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INCREASED EXTRACELLULAR LEVELS OF GLUTAMATE IN THE HIPPOCAMPUS OF CHRONICALLY EPILEPTIC RATS

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Abstract—An increase in the release of excitatory amino acids has consistently been observed in the hippocampus during seizures, both in humans and animals. However, very little or nothing is known about the extracellular levels of glutamate and aspartate during epileptogenesis and in the interictal chronic period of established epilepsy. The aim of this study was to systematically evaluate the relationship between seizure activity and changes in hippocampal glutamate and aspartate extracellular levels under basal and high K^+-evoked conditions, at various time-points in the natural history of experimental temporal lobe epilepsy, using *in vivo* microdialysis. Hippocampal extracellular glutamate and aspartate levels were evaluated: 24 h after pilocarpine-induced status epilepticus (SE); during the latency period preceding spontaneous seizures; immediately after the first spontaneous seizure; in the chronic (epileptic) period. We found that (i) basal (spontaneous) glutamate outflow is increased in the interictal phases of the chronic period, whereas basal aspartate outflow remains stable for the entire course of the disease; (ii) high K^+ perfusion increased glutamate and aspartate outflow in both control and pilocarpine-treated animals, and the overflow of glutamate was clearly increased in the chronic group. Our data suggest that the glutamatergic signaling is preserved and even potentiated in the hippocampus of epileptic rats, and thus may favor the occurrence of spontaneous recurrent seizures. Together with an impairment of GABA signaling (Soukupova et al., 2014), these data suggest that a shift toward excitation occurs in the excitation/inhibition balance in the chronic epileptic state. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: temporal lobe epilepsy, pilocarpine, glutamate, aspartate, microdialysis.

INTRODUCTION

A disorder in glutamate-mediated excitatory neurotransmission has long been a candidate as a central factor in the pathophysiology of at least some forms of human and experimental epilepsy. A number of studies have suggested that an abnormal amplification of glutamate signaling occurs during seizures (Bradford, 1995; Sherwin, 1999). Moreover, an impairment of inhibition due to reduction of GABA release (Soukupova et al., 2014), desensitization of $GABA_A$ receptors (Palma et al., 2007; Mazzuferi et al., 2010) and/or loss of GABAergic interneurons (Huusko et al., 2013; Houser, 2014) may contribute to exaggerate excitatory signals. Altogether, studies carried out in the last two decades both in epileptic patients and in animal models support the notion that, during seizures, epileptic circuitries lack the necessary balance between inhibition and excitation in favor of the latter.

In humans, epilepsy has been found to be associated with increased extracellular levels of glutamate and aspartate. A highly significant increase in glutamate extracellular concentration was observed before and during partial seizures with secondary generalization in mesial temporal lobe epilepsy (mTLE) patients undergoing surgery, using bilateral intrahippocampal microdialysis and the non-epileptogenic hippocampus of each patient as control (During and Spencer, 1993). Microdialysis evidence supports the notion that not only glutamate but also aspartate extracellular concentrations are significantly increased in the epileptogenic brain tissue under basal conditions and during intense seizures in epilepsy surgery patients (Sherwin, 1999; Thomas et al., 2003). Moreover, the interictal extracellular glutamate levels in the non-epileptogenic human hippocampus were found to be much lower compared to those in the epileptogenic area (Cavus et al., 2008). Although these results indicate a strong association between higher glutamate (and maybe also aspartate) and epileptiform activity, for obvious reasons all these human studies lack

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Abbreviations: ANOVA, analysis of variance; EEG, electroencephalogram; HPLC, high-performance liquid chromatography; mTLE, mesial temporal lobe epilepsy; SE, status epilepticus.

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stringent controls and do not provide information on the dynamic changes occurring in the natural history of the disease.

Several animal studies support and integrate the observations made in humans. Increases in hippocampal or cortical extracellular glutamate and aspartate have been consistently observed during different types of chemically and electrically induced seizures in rats. An ictal increase in hippocampal glutamate levels has been described after microperfusion of various chemoconvulsants (namely pilocarpine, picrotoxin and 3,5-dihydrophenylglycine) into the hippocampus (Meurs et al., 2008) or during chronic-phase seizures following intrahippocampal kainate injection in rats (Wilson et al., 1996; Kanamori and Ross, 2011). Hippocampal extracellular aspartate levels were also found to increase during seizures (Wilson et al., 1996). A very small number of studies describe the interictal extracellular levels of glutamate (or aspartate) in chronic models of epilepsy. However, a significant increase in extracellular glutamate concentrations has been observed in the hippocampus of rats 60 days after intra-amygdala kainate injection (Ueda et al., 2001) and in fully kindled as compared with naïve rats (Mazzuferi et al., 2005; Maciejak et al., 2009). Similarly, significantly increased extracellular interictal levels of aspartate have been observed in a model of focal epilepsy induced by intracortical injection of ferrous chloride (Ronne Engstrom et al., 2001).

Altogether, human and animal studies suggest that glutamate and aspartate extracellular levels are increased in the chronically epileptic tissue (and further increased during seizures). Evidence in this respect needs to be strengthened under rigidly controlled conditions. Moreover, no data are available on the changes in these systems that may occur in the course of the disease, from the initial epileptogenic insult to the development and maintenance of spontaneous seizures. In a previous study (Soukupova et al., 2014) we have demonstrated that GABA release undergoes significant changes in the course of TLE development. Here, we used microdialysis to analyze, for the first time in detail, basal and stimulated glutamate and aspartate outflow in the hippocampus at different time-points of TLE.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (250–350 g; Harlan, Milan, Italy) were housed under controlled illumination (12-h light/dark cycle; light on 06.00 am) and environmental conditions (ambient temperature 22–24 °C, humidity 55–65%) beginning at least one week before surgeries. Rat chow and tap water were available *ad libitum*. The experimental procedures were approved by the University of Ferrara Institutional Animal Care and Use Committee and by Italian Ministry of Health (authorization: D.M. 246/2012-B) in accordance with guidelines outlined in the European Communities Council Directive of 24 November 1986 (86/609/EEC). All animals were acclimatized to the microdialysis laboratory conditions for at least 1 h before each experiment. After the last day of microdialysis, they were killed by decapitation under 1.4% isoflurane anesthesia. Dialysates from a subset of the animals employed in this study (two per group) had been also employed in another, previously published study (Soukupova et al., 2014) to measure GABA outflow. All efforts were made to reduce animal numbers and suffering during the experiments.

Pilocarpine protocol. The pilocarpine protocol was identical to one we previously described (Soukupova et al., 2014). Briefly, intraperitoneal injection of pilocarpine (350 mg/kg) 30 min after a single subcutaneous injection of methyl-scopolamine (1 mg/kg) induced in animals the typical behavior: early partial seizures (movements of vibrissae and head nods within 5 min after pilocarpine administration) evolving into recurrent generalized convulsions (status epilepticus, SE) within 25–30 min. Rats that did not develop SE within 30 min received an additional dose of pilocarpine (175 mg/kg, i.p.). SE was interrupted 3 h after onset by administration of diazepam (20 mg/kg, i.p.). Control animals received a single injection of methyl-scopolamine (1 mg/kg) 30 min prior to vehicle (0.9% NaCl solution, pH adjusted to 7.0). Recording of the seizure behavior began immediately after the pilocarpine injection and was continued for at least 6 h thereafter.

To favor recovery from the body weight loss that follows SE, animals were injected with saline (1 ml of 0.9% NaCl solution, pH adjusted to 7.0) and fed with a 10% sucrose solution for 2–3 days. Those animals that did not achieve the initial body weight within the first week after pilocarpine SE were excluded from the study. Rats were randomly assigned to four experimental groups: acute phase (24 h after SE), latency (7–9 days after SE), first spontaneous seizure (approximately 11 days after SE), and chronic period (22–24 days after SE, i.e., about 10 days after the first seizure). Data were collected and processed only from those animals in which the probe was correctly placed. 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. The number of valid animals per group was predetermined as five or more (Soukupova et al., 2014).

Identification of seizures and electroencephalogram (EEG) activity. EEG seizures were defined 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; Paradiso et al., 2011). They were detected using a hard wire system MP150 and AcqKnowledge 4.3 software (both Biopac, Goleta, CA, USA). Severity of behavioral seizures was scored according to Racine (1972): class 1, chewing, lips and facial movements; class 2, head nods; class 3, forelimb clonus; class 4, generalized seizure with rearing; class 5, generalized seizure with rearing and falling.

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