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Benefits of agomelatine in behavioral, neurochemical and blood brain barrier alterations in prenatal valproic acid induced autism spectrum disorder

Hariom Kumar [a], B.M. Sharma [b], Bhupesh Sharma [c, d, *]

[a] CNS Research Lab, Department of Pharmacology, School of Pharmacy, Bharat Institute of Technology, Partapur Bypass, Meerut, Uttar Pradesh, India
[b] School of Pharmacy, Bharat Institute of Technology, Partapur Bypass, Meerut, Uttar Pradesh, India
[c] Amity Institute of Pharmacy, Amity University, Sector-125, Noida, Uttar Pradesh, India
[d] CNS Pharmacology, Conscience Research, Pocket F-233, B, Dilshad Garden, Delhi 110095, India

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ABSTRACT

Valproic acid administration during gestational period causes behavior and biochemical deficits similar to those observed in humans with autism spectrum disorder. Although worldwide prevalence of autism spectrum disorder has been increased continuously, therapeutic agents to ameliorate the social impairment are very limited. The present study has been structured to investigate the therapeutic potential of melatonin receptor agonist, agomelatine in prenatal valproic acid (Pre-VPA) induced autism spectrum disorder in animals. Pre-VPA has produced reduction in social interaction (three chamber social behavior apparatus), spontaneous alteration (Y-Maze), exploratory activity (Hole board test), intestinal motility, serotonin levels (prefrontal cortex and ileum) and prefrontal cortex mitochondrial complex activity (complex I, II, IV). Furthermore, Pre-VPA has increased locomotor activity (actophotometer), anxiety, brain oxidative stress (thiobarbituric acid reactive species, glutathione, and catalase), nitrosative stress (nitrite/nitrate), inflammation (brain and ileum myeloperoxidase activity), calcium levels and blood brain barrier leakage in animals. Treatment with agomelatine has significantly attenuated Pre-VPA induced reduction in social interaction, spontaneous alteration, exploratory activity intestinal motility, serotonin levels and prefrontal cortex mitochondrial complex activity. Furthermore, agomelatine also attenuated Pre-VPA induced increase in locomotion, anxiety, brain oxidative stress, nitrosative stress, inflammation, calcium levels and blood brain barrier leakage. It is concluded that, Pre-VPA has induced autism spectrum disorder, which was attenuated by agomelatine. Agomelatine has shown ameliorative effect on behavioral, neurochemical and blood brain barrier alteration in Pre-VPA exposed animals. Thus melatonin receptor agonists may provide beneficial therapeutic strategy for managing autism spectrum disorder.

1. Introduction

Autism spectrum disorder (ASD) is a cluster of neurodevelopmental disorders characterized by impairment stereotypical behavior, communication and social interest (de Theije et al., 2014). ASD display a diversity of comorbid traits, including seizures, anxiety, aggressive behavior, gastrointestinal problems, motor deficits, abnormal sensory processing and sleep disturbances. These impairments negatively impact daily life and can exaggerate the effects of the core diagnostic traits (social communication deficits and repetitive behaviors) (Argyropoulos et al., 2013). ASD has 1% prevalence over worldwide population and is dramatically increasing. ASD affects more male than female population, having more than 70% comorbidity (Lai et al., 2014).

Maternal use of the valproic acid (VPA) has been found to provide teratogenic effect, which may contribute to the development of ASD in offspring (Kim et al., 2011). Prenatal valproic acid (Pre-VPA) exposure has emerged as a rodent model of ASD, because it causes broad spectrum autistic core and comorbid features in

* Corresponding author. Amity Institute of Pharmacy, Amity University, Sector-125, Noida, Uttar Pradesh, India.
E-mail addresses: sharmaslab7@gmail.com (H. Kumar), sharmaslab1@gmail.com (B.M. Sharma), conscienceresearch@scientist.com, drbhupeshresearch@gmail.com (B. Sharma).

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animals similar to humans (Schneider and Przewłocki, 2005).

Melatonin receptors are expressed throughout the central nervous system (Tardito et al., 2012). They exert their actions through the activation of G protein-coupled receptors, namely MT_1 and MT_2 (Pala et al., 2013). Agomelatine is a dual agonist of melatonin MT_1 and MT_2 receptors (Chenu et al., 2013). The reduction of circulating melatonin has been observed in numerous diseases, including Alzheimer's disease, stress, pain and metabolic disorders (Hardeland, 2012). Centrally, melatonin receptor agonists have been reported to act as anxiolytic (Kopp et al., 2000), anti-oxidants (Singh et al., 2015) and anti-inflammatory activity (De Filippis et al., 2008) etc. These receptors also regulates exploratory activity (Romero-Zerbo et al., 2009), neural gene responses and neuronal differentiation (de Faria Poloni et al., 2011). Circulating melatonin (Jonsson et al., 2014) and its receptor signaling (Hong et al., 2015) have been found to alter in ASD and associated behavior (Tordjman et al., 2013). It has recently been reported that melatonin treatment significantly improve social behavior in animals (Tian et al., 2014). We hypothesized that melatonin receptor modulation may exert a protective effect in ASD. Thus, melatonin receptor modulation deserves investigations for its potential in ASD.

In the light of above, the present study has been designed to investigate the potential of agomelatine, a selective melatonin MT_1 and MT_2 agonist in Pre-VPA induced ASD in rats.

2. Material and methods

2.1. Animals

Adult albino Wistar rats (purchased from Indian veterinary research Institute izatnagar India) were allowed to mate along. Pregnant females and their offspring were utilized in the current study. They were housed in animal house with free access to water and standard laboratory pellet chow diet (Golden Feeds Ltd, New Delhi, India) with exposed to natural light and dark cycle. The various assessments were conducted on the male offspring between 9.00 and 18.00 h. The offspring acclimatized to the laboratory condition five days prior to behavioral study and maintained in the laboratory until the completion of the study. The protocol of the study was duly approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 25/230/2011/AWD/CPCSEA).

2.2. Drugs and reagents

Agomelatine was obtained from Abbott healthcare Pvt. Ltd., India. Sodium valproate was obtained from Sun Pharma Pvt. Ltd., India. Lowry's reagent, 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), Folin-Ciocalteu reagent, bovine serum albumin (BSA) and N-naphthylethylenediamine were purchased from Sigma Aldrich, India. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), ethylene glycol tetra acetic acid (EGTA), mannitol, glycyl glycine buffer, nicotinamide adenine dinucleotide (NADH), nitrazobluetetrazolium (NBT) and cytochrome −c, Evans blue was purchased from SISCO Research Laboratory Pvt. Limited, Mumbai, India. Hydrogen peroxide and pyridine were purchased from Rankem Laboratories Pvt. Ltd., India. Acetic acid, acetylcholine iodide, copper sulfate pentahydrate, disodium hydrogen phosphate, n-butanol, potassium chloride, potassium ferricyanide, sodium bicarbonate, sodium carboxymethyl-cellulose, sodium citrate, sodium dihydrogen phosphate, sodium dodecyl sulfate, sodium hydroxide, sodium phosphate monobasic, sodium phosphate dibasic, sodium tartarate, succinic acid, sucrose, trichloroacetic acid and zinc sulfate were purchased from CDH Laboratories Pvt. Ltd., India.

2.3. Drug administration

All drug solutions were freshly prepared before use. The sodium salt of valproic acid was dissolved in 0.9% saline for a concentration of 250 mg/mL and given intra-peritoneal (500 mg/kg; on embryonic day 12.5th) to the animals (Markram et al., 2008). Agomelatine was suspended in 0.5% carboxy-methylcellulose (CMC) and given orally (2 and 4 mg/kg; from postnatal day 21st to 50th) by oral gavage to the animals (Singh et al., 2015). Both of the doses of agomelatine in different groups were administered at 15:00 (2 h prior to the dark phase), based on the circadian rhythm (Dagytė et al., 2011).

2.4. Prenatal valproic acid (Pre-VPA) induced ASD

Pregnancy was determined by the presence of a vaginal plug on the embryonic 1st day. Sodium valproate was dissolved in 0.9% saline. Pregnant females on embryonic 12.5th day received sodium valproate (500 mg/kg; intra-peritoneal; single dose). Females were housed individually with their offspring till weaning (post-natal day 20) (Markram et al., 2008).

2.5. Assessment of locomotor activity

It has already been reported that valproic acid causes an increase in locomotor activity (Sandhya et al., 2012). On post-natal day 43rd, Actophotometer (INCO, Ambala, India) was used to assess locomotor activity. The apparatus was placed in a darkened, sound attenuated and ventilated testing room during assessment. Locomotor activity was assessed by comparing the number of beam breaks across a 20-min session divided into two 10-min intervals by animals (Sandhya et al., 2012).

2.6. Assessment of social interaction test

Impairment in social interaction is the core feature of the ASD (Kim et al., 2011). Between post-natal day 44th−45th, the social interaction was assessed by three chamber social behavior apparatus (70 cm wide and 30 cm long and divided into three equal chambers) for rat as described by previous researchers with slight modification (Kim et al., 2011). Briefly, before starting first phase the rat being tested was placed in the central chamber and habituated for 5 min for increase rat exploration of side chambers relative to center chamber. After termination of habituation period of 5 min, the first phase was started, which was known as sociability phase. In the sociability phase, age and sex matched a stranger rat (habituated to the wire cage in the apparatus for 30 min, 24 h before the test) was placed under a small wire cage with a radius of 5.5 cm in either left or right chamber. While another side chamber chamber wire cage was remained empty during sociability phase. Both left and right chambers were chosen randomly to avoid side preference chamber. During the sociability phase, the stranger animal wire cage side was called stranger chamber while empty wire cage side was called empty chamber. The testing rat was allowed to freely explore all the three chambers for 10 min. Directly after the termination of the first phase, the second phase was started and conducted for 10 min. This second phase was called social preference phase. In this phase, age and sex matched, a novel rat (habituated to the wire cage in the apparatus for 30 min, 24 h before the test) from another control litter was placed in the empty chamber under the wire cage. In this phase,

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