Lovastatin suppresses hyperexcitability and seizure in Angelman syndrome model

Leeyup Chunga,1, Alexandra L. Beyb, Aaron J. Towersc, Xinyu Caoa, Il Hwan Kimd, Yong-hui Jianga,b,c,*

a Department of Pediatrics, Duke University School of Medicine, Durham, NC 27710, United States
b Department of Neurobiology, Duke University School of Medicine, Durham, NC 27710, United States
c Department of Cell Biology, Duke University School of Medicine, Durham, NC 27710, United States

ABSTRACT

Epilepsy is prevalent and often medically intractable in Angelman syndrome (AS). AS mouse model (Ube3a−/−/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−/−/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−/−/p+. The in vitro model of Ube3a−/−/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 are 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. Valporic 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−/−/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.,...
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 Ube3am−/−/+ and Ube3am−/−−/+ were produced from breedings between Ube3am−/−−/+ 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′-CTCTCTAAGGTAAAGCTGCGTTCG-3′; P2/reverse, 5′-GCTCAAGGTTGTATGCCTTGGTGCT-3′; P3/HPRT forward, 5′-TGCTATGGCAATGTTGAGATTGTGTC-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 NaH2PO4, 26 NaHCO3, 10 glucose, 7 MgCl2, 0.5 CaCl2. Slices were recovered at room temperature (Fig. 1) or 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 μM 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 harderly 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. 3H and I, 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−/−/+ brain slices

Ube3a−/−/+ 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−/−/+ has higher excitability than wild type (Ube3a+/+−/) 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−/−/+ brain slices in response to the sequential increase of extracellular K+ ion concentration (Fig. 1A–B). The experimenter was blind to the genotypes in the experiments where wild type and Ube3a−/−/+ were used. At 6 mM of K+, 4 of 7 Ube3a−/−/+ brain slices (one slice per mouse) showed synchronized discharges but none of Ube3a−/−/+ (0 of 6 slices, 6 mice) (Fisher’s exact test, p = 0.025). The number of synchronized discharges within the 20 min observation period was increased compared to the controls (P < 0.05).
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات