 4Dipartimento di Neuroscienze, Università degli Studi di Roma ‘Tor Vergata’, 00173 Rome, Italy

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D’Antuono, Margherita, Ruba Benini, Giuseppe Biagini, Giovanna D’Arcangelo, Michaela Barbarosie, Virginia Tancredi, and Massimo Avoli. Limbic network interactions leading to hyperexcitability in a model of temporal lobe epilepsy. J Neurophysiol 87: 634–639, 2002; 10.1152/jn.00351.2001. In mouse brain slices that contain reciprocally connected hippocampus and entorhinal cortex (EC) networks, CA3 outputs control the EC propensity to generate experimentally induced ictal-like discharges resembling electrographic seizures. Neuronal damage in limbic areas, such as CA3 and dentate hilus, occurs in patients with temporal lobe epilepsy and in animal models (e.g., pilocarpine- or kainate-treated rodents) mimicking this epileptic disorder. Hence, hippocampal damage in epileptic mice may lead to decreased CA3 output function that in turn would allow EC networks to generate ictal-like events. Here we tested this hypothesis and found that CA3-driven interictal discharges induced by 4-aminopyridine (4AP, 50 μM) in hippocampus-EC slices from mice injected with pilocarpine 13–22 days earlier have a lower frequency than in age-matched control slices. Moreover, EC-driven ictal-like discharges in pilocarpine-treated slices occur throughout the experiment (≤6 h) and spread to the CA1/subicular area via the temporoparietal cortical path; in contrast, they disappear in control slices within 2 h of 4AP application and propagate via the trisynaptic hippocampal circuit. Thus, different network interactions within the hippocampus-EC loop characterize control and pilocarpine-treated slices maintained in vitro. We propose that these functional changes, which are presumably caused by seizure-induced cell damage, lead to recurrent limbic seizures in vivo. This process is facilitated by a decreased control of EC excitability by hippocampal outputs and possibly sustained by the reverberant activity between EC and CA1/subiculum networks that are excited via the temporoparietal cortical path.

INTRODUCTION

Application of 4-aminopyridine (4AP) or Mg2+-free medium to combined hippocampus–entorhinal cortex (EC) slices obtained from rodents induces ictal-like (thereafter termed ictal) epileptiform discharges that originate in EC and propagate to the hippocampus, as well as interictal activity initiating in CA3 (Avoli et al. 1996; Barbarosie and Avoli 1997; Dreier and Heinemann 1991; Wilson et al. 1988). CA3-driven interictal activity exerts an unexpected control on the EC propensity to generate ictal discharges. Accordingly, 1) ictal discharges occur throughout the experiment, but ictal activity disappears within 1–2 h; and 2) Schaffer collateral cut abolishes interictal activity in EC while making ictal discharge reappear in this structure (Barbarosie and Avoli 1997).

Patients suffering from temporal lobe epilepsy present seizures involving the temporal cortex and limbic structures such as the hippocampus and the EC. These patients can manifest a pattern of brain damage (termed mesial temporal sclerosis) characterized by cell loss in CA3 and CA1 subfields and in the dentate hilus (Wieser et al. 1993). A similar pattern of brain damage is reproduced in laboratory animals by injecting kainic acid (Ben Ari 1985) or pilocarpine (Cavalheiro et al. 1996; Liu et al. 1994; Turski et al. 1984) that induces an initial status epilepticus followed 2–3 wk later by recurrent, limbic-type seizures.

Limbic network hyperexcitability in temporal lobe epileptic patients and in animal models mimicking this disorder may result from seizure-induced hippocampal damage leading to synaptic reorganization such as mossy fiber sprouting (Cavazos et al. 1991; Houser et al. 1990; Sutula et al. 1989). However, recurrent limbic seizures can occur in pilocarpine-treated rats when mossy fiber sprouting (but not neuronal damage) is abolished by inhibiting protein synthesis (Longo and Mello 1997, 1998), thus suggesting that cell loss alone may cause a chronic epileptic condition. Since hippocampal output activity controls the EC propensity to generate electrographic seizures in control mouse slices (Barbarosie and Avoli 1997), we predicted that a decrease in hippocampal network activity due to cell damage may lead per se to a chronic epileptic condition in pilocarpine-treated animals and perhaps in patients with temporal lobe epilepsy. Here, we tested this hypothesis by comparing the epileptiform patterns induced by 4AP in hippocampus–EC slices obtained from pilocarpine-treated and age-matched mice.

METHODS

Twenty-two CD-1 mice (29–42 days old) were used in this study. The procedures for injecting animals (n = 12) with pilocarpine were similar to those used in our laboratories with rats (Liu et al. 1994). To
prevented discomfort caused by stimulation of peripheral muscarinic receptors by pilocarpine (60–100 mg/kg), mice were pretreated with subcutaneous scopolamine methyl nitrate (1 mg/kg). The animals’ behavior was monitored ≤4 h after pilocarpine and scored according to Racine’s classification (Racine et al. 1972). Slices defined as "pilocarpine-treated" were obtained 13–24 days following pilocarpine injection from mice with a behavioral response classified as stage 6 (i.e., tonic-clonic seizures occurring for ≥1 h). Control slices were obtained from age-matched mice. Animals were decapitated under halothane anesthesia; their brains were removed and placed in cold oxygenated artificial cerebrospinal fluid (ACSF) (Barbarosie and Avoli 1997). Horizontal, hippocampus–EC slices (500 μm thick) were cut with a vibratome and transferred to a tissue chamber where they lay between oxygenated ACSF and humidified gas (95% O2:5% CO2) at 32–34°C. ACSF composition was as follows (mM): 124 NaCl, 2 KCl, 1.25 KH2PO4, 2 MgSO4, 2 CaCl2, 26 NaHCO3, and 10 glucose. 4AP (50 μM) was bath applied. Chemicals were acquired from Sigma.

Field potential recordings were made with ACSF-filled glass pipettes (tip diameter <10 μm; resistance <5–10 MΩ) positioned in EC, dentate gyrus, CA3 or CA1, and/or the subiculum. Signals were fed to high-impedance DC amplifiers and displayed on a Gould pen recorder. Field potential profiles of the ictal discharges recorded in the CA1/subiculum were performed with two recording electrodes. One electrode was maintained at a fixed position, while the other was moved in 100 μm stepwise increments along an axis normal to the alveus. Signals from the fixed electrode were used for temporal alignment of the field potentials obtained with the moving electrode. Field potential amplitudes at different latencies from the epileptiform discharge onset were calculated by averaging two to four events and plotted in a bidimensional fashion (i.e., amplitude versus space). In any given experiment, this type of analysis was restricted to ictal events that had similar electrographic characteristics (e.g., duration >20 s) when recorded from the fixed electrode. Time delays for discharge onset in different areas of the slice were calculated by taking as reference the first deflection from the baseline in expanded traces. Electrophysiological measurements are expressed as mean ± SD and n represents the number of slices studied. Data were compared with the Student’s t-test or the analysis of variance (ANOVA) test and were considered significantly different if P < 0.05.

At the end of the experiments, some slices were fixed in 4% paraformaldehyde/100 mM phosphate-buffered solution overnight at 4°C and then rinsed several times in 15 and 30% sucrose–phosphate-buffered solutions for cryoprotection, and frozen at −80°C. Slices were cut with a cryostat into 14 μm thick sections and processed for Nissl staining. A blinded collaborator assessed the presence of tissue damage in various hippocampal regions. In pilocarpine-treated slices processed for histology (n = 7), we found a decrease of total neuron number that ranged 36–56 and 63–80% of controls in the CA1 and CA3 area, respectively. These data are in line with previous studies of the effects of ip pilocarpine in albino mice (Cavalheiro et al. 1996; Turski et al. 1984).

RESULTS

Bath application of 4AP (50 μM) to combined hippocampus–EC slices (n = 8) obtained from control mice induced brief, ictal events at 0.5–1.1 Hz and prolonged ictal discharges with intervals of occurrence ranging 50–160 s. These two types of epileptiform activity were recorded in hippocampus and EC after 20–30 min of 4AP application (Fig. 1A). Time delay measurements and pathway cutting demonstrated that the ictal interictal discharges originated in CA3 (Fig. 1A, inset), while the ictal events initiated in the EC (Barbarosie and Avoli 1997). Moreover, ictal discharges disappeared in control slices within about 2 h of continuous 4AP application, while the interictal activity occurred throughout the experiment (Fig. 1, A and F).

Hippocampus–EC slices (n = 17) from pilocarpine-treated mice also responded to 4AP application by generating interictal and ictal discharges (Fig. 1B). However, the interictal activity observed in these experiments had a lower rate of occurrence and a longer duration than in control slices (Fig. 1, B, D, and E). Moreover, ictal discharges generated by pilocarpine-treated slices continued to occur throughout the experiment (≥6 h). Thus, the percentage of slices generating ictal discharges at different times of 4AP application was different when analyzed in control and pilocarpine-treated slices (Fig. 1F). As reported in control slices (Barbarosie and Avoli 1997), ictal discharges in pilocarpine-treated slices initiated in EC (Fig. 1C).

Next, we analyzed the modalities of propagation of the interictal and ictal discharges induced by 4AP in slices obtained from control and pilocarpine-treated mice. This was done by simultaneously recording the field potential activity in the EC, the dentate gyrus, and either the CA3 or the CA1/subiculum. The epileptiform activity occurring in control slices (n = 5) at the beginning of the experiment propagated as previously reported (Barbarosie and Avoli 1997; Barbarosie et al. 2000). Namely, CA3-driven interictal discharges appeared to spread successively to CA1, subiculum, and EC from where they presumably re-entered the hippocampus via the perforant path (Fig. 2, A and C) (cf. Paré et al. 1992). Ictal discharges initiated in EC and propagated to the hippocampus through the perforant path with onset delays, suggesting the involvement of the classic trisynaptic hippocampal circuit (Fig. 2, A and D). CA3-driven interictal discharges in pilocarpine-treated slices (n = 10) also propagated to EC via the CA1/subiculum and re-entered the hippocampus via the perforant path (Fig. 2, B and C). In these experiments, however, ictal discharges initiating in EC were recorded in the dentate gyrus, CA1, and subiculum with similar time delays (Fig. 2, B and D). Hence, they presumably spread from the EC to the CA1/subiculum via the temporoammonic path.

Temporolammonic inputs to CA1/subicular neurons are localized more apically than those provided by the Schaffer collateral system (Soltesz and Jones 1995). Therefore, we analyzed the depth profile characteristics of the ictal discharges recorded in the subiculum of control (n = 5) and pilocarpine-treated slices (n = 4). In both types of tissue, the steady shift associated with the ictal discharge was positive-going at or near the alveus, inverted in polarity when the electrode was moved toward the depth, and increased in amplitude as the electrode was further lowered toward the dentate upper blade (Fig. 3B). However, in pilocarpine-treated slices, it displayed maximal negative values at sites that were deeper (and thus more apical) than in control slices. Moreover, the peak-to-peak amplitude of the population spikes occurring during the ictal discharge attained maximal amplitude at approximately 500 and 700 μm in control and pilocarpine-treated slices, respectively. The depth-profile data obtained from three control and four pilocarpine-treated slices are summarized in Fig. 3, C and D.

DISCUSSION

Hippocampal cell loss is found in patients with temporal lobe epilepsy (Wieser et al. 1993) and in laboratory animals...
treated with convulsants such as kainic acid (Ben Ari 1985) or pilocarpine (Liu et al. 1994; Turski et al. 1983, 1984). The neuronal damage induced by the initial status epilepticus leads to sprouting along with synaptic reorganization (Cavazos et al. 1991; Gorter et al. 2001; Houser et al. 1990; Sutula et al. 1989). In addition, structural and functional impairment of GABA-mediated inhibition has been documented in these animal models (Doherty and Dingledine 2001; Fountain et al. 1998; Gorter et al. 2001; Williams et al. 1993). However, it is unclear how these changes in network function produce a chronic epileptic condition.

Previous work performed in nonepileptic mouse hippocampus–entorhinal cortex (EC) slices has revealed that CA3-driven interictal activity controls the expression of ictal discharges in the EC, presumably by perturbing the ability of EC networks to reverberate (Barbarosie and Avoli 1997). Here, we have found that CA3-driven interictal activity in pilocarpine-treated slices occurs at lower rates than in control slices. Note also that ictal discharges continue to occur after 2 h of 4AP application. C: expanded interictal-ictal discharge recorded in a pilocarpine-treated slice shows that the ictal event initiates in EC and propagates to CA3 with a 90 ms latency. D and E: duration and rate of occurrence of interictal and ictal discharges in control (n = 10) and pilocarpine-treated (n = 11) slices. Values that were significantly different (P < 0.05) are indicated by the asterisks. F: percentage of slices generating ictal discharges at different times of 4AP application in control and pilocarpine-treated slices. Values were obtained from 6, 5, and 4 control slices for the periods of 1, 2–3, and 4–5 h, respectively, as well as from 8, 7, and 6 pilocarpine-treated slices for the periods of 1–3, 4, and 5 h, respectively.
output activity, may release its control on EC network excitability. In line with this view, similar data are obtained in control mouse slices by cutting the Schaffer collateral, a procedure that prevents CA3-driven interictal discharges from reaching the CA1/subiculum and thus from activating the EC (Barbarosie and Avoli 1997).

We have also found that in intact, pilocarpine-treated slices the spread of ictal discharges from the EC to the CA1/subiculum occurs through the temporoammonic path (cf. Soltesz and Jones 1995). In contrast, in control slices, this activity propagated to the CA1 through the classic trisynaptic circuit (cf. Paré et al. 1992). This conclusion is supported by the depth profile analysis of the ictal discharges recorded in the subiculum of control and pilocarpine-treated mice. We have previously shown in nonepileptic mouse slices that the temporoammonic path becomes involved in the propagation of 4AP-induced ictal discharges after cutting the Schaffer collateral and thus after blocking the activation of CA1 and subicular networks (Barbarosie et al. 2000). Under normal conditions, depressing synaptic transmission between CA3 and CA1 makes the temporoammonic projection from the EC to CA1 operative (Maccaferri and McBain 1995). In pilocarpine-treated tissue, this effect may also be contributed by a use-dependent reduction of the excitatory drive onto interneurons (Doherty and Dingledine 2001). The functional consequence of this change in modality of propagation is that the ictal activity originating in the EC short-circuits the trisynaptic hippocampal route and thus can monosynaptically activate CA1 and subicular neurons, thus ensuring a high-fidelity synaptic transfer that increases epileptiform synchronization. Indeed, it may be hypothesized that in the pilocarpine-treated brain, subicular networks play a unique role in sustaining limbic seizures.

In conclusion, we have identified some differences in the way(s) limbic networks obtained from pilocarpine-treated and age-matched control mice interact in vitro during 4AP application. Our data provide some novel explanations for why pilocarpine-treated mice, and perhaps temporal lobe epilepsy patients, are susceptible to generating seizures in vivo. In
FIG. 3. Depth profile characteristics of the field potentials associated with the ictal discharges recorded in the CA1/subicular area of control and pilocarpine-treated slices. A: schematic representation of the experimental procedure indicating the area where recordings were obtained with two microelectrodes: one was maintained at a fixed position, while the other was moved in 100 μm stepwise increments along an axis normal to the pial aspect of the subiculum. B: field potential recordings obtained at different depths in control and pilocarpine-treated slices. Depth values were measured relative to the pia and are indicated on the left of each sample. Asterisks indicate the recording obtained from the stationary electrode in each of the two experiments. C and D: depth distribution of the amplitudes of the fast events and of the DC shifts associated with the ictal discharges in control (n = 3) and pilocarpine-treated slices (n = 4). Values were grouped in increments of 100 μm. Note that the amplitudes of the fast transients in pilocarpine-treated slices attain maximal values at depths that are greater than control slices (P < 0.05). A similar pattern of distribution is also evident for the DC shift negative values.

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particular, our findings emphasize the role played by cell loss in excitability and also make the temporoammonic path operative.

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