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Research report

Alterations in the endocannabinoid system in the rat valproic acid model of autism

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HIGHLIGHTS

- Prenatal VPA exposure elicits autistic-like behaviour during adolescence.
- Social exposure increases hippocampal anandamide levels in VPA exposed rats.
- DAGLα and MAGL expression is reduced in the cerebellum and hippocampus of VPA exposed rats.
- PPARα and GPR55 mRNA expression in the cortex is reduced in VPA exposed rats.
- VPA exposed rats exhibit reduced PPARγ and GPR55 mRNA expression in the hippocampus.

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ABSTRACT

The endocannabinoid system plays a crucial role in regulating emotionality and social behaviour, however it is unknown whether this system plays a role in symptoms associated with autism spectrum disorders. The current study evaluated if alterations in the endocannabinoid system accompany behavioural changes in the valproic acid (VPA) rat model of autism. Adolescent rats prenatally exposed to VPA exhibited impaired social investigatory behaviour, hypoalgesia and reduced lococmotor activity on exposure to a novel aversive arena. Levels of the endocananbinoids, anandamide (AEA) and 2-arachidonylglycerol (2-AG) in the hippocampus, frontal cortex or cerebellum were not altered in VPA- versus saline-exposed animals. However, the expression of mRNA for diacylglycerol lipase α, the enzyme primarily responsible for the synthesis of 2-AG, was reduced in the cerebellum of VPA-exposed rats. Furthermore, while the expression of mRNA for the 2-AG-catabolising enzyme monoacylglycerol lipase was reduced, the activity of this enzyme was increased, in the hippocampus of VPA-exposed animals. CB_1 or CB_2 receptor expression was not altered in any of the regions examined, however VPA-exposed rats exhibited reduced PPARα and GPR55 expression in the frontal cortex and PPARγ and GPR55 expression in the hippocampus, additional receptor targets of the endocannabinoids. Furthermore, tissue levels of the fatty acid amide hydrolase substrates, AEA, oleoylethanolamide and palmitoylethanolamide, were higher in the hippocampus of VPA-exposed rats immediately following social exposure. These data indicate that prenatal VPA exposure is associated with alterations in the brain's endocannabinoid system and support the hypothesis that endocannabinoid dysfunction may underlie behavioural abnormalities observed in autism spectrum disorders.

1. Introduction

Autism is a neurodevelopmental disorder characterised by impaired social interaction, deficits in communication and restrictive, repetitive stereotyped patterns of behaviours. The aetiology of this disorder remains unknown, although several genetic and environmental factors have been identified which play a role in this spectrum of disorders. Prenatal exposure to teratogenic agents such as valproic acid (VPA) has been implicated in the pathogenesis of autism [1–3] and knowledge of this association has led to the development of a widely used and validated preclinical model of autism.

Abbreviations: 2-AG, 2-arachidonyl glycerol; AEA, anandamide; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; GPR55, G protein-coupled receptor 55; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D; OEA, *N*-oleoylethanolamide; PEA, *N*-palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; VPA, valproic acid.

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Exposure of prenatal rats to VPA impairs neural tube closure and results in behavioural aberrations such as reduced social behaviour, lower sensitivity to pain and increased anxiety and fear in adolescent and adult rats [4–7], behaviours analogous to those observed clinically. Anatomical alterations such as diminished number of cerebellar purkinje and cranial neurons [8,9], enhanced synaptic plasticity of the prefrontal cortex [10] and amygdala [7,11], alterations in monoamine and amino acid neurotransmission [6,12,13] and immunological alterations [14] have also been reported in the model.

Increasing evidence suggests a role for the endocannabinoid system in social and emotional processing [15,16], however there is a paucity of studies directly examining the role of this system in autism. Comprised of the G-protein coupled CB_1 and CB_2 receptors, the endogenous cannabinoid ligands (endocannabinoids) including anandamide (AEA) and 2-archidonylglygerol (2-AG) and the enzymes responsible for the synthesis and catabolism of the endocannabinoids, the neuroanatomical distribution of this system means that it is well positioned to modulate affective and social responding. A recent review has suggested metabolism of acetaminophen (paracetamol) to *N*-arachidonoylphenolamine (AM404) [17], an AEA reuptake inhibitor, results in enhanced AEA tone which may alter neuronal development and immunological function during critical neurodevelopmental phases possibly predisposing certain children to developing autism [18]. However, to date no detailed studies have been carried out investigating the link between acetaminophen, the endocannabinoid system and the development of autism. Polymorphisms in the gene encoding the CB_1 receptor, *CNR1*, have been shown to modulate striatal responses [19] and gaze duration [20] to social reward cues, indicating that subtle changes in endocannabinoid affinity at the CB_1 receptors due to these polymorphisms may underlie deficits in social reward processing such as observed in autism. Preclinical studies have indicated that social play behaviour enhances AEA levels in several brain regions including the amygdala, nucleus accumbens [21] and striatum [22] and that enhancing endogenous AEA tone following pharmacological inhibition of fatty acid amide hydrolyse (FAAH), the enzyme primarily responsible for the catabolism of this endocannabinoid [23], or inhibition of AEA reuptake, and subsequent CB_1 receptor activation results in enhanced social play behaviour [24,25]. In comparison, direct activation of CB_1 receptors with the potent agonist WIN55,212-2 reduces social behaviour [24]. The differential effects of global CB_1 receptor activation and enhancing AEA tone on social play behaviour have been proposed to be due to the selective activation of CB_1 receptors in brain regions involved in social and emotional responding following FAAH inhibition [21,24]. However, it should be noted that in addition to increasing AEA levels, FAAH inhibition also increases *N*-acylethanolamines such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), although the role of these *N*-acylethanolamines on social and emotional behavioural responding remains to be been investigated. Recent studies have demonstrated enhanced cortical levels of AEA, but not 2-AG, following social exposure in BTBR mice, [26], a mouse strain known to exhibit an autistic-like behavioural phenotype [27]. Agonist-induced GTPγS binding of CB_1 receptors is enhanced in the BTBR mouse [26] and pharmacological activation of CB1/2 receptors has been shown to attenuate the hyperlocomotor activity displayed by these mice [26,28]. Central activity of diacylglycerol lipase (DAGL)α and monoacylglycerol lipase (MAGL), the enzymes responsible for the synthesis and catabolism of 2-AG respectively [29,30], have been reported to be enhanced in the *fmr*−/− mouse [31,32], a model of fragile X syndrome, the most common genetic form of autism. In addition, pharmacological inhibition of MAGL and subsequent augmentation of endogenous 2-AG levels, results in the normalisation of locomotor and anxiety-related behavioural changes in *fmr*−/− mice [32]. As highlighted, several lines of evidence suggest a potential role for the endocannabinoid system in autism, however a detailed profile of the system in a validated preclinical model is lacking.

The aim of the present study was to examine if the autistic-like behavioural changes exhibited by adolescent rats prenatally exposed to VPA are associated with endocannabinoid dysfunction in discrete brain regions known to modulate emotional and social behaviour. In addition to examining changes in endocannabinoid and *N*-acylethanolamine levels, and the expression of genes regulating the synthesis and catabolism of AEA and 2-AG, the expression of CB_1 and CB_2 receptors and other targets of the endocannabinoid system including peroxisome proliferator-activated receptor (PPAR)α, PPARγ and GPR55 [33,34] were examined.

2. Materials and methods

2.1. Animals

Male and female Sprague-Dawley rats (200–300 g; Charles River Laboratories, UK) were mated following determination of the oestrus phase of the reproductive cycle. The presence of spermatozoa in vaginal smears indicated the first day of gestation (G0.5). Following copulation, female rats were housed singly and maintained at constant temperature (21 ± 2 °C) and humidity (30–35%) under standard lighting conditions (12:12 h light–dark, lights on from 07:00 to 19:00 h). Food and water were available ad libitum. Experimental protocols were carried out under approval from the Animal Care and Research Ethics Committee at NUI Galway and under licence from the Irish Department of Health and Children, in compliance with the European Communities Council directive 86/609.

On gestational day 12.5 (G12.5), female rats received a single subcutaneous injection of sodium valproate (VPA) (Sigma, Dublin, Ireland) (600 mg/kg) or saline vehicle. The dose and time of administration was chosen based on studies demonstrating that this regime elicits autistic-like behavioural changes in offspring [5]. Females were allowed to raise their own litters and pups which were weaned on postnatal day (PND) 21. Following weaning, rats of either sex were housed separately in groups of 3–6 per cage.

2.2. Experimental design

A schematic representation of the experimental design is presented in Fig. 1.

2.2.1. Experiment 1: behavioural profile of the VPA model and associated changes in the endocannabinoid system

Behavioural testing was carried out during adolescence between PND 33 and 40. The sequence of testing remained constant, and involved the sociability test (saline-treated $n = 16$; VPA treated $n = 14$) followed by the hot plate test, followed by the open field and elevated plus maze test (saline-treated $n = 10$; VPA treated $n = 8$) and was modelled on the study design described by Schneider and colleagues [5]. All behavioural testing was carried out by an experimenter blinded to treatment. Seventy-two hours following the final behavioural test (PND 43) animals were killed by decapitation, the brain removed and discrete brain regions including the frontal cortex, hippocampus and cerebellum dissected out and snap frozen on dry ice. The frontal cortex was considered cortical tissue rostral to the central sulcus and included regions such as the prefrontal cortex, premotor cortex and motor cortex. All regions of the cerebellum (cerebro-, spino- and vertibular) were included in the cerebellar tissue samples that were processed. The aforementioned regions have been implicated in autistic-like symptoms and alterations in these regions have previously been demonstrated in the VPA model of autism [8,13,35]. Brain regions were stored at −80 °C until assayed for endocannabinoid and *N*-acylethanolamine levels, and mRNA expression of endocannabinoid related genes.

2.2.2. Experiment 2: endocannabinoid and N-acylethanolamine levels in discrete brain regions in VPA-exposed animals following exposure to the sociability test

Immediately following the sociability test, a subset of animals (saline-treated $n = 6$; VPA treated $n = 6$) were killed by decapitation, the frontal cortex, hippocampus and cerebellum excised, snap frozen on dry ice and stored at −80 °C until assayed for endocannabinoid and *N*-acylethanolamine levels.

2.3. Behavioural testing

2.3.1. Sociability test

The sociability test was conducted in a novel 3-chamber apparatus which allows for the measurement of social approach and social preference [36,37]. In brief, animals were placed into a novel arena (80 cm × 31.5 cm) composed of three communicating chambers separated by Perspex walls with central openings allowing access to all chambers for 5 min. Distance moved (cm) and time spent (s) in the