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Cholinergic modulation of status epilepticus in the rat barrel field region of primary somatosensory cortex

Eduardo D. Martín[a,*], Valentín Ceña[a], Miguel A. Pozo[b]

[a]*Unidad Asociada Neurodeath, UCLM-CSIC, Departamento de Ciencias Médicas, Universidad de Castilla-La Mancha, Avda. de Almansa s/n, 02006 Albacete, Spain*

[b]*Brain Mapping Unit, Instituto Pluridisciplinar, UCM. Paseo Juan XXIII, 1. 28040 Madrid, Spain*

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Abstract

Status epilepticus (SE) represents a serious medical emergency that can produce long-lasting brain damage as well as cognitive and memory deficits. However, the mechanisms that determine the emergence of SE from a single seizure and the prolonged duration of SE are unknown. Therefore, we used pharmacological tools to investigate the cellular mechanisms that underlie this prolonged epileptic activity in the rat barrel field region of somatosensory cortex (S1BF). Electrocortical and unitary extracellular field recording in the rat S1BF region was used to assess abnormal epileptiform activity induced by intracerebral application of 4-aminopyridine (4-AP). Simultaneously, electromyographic (EMG) activity was recorded from mystacial pad musculature. Intracerebral injection of 4-AP induced an SE that was paralleled by an increase of whisker activity that was not synchronized with the electrocortical recording. The seizures were originated ipsilaterally in the cortex of the injected hemisphere and propagated to the contralateral cortex with lower amplitude. The application of the glutamatergic NMDA receptor antagonist D (−)-2-amino-5-phosphonopentanoic acid (AP5) strongly increased the seizure-onset latency. The muscarinic receptor antagonist atropine changed the continuous rapid spiking pattern of SE to periodic discharges, while glutamatergic or GABAergic antagonist did not modify the electrographic features of SE. Our data suggest that the muscarinic cholinergic system plays an important role in the seizure modulation during SE in the somatosensory cortex, while their emergence is controlled, in part, by glutamatergic NMDA receptors.
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Keywords: Epileptogenesis; Cholinergic pathway; Muscarinic receptors; Glutamatergic receptors; 4-aminopyridine

Introduction

Status epilepticus (SE) is a serious medical emergency characterized by high morbidity and mortality (Lowenstein and Alldredge, 1998). Generalized tonic–clonic SE can produce brain damage (Treiman, 1993) while nonconvulsive SE has been reported to cause brain damage as well as cognitive and memory deficits (Engel et al., 1978; Privitera, 1997). The operational definition of SE is a continuous, generalized, convulsive seizure lasting longer than 5 min (in an adult or child older than 5 years), or two or more seizures during which the patient does not return to baseline consciousness (Lowenstein et al., 1999). For the purposes of clinical research, SE has been defined as continuous seizures that last at least 30 min or two or more seizures with an incomplete recovery of consciousness between episodes (Lowenstein, 1999; Kapur, 1999). The difficulties setting up SE studies in emergency conditions are obvious; therefore, reliable animal models of SE are necessary to address questions concerning the pathophysiology of SE (Martín and Pozo, 2003).

The rat somatosensory cortex as an animal model in epilepsy has recently received considerable attention because trigeminal nerve stimulation can cause cortical and thalamic desynchronization, resulting in a reduction of seizure activity (Fanselow et al., 2000). In addition, a

* Corresponding author. Fax: +34 967 599 320.
E-mail address: eduardo.martin@uclm.es (E.D. Martín).

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synchronization over the entire neocortex, accompanied by an important loss of muscular electrical activity, was obtained by acute and massive vibrissal deafferentation (Prchal and Decima, 2004). Thus, we thought the rat somatosensory cortex to be a good model to investigate the pathophysiology of epileptiform activity. Since epileptiform activity is associated with an imbalance between excitatory and inhibitory synaptic mechanisms (Prince, 1978), the current study was designed to investigate, using electrophysiological techniques and pharmacological tools, the synaptic mechanisms that may be involved in the emergence and prolonged duration of SE in the rat barrel cortex region. Our results suggest that cholinergic pathways modulate the seizure pattern during SE while NMDA receptors contribute to their emergence.

Materials and methods

All experiments in this study were performed in accordance with guidelines of the European Union (86/609/EU) for the use of laboratory animals and every effort was made to minimize the suffering and number of animals used. Adult male Wistar rats (285 ± 20 g) were anesthetized with an intraperitoneal (i.p.) injection of urethane (1.3 g/kg). The animal was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) and the body temperature was maintained at 37 ± 1°C with a heating blanket (Harvard Apparatus, Millis, MA, USA). Stereotaxic coordinates were determined according to the Paxinos and Watson (1982) atlas. Appropriate holes were drilled in the skull and needle EEG electrodes (Meditec, Parma, Italy), insulated except at the tips, were placed symmetrically in each hemisphere (1.5 mm posterior, 5 mm lateral to bregma) to record electrocortical activity (ECoG). Fixation of the electrodes on the skull was performed with dental acrylic cement. A subcutaneous stainless steel needle in the nose served as ground and reference electrode. ECoG activity from each hemisphere was amplified (Iso-DAM8, WPI, Sarasota, FL, USA) and band-pass filtered between 10 Hz and 100 Hz. Monopolar tungsten electrodes were implanted into the left and right mystacial pads to record simultaneously the facial muscle activity. The extramuscular potential from the mystacial pad was amplified (P55, Grass Instruments, West Warwick, RI, USA) and band-pass filtered between 10 Hz and 200 Hz to form the electromyographic (EMG) signal. In some experiments, a monopolar tungsten electrode was placed in the barrel field region of primary somatosensory cortex (S1BF; 1.3 mm posterior, 5 mm lateral to bregma, and depth of 3 mm) to record extracellular field responses. S1BF activity was amplified (Iso-DAM8, WPI) and band-pass filtered between 10 Hz and 500 Hz. The amplified signals were stored in a Pentium-based PC through a DigiData 1322A interface board (Axon Instruments, Foster City, CA, USA). The pClamp 9.0 software (Axon Instruments) was used for acquisition, storage, and analysis of data.

To induce continuous epileptiform activity, 5 μl of a solution of 0.9% NaCl containing 10 mM 4-aminopyridine (4-AP) were slowly (≈2 min) injected with a Hamilton 7101N syringe and needle (5 mm posterior and 3 mm lateral to bregma and 2.5 mm vertical from the dura). Five control rats were injected only with saline (5 μl). Antagonists for different neurotransmitters were stored as frozen concentrated stock solutions and dissolved in 4-AP solution before application. In all situations, the total volume injected was 5 μl. All drugs were purchased from Sigma except 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) and D (−)-2-amino-5-phosphonopentanoic acid (AP5) that were from Tocris (Ballwin, MO, USA). Differences among group means were assessed either with proper t test or appropriately designed analyses of variance (ANOVAs) for independent and/or repeated measures. For post hoc evaluations using ANOVAs, the Bonferroni t test was used and differences were considered statistically significant if $P < 0.05$. Differences and values are given as the mean ± SEM unless stated otherwise.

Results

The intracerebral injection of 5 μl of 10 mM 4-AP induced intense epileptiform discharges in all (n = 10) rats tested (Fig. 1A). The ECoG recorded discharges, that started suddenly, appeared with a latency of 389 ± 62 s (Fig. 1C, n = 10) after the 4-AP injection, and lasted as long as the recordings (at least 60 min) (on average 81.4 ± 28 min; SDM; n = 10). Seizures were characterized by hypersynchronic activity of high-amplitude spikes that occurred with a frequency of 5.7 ± 0.8 Hz (Fig. 1A, 4-AP; LCx). The epileptiform activity was propagated to the contralateral cortex (Fig. 1A, 4-AP; RCx) with a lower amplitude. Therefore, these abnormal discharges corresponded to a continuous epileptiform activity that resembled status epilepticus (Kapur, 1999; Lowenstein, 1999; Martín and Pozo, 2003). Simultaneously, a marked increase in the whisker activity was clearly revealed by the corresponding EMG recorded from mystacial pad musculature (Fig. 1A, 4-AP; LWhi and RWhi). Given the parallelism between experimental seizures and whisker activity, we performed a cross-correlation analyses of simultaneously recorded cortical ECoG and EMG to test whether temporal correlations could be detected between both types of activity. No significant peak was found in the cross-correlogramms of ECoG vs. EMG activities recorded either ipsilateral (Fig. 1B, LCx–LWhi, black solid line) or contralaterally (data not shown) to the injected hemisphere, indicating that the EMG activity was not synchronized with the electrocortical recording. However, we observed a correlation between ECoG recording that has main peaks centered around zero (Fig. 1B, LCx–RCx, gray solid line),

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