Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue1abst036.pdf

Alterations In The Endocannabinoid System In The Valproic Acid Rat Model Of Autism

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Prenatal exposure of rodents to valproic acid (VPA) induces symptoms in offspring which resemble those observed in autistic patients and, as such, has been proposed as a preclinical model to study this disorder[1]. The endocannabinoid system modulates emotionality and social behaviours[2], however it is unknown if alterations in this system occur in autism. The current study aimed to evaluate if alterations in the endocannabinoid system accompany behavioural changes in the VPA rat model of autism. Pregnant female Sprague Dawley rats received VPA (600mg/kg s.c.) or saline vehicle on gestational day G12.5 and behavioural testing was carried out on the offspring during adolescence (PND33-40). Behaviours assessed included social investigatory behaviour (3-chamber sociability test), thermal nociception (hot plate test), anxiety-like behaviour (open field and elevated plus maze) and locomotor activity. Seventy-two hours following the final behavioural test, animals were sacrificed by decapitation, the brain removed and discrete brain regions including the frontal cortex, hippocampus and cerebellum dissected out, snap frozen and stored at -80^{o}C. The expression levels of the genes encoding enzymes and receptors of the endocannabinoid system were determined in each region using quantitative RT-PCR. Concentrations of the endocannabinoids, anandamide and 2-arachidonylglycerol (2-AG), and the N-acylethanolamines, N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA), were measured using LC-MS/MS. Data were analysed by unpaired t-test and $P<0.05$ was deemed significant. N = 8-12 per group.

Rats prenatally exposed to VPA exhibited reduced time spent in (134.7 ± 11.1s vs. 173.5 ± 16.7s, $P<0.05$) and frequency of (No of investigatory events 51.9 ± 3.3 vs. 65.7 ± 5.2, $P<0.05$) social investigatory behaviour and an increased latency to respond in the hot plate test (13.6 ± 1.3s vs. 8.9 ± 0.6s, $P<0.01$), when compared to saline-treated counterparts. Although locomotor activity in the sociability arena did not differ between the groups, VPA exposed animals exhibited reduced distance moved (2741 ± 362cm vs 3741 ± 285cm, $P<0.05$) and time in the inner zone (4.81 ± 2.91s vs. 12.26 ± 2.65s, $P<0.05$) on exposure to a novel aversive open field arena. Levels of the endocananbinoids, anandamide and 2-AG, or the N-acylethanolamines in the hippocampus, cortex or cerebellum did not differ between VPA and saline-treated animals. In comparison, the expression of diacylglycerol lipase-α (18% decrease, $P<0.01$) and monoacylglycerol lipase (MAGL) (20% decrease, $P<0.05$), the enzymes primarily responsible for the synthesis and metabolism of 2-AG, were reduced in the cerebellum and hippocampus of VPA treated rats respectively. MAGL activity was enhanced in the hippocampus of VPA- vs saline-treated rats (786 ± 64 vs. 588 ± 36nmol/min/g, $P<0.05$). Although CB_1 receptor mRNA expression was not altered, VPA exposed rats exhibited reduced PPARα (27% decrease, $P<0.05$) and GPR55 (50% decrease, $P<0.05$) expression in the cortex and PPARγ (40% decrease, $P<0.01$) and GPR55 (34% decrease, $P<0.05$) expression in the hippocampus, further receptor targets of the endocannabinoids. These data indicate that a rat model of autism 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.

Acknowledgements: Research was ethically approved by NUI Galway ACREC and preformed under licence from the Irish Department of Health and Children and in compliance with the European Communities Council directive 86/609. provided by the NUI Galway Millennium Fund and disciplines of Physiology and Pharmacology and Therapeutics, NUI Galway, Ireland

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