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Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid

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ABSTRACT

Autism spectrum disorders are a group of neurodevelopmental disorders characterised by impaired social interaction, deficits in communication and repetitive stereotyped behaviours. The endocannabinoid system plays an important role in modulating emotionality and social responding, however there have been a paucity of studies investigating this system in autistic animal models. This study investigated the effect of inhibiting fatty acid amide hydrolyase (FAAH), the anandamide catabolic enzyme, on behavioural responding in the valproic acid (VPA) rat model of autism. Male rats prenatally exposed to VPA exhibit an autistic-like behavioural phenotype exemplified as thermal hypoalgesia, reduced social and exploratory behaviour, and enhanced repetitive behaviour. Systemic administration of the FAAH inhibitor PF3845 (10 mg/kg) attenuated the deficit in social behaviour observed in VPA exposed male animals without altering nociceptive, repetitive or exploratory behaviour. In comparison, female VPA exposed rats displayed enhanced repetitive and reduced exploratory behaviour, but no change in social behaviour or thermal nociceptive responding. PF3845 did not alter social, repetitive or thermal nociceptive responding, but reduced exploratory behaviour in a social context in VPA-, but not saline-, exposed females. These data indicate that FAAH inhibition elicits sexual dimorphic effects on behavioural responding in VPA exposed rodents, and support an important role for FAAH in the regulation of social behavioural deficits in autistic males.

1. Introduction

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterised by impaired social interaction, deficits in communication and restrictive, repetitive stereotyped patterns of behaviours. The aetiology of this spectrum of disorder remains largely unknown, and although several genetic factors have been identified which play a role, known genetic factors are estimated to account for only 5–15% of autism cases. As such, environmental factors and subsequent epigenetic alterations are considered to play particularly important roles in the aetiology of this disorder. Environmental factors identified have included the prenatal exposure to teratogenic agents such as valproic acid (VPA) [1–3] and knowledge of the association between VPA and ASD has lead to the development of a widely used and validated preclinical model [4]. Exposure of prenatal rats to VPA has been shown to impair neural tube closure resulting in anatomical and neurochemical alterations and consequently behavioural aberrations such as reduced social and exploratory behaviour, enhanced repetitive behaviour and communication deficits, core changes associated with ASD. In addition, VPA exposed animals also exhibit additional behavioural alterations similar to the co-morbid symptoms observed clinically such as altered sensitivity to noxious stimuli (hypo- or hyper-algesia) and increased anxiety [for review see [4]]. Thus, the VPA rodent model provides a useful tool for evaluating the neurobiology underlying ASD and identifying novel therapeutic targets for this neurodevelopmental disorder.

The endocannabinoid system is comprised of the G-protein coupled CB_1 and CB_2 receptors, the endogenous cannabinoid

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ligands (endocannabinoids) including anandamide (AEA) and 2-archidonylglygerol (2-AG) and the enzymes responsible for the synthesis and catabolism of the endocannabinoids. In addition to CB_1 and CB_2 receptors, endocannabinoids are also known to have affinity for and activity at other receptor targets including the transient receptor potential vanilloid 1 (TRPV1), PPARs, GPR55 and GPR119 [5–8]. Several lines of evidence have demonstrated that the endocannabinoid system plays an important role in social and emotional processing [9–11]. For example, gene analysis profiling has demonstrating reduced expression of the gene encoding the CB_1 receptor *CNR1* in postmortem brain tissue of people with ASD [12]. Polymorphisms in *CNR1*, (in particular 2 SNP: rs806377 and rs806380) have been shown to be associated with increased striatal activity [13] and gaze duration [14] to social reward cues happy faces). Given that ASD is associated with atypical eye contact and facial emotion processing, this data suggest that polymorphisms in CNR1 may, in part, underlie the deficits in social reward processing. In accordance, preclinical studies have demonstrated that CB_1 receptors on specific neuronal subtypes (GABA vs Glutamate) modulate social investigatory behaviour of female mice [15]. Social play behaviour has been shown to enhance AEA levels in several brain regions including the amygdala, nucleus accumbens [16,17] and striatum [11]. Furthermore, enhancing endogenous AEA tone or inhibition of AEA reuptake, results in enhanced social behaviour [17–20]. However, there is a paucity of studies directly examining alterations or the effects of modulating the endocannabinoid system in preclinical models of ASD. Cortical levels of AEA, but not 2-AG, have been shown to be elevated following social exposure in BTBR T^+ $Itpr3^{tf}$/J (BTBR) mice, [21], an in-bred mouse strain known to exhibit an autistic-like behavioural phenotype. Agonist-induced GTPγS binding of CB_1 receptors is enhanced in the BTBR mouse [21] and pharmacological activation of $CB_{1/2}$ receptors (using Δ^9-THC or WIN55,212-2) has been shown to attenuate the hyperlocomotor activity displayed by these mice [21,22]. Fragile X syndrome is one of the leading genetic cause of ASD and the *FMR1* knockout mouse has provided a useful model in investigating the neurobiology underlying this condition [23]. Recent studies have demonstrated that CB_1 receptor antagonism [24] and FAAH inhibition [25] attenuated cognitive impairments in *FMR1*-/- mice, in the novel object recognition and passive avoidance test respectively. In comparison, CB_2 receptor antagonism attenuates anxiety-related behaviour, in these animals [24]. However to date, there have been no studies evaluating the impact of endocannabinoid modulation on behavioural responding in non-genetic animal models of ASD. Recent work from our laboratory has demonstrated that on exposure to a social stimulus levels of AEA, and the related *N*-acylethanolamines, oleoylethalomine and palmitoylethanolamine, are increased in the hippocampus of adolescent rats prenatally exposed to VPA [26]. In addition, while the expression of $CB_{1/2}$ receptors are not altered, rats prenatally exposed to VPA exhibited reduced cortical expression of PPARα and GRP55 and reduced hippocampal expression of PPARγ and GPR55 [26], additional receptors targets for endocannabinoids and *N*-acylethanolamines. Thus, alterations in the endocannabinoid system may underlie the pathophysiology and behaviour alterations observed in this model of ASD. Thus the aim of the present study was to examine the effect of enhancing AEA tone, by pharmacologically inhibiting the enzyme primarily responsible for its catabolism fatty acid amide hydrolyse (FAAH), on social, repetitive and exploratory behaviour in the VPA rat model of autism. In addition, we also evaluated the effect of FAAH inhibition on thermal nociceptive responding, given previous data from our group demonstrating that VPA-exposed animals exhibit thermal hypoalgesia [26], an effect similar to that reported clinically in ASD patients, and the increasing evidence to support a role for the endocannabinoid system in nociceptive processing [27]. Clinical literature indicates a higher prevalence of ASD in males vs female (5:1 ratio) with recent data indicated that ASD females exhibit excessive mutational alterations when compared to males [28], highlighting that females may be somewhat protected against neurodevelopmental disorders such as ASD. Accordingly, VPA exposed males have been shown to exhibit more pronounced behavioural alterations when compared to females; and several studies have now demonstrated sexual dimorphic effects on the cytoarchitecture, neurotransmission and inflammatory mediators in the model [29–32]. Thus, a further aim of this study was thus to examine if FAAH inhibition elicits sexual dimorphic behavioural changes in rats prenatally exposed to VPA or saline.

2. Material 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 reverse lighting conditions (12:12 h dark–light, lights on from 1900 to 0700 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 2010/63/EU. All sections of the study adhered to the ARRIVE Guidelines for reporting in animal research [33]

On gestational day 12.5 (G12.5), 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 [26,34]. There was no effect of VPA administration on maternal health, gestational time (21–22 days) or litter size (7–12 rats). Litters were not culled and females were allowed to raise their own litters and pups which were weaned on postnatal day (PND) 21. All pups survived to weaning apart from one VPA exposed litter where 3 out of 7 pups died and thus this litter was not used for further testing due to possible differences in maternal care between this group and the other litters. Following weaning, rats of either sex were housed separately in litter groups of 3–5 siblings per cage. All animals underwent all behavioural tests which occurred during the dark phase (0900–1800 h) under red light illumination.

2.2. Experimental design

Behavioural testing was carried out during adolescence between PND 42–44. 24 h prior to the test day, animals were housed individually as this has been shown to enhance social behavioural responding [35]. On the experimental day, animals received an intraperitoneal injection of the FAAH inhibitor PF3845 (10 mg/kg; NIMH drug synthesis program, US) or vehicle (1:1:18 ethanol:chremaphore:saline) and were returned to their home cage for a period of 2 h. The dose and time of administration were chosen based on published studies [36–39] and pilot data demonstrating enhanced AEA levels in the brain from 1hr post administration. After the 2hr period, animal were then removed from their home cage and tested individually in the hotplate test, followed 2 min later by exposure to the 3-chamber sociability arena. All behavioural testing was carried out by an experimenter blinded to treatment regime.