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Research report

Reduced prefrontal dopaminergic activity in valproic acid-treated mouse autism model

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HIGHLIGHTS

- Prefrontal DA system was studied in VPA-treated mouse autism model.
- Prenatal VPA reduced METH-induced hyperlocomotion in male offspring.
- VPA reduced METH-induced increases in prefrontal DA release and c-Fos expression.
- VPA reduced the mRNA levels of DA D_1 and D_2 receptors in the prefrontal cortex.
- The effects of VPA were not observed in female offspring.

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ABSTRACT

Previous studies suggest that dysfunction of neurotransmitter systems is associated with the pathology of autism in humans and the disease model rodents, but the precise mechanism is not known. Rodent offspring exposed prenatally to VPA shows autism-related behavioral abnormalities. The present study examined the effect of prenatal VPA exposure on brain monoamine neurotransmitter systems in male and female mice. The prenatal VPA exposure did not affect the levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT) and their metabolites in the prefrontal cortex and striatum, while it significantly reduced methamphetamine (METH) (1.0 mg/kg)-induced hyperlocomotion in male offspring. *In vivo* microdialysis study demonstrated that prenatal VPA exposure attenuated METH-induced increases in extracellular DA levels in the prefrontal cortex, while it did not affect those in extracellular NA and 5-HT levels. Prenatal VPA exposure also decreased METH-induced c-Fos expression in the prefrontal cortex and the mRNA levels of DA D_1 and D_2 receptors in the prefrontal cortex. These effects of VPA were not observed in the striatum. In contrast to male offspring, prenatal VPA exposure did not affect METH-induced increases in locomotor activity and prefrontal DA levels and the D_1 and D_2 receptor mRNA levels in the prefrontal cortex in female offspring. These findings suggest that prenatal VPA exposure causes hypofunction of prefrontal DA system in a sex-dependent way.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social, communicative, and behavioral impairments in children [1,2]. Previous studies suggest that certain neurotransmitter systems are involved in the etiology of ASD, but the details of their involvement are not known. Regarding the

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dopamine (DA) system, genetic studies have reported that mutations of DA-associated genes—such as the DA transporter [3], DA receptors [4–7], and enzymes of DA synthesis [8]—were related to ASD. Furthermore, positron emission tomography demonstrated that the DA transporter binding was significantly higher in the orbitofrontal cortex of high-functioning autistic adults compared with controls [9]. These findings suggest that brain DA system is involved in the etiology ASD, but the precise involvement is not known.

Rodents prenatally exposed to VPA have been used as an animal model of ASD [10–13]. Narita et al. [14] reported that prenatal VPA exposure increased hippocampal serotonin (5-HT) and prefrontal DA levels in rats. Furthermore, a microdialysis study by Nakasato et al. [15] showed that the basal DA level in the prefrontal cortex was significantly higher in VPA-exposed rats relative to the controls. These findings suggest that the DA system is hyperactive in VPA-treated rats. In these studies, however, VPA was exposed at embryonic day 9. Kim et al. [16] reported that embryonic day 12 is the critical period in rats when VPA exposure has prominent effects for inducing the altered social behavior similar to human autistic behavior. We have also shown that mice prenatally exposed to VPA at embryonic day 12.5 displayed ASD-like hypolocomotion, anxiety-like behavior, social interaction deficits, and memory impairment at 8 weeks old [17]. Our subsequent study shows that chronic treatment with VPA or sodium butyrate, a histone deacetylase inhibitor, attenuates the prenatal VPA exposure-induced cognitive dysfunction and changes in dendritic spine morphology [18], but it is not known whether brain DA system is altered in VPA-treated autism model rodents. In the present study, we examined the effects of prenatal VPA exposure to mice at embryonic day 12.5 on brain dopaminergic activity of male and female offspring. The present study demonstrates that prenatal VPA exposure reduces methamphetamine-induced increase in locomotor activity with decreases in methamphetamine-induced increases in prefrontal DA release and the expression of prefrontal DA D_1 and D_2 receptors in male offspring, while the effects of VPA were not observed in female offspring.

2. Materials and methods

2.1. Animals

Eight week-old male and female ICR (CD1) mice were purchased from Japan SLC Inc., Hamamatsu, Japan 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.

Vaginal smears were taken daily from all female mice to determine their estrous cycle. Vaginal cell specimens were spotted onto glass slides by gently pipetting with a small amount of water. The air-dried smears were stained with Giemsa's solution. The stage of the estrous cycle was determined from the cell types observed in the vaginal smear. When a vaginal smear indicated proestrus or early estrus, male and female mouse were mated overnight, and next day was defined as gestation day 0.

2.2. Preparation of VPA-induced mouse model of autism

The pregnant mice were injected with either 500 mg/kg of VPA (Sigma–Aldrich, St. Louis, MO, USA) (i.p.) or saline on embryonic day 12.5 [17]. VPA was dissolved in 0.9% NaCl solution (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan), and the volume of injection was 10 mL/kg. All animals were returned to their home cages immediately after the injection and left undisturbed until weaning of the offspring. Offspring born from VPA and saline-treated mothers were weaned, sexed, and caged in groups of 5–6 mice of the same sex at postnatal day 21, and then the male and female offspring were used for the experiments.

2.3. Drug administration

(+)-Methamphetamine hydrochloride (METH) was dissolved in 0.9% NaCl solution. METH (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) (0.3, 1 or 3 mg/kg) was injected intraperitoneally at 10 mL/kg body weight.

2.4. Behavioral analysis

Locomotor activity was measured according to the method previously reported [17]. Each mouse was placed in the center of an open cubic transparent acrylic box with a black Plexiglas floor (45 cm × 45 cm × 30 cm), and allowed to freely explore the environment for 90 min under illumination by 100 lx of white light. After habituation for 90 min, each mouse was injected with saline or METH (0.3, 1 or 3 mg/kg, i.p.), returned immediately to the same box, and then allowed to freely explore for 90 min. The ambulations of the mice were monitored using a Panlab Infrared Actimeter System (LE8815) with acquisition software Acti-Track® 2.65 for Windows (Panlab, Barcelona, Spain). The paths taken by each mouse were stored permanently as x–y coordinate sequences, and the total traveling distance was assessed as horizontal locomotor activity. The open-field was wiped clean with 70% ethanol before each mouse was placed in it.

2.5. Measurement of monoamine levels

The concentrations of monoamines in the prefrontal cortex and striatum were quantified by high-performance liquid chromatography (HPLC) with an electrochemical detector (HTEC-500; Eicom, Kyoto, Japan) as previously reported with minor modifications [19,20]. Briefly, the prefrontal cortex and striatum were individually isolated, frozen on dry ice, and stored at −80 °C until assay. Tissue samples were homogenized in 0.2 M perchloric acid containing 100 μM EDTA and isoproterenol as an internal standard. The homogenate was centrifuged at 15,000 × *g* for 15 min at 0 °C. The supernatant was filtered through a 0.22 μm membrane filter (Millipore, Tokyo, Japan), and then a 10-μl aliquot of the sample was injected onto the HPLC column every 45 min for monoamines and their metabolites assay. An Eicompak SC-5ODS column (3.0-mm i.d. × 150 mm: Eicom) was used, and the potential of the graphite electrode (Eicom) was set to +750 mV against an Ag/AgCl reference electrode. The mobile phase contained 0.1 M sodium acetate/0.1 M citrate buffer, pH 3.5, 190 mg/l octanesulfonic acid, 5 mg/l EDTA, and 11.8% (v/v) methanol. Values are expressed as ng/g of tissue (wet weight).

2.6. In vivo *microdialysis*

In vivo microdialysis studies were performed as previously reported [21,22]. Each mouse was anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and stereotaxically implanted with a guide cannula (one site per animal) for a dialysis probe (Eicom Corp., Kyoto, Japan) in the prefrontal cortex (*A* +1.9 mm, *L* ±0.5 mm, *V* −0.8 mm, from the bregma and skull) or striatum (*A* +0.4 mm, *L* ±1.7 mm, *V* −2.5 mm). Postoperative analgesia was performed with