Reproductive Toxicology 77 (2018) 53–61

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Transcriptional and splicing dysregulation in the prefrontal cortex in valproic acid rat model of autism

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ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form 28 January 2018
Accepted 30 January 2018
Available online 7 February 2018

Keywords:
Valproic acid (VPA)
RNA sequencing (RNA-seq)
Transcriptome
Alternative splicing gene
Autism

ABSTRACT

Gene-environmental interaction could be the major cause of autism. The aim of the current study is to detect the effects of valproic acid on gene expression profiles and alternatively spliced genes in the prefrontal cortex in rat models of autism. Female rats received a single intraperitoneal injection of 600 mg/kg valproic acid at day 12.5 post-conception, and controls were injected with saline. Only male offspring were employed in the current study. RNA sequencing was used to investigate transcriptome in the prefrontal cortex of VPA-exposed rats. There were 3228 differently expressed genes and 637 alternative spliced genes, in VPA rats compared to controls. Pathways enrichment among the differently expressed genes and alternatively spliced genes were associated with neurological diseases and neural system development. The results implied VPA affected transcriptional and splicing events genome-wide and the transcriptional and splicing events may be associated with the autistic behaviors of VPA rats.

1. Introduction

Autism is a spectrum of neurodevelopmental disorders characterized by impairment of communication, social interaction, abnormally restricted and repetitive behavior [1–3]. Although 10–20% of autism spectrum disorders were attributed to genetic factors, the genetic bias of the remaining cases remains unclear. The etiology of autism may well involve a complex interplay of environmental and genetic factors that determine susceptibility [4,5]. Gene-environment interactions may influence epigenetic programming and ultimately gene expression profiles during brain development [6]. Transcriptome profiles differ in blood and frontal and temporal cortex in autism patients compared to healthy controls [2,7]. This raises the question of environmental factors that could affect transcriptional and splicing regulation leading to an autism phenotype.

Valproic acid (VPA) was widely used as an anti-epileptic because of its ability to potentiate GABA signaling in the brain [8], but it is teratogenic. Maternal administration of VPA during pregnancy increases fetal susceptibility to autism-like consequences [9]. Administration of VPA (600 mg/kg) to rats at gestational day 11–12.5 also causes severe autism-like behaviors, such as increased impaired social interaction and repetitive behavior, in their male offspring [3,9–16].This frequently studied model of an environmental trigger [9–12] could be a useful model of the developmental injury that initiates autism [17].

The Strength of the VPA induced models of autism has been evaluated on three criteria including construct, face, and predictive validity. As we already know, VPA induces autism both in human and animals, this partly certifies that VPA induced model effectively mimics an aspect of etiology of autism (construct validity). The VPA induced model showed all two core symptoms which include impaired social communication and interaction, restricted, repetitive patterns of behaviors in their male offspring (face validity). Furthermore, known drugs (i.e., Risperidone) and many drug candidates have been assessed for the applicability as potential therapeutics in this model (predictive validity). This model also has good replicability, it can be reproducible in many laboratories. Thus,

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https://doi.org/10.1016/j.reprotox.2018.01.008
0890-6238/

the VPA induced animal model of autism is one of the most widely used animal model in the field [12].

VPA is a histone deacetylase inhibitor (HDACi). Histone acetylation decreases the interaction between histones and DNA, transforming condensed chromatin to a more relaxed structure and it is a global marker and facilitator of gene activity [10,11]. It has been reported that VPA could regulate gene expression in rat whole embryo culture and embryonic stem cell [18,19]. Furthermore, the downstream signaling pathways involved in the HDACi action of VPA could be evoked, and affect oxidative stress and the synaptic excitatory/inhibitory balance in brain development [12,20]. Thus, maternal VPA exposure might result in long-term effects following altered gene expression invoking autism-like behaviors in rats.

The prefrontal cortex is the brain region related to social recognition, and is considered one of the major brain regions in autism [21–23]. Excess neuron numbers and cortical overgrowth are pronounced in prefrontal cortex tissue in the majority of autism patients [22,24]. Morphological and functional changes had been observed in VPA rats within the prefrontal cortex [22,23].

We therefore postulated that VPA exposure (600 mg/kg) can affect the transcriptome in prefrontal cortex of rat and ultimately produce more sustained effects including those leading to autism-like behaviors. In our current study, VPA-induced rat models of autism were constructed by a single intraperitoneal injection of 600 mg/kg sodium VPA to female rats at day 12.5 post-conception, and RNA sequencing (RNA-seq) was used to investigate the effects of VPA on gene expression and alternatively spliced gene profiles in prefrontal cortex of VPA exposure at pups. To elucidate the molecular mechanism of autism, pathway enrichment was performed in differentially expressed genes and alternatively spliced genes in VPA-induced rat models of autism. Real-time quantitative PCR (qPCR) was used to validate the selected differentially expressed genes.

2. Materials and methods

2.1. Experimental animal groups

Sprague-Dawley (SD) rats were purchased from Xi'an Jiaotong University animal center (Xi'an, China). All rats were housed under a 12-h light/dark cycle at 18–22 °C, relative humidity at 50–60%, and allowed free access to food and water. 12-week-old female rats (250–300 g), with controlled fertility cycles, were mated overnight. The vaginal secretion was collected in the next morning, and the day in which spermatozoa were detected was designated the first day of gestation (GD1). Randomly, dams were assigned to either the VPA treatment or the control group, and the females in VPA group received a single intraperitoneal injection of 600 mg/kg VPA (250 mg/ml in saline, pH 7.3. Sigma, Oakville, CA) while the control ones were injected with saline on GD 12.5, which is the critical period for the end of neural tube closure phase and the beginning of neurogenesis phase [13]. The dose of VPA administered here was based on previous reports [14,16]. The female rats were housed individually, and were allowed to raise their own litters. Since the growth and behavioral development were conducted in the current study, the litters were culled to eight animals per litter (four males, four females). The offspring were weaned on postnatal day (PND) 23 and separated by sex. Because of the higher male incidence of autism, and impaired social interaction and repetitive behaviors were only observed in all male offspring after VPA exposure, only male offspring were used in developmental and behavioral test (3 males/3 litters) and RNA-seq (1 male/3 litters) in the current study.

All animal experiments were performed in accordance with the National Institutional Animal Care and the guidelines approved by the Medicine Animal Care and Use Committee of Shaanxi Normal University. All efforts were made to minimize the number of animals used and their suffering, and the rats were euthanized by carbon dioxide asphyxiation.

2.2. Postnatal developmental and behavioral tests

The developmental and behavioral tests except three-chamber test were performed as described previously by Schneider et al [14] with modifications. All tests were conducted on groups of 9 male animals (three rats/three litters/group).

Body weight gain was measured on PND 7, 14, 21, 56 and 70. Eye opening was observed once daily from PND 12–16 and rated as follows: 0- both eyes closed; 1-one eye open; and 2- both eyes open.

2.2.1. Behavioral tests

Negative geotaxis: This behavioral test reflects vestibular function, motor development, and activity [14]. It was observed once daily from PND 7–10. Pups were placed on a 25° inclined surface in a head down position and timed for completion of a 180° turn.

Swimming performance: The swimming test measures motor development and integration of coordinated series of reflex responses [14]. It was conducted on PND 8, 10, 12 and 16. Each animal was put at the center of an container filled with water (28–29 °C) and was observed for 5–10 s. Swimming performance was rated as follows: 0-head and nose below the surface; 1- nose below the surface; 2- nose and top of head at or above the surface, but ears still below the surface; 3- the same as in 2 except that water line was at mid-ear level; and 4- the same as in 3 except that water line was at the bottom of ears.

Olfactory discrimination: This test reflects a nest-seeking response mediated by the olfactory system [14]. It was conducted daily on PND 9–11. The apparatus is a container with a clear plastic cover. One side of the container contained a bin filled with clean bedding, while at the other end, there was a bin filled with home cage bedding. Each pup was placed in the centrally demarcated region with its head facing to or away from the experimenter, and the latency to enter the home bedding side by crossing the designed line with the front paws and head was timed.

Three-chamber test: The social behavioral test was performed in a three-chamber apparatus on PND 30–35 [17]. The apparatus is an acrylic plastic box with dimensions (length/width/height in cm) 120/40/50. The box is separated into three chambers, the central chamber being 60 cm in length and each side 30 cm. There is an identical cage in each side chamber, with a stranger rat in one of the cages. The subject rat was allowed free exploration of the different chambers. Rats were individually acclimated for 5 min into the three-chamber apparatus on the day before the experiment. The sociability test was performed by putting the test rat in the central chamber, and the experiment was performed for 10 min. Time duration and the number of entrances into the chamber with the empty cage, as well as into the chamber with the stranger rat, was analyzed. The strange rats were SD males of the same age and have had no previous contact with the test rats.

2.3. RNA-seq

2.3.1. Sampling and RNA isolation

Male offspring of VPA- or saline-injected dams from different litters were euthanized with 10 g/ L with pentobarbital sodium (40 mg/ kg) intraperitoneally on PND 35 (n = three rats/three litters/group). The rats used for RNA-seq analysis were from the same litters from which three other pups each were derived for the neurobehavioral tests. Prefrontal cortex was dissected from both hemispheres, the anterior 1 mm was cut from the two hemispheres to remove the olfactory tubercle, then tissue was dissected from