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Lovastatin suppresses hyperexcitability and seizure in Angelman syndrome model

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ABSTRACT

Epilepsy is prevalent and often medically intractable in Angelman syndrome (AS). AS mouse model ($Ube3a^{m-/p+}$) shows reduced excitatory neurotransmission but lower seizure threshold. The neural mechanism linking the synaptic dysfunction to the seizure remains elusive. We show that the local circuits of $Ube3a^{m-/p+}$ *in vitro* are hyperexcitable and display a unique epileptiform activity, a phenomenon that is reminiscent of the finding in fragile X syndrome (FXS) mouse model. Similar to the FXS model, lovastatin suppressed the epileptiform activity and audiogenic seizures in $Ube3a^{m-/p+}$. The *in vitro* model of $Ube3a^{m-/p+}$ is valuable for dissection of neural mechanism and epilepsy drug screening *in vivo*.

1. Introduction

Angelman syndrome (AS) is a neurodevelopmental disorder caused by maternal deficiency of the E6-AP ubiquitin ligase (UBE3A) associated with a deletion of chromosome 15q11–q13 region, paternal uniparental disomy, point mutation in *UBE3A* or an imprinting defect (Buiting et al. 2016; Jiang et al. 1999). Clinically, epilepsy or seizure disorder is one of the most common (80 to 95%) and devastating features in AS, which starts before 3 years of age in most cases (Fiumara et al. 2010; Thibert et al. 2013). The seizure phenotype is reported to be more prominent in AS patients with a maternal deletion of chromosomal 15q11-q13 region, presumably due to the haploinsufficiency of a cluster of GABA receptors including GABRB3 in the distal end (Dan 2009; DeLorey et al. 1998; Egawa et al. 2008). The electroencephalogram (EEG) in AS patients has a characteristic pattern of large-amplitude slow-spike waves at 1–2 or 4–6 Hz (Sidorov et al. 2017; Vendrame et al. 2012). A significant fraction of clinical seizures is medically intractable and the quality of life is significantly compromised in these individuals (Tan and Bird 2016).

The epilepsy in AS is mostly generalized but partial epilepsy has also been frequently observed. The specific clinical seizures may vary from atypical absence, myoclonic, generalized tonic-clonic, tonic and atonic seizures (Dan 2009; Tan and Bird 2016). The genotype and phenotype correlation for seizure presentation has been described. Chromosomal deletion results in more severe seizure than other causes including uniparental disomy, point mutation in *UBE3A* gene, and an imprinting defect (Thibert et al. 2013). Current treatment is symptomatic with one or multiple drugs at a time. Valproic acid and clonazepam are most commonly prescribed but others such as levetiracetam, lamotrigine and clobazam have also been used frequently (Shaaya et al. 2016; Thibert et al. 2009). The molecular target therapy of reactivating *UBE3A* from the paternal chromosome has been investigated in animal model but remains to be seen if this is feasible in human (Buiting et al. 2016; Huang et al. 2012; Meng et al. 2015).

Despite the substantial progress in understanding the molecular basis and synaptic mechanism of AS, the mechanism underlying seizure caused by the *UBE3A* deficiency remains poorly understood. The AS mouse model ($Ube3a^{m-/p+}$) recapitulates the major clinical features of AS including abnormal EEG in hippocampus and neocortex (Jiang et al. 1998; Mandel-Brehm et al. 2015; Miura et al. 2002). Increased susceptibility of audiogenic seizures has been reported in AS model and they are mouse strain and age dependent (Jiang et al. 1998; Jiang et al.,

Abbreviations: AS, Angelman syndrome; BMI, (−)-bicuculline methiodide; DHPG, (*RS*)-3,5-dihydroxyphenylglycine; EEG, electroencephalogram; FXS, fragile X syndrome; LTD, long-term depression; mGluR, metabotropic glutamate receptor; MPEP, 2-Methyl-6-(phenylethynyl)pyridine hydrochloride; UBE3A, E6-AP ubiquitin ligase

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2010; Mandel-Brehm et al. 2015). The spontaneous seizure is also observed in AS model with a maternal deletion from *Ube3a* to *Gabrb3* but at a low frequency (Jiang et al., 2010). The excitatory neurotransmission was decreased in neocortex and hippocampal CA1 region (Greer et al. 2010; Kaphzan et al. 2011; Wallace et al. 2012; Yashiro et al. 2009). The increased SK2 potassium channel levels for after-hyperpolarization in *Ube3a*$^{m-/p+}$ might suggest a decrease of excitability as well (Sun et al. 2015). Interestingly, GABAergic neuron specific loss of *Ube3a* results in abnormal EEG and enhanced seizure susceptibility (Judson et al. 2016; Santini and Klann 2016).

While these findings provide a plausible mechanistic link between the deficiency of *Ube3a* and hyperexcitability, a significant gap is present to translate this knowledge to the development of novel molecular targets for treatment of seizure. Because of the spontaneous nature of seizure activity *in vivo*, a unique local circuit phenomenon that recapitulates the hyperexcitability would be valuable for anti-seizure drug screening and understanding the epileptogenesis in AS. In this study, we attempted to delineate a hyperexcitable local circuit activity in the hippocampus of *Ube3a*$^{m-/p+}$ *in vitro*, a brain region that has been studied extensively for synaptic function and where abnormal EEG was also observed analogous to those in cortex (Greer et al. 2010; Miura et al. 2002). While the brainstem has been frequently implicated in the initiation of audiogenic seizure in rodent model, the involvement hippocampus has also been reported (Reid et al. 1983). We expect that the knowledge learned from the hippocampus of *Ube3a*$^{m-/p+}$ will be applicable to other brain regions (McNamara et al. 2006). In this study, we demonstrate the value of this platform for the development of anti-epileptic drugs for AS.

2. Materials and methods

2.1. Animals

All experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee at Duke University. Animals were housed on a 12 h light/dark cycle. Mice of *Ube3a*$^{m+/p+}$ and *Ube3a*$^{m-/p+}$ were produced from breedings between *Ube3a*$^{m+/p-}$ females and wild-type C57BL/6 males. Littermates were used as controls for all experiments. Primers used for genotyping are as following: P1/genomic forward, 5′-CTTCTCAAGGTAAGCTGAGCTTGC-3′, P2/reverse, 5′-GCTCAAGGTTGTATGCCTTGGTGCT-3′ and P3/HPRT forward, 5′-TGCATCGCATTGTGTGAGTAGGTGTC-3′. PCR cycle conditions were 95 °C for 30 s, 56 °C for 60 s and 70 °C for 45 s for 35 cycles.

2.2. Brain slice preparation

Transverse hippocampal slices (400 μm) were prepared from postnatal day (P)17-P27 mice for excitability experiments or from P20 to P35 mice for LTD (long-term depression) experiments. For LTD experiments, CA3 was removed. Ice cold slicing solution contained in mM: 75 sucrose, 87 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 7 $MgCl_2$, 0.5 $CaCl_2$. Slices were recovered at room temperature (Fig. 1) or at 30 °C (Figs. 2 and 3) for at least 2 h in artificial cerebrospinal fluid (ACSF). ACSF contained in mM: 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 1 $MgCl_2$, and 2 $CaCl_2$.

2.3. Field potential recording

Hippocampal slices were placed in the submersion chamber maintained at 30 °C. After acclimation period (> 30 min), glass recording electrodes (1–3 MΩ) filled with ACSF, were placed in CA1 or CA3 pyramidal layer. For trains of stimulation experiment, stimulating electrode was placed at the border between CA1 and CA3. Vehicle or drugs were bath applied. The input-output relationship was obtained by 5, 10, 15, 20, 25, 30, 40, 50, 60, 100 μA stimulation (200 μsec, DS301 or Isoflex). Paired-pulse ratios were obtained from the ratio of the second field excitatory postsynaptic potential (fEPSP) slope to the first, for a range of inter-stimulus intervals (25–2000 ms). For LTD, *(RS)*-3,5-dihydroxyphenylglycine (DHPG) (100 μM, 10 min) was applied after stable baseline for 20 min (< 5% drift). The slope at 55–60 min was compared to the pre-conditioning baseline response (last 5 min of baseline).

The criteria for the "long burst" in this study was set at burst duration of longer than 2 s. This is based on the previous studies where "short discharges" (interictal-like activity) is hardly longer than 1.5 s and "prolonged synchronized discharges" in stable condition (30 to 60 min after DHPG) is longer than 2 s (Chuang et al. 2005; Taylor et al. 1995; Zhao et al. 2004). The duration measurement in extracellular recording in this study is based on extracellular and intracellular recordings in previous "prolonged synchronized discharges" studies (Taylor et al. 1995; Young et al. 2013). In Fig. 3**H** and **L**, the duration of individual long bursts were averaged for each slices for the mean duration of 10 min interval. If no long bursts were present within the interval in a slice, zero was assigned.

2.4. Drug treatment

Lovastatin (sodium salt) were obtained from Millipore. (*RS*)-3,5-Dihydroxyphenylglycine (DHPG), 2-Methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP) and (−)-bicuculline methiodide (BMI) and nimodipine were acquired from Tocris Bioscience. Other chemicals were from Sigma. All drugs were bath applied. Extracellular K^+ concentration was raised by adding 1.0 M KCl stock solution to ACSF.

2.5. Lovastatin treatment and audiogenic seizure

Mice of P15 to P23 were injected i.p. with: (1) 10 mg/kg lovastatin acid or vehicle (DMSO 100%); (2) 100 mg/kg lovastatin acid or vehicle (DMSO 100%). Mice were housed in home cage for one hour before evoking audiogenic seizure. Each mouse was transferred to a plastic test chamber (17 × 22 × 20 cm) and habituated for 1 min. A loud sound (about 130 dB) was generated for 2 min from a personal alarm (Radioshack model 49-1010). The behavior was monitored with digital video camera recorder (Sony DCR-SR45). The positive for seizure was scored only when tonic-clonic movement was observed (van Woerden et al. 2007).

2.6. Statistical analysis

Fisher's exact test (two-tailed) for categorical data, *t*-test and paired *t*-test for independent and repeated two group comparisons and repeated one-way ANOVA with Tukey post-hoc test for 3 repeated measurements were used. The level of significance was set at $p < 0.05$.

3. Results

3.1. Lower threshold for hyperexcitability in *Ube3a*$^{m-/p+}$ brain slices

Ube3a$^{m-/p+}$ mice displayed a lower threshold for audiogenic seizures (Jiang et al. 1998; Mandel-Brehm et al. 2015; Miura et al. 2002; van Woerden et al. 2007). This would predict that the neuronal circuit in *Ube3a*$^{m-/p+}$ has higher excitability than wild type (*Ube3a*$^{m+/p+}$) in *in vitro* model. Because the abnormal EEG and abnormal synaptic function has been well characterized in hippocampus of AS model (Miura et al. 2002), we monitored the neuronal excitability in the CA1 of *Ube3a*$^{m-/p+}$ bran slices in response to the sequential increase of extracellular K^+ ion concentration (Fig. 1**A–B**). The experimenter was blind to the genotypes in the experiments where both wild type and *Ube3a*$^{m-/p+}$ were used. At 6 mM of K^+, 4 of 7 *Ube3a*$^{m-/p+}$ brain slices (one slice per mouse) showed synchronized discharges but none of *Ube3a*$^{m+/p+}$ (0 of 6 slices, 6 mice) (Fisher's exact test, $p = 0.025$). The number of synchronized discharges within the 20 min observation