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Research Report

Early maternal deprivation induces changes on the expression of 2-AG biosynthesis and degradation enzymes in neonatal rat hippocampus

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ABSTRACT

Early maternal deprivation (MD) in rats (24 h, PND 9–10) is a model for neurodevelopmental stress. Our previous data showed that MD altered the hippocampal levels of the endocannabinoid 2-AG and the expression of hippocampal cannabinoid receptors in 13-day-old rats, with males being more markedly affected. The aim of this study was to analyze the impact of MD on the enzymes involved in 2-AG biosynthesis (DAGLα and DAGLβ) and degradation (MAGL) in relevant areas (DG, CA1, CA3) of the hippocampus in 13-day-old neonatal rats. The expression of the enzymes was evaluated by quantitative RT–PCR, immunohistochemistry, and densitometry. MD induced a significant increase in DAGLα immunoreactivity in both males and females, which was mainly associated with fibers in the polymorphic cell layer of the dentate gyrus and in the stratum pyramidale of CA3. In contrast, the molecular layer of the dentate gyrus showed a significant decrease in DAGLα immunoreactivity in MD males and females. No changes were observed in DAGLβ immunoreactivity. MD induced a significant decrease in MAGL immunoreactivity in hippocampal CA3 and CA1 areas, more marked in males than in females, and that was mainly associated with fibers in all strata of CA3 and CA1. The results also showed a significant decrease of MAGL mRNA levels in MD males. These data support a clear association between neurodevelopmental stress and dysregulation of the endocannabinoid system. This association may be relevant for schizophrenia and other neurodevelopmental psychiatric disorders.

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1. Introduction

Exposing neonatal rats [postnatal day (PND) 9] to a single prolonged 24-h episode of maternal deprivation (MD) has been shown to induce long-term behavioral alterations that resemble psychotic-like symptoms. Thus, MD rats showed, in the adulthood, disturbances in prepulse inhibition, latent inhibition, and auditory sensory gating and startle habituation. Moreover, MD led to a significant reduction in hippocampal levels of polysialylated-neuronal cell adhesion molecule (PSA-NCAM) and brain-derived neurotrophic factor (BDNF) as well as a significant decrease in the mRNA levels of glutamate *N*-methyl-D-aspartate (NMDA) receptor subunits, NR- 2A and NR-2B. These changes are suggestive of a loss of synaptic plasticity and hypofunctionality of the glutamatergic system, as recently postulated for schizophrenia (see for review, Ellenbroek and Riva, 2003; Ellenbroek et al., 2004).

Several lines of evidence support an association between an altered endocannabinoid system (ECS) and the pathogenesis of schizophrenia. For example, increases in CB1 cannabinoid receptor expression have been found in the prefrontal cortex (Dean et al., 2001) and cingulate cortex (Zavitsanou et al., 2004) of schizophrenic patients. Also, elevated levels of the endocannabinoid anandamide have been detected in the cerebrospinal fluid of schizophrenics (Leweke et al., 1999, 2007; Giuffrida et al., 2004). Furthermore, frequent cannabis use significantly increases the risk for psychotic symptoms and schizophrenia (see for review, Di Forti et al., 2007; Leweke and Koethe, 2008). In addition, glutamatergic transmission is regulated by retrograde endocannabinoid signaling, and both the endocannabinoid and glutamatergic systems are involved in the pathophysiology of major symptoms of schizophrenia (Leweke and Koethe, 2008). Previous data from our group indicated that MD resulted in alterations of the hippocampal endogenous cannabinoid system (Llorente et al., 2008; Suárez et al., 2009), as it has been described for the glutamatergic transmission (Roceri et al., 2002; Pickering et al., 2006), further supporting the view that MD modifies neurochemical systems that are also altered in schizophrenia. We first reported an increase of the endocannabinoid 2-arachidonylglycerol (2-AG) in the hippocampus of MD male but not in MD female rats at PND 13 (Llorente et al., 2008). More recently, we demonstrated that, at this same age, MD male rats showed a significantly decreased expression of hippocampal CB_1 receptor, whereas hippocampal CB_2 receptor expression was significantly increased in MD animals of both genders (Suárez et al., 2009). These findings provided the first direct evidence for a link between MD stress and the ECS that appeared to be expressed in a gender-dependent manner.

One of the most prevalent hypotheses for the pathogenesis of schizophrenia states that the disease is a neurodevelopmental disorder associated with early brain developmental abnormalities (Weinberger, 1987; Lewis and Levitt, 2002; Marek and Merchant, 2005). From this perspective, behavioral deficit observed in mature MD animals might be related to altered neurodevelopmental processes triggered by stress-induced increases in glucocorticoid levels. In fact, MD, acutely, leads to high levels of corticosterone that persist elevated at PND 13 (Llorente et al., 2008; Viveros et al., 2009) and the developmental hippocampus that shows a high density of glucocorticoid receptors and appears to be particularly sensitive to the detrimental effects of glucocorticoids (Sapolsky et al., 1988; Gould, 1994; Sapolsky, 2000) is clearly affected by MD (both neurons and glia) (Llorente et al., 2009), supporting the hypothesis that MD might be a model of neuropsychiatric symptoms with a neurodevelopmental basis. There is evidence for a crucial implication of the ECS in brain developmental processes such as neural progenitor proliferation, lineage segregation, migration and phenotypic specification of immature neurons, axonal elongation, and synaptogenesis (see for reviews, Fernández-Ruiz et al., 2000; Berghuis et al., 2007; Harkany et al., 2008). Retrograde signaling involving endocannabinoids appears to be responsible for the homeostatic control of synaptic transmission and the resulting network patterns in the immature hippocampus (Bernard et al., 2005). Thus, it is likely that there is a functional link between the hippocampal neuronal and glial developmental alterations observed in the MD neonatal rats and their altered ECS.

Studies on the role of the ECS in the control of neurodevelopmental processes have mainly focused in CB_1 receptors. However, there is scarce information on the presence and functions of other components of the ECS, such as enzymes involved in 2-AG biosynthesis, diacylglycerol lipase α and β (DAGLα and DAGLβ), and degradation, monoacylglyceride lipase (MAGL), whose functions point to a spatial and temporal regulation of the 2-AG level in the brain (Dihn et al., 2002; Bisogno et al., 2003; Suárez et al., 2008). In addition, DAGL activity is related to axonal growth and guidance during development (Brittis et al., 1996; Williams et al., 2003). Supporting this hypothesis, the expression of DAGL isozymes (α and β) changes during development of the mouse brain, from axonal tracts of the embryo to dendritic fields of the adult (Bisogno et al., 2003). In turn, this correlates with the developmental change in the requirement for 2-AG synthesis from the pre- to the postsynaptic compartment, for instance, in the hippocampal pyramidal dendritic field (Yoshida et al., 2006). Moreover, the substantial down-regulation of DAGLβ contrasts with the high DAGLα expression in the adult mouse cerebellum (Bisogno et al., 2003). Finally, most brain 2-AG hydrolase activity can be ascribed to MAGL (Blackman et al., 2007), where it localizes to presynaptic terminals (Gulyas et al., 2004) and regulates the inhibitory postsynaptic currents (IPSCs) by the modulation of the basal 2-AG tone (Hashimotodani et al., 2007). Its role in development is linked to its focused distribution on the presynaptic compartment where it helps to establish effective retrograde signaling. These findings provide additional evidence supporting relevance for the regulation of 2-AG/cannabinoid receptor signaling in neurodevelopmental alterations. However, the effect of dysregulated MAGL activity on development and adult phenotype remains to be determined conclusively. As above mentioned, we have shown that precisely this endocannabinoid is significantly increased in the developmental hippocampus of MD rats. Thus, in the present study, we have focused on the impact of MD on the enzymes involved in 2-AG biosynthesis (DAGLα and DAGLβ) and degradation (MAGL), in the hippocampus of 13-day-old rats, the same age at which we have previously reported the hippocampal cellular, biochemical (endocannabinoid levels), and immunohistochemical (cannabinoid CB_1