ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 29 (2008) 368–380

Long-term potentiation of high-frequency oscillation and synaptic transmission characterize in vitro NMDA receptor-dependent epileptogenesis in the hippocampus

Christos Moschovos, George Kostopoulos, and Costas Papatheodoropoulos*

Department of Physiology, Medical School, University of Patras, Rion, 26504, Greece

Received 14 June 2007; revised 19 September 2007; accepted 19 September 2007
Available online 11 October 2007

The implication of high-frequency network oscillations (HFOs) in brain pathology resides in as yet unclear mechanisms. Employing field recordings from ventral hippocampal slices and two models of epileptogenesis (i.e. establishment of interictal-like persistent bursts), we found that HFOs associated with epileptiform bursts and excitatory synaptic transmission were co-modulated during epileptogenesis. NMDA receptor-dependent epileptogenesis in CA3 was consistently accompanied by long-lasting strengthening in synaptic transmission (by 94 ± 17%, $n=5$) and HFOs (frequency, power and duration increased by 24 ± 8%, 57 ± 18% and 33 ± 10%, respectively). Co-modulation of synaptic transmission and HFOs was also observed in NMDA receptor-independent epileptogenesis, although in individual experiments either enhancement or depression of both phenomena was observed. Pathological HFOs >200 Hz were unequivocally present in persistent bursts induced by NMDA receptor-dependent but not NMDA receptor-independent mechanisms. The duration of pathological HFOs associated with persistent bursts but not of HFOs associated with bursts before the establishment of epileptogenesis was linearly and strongly correlated with the duration of bursts ($r=0.58$, $P<0.0001$). We propose that interplay between spontaneous synchronous bursting and long-lasting synaptic potentiation accompanying certain forms of epileptogenesis may underlie long-lasting potentiation of HFOs, whose quantitative aspects may reliably signal the degree of network changes involved in epileptogenesis.

Keywords: Synaptic transmission; High-frequency oscillation; Fast ripples; Epileptogenesis; Persistent interictal burst; CA3; Long-term potentiation; NMDA receptors; In vitro hippocampal slices

Introduction

Cortical circuits can oscillate at high frequency (80–500 Hz) under normal and pathological conditions. In hippocampus, high-frequency oscillations (HFOs) at the range of 100–200 Hz, called ripples (normal HFOs), are observed during physiological states like slow wave sleep and quiet sitting (Buzsaki et al., 1992; O'Keefe and Conway, 1978; Staba et al., 2004). Oscillations at relatively higher frequencies (usually 200–500 Hz, pathological HFOs), often called fast ripples, have been recorded in human and animal epileptic brains (Bragin et al., 1999a,b; Staba et al., 2004). The discovery of reliable "surrogate markers" of epileptogenicity and epileptogenesis would help localize epileptogenic areas, predict whether seizures will develop following a certain insult and assess therapeutic efficacy. Pathological HFOs are recently considered as best available candidate such marker (Engel and Schwartzkroin, 2006). Data from in vivo studies suggests that HFOs at the frequency range of fast ripples (≥200 Hz) can be used as a valid functional indicator of the epileptogenic areas (Bragin et al., 1999b; Jirsch et al., 2006), as well as of the severity of seizures (Bragin et al., 2004, 2003). Furthermore, a trend towards higher frequency oscillations has been observed during the transition from interictal to seizure activity either in vivo (Fisher et al., 1992; Grenier et al., 2003; Traub et al., 2001; Worrell et al., 2004) or in vitro (Dzhala and Staley, 2003; Khosravani et al., 2005; Lasztoczi et al., 2004). It has been hypothesized that in the adult brain oscillations related to epileptic activity may arise from a network of pathologically interconnected neurons (Bragin et al., 2002; Bragin et al., 2000; Staba et al., in press), which may underlie epileptogenesis. Some kinds of epileptogenesis involve long-term strengthening of synaptic connections (Leite et al., 2005). However, the mechanisms underlying the proposed relationships between HFOs and epileptogenesis are not known. Epileptogenesis can be studied in vitro employing models which involve induction of long-term establishment of epileptiform activity persisting in normal conditions long after the removal of epileptogenic factors (Alzheimer et al., 1989; Anderson et al., 1987; Delorenzo et al., 2005; Papatheodoropoulos et al., 2005; Schneiderman et al., 1994; Stoop et al., 2003; Wong et al., 2002). In

* Corresponding author. Fax: +30 610 997215.
E-mail address: cepapath@med.upatras.gr (C. Papatheodoropoulos).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2007.09.007

this study, employing ventral hippocampal slices we made use of a model of *N*-methyl-D-aspartate receptor (NMDAR)-dependent epileptogenesis (Papatheodoropoulos et al., 2005) as well as of a model of epileptogenesis induction independent of NMDARs activation (Alzheimer et al., 1993). We demonstrate that long-term enhancement of both HFOs associated with epileptiform bursts and excitatory synaptic transmission reliably accompanies NMDAR-dependent but not NMDAR-independent epileptogenesis, in which only in a fraction of experiments this correlation was observed. Furthermore, we confirm previous in vivo observations on pathological HFOs with a frequency greater than 200 Hz as marker of epileptogenesis.

Methods

Slice preparation and recording procedure

Transverse hippocampal slices were prepared from 35 male Wistar adult rats, 2–5 months old (Athens Pasteur Institute). Adequate measures were taken to minimize pain and the number of animals used. Experiments were conducted according to the European Communities Council Directive Guidelines (86/609/EEC) and the Greek National Laws (Animal Act, PD 160/91) for the care and use of Laboratory animals. Animals were deeply anesthetized with diethyl-ether and decapitated. The brain was removed and placed in chilled (2–4 °C) artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 4 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, at pH 7.4. The two hippocampi were excised free and slices, 550 μm thick, were prepared from the ventral hippocampus using a McIlwain tissue chopper as previously (Papatheodoropoulos and Kostopoulos, 2000). The slices were immediately transferred on the two independent channels of an interface type recording chamber and maintained at a constant temperature of 32±0.2 °C, continuously humidified with mixed gas 95% O_2 and 5% CO_2 and perfused with ACSF at a rate of ~1 ml/min. Spontaneous activity was recorded from the st. pyramidale or st. radiatum of CA3 and from the st. pyramidale of CA1 fields using carbon fiber electrodes (7–10 μm of diameter, WPI or Kation Scientific, USA). Evoked responses were obtained from st. radiatum or st. pyramidale of CA3 field following electrical stimulation delivered using a bipolar wire-made electrode (platinum/iridium with 25 μm of diameter) positioned at the CA3 st. radiatum.

Experimental protocols for induction of persistent population bursts

In the present study, we made use of in vitro models of induction of spontaneous interictal-like population bursts, the generation of which persists under normal conditions. The persistent generation of interictal-like bursts under normal in vitro conditions, in the absence of any experimental induction factor, directly indicates the hyperexcitability of the tissue and resembles in vivo activity occurring in

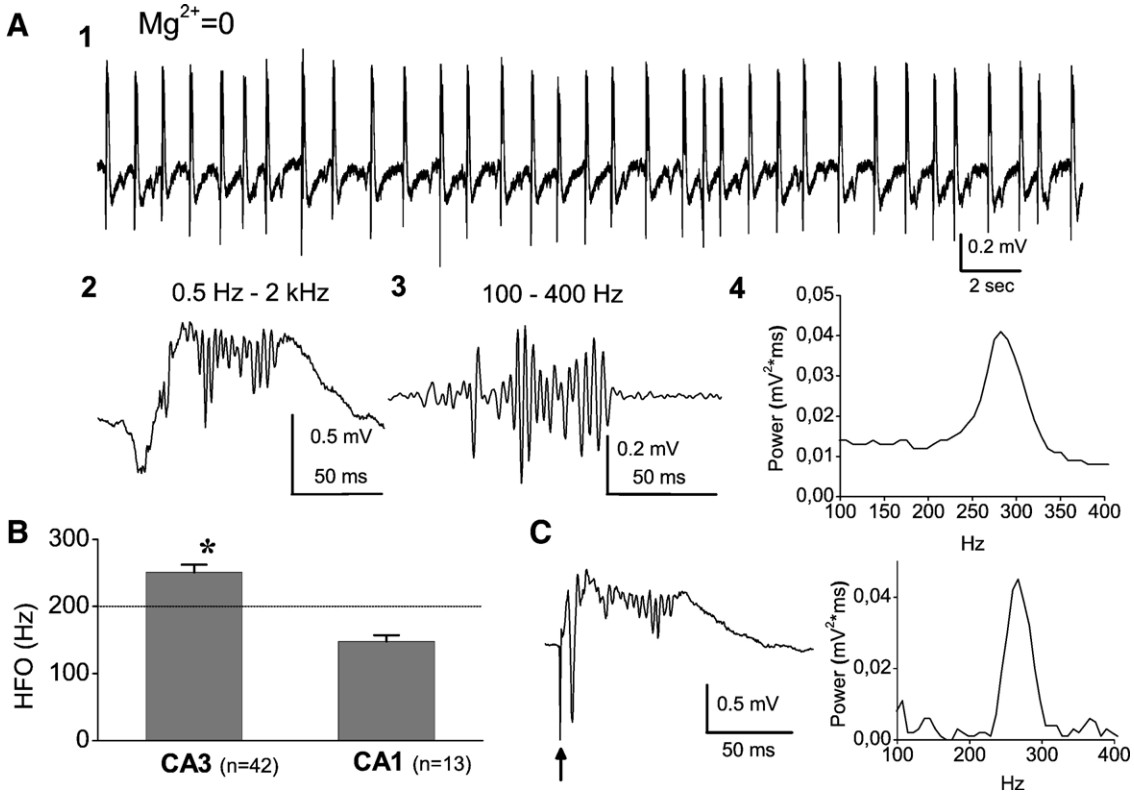

Fig. 1. HFOs associated with SBs induced in Mg^{2+}-free medium. (A) Continuous recording of SBs from the CA3 st. pyramidale of a ventral hippocampal slice is shown (1). The bottom panel shows wide-band (2) and band-pass filtered sweep (3) of a single SB illustrating the SB-associated HFO. The corresponding FFT illustrating the high frequency peak at 280 Hz is shown (4). (B) Comparison of the frequency of SB-associated HFO between CA3 and CA1. HFOs in CA3 displayed significantly higher frequency. Also, note that the mean frequency of the oscillation in CA3 was greater than 200 Hz, whereas the frequency in the CA1 was lower than 200 Hz. Data were collected from 42 slices. In 17 of those slices, simultaneous recordings from CA3 and CA1 were made. In four slices, HFO in CA1 could not be detected. (C) Example of evoked burst in CA3 st. pyramidale (trace on the left) and the corresponding FFT with the high-frequency peak (diagram on the right). Arrow points to the stimulation artifact.