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Impairment of GABA release in the hippocampus at the time of the first spontaneous seizure in the pilocarpine model of temporal lobe epilepsy

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ABSTRACT

The alterations in GABA release have not yet been systematically measured along the natural course of temporal lobe epilepsy. In this work, we analyzed GABA extracellular concentrations (using in vivo microdialysis under basal and high K^+-evoked conditions) and loss of two GABA interneuron populations (parvalbumin and somatostatin neurons) in the ventral hippocampus at different time-points after pilocarpine-induced status epilepticus in the rat, i.e. during development and progression of epilepsy. We found that (i) during the latent period between the epileptogenic insult, status epilepticus, and the first spontaneous seizure, basal GABA outflow was reduced to about one third of control values while the number of parvalbumin-positive cells was reduced by about 50% and that of somatostatin-positive cells by about 25%; nonetheless, high K^+ stimulation increased extracellular GABA in a proportionally greater manner during latency than under control conditions; (ii) at the time of the first spontaneous seizure (i.e., when the diagnosis of epilepsy is made in humans) this increased responsiveness to stimulation disappeared, i.e. there was no longer any compensation for GABA cell loss; (iii) thereafter, this dysfunction remained constant until a late phase of the disease. These data suggest that a GABAergic hyperresponsiveness can compensate for GABA cell loss and protect from occurrence of seizures during latency, whereas impaired extracellular GABA levels can favor the occurrence of spontaneous recurrent seizures and the maintenance of an epileptic state.

Introduction

In temporal lobe epilepsy (TLE), the most frequent epilepsy syndrome in adults, the hippocampal formation often displays distinct neuropathological features, such as neuronal death, neurogenesis, gliosis, axonal sprouting and reorganization of neuronal interconnections. These abnormalities develop in a previously healthy tissue, often after an initial "epileptogenic" event that can produce damage, for example an episode of prolonged, uncontrolled seizures (status epilepticus, SE). Only after a latent period of weeks to years epileptogenic events may be followed by spontaneous recurrent seizures, i.e. by the diagnosis of epilepsy (Pitkanen and Sutula, 2002).

The control of excitability in the mammalian brain, including epileptic hyper-excitability, is largely dependent on the main inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Indeed, many drugs potentiating GABA transmission are effective antiseizure agents (Treiman, 2001). Unfortunately, however, little is known on the dynamic changes in the GABAergic system in natural course of TLE and in its progression toward pharmaco-resistance. In the epileptic tissue, seizures are not generated in a normal circuit but in a profoundly rewired network (Cossart et al., 2005). Only some aspects of the alterations specifically affecting the GABA system have been identified. For example, a substantial loss of glutamic acid decarboxylase (GAD) mRNA-containing (i.e. GABAergic) neurons has been found in the hilus of dentate gyrus (Obenaus et al., 1993) and in the stratum oriens of CA1 (Houser and Esclapez, 1996). Moreover, reduced number of specific GABAergic neurons, including parvalbumin- (Drexel et al., 2011; Kuruba et al., 2011; Pavlov et al., 2011) and somatostatin-positive interneurons (Paradiso et al., 2009; Sperk et al., 1992; Sun et al., 2007), has been found in the epileptic hippocampus. Another interesting alteration is that repetitive activation leads to profound post-synaptic $GABA_A$ receptor desensitization (run-down) in the human epileptic tissue (Ragozzino et al., 2005) and in chronically epileptic rats (Mazzuferi et al., 2010; Palma et al., 2007).

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The pre-synaptic counterpart of these alterations in the GABA system has not been systematically studied yet. Microdialysis studies in pharmaco-resistant epileptic patients undergoing depth electrode investigation prior to surgery have shown an increased outflow of GABA in the hippocampus in response to seizures, even if this increase was not as dramatic as that of the excitatory neurotransmitters glutamate and aspartate (During and Spencer, 1993; Thomas et al., 2005; Wilson et al., 1996), whereas the basal, interictal GABA outflow was non-significantly reduced in the epileptogenic hippocampus (Pan et al., 2008). For obvious reasons, these works lack stringent controls apart from the apparently non-epileptogenic contralateral hippocampus (During and Spencer, 1993; Thomas et al., 2005) or hippocampus of patients with neo-cortical epilepsy (Pan et al., 2008). Unfortunately, studies in animal models also did not provide insight on this issue, because they revealed transient or non-significant increases in hippocampal GABA outflow during pilocarpine-induced SE (Khongsombat et al., 2008; Meurs et al., 2008; Smolders et al., 1997), but did not yet explore the possible subsequent adaptive changes in GABA neurotransmission.

Here, we used microdialysis to analyze the basal and potassium stimulated GABA outflow in the ventral hippocampus at different time-points after pilocarpine induced SE in the rat. In parallel, we measured the loss of parvalbumin- and somatostatin-expressing GABA interneurons. We found that the loss of GABA neurons is compensated by hyper-responsiveness of the system in the latency period, whereas GABA outflow is dramatically reduced when spontaneous seizures begin to occur.

Materials and methods

Animals

Male Sprague–Dawley rats (250–350 g; Harlan, Italy) were used for all experiments. They were housed under standard conditions: constant temperature (22–24 °C) and humidity (55–65%), 12 h light/dark cycle, free access to food and water. Procedures involving animals and their care were carried out in accordance with European Community (EU Directive 2010/63/EU), national and local laws and polices (authorization: D.M. 83/2009-B and D.M. 246/2012-B). All animals were acclimatized to the microdialysis laboratory conditions for at least 1 h before each experiment and euthanized immediately after the last day of microdialysis by an anesthetic overdose. Rats were killed by decapitation under 1.4% isoflurane anesthesia. The number of animals was kept as small as possible.

Pilocarpine

Rats were randomly assigned to groups that received a single injection of methyl-scopolamine (1 mg/kg, s.c.) 30 min prior to pilocarpine (370 mg/kg, i.p.) or a single injection of methyl-scopolamine 30 min prior to vehicle (0.9% NaCl solution; control animals), and their behavior was observed by experienced researchers for at least 6 h thereafter. Within the first 20–25 min after pilocarpine injection, 83% of the animals developed seizures evolving into recurrent generalized convulsions (status epilepticus, SE). SE was interrupted 3 h after onset by administration of diazepam (20 mg/kg, i.p.). One fourth of the animals that entered SE (i.e. 21% of those administered pilocarpine) died during SE or within 1–2 days. Test and interspersed control animals were then randomly assigned to five experimental groups representing different phases of the natural history of the disease (Fig. 1A): acute phase (24 h after SE), latency (7–9 days after SE), first spontaneous seizure (11 ± 1 days after SE), early chronic (22–24 days after SE, i.e. about 10 days after the first seizure) and late chronic (2 months after SE, i.e. about 50 days after the first seizure). Animals that did not fully recover (i.e. no increase in body weight within the first week) after pilocarpine SE were excluded from the study. Data were collected and processed only from those animals in which the probe was correctly placed, as estimated using a hematoxylin-eosin staining (see below). Experiments were considered completed only when the number of animals in the various groups achieved five or more. In summary: inclusion/exclusion criteria were development of convulsive SE within 1 h after pilocarpine administration; weight gain in the first week after SE; correct positioning of the microdialysis probe.

Analysis and statistics

Convulsive seizures were assessed by 24/24-h video monitoring, performed using a digital video surveillance system DSS1000 (V4.7.0041FD, AverMedia Technologies, USA). Video monitoring was started approximately 6 h after pilocarpine administration (i.e. at the end of direct observation by the researchers – see above) and continued until day 5. For proper identification of the first spontaneous seizure, continuous video-EEG monitoring was started from day 5 after SE until the day of the first spontaneous seizure (Fig. 1A). Video-EEG monitoring (hardware system MP150 and software AcqKnowledge 4.3, all from Biopac, USA) was started at day 5 because, as previously reported (Mazzuferi et al., 2010; Paradiso et al., 2009), we do not observe spontaneous seizures earlier than 8–9 days after pilocarpine administration under the experimental conditions employed in this study. Video-EEG was also performed in the course of microdialysis for assessment of absence of seizures in the 3 h prior to microdialysis and of seizures evoked by high K^+; video-monitoring was also performed in two 2-weeks epochs in the early (days 11–24) and late (days 49–62) chronic phase for assessment of generalized seizure progression (Fig. 1A).

EEG seizures were categorized and measured as periods of paroxysmal activity of high frequency (>5 Hz) characterized by a >3-fold amplitude increment over baseline with progression of the spike frequency that lasted for a minimum of 3 s (Williams et al., 2009). Seizure severity was scored using the scale of Racine (Racine, 1972): 1, chewing or mouth and facial movements; 2, head nodding; 3, forelimb clonus; 4, generalized seizure with rearing; 5, generalized seizure with rearing and falling. Video-EEG analysis was performed by two independent investigators that were blind for the group to which the rats belonged. In case of differential evaluation, data were reviewed together to reach a consensus (Paradiso et al., 2011). In the early and late chronic period, animals were continuously video recorded for two weeks before and during microdialysis, to identify frequency and duration of generalized seizures (class 4 or 5), which were statistically examined using the Student's t-test for unpaired data. The Kruskal-Wallis and post hoc the Dunn's multiple comparison test were used for evaluation of high K^+-evoked seizures.

Microdialysis

Surgery

Under initial ketamine/xylazine (43 and 7 mg/kg, i.p.) anesthesia, rats were secured to a stereotaxic apparatus with the nose bar positioned at +5 mm. Anesthesia was then maintained using isoflurane (1.4% in air; 1.2 ml/min). A 15 mm long guide cannula (MAB 4.15.iC; Agn Tho's, Lidingö, Sweden, outer diameter 500 ± 5 μm) equipped with a dummy cannula (Plastics One, Roanoke, Virginia, USA) and with a recording electrode glued at its outside (0.5 mm longer than the cannula, 0.3 mm in diameter) was implanted into the right ventral hippocampus (A −3.4 mm; L +4.5 mm; P +6.5 mm to bregma; Fig. 1B). A reference electrode was placed on the skull. The guide cannula was fixed to the skull with four stainless screws and methacrylic cement. Rats were allowed 7 days to recover.

In vitro recovery

To optimize the experimental conditions of microdialysis, we estimated the in vitro recovery of GABA when using two perfusion rates and five different home-made or manufactured probes, all of them of 1 mm membrane length. We compared two home-made probes endowed with a polyacrylonitrile membrane (molecular weight cut-