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Chronic treatment with valproic acid or sodium butyrate attenuates novel object recognition deficits and hippocampal dendritic spine loss in a mouse model of autism ☆

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ABSTRACT

We recently showed that prenatal exposure to valproic acid (VPA) in mice causes autism-like behavioral abnormalities, including social interaction deficits, anxiety-like behavior and spatial learning disability, in male offspring. In the present study, we examined the effect of prenatal VPA on cognitive function and whether the effect is improved by chronic treatment with VPA and sodium butyrate, histone deacetylase inhibitors. In addition, we examined whether the cognitive dysfunction is associated with hippocampal dendritic morphological changes. Mice given prenatal exposure to VPA exhibited novel object recognition deficits at 9 weeks of age, and that the impairment was blocked by chronic (5-week) treatment with VPA (30 mg/kg/d, i.p.) or sodium butyrate (1.2 g/kg/d, i.p.) starting at 4 weeks of age. In agreement with the behavioral findings, the mice prenatally exposed to VPA showed a decrease in dendritic spine density in the hippocampal CA1 region, and the spine loss was attenuated by chronic treatment with sodium butyrate or VPA. Furthermore, acute treatment with sodium butyrate, but not VPA, significantly increased acetylation of histone H3 in the hippocampus at 30 min, suggesting the difference in the mechanism for the effects of chronic VPA and sodium butyrate. These findings suggest that prenatal VPA-induced cognitive dysfunction is associated with changes in hippocampal dendritic spine morphology.

1. Introduction

Valproic acid (VPA), a widely used drug to treat epilepsy and bipolar disorder (Henry, 2003), inhibits histone deacetylases (HDAC) (Göttlicher et al., 2001). Clinical studies indicate that maternal use of VPA during pregnancy is associated with an increased rate of autism spectrum disorders (ASD) (Williams and Hersh, 1997; Williams et al., 2001), which are classified as neurodevelopmental disorders. In line with this finding, a growing number of animal studies has revealed that in utero VPA exposure in both rats and mice leads to ASD-like behaviors in the offspring, and the rodents exposed to VPA prenatally has been proposed as an ASD animal model (Wagner et al., 2006; Yochum et al., 2008; Gandal et al., 2010; Mehta et al., 2011; Edalatmanesh et al., 2013; Roullet et al., 2013). We have also shown that prenatal exposure to VPA at embryonic day 12.5 (E12.5) causes ASD-like behavioral abnormalities, including social interaction impairment, anxiety-like behavior and spatial learning disability, in male mice (Kataoka et al., 2013). The study showed that inhibition of HDAC increases acetylated levels of histones H3 and H4 in the embryonic brain and plays a critical role in the expression of ASD-like behavioral abnormalities (Kataoka et al., 2013). With respect to a sex difference in the clinical manifestation of ASD (Carter et al., 2007; Yeargin-Allsopp et al., 2003), we found that the VPA-induced social interaction impairment was not observed in female mice (Hara et al., 2012), while other behavioral abnormalities, such as anxiety-like behavior and spatial learning disability, were observed in both male and female mice.

Several lines of evidence suggest that HDAC inhibitors have great potential for treating cognitive impairment resulting from neurodegenerative and neurodevelopmental disorders (Gräff and Tsai, 2013a,b; Fischer et al., 2010). Ricobaraza et al. (2009, 2012) demonstrated that the HDAC inhibitor 4-phenylbutyrate ameliorates spatial learning and memory deficits and rescues dendritic spine loss associated with fear memory deficits in the Tg2576 mouse model of Alzheimer's disease.

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However, it is not known whether HDAC inhibitors affect memory deficits in VPA-induced ASD model.

In this study, we first examine the effects of prenatal exposure to VPA at E12.5 on cognitive function and dendritic spine density in the hippocampus of male offspring. Then, we examine the effects of chronic treatment with the HDAC inhibitors sodium butyrate and VPA on the prenatal VPA-induced changes in behavior and dendritic spine morphology. Finally, we examine the effects of VPA and sodium butyrate on acetylated levels of histone H3 to clarify whether the effects of these drugs are associated with the HDAC inhibition. This study suggests that the change in hippocampal dendritic spine morphology is involved in prenatal VPA-induced cognitive dysfunction.

2. Materials and methods

2.1. Animals

Female ICR (CD1) mice (Japan SLC Inc., Hamamatsu, Japan) were obtained at 8 days of gestation and housed individually in plastic cages under a standard light/dark cycle (12-h light cycle starting at 8:00) at a constant temperature of 22 ± 1 °C. The animals had ad libitum access to food and water, and they were handled in accordance with the guidelines established by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences, Osaka University, the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Chemicals and antibodies

The principal reagents used in this study are as follows: 2-propylpentanoic acid sodium salt (the sodium salt form of VPA) and sodium butyrate (Sigma-Aldrich, St. Louis, MO); FD Rapid GolgiStain™ Kit (FD Neuro Technologies, Ellicott, MD); rabbit anti-histone H3 IgG (Cell Signaling Technologies, Danvers, MA); rabbit anti-acetyl-histone H3 IgG (Millipore, Billerica, MA); HRP-conjugated anti-rabbit IgG (KPL, Gaithersburg, MD). All other chemicals used in this study were of the highest purity commercially available.

2.3. Drug administration

VPA and sodium butyrate were dissolved in isotonic 0.9% NaCl solution (saline), and the volume of injection was 10 ml/kg. Pregnant mice were intraperitoneally injected with either VPA (500 mg/kg) or saline on embryonic day 12.5 (E12.5) (Kataoka et al., 2013) and returned to their home cages. Offspring born to the 16 VPA- and 15 saline-treated mothers were weaned, sexed at 3 weeks of age, and then randomly divided into several cages (5–6 mice/cage). Male mice (65 saline- and 66 VPA-exposed offspring, and 18 naïve mice) were used in behavioral analysis (Fig. 1: 22 saline-exposed offspring from 5 mothers and 18 VPA-exposed offspring from 4 mothers; Fig. 2: 34 saline-exposed offspring from 8 mothers and 39 VPA-exposed offspring from 9 mothers), spine morphological analysis (9 saline-exposed offspring from 2 mothers and 9 VPA-exposed offspring from 2 mothers) and biochemical analysis (18 naïve mice purchased from Japan SLC Inc.). One week after weaning, the mice in each group were further divided into two subgroups (postnatal drug- and vehicle-treated groups), and then the mice in each subgroup were intraperitoneally treated with 30 mg/kg of VPA (Qing et al., 2008), 1.2 g/kg of sodium butyrate (Fischer et al., 2007) or vehicle (saline) once daily for 5 consecutive weeks. The previous studies (Qing et al., 2008; Fischer et al., 2007) have demonstrated that the treatment duration and drug doses are effective to alleviate memory deficits in mouse models of neurodegenerative diseases. Each mouse was subjected to behavioral test once. Samples for morphological analysis were prepared from mice without subjecting to the behavioral test. Observers, who were blinded to the experimental groups, were performed the behavioral and biochemical tests.

2.4. Novel object recognition test

The novel object recognition test was performed between 12:00 and 15:00 as previously reported (Ito et al., 2007; Mizoguchi et al., 2008) with a minor modification. Each mouse was habituated to a Plexiglas open box (30 cm × 30 cm × 35 cm) with a sawdust-covered floor, which was located in a sound-attenuated room under dim light conditions (30 lx), for 10 min per day for 3 consecutive days. In the training session, two novel objects 'a' and 'b' were symmetrically fixed to the floor of the box, 8 cm from the walls, and the mouse was allowed to explore freely for 10 min. After training, mice were returned to their home cages. In the retention session at 1 or 24 h after the training session, the mouse was allowed to explore in the same field, in which one of the familiar objects 'b' used in the training was replaced by a novel object 'c', for 5 min. The objects were different in shape and color, but similar in size, including a golf ball, a plastic column a toy block, and a plug adapter. The sawdust in the floor was renewed, and the objects were cleaned and wiped thoroughly with 70% ethanol between trials to ensure the absence of olfactory cues. The objects had been previously tested with naïve mice to ensure an equivalent level of spontaneous preference. The performance of the mice during the training and retention sessions was videotaped using a digital camera, and the time spent exploring each object was measured. The discrimination index, which is defined as the difference between the exploration time for the novel object 'c' and that for the familiar object 'a', respectively, divided by total exploration time, was used to measure recognition memory (Wang et al., 2014).

2.5. Dendritic spine analysis

Golgi-Cox impregnation was performed using the FD Rapid GolgiStain™ Kit according to the manufacturer's instructions. Briefly, male mice (9 weeks of age) were deeply anesthetized with pentobarbital (40 mg/kg, i.p.) at 24 h after final drug administration, and their brains were removed, rinsed with Nanopure water, and immersed in the impregnation solution composed of potassium dichromate, mercuric chloride and potassium chromate. The brains were stored at room temperature for 2 weeks and then transferred and stored in the cryoprotectant solution for 2.5 days in the dark. Coronal sections of 100 μm thickness were cut on a cryostat (CM3050S; Leica Microsystems GmbH) at −22 °C, mounted on MAS-coated glass slides (Matsunami Glass Ind., Kishiwada, Japan), and allowed to air dry at room temperature in the dark for up to 3 days. After drying, the sections were rinsed with Nanopure water, reacted in the working solution, and dehydrated with a 50, 75, 95 and 100% graded ethanol series. Finally, the sections were defatted in xylene and coverslipped using Mount Quick (Daido Sangyo, Saitama, Japan).

Digitized images from the stratum radiatum and lacunosum-moleculare subregions of the hippocampal CA1 region were obtained with an upright light microscope with a cooled CCD digital camera system (Axio Imager.M2/AxioCam MRc5; Carl Zeiss, Jena, Germany) using a 100× oil immersion lens. The 30–40 serial Z-stack images were collected at 0.2 μm intervals to cover the full depth of the dendritic arbors (25–50 μm) and then projected into a single TIFF image using the Z-Stack and Extended Focus modules (AxioVision 4.8.2; Carl Zeiss). The projected images were transferred to NIH ImageJ 1.46 for Mac OS X (http://rsb.info.nih.gov/ij), and all dendritic protrusions were assigned and counted as spines. At least 4 secondary dendrites branching from the apical dendrite and most proximal to the cell soma were imaged per section, and the spine density was averaged from at least 3 sections per mouse. In addition, the ratios of mature/immature spines were calculated according to spine classification (Irwin et al., 2000), in which dendritic protrusions were categorized