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Short communication

Enhanced endogenous activation of NMDA receptors in pyramidal neurons of hippocampal tissues from patients with mesial temporal lobe epilepsy: A mechanism of hyper excitation

Jyotirmoy Banerjee[a,**], Aparna Banerjee Dixit[a], Manjari Tripathi[a,b], Chitra Sarkar[c], Yogendra Kumar Gupta[d], P. Sarat Chandra[a,e,*]

[a] Center of Excellence for Epilepsy, National Brain Research Center, Manesar, India
[b] Department of Neurology, All India Institute of Medical Sciences, New Delhi, India
[c] Department of Neuropathology, All India Institute of Medical Sciences, New Delhi, India
[d] Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India
[e] Department of Neurosurgery, All India Institute of Medical Sciences, New Delhi, India

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ABSTRACT

Altered excitatory synaptic transmission is one of the primary causes of seizure generation in patients with mesial temporal lobe epilepsy (MTLE). The present study is designed to delineate the contribution of glutamatergic tone under resting conditions to the hyper excitability in patients with MTLE. Resected hippocampal tissues were obtained from patients with MTLE. In these samples spontaneous excitatory postsynaptic currents (EPSCs), sensitive to NMDA receptor antagonist APV (50 μM) and AMPA receptor antagonist CNQX (10 μM) were recorded from pyramidal neurons at −70 mV. We observed that frequency of EPSCs were 28.2% higher in slices obtained from patients with MTLE compared to that in case of non-epileptic controls. We also examined spontaneous fast current transients (CTs) recorded from these pyramidal neurons under cell-attached configuration. The frequency of CTs increased in the absence of extracellular Mg^{2+} in brain slice preparations and was completely blocked by APV. We found that the frequency of CTs in pyramidal neurons were higher in case of MTLE samples compared to non-epileptic controls. This study suggests that enhanced endogenous activity of NMDA receptor contributes to excitability in pyramidal neurons of slice preparations obtained from patients with MTLE.

1. Introduction

The most common form of drug refractory temporal lobe epilepsy is mesial temporal lobe epilepsy (MTLE; Shinnar, 2003), where the hippocampus is involved in seizure generation (Wieser, 2004). Seizure generation in patients with MTLE can be mediated by multiple ways, but one common principle that pertains is the interruption of processes that normally create a balance between excitation and inhibition (Banerjee et al., 2013). This concept of balance is useful model for understanding the mechanisms that leads to generation of hyper excitable neuronal network in patients with MTLE. In the cortex, glutamate-dependent excitatory neurotransmission can be enhanced primarily through NMDA receptor and AMPA receptor (Traynelis et al., 2010). As glutamate receptor-mediated excitatory postsynaptic currents (EPSCs) is the major stimulus to pyramidal neurons we pharmacologically tested the role of endogenous activation of glutamate receptors on pyramidal neurons in slice preparations obtained from patients with MTLE. Specifically, we analysed the effects of the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid (APV), and the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on the frequency of EPSCs. We also examined if the frequency of spontaneous fast current transients (CTs) recorded from pyramidal neurons in samples from MTLE patients is altered compared to that in case of non-epileptic control samples. Whole-cell current-clamp technique is used to record action potential generated from a neuron (Cohen and Miles, 2000), but there are studies which suggest that action potentials can also be detected as CTs in single neurons under cell-attached voltage-clamped condition (Williams and

* Corresponding author at: Room No. 607, Department of Neurosurgery, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi 110029, India.
** Corresponding author at: Center of Excellence for Epilepsy, National Brain Research Centre, NH-8, Manesar, Gurgaon, Haryana 122051, India.
E-mail addresses: jyotirmoybanerjee1@gmail.com (J. Banerjee), aparnabanerjeedixit@gmail.com (A. Banerjee Dixit), manjari.tripathi1@gmail.com (M. Tripathi), sarkar.chitra@gmail.com (C. Sarkar), yk.ykgupta@gmail.com (Y.K. Gupta), saratpchandra3@gmail.com (P.S. Chandra).

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Stuart, 1999; Alkondon et al., 2011). In brain slice preparations, investigation of spontaneous CT's in a morphologically identified neuron type can determine the contribution of specific neurotransmitter system through intervention of pharmacological agents. Thus, here, we attempted to determine pharmacologically the role of NMDA receptors on the frequency of CTs on pyramidal neurons of resected brain specimens obtained from patients with MTLE. We for the first time have demonstrated that in the absence of external stimuli, basal levels of glutamate contribute to the excitability of pyramidal neurons and it is enhanced in hippocampal tissue samples obtained from patients with MTLE.

2. Materials and method

The experiments mentioned in this study have been performed as per the guidelines of institutional ethics committee (IEC), All India Institute of Medical Sciences (AIIMS), New Delhi, India. Six patients with MTLE who underwent surgery were included in this study. The pathology in each patient was demonstrated by documenting convergent data on video EEG magnetic resonance imaging (MRI), fluoro-2-deoxyglucose positron emission tomography (FDG-PET) evaluations and electrocorticography (ECoG) and verified by histopathologic examinations. The patients with MTLE were on a combination of anti-epileptic drugs (AEDs) which include valproic acid, carbamazepine, phenytoin and levetiracetam. As it was not possible to ethically obtain control human hippocampal specimens cortical tissues resected from the tumour margin during brain tumour surgery of seizure-free patients were considered as non-epileptic control specimens (six patients). Resected hippocampal specimens, which are part of the epileptogenic foci, were excised from patients with MTLE based on abnormal electrocorticography and neuroimaging assessments. Samples were further processed for cellular electrophysiological experiments as per the protocol reported by Alkondon et al. (2000). Briefly, samples were placed in well carbogenated, ice-cold ACSF within 2 min of resection. ACSF is composed of $CaCl_2$, 2 mM; $NaHCO_3$, 25 mM; NaH_2PO_4, 1.25 mM; NaCl, 125 mM; KCL, 2.5 mM; $MgCl_2$, 1 mM; and glucose, 25 mM. Within 15 min of resection, slices (350-μm thick) were prepared using vibrating blade microtome. Infrared-assisted videomicroscopy with differential interference contrast (IR-DIC) was used to morphologically identify pyramidal neurons located in slice preparations. Neurons showing pyramid-like soma and a single thick apical dendrite were used for this study. Passive membrane properties of neurons on both epileptic and non-epileptic samples were determined by using membrane test function integrated in pCLAMP 10 software (Molecular Devices, Sunnyvale, USA). Cell-attached and whole-cell recordings were obtained from the soma of visually identified pyramidal neurons in slice preparations using an Axopatch 200B amplifier (Molecular Devices, USA). Patch pipettes were filled with internal solution containing HEPES, 10 mM; Cs-methanesulfonate, 130 mM; EGTA, 10 mM; CsCl, 10 mM; $MgCl_2$, 2 mM (pH adjusted to 7.3 with CsOH; 340 mOsm). To record spontaneous EPSCs from neurons under whole-cell configuration at a holding potential of −70 mV, 5 mM QX-314 was added in the internal pipette solution, but QX314-free pipette solution was used for all cell-attached recordings, for CT measurement. All experiments were carried out at room temperature (20–22 °C). Experiments on non-epileptic control samples were performed in the same way as in case of MTLE samples. We have studied eight neurons from hippocampal specimens of six MTLE patients and 10 neurons from cortical samples of six tumour patients for non-epileptic control.

Adult male Wistar rats were obtained from the animal facility at AIIMS, New Delhi. Animal care and handling were done strictly in accordance with the guidelines set forth by the Institutional Animal Ethics Committee of the AIIMS. Rats were euthanized by CO_2 narcosis followed by decapitation. Their brains were removed and placed in ice-cold ACSF. The hippocampi and neocortical samples were dissected out and mounted on the stage of vibrating blade microtome, which was used to cut transversal slices of 300–350 μm thickness. Slices were stored at room temperature for at least 45 min in an immersion chamber containing ACSF continuously bubbled with 95% O_2 and 5% CO_2 before recordings. Spontaneous EPSCs were recorded from CA1 pyramidal neurons in hippocampal slices and pyramidal neurons from neocortical slices as mentioned previously.

EPSCs and CTs were analysed using pCLAMP 10.0 software. Frequency, peak amplitude, rise time (10–90%), and decay-time constant (τ_d) of the EPSCs were measured using clampfit module of pCLAMP 10.0 software. EPSCs showing double- and multiple-peaks were excluded from analysis of kinetic properties, but utilised for calculation of frequency of events as multiple events. Data are expressed as mean ± S.E.M. of results obtained from various patients. Statistical tests included *t*-test or one-way ANOVA in Sigmaplot 12.0 (Systat Software, Inc., Chicago, IL) to determine any significance in the data.

3. Results and discussion

Passive membrane properties of pyramidal neurons on slice preparation obtained from hippocampal samples of patients with MTLE and that in cortical samples from non-epileptic controls were comparable. The cell capacitance was 176 ± 13 pF and 161 ± 10 pF in epileptic and non-epileptic control, respectively. The input resistance was 131 ± 11 MΩ and 122 ± 10 MΩ in epileptic and non-epileptic control, respectively. In the presence of $GABA_A$ receptor blocker, bicuculline (10 μM), spontaneous EPSCs were recorded at a holding potential of −70 mV from pyramidal neurons in slices obtained from patients with MTLE ($n = 8$) and also from non-epileptic controls ($n = 10$). EPSCs appeared as short duration inward current (inset Fig. 1A) and were completely blocked following 10 min superfusion of the slices with ACSF containing the admixture of AMPA receptor antagonist CNQX (10 μM) and NMDA receptor antagonist APV (50 μM). When the slice preparations are superfused with ACSF containing APV alone, there was a significant reduction in the frequency of EPSCs in both non-epileptic control and MTLE (Fig. 1B). We observed that percent reduction in the frequency of EPSCs caused by APV alone was higher ($p < 0.01$, according to one-way ANOVA followed by Dunnett post hoc test) in case of MTLE (68.1 ± 5.2%) compared to that in non-epileptic controls (53.6 ± 3.9%). To determine the contribution of glutamatergic transmission to the hyperexcitablity in patients with MTLE, we compared the EPSC kinetics of these samples with that of non-epileptic control. The mean frequency in case of MTLE samples was 0.95 ± 0.2 Hz, which is significantly higher than that of non-epileptic controls (Fig. 1B). We also observed a significant increase in the peak amplitude recorded from pyramidal neurons in case of MTLE compared to the values in non-epileptic controls (inset Fig. 1B). The kinetics of the spontaneous events were also altered in case of MTLE (inset Fig. 1B).

Spontaneous CTs were recorded from pyramidal neurons of non-epileptic control samples under cell-attached voltage-clamp configuration at −60 mV in the presence of bicuculline (10 μM). During voltage-clamp recordings the fast CTs appear as inverted action potentials. Individual CTs had a fast inward component and a large slow outward component (see expanded single event in Fig. 1A) and represented action potentials recorded under voltage-clamp (Williams and Stuart, 1999; Alkondon et al., 2011). Recording of CTs under cell-attached mode helps to examine intact pyramidal neurons without the problems of dialysis and subsequent loss of intracellular contents associated with conventional whole-cell recordings. In Mg^{2+} containing ACSF, the frequency of CTs