Epilepsy Research 119 (2016) 30–33

Contents lists available at www.sciencedirect.com

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Short communication

The pervasive reduction of GABA-mediated synaptic inhibition of principal neurons in the hippocampus during status epilepticus

Hua Yu Sun[a], Howard P. Goodkin[a,b,*]

[a] Department of Neurology, University of Virginia Health System, Charlottesville, VA 22908, USA
[b] Department of Pediatrics, University of Virginia Health System, Charlottesville, VA 22908, USA

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 19 August 2015
Accepted 9 November 2015
Available online 12 November 2015

Keywords:
Status epilepticus
Pyramidal neuron
Dentate granule cell
$GABA_A$ receptors
Miniature inhibitory postsynaptic currents

ABSTRACT

The goal of this study was to determine whether there are region-specific or time-dependent changes in GABA-mediated synaptic inhibition of principal neurons in the hippocampus during in vivo status epilepticus. Standard whole cell patch clamp electrophysiological techniques were used to characterize miniature inhibitory postsynaptic currents (mIPSCs) in recordings from the principal neurons (PNs) of the dentate gyrus, CA1, and CA3 in acutely-obtained hippocampal slices from control and lithium/pilocarpine-induced status epilepticus(SE)-treated animals. The reduction in mIPSC amplitude was pervasive across the 3 regions examined in hippocampal slices obtained after 60 min (late) or just 15 min after the onset of SE. The mIPSC frequency was reduced in all 3 regions after 60 min and 2 regions (dentate, CA1) after 15 min. These findings lend further support to the hypothesis that a rapid modification of the postsynaptic $GABA_A$ receptor population leads to a widespread decline in GABA-mediated inhibition that, in part, contributes to both the self-sustaining nature of SE and to the decrease in the efficacy of benzodiazepines.

1. Introduction

Status epilepticus (SE) is a neurological emergency characterized by a prolonged, self-sustaining seizure that can result in death or neurological sequelae. There is general agreement that the genesis and maintenance of SE is, in part, the result of rapid modifications in GABA-mediated inhibition that includes changes in the surface expression of the postsynaptic receptor population (Goodkin and Kapur, 2009).

The hippocampal CA3 region is activated and injured during SE (e.g., Motte et al., 1998). Although previous studies demonstrated changes in the characteristics of miniature inhibitory postsynaptic currents (mIPSCs) recorded from dentate granule cells (DGCs) (Naylor et al., 2005; Goodkin et al., 2008) and CA1 Pyramidal Neurons (CA1 PyNs) (Terunuma et al., 2008), no study has evaluated for changes in GABA-mediated synaptic inhibition of CA3 PyNs during in vivo SE.

Given the recent demonstration of region-specific, time-dependent differences in AMPA receptor-mediated currents during SE (Rajasekaran et al., 2012), we choose to confirm and extend the previous findings of the effect of SE on the characteristics of mIPSCs recorded from DGCs, CA1 PyNs, and CA3 PyNs in hippocampal slices acutely obtained from animals in SE induced using the combination of lithium and pilocarpine.

2. Methods

Male Sprague-Dawley rats postnatal day 15–25 were used. Animals were maintained with the dame. All animals were treated in accordance with the guidelines set by the University of Virginia Animal Care and Use Committee.

SE was induced via pretreatment with LiCl 3 mEq/Kg intraperitoneal followed 20–24 h later by pilocarpine hydrochloride 50 mg/Kg intraperitoneal. After pilocarpine administration, animals were continuously monitored for behavioral seizures (SE-treated animals). Sixty (*late* SE) or 15 (*early* SE) minutes after the first observed Racine grade 5 seizure, the animals were anesthetized with isoflurane. Following decapitation, the brain was promptly removed for hippocampal slice preparation. Age-matched naïve animals (controls) were treated with saline. Coronal hippocampal slices (300 or 400 μm) were prepared using a vibrating microtome (VT1200S: Leica, Wetzlar, Germany) with the brain immersed in an ice-cold (1–3 °C) dissection buffer equilibrated with 95% O_2–5% CO_2. The dissection buffer was composed of the following: 120 mM NaCl, 3.5 mM KCl, 4.0 mM $MgCl_2$, 0.7 mM

* Corresponding author. Department of Neurology, PO Box 800394, Charlottesville, VA 22908, USA. Tel.: +1 434 924 5312; fax: +1 434 982 1728.
E-mail address: hpg9v@virginia.edu (H.P. Goodkin).

http://dx.doi.org/10.1016/j.eplepsyres.2015.11.006
0920-1211/

$CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose (300 mOsm). All chemicals were obtained from Sigma (St. Louis, MO). Slices were maintained for a minimum of 30 min at 30 °C prior to commencing electrophysiological recordings.

Whole cell patch clamp recordings of GABA-mediated mIPSCs in hippocampal slices and off-line analysis were performed using standard techniques as previously described (Goodkin et al., 2005, 2008). Superficial cells were avoided. All recordings commenced within 2 h of hippocampal acute-slice preparation.

DGCs were visually identified as small- and medium-sized neurons with typical oval-shaped somas and a single process located within the dentate granule layer. PyNs were visually identified as cells with typical triangular shaped somas within the pyramidal layer.

Data is presented as means ± standard error (SEM). Error bars represent SEM. Statistical comparisons of the control, *early* SE-treated, and *late* SE-treated populations were performed using a one-way ANOVA followed by a Holm–Sidak test (Prism v6.05, GraphPad Software, Inc).

3. Results

3.1. GABA-mediated synaptic inhibition of CA1 PyNs and DGCs was reduced after late SE

Recordings from CA1 PyNs and DGCs in acutely obtained control and *late* SE-treated slices confirmed previously published findings (Naylor et al., 2005; Goodkin et al., 2008; Terunuma et al., 2008) that mIPSC amplitude was reduced after *late* SE (Figs. 1–2; Figs. 3B and C). In these recordings, mIPSC frequency was also reduced. No changes were observed in rise time (date not shown) or decay (Fig. 1A).

3.2. GABA-mediated synaptic inhibition of CA3 PyNs was altered after late SE

Recording from CA3 PyNs in acutely obtained control and *late* SE-treated slices demonstrated that the reduction in GABA-mediated synaptic inhibition of principal neurons was pervasive within the hippocampus after *late* SE. In Fig. 2, traces recorded from a control CA3 PyN and *late* SE-treated CA3 PyN are displayed. The mIPSC frequency and median mIPSC amplitude for the control neuron was 0.41 Hz and 31.5 pA, respectively, compared to 0.13 Hz and 24.1 pA for the *late* SE-treated CA3 PyN. For the population of control CA3 PyNs ($n = 9$ cells, 6 animals), the mean of the median mIPSC amplitudes was 33.4 ± 2.8 pA. In comparison, the mean of the median mIPSC amplitudes for the *late* SE-treated CA3 PyNs ($n = 9$ cells, 7 animals) was 24.6 ± 1.9 pA ($p < 0.05$; Fig. 2D). This ~25% decrease is similar to the reduction in recordings obtained from DGCs (29% decrease) and CA1 PyN (~35% decrease).

In addition to the decline in mIPSC amplitude, a decrease in mIPSC frequency (0.36 ± 0.09 Hz vs. 0.14 ± 0.02 Hz, $p < 0.05$) was present (Fig. 2E). The rise time (data not shown) and decay (Fig. 2c') were unchanged.

3.3. GABA-mediated synaptic inhibition was reduced after early SE

The reduction in GABA-mediated synaptic inhibition observed following the 1 h SE time point in recordings from CA1 PyNs and DGCs, and now CA3 PyNs, has been posited to contribute to the pathogenesis of SE and benzodiazepine pharmacoresistance. As benzodiazepine pharmacoresistance is established rapidly after SE onset (Kapur and Macdonald, 1997; Jones et al., 2002; Goodkin et al., 2003), we chose to record mIPSCs from principal neurons in *early* SE-treated slices.

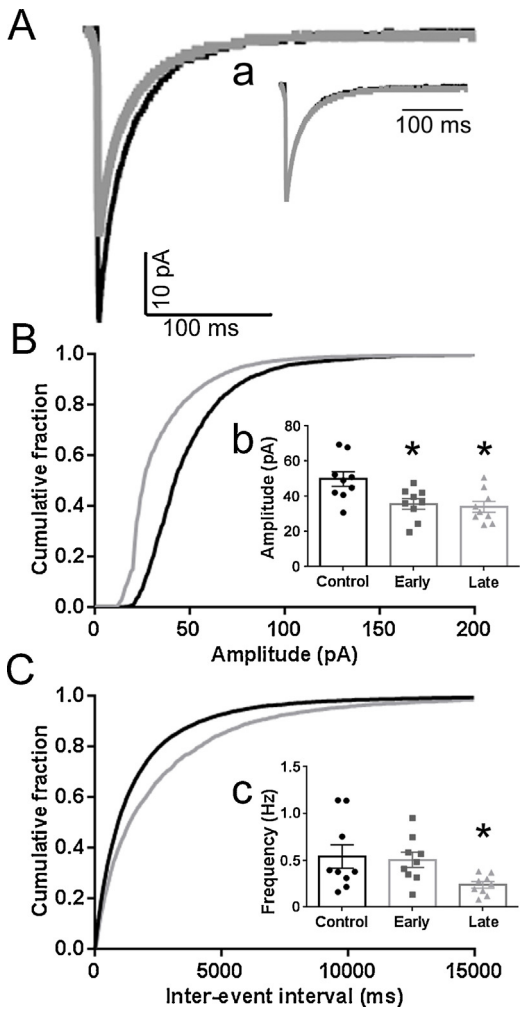

Fig. 1. Diminished GABAergic synaptic transmission in CA1 PyNs following SE. (**A**), Averaged mIPSC traces from a control (black) CA1 PyN and a *late* SE-treated (gray) CA1 PyN voltage clamped to a holding potential of −60 mV. The mIPSC frequency, mean mIPSC amplitude, and median mIPSC amplitude for the control CA1 PyN was 0.38 Hz, 52.4 pA, and 47.6 pA, respectively, compared to 0.25 Hz, 38.8 pA, and 24.4 pA for the *late* SE-treated CA1 PyN. (a), Normalized averaged mIPSC traces for the control and *late* SE-treated CA1 PyNs displayed in (A) demonstrating similar decays for these neurons (control 33 ms vs. SE-treated 28 ms). The mean weighted decay for the population of *late* SE-treated CA1 PyNs (31.0 ± 0.6 ms) was similar to the controls (35.3 ± 2.9 ms; $p > 0.05$). (**B**), (**C**), Cumulative probability plots of mIPSC amplitude (B) and inter-event interval (C) obtained by pooling the data from 9 control CA1 PyNs from 6 animals (black) and 9 *late* SE-treated CA1 PyNs from 6 animals (gray). (b), (c), Inserted 3-bar bar graphs display the mean of the median mIPSC amplitudes (b) and mean mIPSC frequency (c) for the population of control (black circles), *early* (gray squares; $n = 9$ from 6 animals) SE-treated CA1 PyNs, and *late* (gray triangles) SE-treated CA1 PyNs. For each inset, the median mIPSC amplitude and mean frequency for each individual neuron is represented by a single point. The mean of the median mIPSC amplitudes was 49.9 ± 4.2 pA for the control CA1 PyNs, 35.8 ± 3.0 pA for the early SE-treated CA1 PyNs, and 34.0 ± 3.1 pA for the *late* SE-treated CA1 PyNs. The mean mIPSC frequency was 0.54 ± 0.13 Hz for the control CA1 PyNs, 0.50 ± 0.10 Hz for the *early* SE-treated CA1 PyNs, and 0.24 ± 0.04 Hz for the *late* SE-treated CA1 PyNs. Compared to control population, the amplitude for the *early* and *late* SE-treated CA1 PyNs was reduced as was the frequency for the *late* SE-treated CA1 PyNs ($^* = p < 0.05$).

When compared to controls, both mIPSC frequency and amplitude were significantly decreased in recordings from DGCs in *early* SE-treated hippocampal slices. In Fig. 3A, averaged mIPSC traces from a control DGC and *early* SE-treated DGC are displayed. The mIPSC frequency and median amplitude for the control neuron was 0.33 Hz and 56.8 pA, respectively, compared to 0.13 Hz and 39.9 pA for the *early* SE-treated neuron. For the population of control DGCs ($n = 9$ cells, 5 animals), the mean mIPSC frequency