Neuroscience 204 (2012) 90–103

REVIEW

MATERNAL DEPRIVATION AND ADOLESCENT CANNABINOID EXPOSURE IMPACT HIPPOCAMPAL ASTROCYTES, CB1 RECEPTORS AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN A SEXUALLY DIMORPHIC FASHION

M. LÓPEZ-GALLARDO,[a,e] A. B. LÓPEZ-RODRÍGUEZ,[b,e] Á. LLORENTE-BERZAL,[b,e] D. ROTLLANT,[c] K. MACKIE,[d] A. ARMARIO,[c] R. NADAL[c] AND M.-P. VIVEROS[b,e]*

[a]*Departmento de Fisiología, Fac. Medicina, Universidad Complutense, Madrid, Spain*

[b]*Departmento de Fisiología (Fisiología Animal II) Fac Biología, Universidad Complutense, Madrid, Spain*

[c]*Unidad de Fisiología Animal, Unidad de Psicobiología, Instituto de Neurosciencias, Universidad Autónoma de Barcelona, Spain*

[d]*Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA*

[e]*Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain*

Abstract—We have recently reported that early maternal deprivation (MD) for 24 h [postnatal day (PND) 9–10] and/or an adolescent chronic treatment with the cannabinoid agonist CP-55,940 (CP) [0.4 mg/kg, PND 28–42] in Wistar rats induced, in adulthood, diverse sex-dependent long-term behavioral and physiological modifications. Here we show the results obtained from investigating the immunohistochemical analysis of CB1 cannabinoid receptors, glial fibrillary acidic protein (GFAP) positive (+) cells and brain-derived neurotrophic factor (BDNF) expression in the hippocampus of the same animals. MD induced, in males, a significant increase in the number of GFAP+ cells in CA1 and CA3 areas and in the polymorphic layer of the dentate gyrus (DG), an effect that was attenuated by CP in the two latter regions. Adolescent cannabinoid exposure induced, in control non-deprived males, a significant increase in the number of GFAP+ cells in the polymorphic layer of the DG. MD induced a decrease in CB1 expression in both sexes, and this effect was reversed in males by the cannabinoid treatment. In turn, the drug "per se" induced, in males, a general decrease in CB1 immunoreactivity, and the opposite effect was observed in females. Cannabinoid exposure tended to reduce BDNF expression in CA1 and CA3 of females, whereas MD counteracted this trend and induced an increase of BDNF in females. As a whole, the present results show sex-dependent long-term effects of both MD and juvenile cannabinoid exposure as well as functional interactions between the two treatments.

This article is part of a Special Issue entitled: Stress, Emotional Behavior and the Endocannabinoid System.

Key words: maternal deprivation, adolescent cannabinoid exposure, CB1 cannabinoid receptor, GFAP, BDNF, sex differences.

Contents

Traumatic experiences during early developmental periods might be associated with psychopathology (such as depression or schizophrenia) and altered neuroendocrine function later in life (Levine, 2005; Moffett et al., 2007; Tyrka et al., 2008). Several experimental models attempt to mimic diverse types of early-life stress. Notably, rats submitted to a single 24-h episode of maternal deprivation (MD) at postnatal day (PND) 9 exhibit, as adults, behavioral abnormalities resembling psychotic-like symptoms, including disturbances in pre-pulse inhibition, latent inhibition, auditory sensory gating and startle habituation. Possible underlying neurochemical correlates in adult MD animals include reduced hippocampal levels of neuropeptide Y (NPY), calcitonin-gene-related peptide, polysialylated neural cell adhesion molecule and brain-derived neurotrophic factor (BDNF) and a decrease in NMDA receptor

*Correspondence to: M.-P. Viveros, Departamento de Fisiología (Fisiología Animal II), Facultad de Biología, Universidad Complutense, Ciudad Universitaria, C/Jose Antonio, Novais n° 2, 28040 Madrid, Spain. Tel: +34-913944993; fax: +34-913944935.
E-mail address: pazviver@bio.ucm.es (M.-P. Viveros).

Abbreviations: ANOVA, analysis of variance; Co, control non deprived animals; CP, CP-55,940; BDNF, brain-derived neurotrophic factor; DG, dentate gyrus; ECS, endocannabinoid system; GFAP, glial fibrillary acidic protein; HPA, hypothalamic–pituitary–adrenal; IB, immunohistochemistry buffer; MD, maternal deprivation; ml–gcl, molecular layer together with the granular cell layer; PND, postnatal day; SL–M, stratum lacunosum together with moleculare; SO, stratum oriens; SR–SP, stratum pyramidale together with radiatum; Vh, vehicle; 2-AG, 2-arachidonylglycerol.

0306-4522/12 $36.00
doi:10.1016/j.neuroscience.2011.09.063

subunits NR-2A and NR-2B (see for review (Ellenbroek and Riva, 2003; Ellenbroek et al., 2004)). In addition, adolescent MD rats showed depressive-like behaviors and altered responses to a cannabinoid agonist (Llorente et al., 2007), as well as a trend towards increased impulsivity (Marco et al., 2007). We have also shown that MD induced neuronal degeneration and increased number of glial fibrillary acidic protein (GFAP)+ cells in the hippocampus and cerebellar cortex of neonatal rats. Interestingly, these effects were often more marked in males. MD also produced an enduring decrease in leptin and an increase in corticosterone for both sexes (López-Gallardo et al., 2008; Llorente et al., 2008, 2009; Viveros et al., 2009). These alterations support our hypothesis that the neonatal stress accompanying MD may be a useful model to examine behavioral symptoms with a neurodevelopmental etiology.

The CB1 cannabinoid receptor is a key component of the endocannabinoid system (ECS). The ECS consists of endogenous ligands called endocannabinoids, typically anandamide (AEA) and 2-arachidonylglycerol (2-AG), which activate cannabinoid receptors (primarily CB1 and CB2 receptors, respectively). The CB1 receptor is the predominant cannabinoid receptor within the central nervous system, and is highly expressed in brain regions involved in emotional processing, motivation, motor activation and cognitive function (Mackie, 2005). Among the multiple functions of the endocannabinoid system (Viveros et al., 2005, 2007; Wotjak, 2005; Cota, 2008; Moreira and Lutz, 2008; Bermudez-Silva et al., 2010) it also plays a role in neural development (Keimpema et al., 2011).

Previously we found that neonatal MD animals had increased levels of 2-AG and decreased CB1 immunoreactivity in the hippocampus, with these alterations being more marked in males (Llorente et al., 2008; Suárez et al., 2009). Concordant with increased 2-AG levels, we recently found that MD also significantly decreased hippocampal monoacylglycerol lipase, the major 2-AG degrading enzyme, as reflected by RT-PCR and immunohistochemistry. This decrease, again, was more marked in males than in females (Suárez et al., 2010). Moreover, two inhibitors of endocannabinoid inactivation modulated the above-indicated cellular effects induced by MD stress (Llorente et al., 2007). As a whole, these data support a clear association between neurodevelopmental MD stress and dysregulation of the ECS.

Adolescence represents a critical phase in development during which the nervous system shows a unique plasticity. During this period, maturation and rearrangement of major neurotransmitter pathways are still taking place (Spear, 2000; Romeo, 2003; Laviola and Marco, 2011) including the endocannabinoid system (Rodríguez de Fonseca et al., 1993; Viveros et al., 2011a, b). The ages associated with adolescence are commonly considered to be approximately between 12 and 20–25 years in humans and around PND 28–42 in rodents (Spear, 2000; Adriani and Laviola, 2004). The early adolescent period has been identified as a phase of development particularly vulnerable to some of the adverse effects of exposure to cannabinoid compounds. Notably, in predisposed people, early exposure to cannabis increases the risk of developing schizophrenia and may exacerbate symptoms in psychotic patients (Di Forti et al., 2007; Leweke and Koethe, 2008; Fernandez-Espejo et al., 2009; Sewell et al., 2009). In addition, both human and animal studies indicate that cannabis use during adolescence, perhaps in a sex-dependent fashion, may produce additional negative effects such as cognitive impairment, depressive symptoms and increased risk of further developing substance-abuse disorders (Sundram, 2006; Rubino et al., 2008; Schneider, 2008; Fernandez-Espejo et al., 2009; Wegener and Koch, 2009; Viveros et al., 2011a, b).

Despite neonatal MD and adolescent cannabinoid exposure having been demonstrated to induce important long-lasting behavioral and neuroendocrine effects, the combination of the two treatments has been scarcely investigated (Macrì and Laviola, 2004; Llorente-Berzal et al., 2011). In our recent study on this topic (Llorente-Berzal et al., 2011), the psychophysiological effects of MD and/or adolescent exposure to CP-55,940 (CP) were studied at adulthood. MD induced a compensatory increase in maternal behavior after reunion with the dam and, in the long-term, we observed, in males, an increase of open-arm exploration in an elevated plus-maze that could be related to risk-taking behavior. Adolescent exposure to cannabinoids exerted more pronounced long-lasting behavioural effects that were evident only in females. The main behavioral alterations consisted in an increased exploration in novel environments (holeboard), which suggests an increase in novelty-seeking and in a decreased prepulse inhibition of the acoustic startle reflex, suggestive of an impairment in sensoriomotor gating (attentional processes) that could increase vulnerability to psychiatric disorders such as drug addiction and schizophrenia. The reactivity of the hypothalamic–pituitary–adrenal (HPA) axis to an intermediate stressor was also studied and results indicated that in males the adolescent treatment with the cannabinoid increased HPA axis response. Thus, overall data supported the view that early MD and adolescent cannabinoid exposure exerted distinct sex-dependent long-term behavioural and physiological modifications that could predispose to the development of certain neurobehavioral disorders.

In the present study we have analyzed hippocampal CB1 cannabinoid receptors and GFAP positive (+) cells in the rats used in our recent paper (Llorente-Berzal et al., 2011). Our interest in these parameters and in this specific brain area was based on the following premises: the high density of hippocampal CB1 receptors (Herkenham et al., 1990), intrahippocampal cannabinoids impairs memory (Lichtman et al., 1995), and cannabinoids desynchronize hippocampal neuronal assemblies, possibly accounting for cannabinoid-induced memory impairment (Robbe et al., 2006). Moreover, morphological changes in the hippocampus have been observed following chronic administration of cannabinoids (Lawston et al., 2000; Tagliaferro et al., 2006). We had previously shown that MD induced rapid changes in the number of GFAP+ cells as well as in CB1 receptors in the hippocampus of neonatal rats (PND 13), and here we aimed to investigate whether these changes