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Enhanced AMPA receptor-mediated neurotransmission on CA1 pyramidal neurons during status epilepticus

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ABSTRACT

Status epilepticus (SE) is a common neurological emergency that results from the failure of the mechanisms responsible for seizure termination or the initiation of mechanisms that lead to abnormally prolonged seizures. Although the failure of inhibitory mechanisms during SE is well understood, the seizure-initiating mechanisms are poorly understood. We tested whether hippocampal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated transmission was enhanced during SE and assessed the underlying molecular mechanism. In animals in self-sustaining limbic SE the amplitudes of the miniature, spontaneous, and AMPA-evoked excitatory currents recorded from the CA1 pyramidal neurons were larger than those recorded in the controls. The evoked EPSCs rectified inwardly. In these animals, the surface expression of GluA1 subunit-containing AMPARs was increased in the CA1 pyramidal neurons. The phosphorylation of the GluA1 subunit on S831 and S845 residues was reduced in animals in SE. In contrast, the GluA1 subunit surface expression and AMPAR-mediated neurotransmission of dentate granule cells (DGCs) was not altered. Treating animals in SE with the NMDAR antagonist MK-801 or with diazaepam blocked the increased surface expression of the GluA1 subunits. NMDAR blockade also prevented the dephosphorylation of the S845 residue but not that of S831. Targeting NMDARs and AMPARs may provide novel strategies to treat benzodiazepine-refractory SE.

1. Introduction

Most seizures are brief and end spontaneously, but some trigger neuronal plasticity such that they transform into a self-perpetuating state. This is referred to as status epilepticus (SE), which is a common neurological emergency that affects 10–41 per 100,000 individuals. The overall mortality associated with SE approaches 20%, and in those who survive, it can cause neuronal injury and death (Betjemann and Lowenstein, 2015; DeLorenzo et al., 1996). SE is initiated in part by repeated or prolonged seizures reducing γ-aminobutyric acid type-A receptor (GABAR)-mediated inhibition, and benzodiazepines, which modulate GABA-A receptors, are the most effective treatment for SE (Alldredge et al., 2001; Silbergleit et al., 2012; Treiman et al., 1998; Chamberlain et al., 2014; Glauser et al., 2016). However, benzodiazepines lose their efficacy as continuous seizure activity occurs, as determined by EEG findings, and SE becomes self-sustaining (Goodkin et al., 2008; Kapur and Macdonald, 1997; Naylor et al., 2005; Terunuma et al., 2008). Studies have revealed that GABAR-mediated inhibitory synaptic transmission is reduced in the hippocampi of animals in SE, due in part to the internalization of synaptic GABARs (Goodkin et al., 2008; Kapur and Coulter, 1995; Kapur and Macdonald, 1997; Naylor et al., 2005; Terunuma et al., 2008).

There is growing evidence that AMPAR plasticity may also play a role in maintaining SE in that, the internalization of the GluA2 subunit is accelerated during SE (Rajasekaran et al., 2012). Furthermore, the benzodiazepine-refractory SE in experimental animals can be terminated by attenuating the fast excitatory transmission mediated by AMPAR (Fritsch et al., 2010; Pitkänen et al., 2007; Rajasekaran et al., 2012; Hanada et al., 2014). However, it is unknown whether AMPAR-mediated transmission is enhanced during SE and the mechanisms that cause this enhancement are unclear.

In the hippocampus, AMPARs are dimers of GluA1/GluA2 and GluA2/GluA3 subunits (Wenthold et al., 1996). The GluA1 subunits play a dominant role in the activity-dependent recruitment of AMPARs at the synapses (Henley and Wilkinson, 2016). Here, we demonstrate the enhanced AMPAR-mediated excitatory transmission of CA1 pyramidal neurons accompanied by the increased surface expression and dephosphorylation of the GluA1 subunit of AMPARs. AMPAR plasticity can be blocked by treatment with agents that dampen the neuronal activity.

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2. Materials and methods

All studies were performed in accordance with protocols approved by the Animal Care and Use Committee of the University of Virginia. All chemicals were obtained from Sigma (St. Louis, MO, USA) unless otherwise stated. Adult male Sprague-Dawley rats (200–250 g) were used.

2.1. Electrode implantation and induction of status epilepticus

Bipolar insulated stainless steel electrodes were stereotaxically implanted over the hippocampus and cortex in adult male rats as described previously (Rajasekaran et al., 2012). The animals were given methyl scopolamine (1 mg/kg, intraperitoneal, ip) 30 min prior to the induction of SE. SE was induced by continuous hippocampal stimulation (CHS) (Lothman et al., 1989) or by the administration of pilocarpine (320 mg/kg, ip) (Rajasekaran et al., 2012). For the studies on SE duration, animals were monitored continuously by video-EEG. For biochemical and electrophysiological studies, the animals were visually monitored, and the time of first grade 5 (Racine score) seizure, which corresponded to the onset of continuous electrographic activity, was noted (Racine, 1972). Continuous seizure activity marks the development of benzodiazepine resistance and the establishment of benzodiazepine refractory SE or established SE (ESE) (Treiman, 2007).

In some of the experiments, animals were treated with MK-801 (2 mg/kg, ip) alone or in combination with diazepam (10 mg/kg, ip) at 10 min after the onset of ESE.

2.2. Hippocampal slice preparation

For biochemical studies the animals were decapitated under halothane anesthesia 60 min after the first stage 5 behavioral seizure was identified using the Racine scale criteria (Racine, 1972), whereas for electrophysiological studies, the animals were sacrificed 40 min after the first tonic-clonic seizure. The brains were removed and immersed in ice-cold oxygenated dissection buffer (4 °C, 95% O_2, and 5% CO_2) containing (in mM) 65.5 NaCl, 2 KCl, 5 $MgSO_4$, 1.1 KH_2PO_4, 1 $CaCl_2$, 10 dextrose, and 113 sucrose (300 mOsm), and horizontal hippocampal slices (300 μm) were prepared with a vibratome (VT1200S; Leica, Wetzlar, Germany).

2.3. Electrophysiology

Following slicing, the slices were placed in an interface chamber containing oxygenated artificial cerebrospinal fluid (aCSF) at room temperature (25 °C) and allowed to equilibrate for 20 min. The aCSF contained (in mM) 124 NaCl, 4 KCl, 1 $MgCl_2$, 25.7 $NaHCO_3$, 1.1 KH_2PO_4, 10 dextrose, and 2.5 $CaCl_2$ (300 mOsm). AMPA (2.5 μM)-evoked whole-cell currents were recorded from the pyramidal neurons in the hippocampal slices in the ESE and control animals (Rajasekaran et al., 2012). The slices were perfused with oxygenated aCSF containing DL-AP5 (50 μM) and picrotoxin (50 μM) to block the NMDA and $GABA_A$ receptors, respectively, at a rate of 2–3 ml/min. The patch electrode was filled with an internal solution containing (in mM) 115 cesium methane sulfonate, 20 CsCl, 10 KCl, 10 HEPES, 0.5 sodium-EGTA, 2.5 $MgCl_2$, 5 Mg-ATP and 5 lidocaine, pH 7.3, 285 mOsm. The neurons were voltage-clamped at −65 mV. Action potential-independent miniature excitatory post-synaptic currents (mEPSCs) were recorded from CA1 pyramidal neurons (Supplementary Fig. 1) and DGCs, spontaneous EPSCs (sEPSCs) were also recorded from CA1 pyramidal neurons. Synaptic currents were analyzed using the MiniAnalysis software as described previously (Sun and Kapur, 2012). The threshold for detecting synaptic currents was set at 5 times the root mean square noise.

Evoked excitatory postsynaptic currents (eEPSCs) were recorded from CA1 pyramidal cells in response to the electrical stimulation of Schaffer collateral axons by a bipolar tungsten microelectrode (FHC, Bowdoin, ME, USA) placed in stratum radiatum.

The plasticity of AMPARs during SE is rapid and transient. Hence, all recordings were completed within 2 h of slicing. To avoid issues associated with nesting of the data, only one cell per animal was recorded in most instances (Aarts et al., 2014).

2.4. Biotinylation and BS^3 cross-linking assay

GluA1 subunit surface expression was determined using a biotinylation assay (Goodkin et al., 2008) or a BS^3 cross-linking assay (Grosshans et al., 2002). Four to six hippocampal slices from one hemisphere were incubated in ice-cold (2–4 °C) artificial CSF (aCSF) containing BS^3 (1 mg/ml) at 4 °C for 40 min with constant shaking, and 4–6 hippocampal slices from the other hemisphere were simultaneously incubated in ice-cold aCSF alone. Following incubation, the slices were washed twice with ice-cold aCSF containing 10 mM Tris-Cl pH 7.4 to stop the cross-linking reaction and remove the remaining BS^3 reagent. The regions of interest were microdissected, and the tissue was lysed in RIPA lysis buffer as described before and was resolved by SDS-PAGE (Goodkin et al., 2008; Rajasekaran et al., 2012). A phosphatase inhibitor cocktail (Thermo Fisher Scientific) was included in the lysis buffer when pGluA1 subunit expression was studied. The antibodies used in this study include anti-GluA1 subunit antibody (MAB2263, Millipore, 1:1000 dilution), anti-pGluA1 S831 (04-823 Millipore, 1:1000), anti-pGluA1 S845 (04-1073 Millipore, 1:1000).and anti-β-actin antibody (A2228, Sigma Aldrich, 1:5000). Horseradish peroxidase conjugated anti-mouse or anti-rabbit secondary antibodies (Biorad, 1:5000 dilution) were used. The Western blot signal was detected using a chemiluminescent reagent (Perkin Elmer), and the blots were exposed on a Chemidoc Touch imaging system (BioRad). The signal intensity was quantified by densitometric scanning, and the expression of the protein of interest was normalized to that of β-actin in each sample.

2.5. Power spectrum analysis

The power analysis of EEG was performed as previously described (Raol et al., 2009). The EEG data were digitized at 400 Hz. Power calculations were performed and graphically displayed using the Scilab software. For spectral analysis, the EEGs were filtered using 1-Hz high-pass, 70-Hz low-pass and 60-Hz notch filters and binned in 5-s intervals, and the power within each period was determined.

2.6. Statistical analysis

The data are expressed as the mean ± SEM. Statistical analysis was performed in the GraphPad Prism software using the *t*-test or Wilcoxon matched-pairs signed-rank test, or ANOVA with post hoc Dunn's multiple comparison test or Kruskal-Wallis test followed by Dunn's multiple comparison test and a p value < 0.05 was considered significant.

3. Results

3.1. Increased AMPAR-mediated synaptic transmission of CA1 pyramidal neurons of animals in SE

To determine whether AMPAR-mediated synaptic transmission was enhanced during SE, hippocampal slices were prepared from rats that had undergone 40 min of pilocarpine-induced SE and were allowed to recover for 20 min. The miniature AMPAR-mediated EPSCs were recorded from CA1 pyramidal neurons of SE animals (Fig. 1A). The mEPSCs recorded from the CA1 pyramidal neurons of SE animals were larger than those recorded from control animals (Fig. 1B, 22.8 ± 1.1 pA, $N = 11$ neurons from 9 SE animals vs 18.5 ± 0.7 pA, $N = 13$ neurons from 7 naïve animals, $p < 0.05$, *t*-test). However, the frequency (0.59 ± 0.12 Hz vs 0.47 ± 0.094 Hz), rise time (0.61 ± 0.04 ms vs 0.61 ± 0.07 ms), and decay (4.99 ± 0.52 ms, vs 5.4 ± 0.66 ms) of mEPSCs did not change ($p > 0.05$). The spontaneous EPSCs recorded from CA1