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Enriched environment decreases microglia and brain macrophages inflammatory phenotypes through adiponectin-dependent mechanisms: Relevance to depressive-like behavior

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ABSTRACT

Regulation of neuroinflammation by glial cells plays a major role in the pathophysiology of major depression. While astrocyte involvement has been well described, the role of microglia is still elusive. Recently, we have shown that Adiponectin (ApN) plays a crucial role in the anxiolytic/antidepressant neurogenesis-independent effects of enriched environment (EE) in mice; however its mechanisms of action within the brain remain unknown. Here, we show that in a murine model of depression induced by chronic corticosterone administration, the hippocampus and the hypothalamus display increased levels of inflammatory cytokines mRNA, which is reversed by EE housing. By combining flow cytometry, cell sorting and q-PCR, we show that microglia from depressive-like mice adopt a pro-inflammatory phenotype characterized by higher expression levels of IL-1β, IL-6, TNF-α and IκB-α mRNAs. EE housing blocks pro-inflammatory cytokine gene induction and promotes arginase 1 mRNA expression in brain-sorted microglia, indicating that EE favors an anti-inflammatory activation state. We show that microglia and brain-macrophages from corticosterone-treated mice adopt differential expression profiles for CCR2, MHC class II and IL-4recα surface markers depending on whether the mice are kept in standard environment or EE. Interestingly, the effects of EE were abolished when cells are isolated from ApN knock-out mouse brains. When injected intra-cerebroventricularly, ApN, whose level is specifically increased in cerebrospinal fluid of depressive mice raised in EE, rescues microglia phenotype, reduces pro-inflammatory cytokine production by microglia and blocks depressive-like behavior in corticosterone-treated mice. Our data suggest that EE-induced ApN increase within the brain regulates microglia and brain macrophages phenotype and activation state, thus reducing neuroinflammation and depressive-like behaviors in mice.

1. Introduction

Inflammation is an important factor in the initiation and development of depression (Maes, 1999; Dickens et al., 2003; Siegert and Abernethy, 2005; Eisenberger et al., 2010; Pasco et al., 2010; Capuron and Miller, 2011). Patients with major depression often exhibit increased markers of innate immune system activation and inflammation associated with elevated levels of pro-inflammatory cytokines in the plasma and cerebrospinal fluid, mainly IL-1β, IL-6 and TNFα (Maes et al., 1997; Schiepers et al., 2005; Howren et al., 2009; Dowlati et al., 2010; Haapakoski et al., 2015). Inversely, both cytokines and lipopolysaccharide (LPS) can induce depressive-like behaviors in rodents after peripheral (Yirmiya, 1996; Swiergiel and Dunn, 2007; Goshen et al., 2008) and central administration (O'Connor et al., 2009; Kaster et al., 2012; Fu et al., 2010). In addition, many clinical investigations showed that antidepressant treatments have anti-inflammatory effects. Indeed, selective serotonin reuptake inhibitors (SSRIs) such

Abbreviations: ApN, adiponectin; EE, enriched environment; SE, standard environment; LPS, lipopolysaccharide; CNS, central nervous system; CBA, cytometric bead array™; IL, interleukin; TLR4, toll like receptor-4; CCR2, C-C chemokine receptor type 2; CCL2, chemokine ligand 2; MHC class II, major histocompatibility complex class II; TNF, tumor necrosis factor; CSF, cerebrospinal fluid; HPA, hypothalamic–pituitary–adrenal; i.c.v., intracerebroventricular; FSC, forward scatter; SSC, side scatter; GCs, glucocorticoids; NF-κB, nuclear factor-kappa B; FST, forced swim test; TST, tail suspension test.

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as fluoxetine, tricyclic anti-depressants such as imipramine or inhibitors of mono-amino-oxidase such as moclobemide all have anti-inflammatory properties by decreasing pro-inflammatory cytokine production and/or by increasing the anti-inflammatory IL-10 production (Swiergiel and Dunn, 2007; Goshen et al., 2008; O'Connor et al., 2009; Kaster et al., 2012). Further evidence of the close link between inflammation and depression comes from clinical observations showing that immunotherapy with the pro-inflammatory cytokines IL-2 and interferon-α increases the risk to develop major depression symptoms (Beishuizen and Thijs, 2003; Dantzer, 2004). Together, these observations provide evidence that prevention of chronic neuroinflammation could be a therapeutic target for depression.

Various studies have shown that enriched environment (EE) has beneficial effects in rodent models of neurological and psychiatric disorders including schizophrenia, depression and post-traumatic stress disorders (Takuma et al., 2011). For laboratory animal models, an enriched environment is "enriched" in relation to standard laboratory housing. "Enriched" animals are housed in larger cages and in larger groups with a variety of objects frequently changed (houses, toys, tunnels, hammocks, nesting materials…). In addition, animals are given the opportunity for voluntary physical activity on running wheels (van Praag et al., 2000; Nithianantharajah and Hannan, 2006). Very recently, we have shown that EE efficiently reverses anxiety/depressive-like state induced by long-term treatment of corticosterone. Moreover, we demonstrated that the adipokine Adiponectin (ApN) plays a crucial role in the neurogenesis-independent antidepressant effects of EE (Nicolas et al., 2015).

ApN is a circulating hormone released by adipocytes that circulates at high concentrations in the bloodstream as either full-length or a globular form that is generated by proteolytic cleavage of full-length ApN. ApN acts by binding to its receptors, AdipoR1 and AdipoR2, which are expressed in skeletal muscle, liver and in different areas of the central nervous system (CNS) such as cortex, the hippocampus, the hypothalamus, amygdala and vascular endothelial cells of the brain (Liu et al., 2012; Fry et al., 2006; Kubota et al., 2007; Psilopanagioti et al., 2009; Qi et al., 2004; Spranger et al., 2006; Zhang et al., 2011). Although 1000-fold less concentrated than in plasma, ApN has been shown to be present in the cerebrospinal fluid (CSF) of rodents (Nicolas et al., 2015; Liu et al., 2012; Qi et al., 2004; Yau et al., 2014) and humans (Neumeier et al., 2007; Ebinuma et al., 2007). In humans, the correlation between plasma levels of ApN and depression has not been formally demonstrated. Indeed, some reports indicate that ApN levels is higher in serum from subjects with major depression compared with healthy controls (Jow et al., 2006), while other researchers found lower (Cizza et al., 2010; Zeman et al., 2009) or unchanged levels (Kotani et al., 2012; Carvalho et al., 2014). In rodents, the results are more homogeneous. Indeed, Liu et al. have shown that levels of ApN in plasma are reduced in a mouse model of depression induced by chronic stress (Liu et al., 2012). In heterozygous $ApN^{+/-}$ mice, reduced rate of ApN exacerbates depressive-like behaviors. Moreover, the neutralization of central effects of ApN by i.c.v. injection of antibodies causes depressive-like behaviors in rodents. Conversely, exogenous ApN i.c.v. injection produces antidepressant effects in normal or diabetic and obese mice (Liu et al., 2012). Interestingly, while plasma concentrations of ApN remained unchanged, CSF ApN levels are increased by physical activity and EE housing, leading to anxiolytic and antidepressant properties (Nicolas et al., 2015; Yau et al., 2014). Indeed, Yau et al. recently showed that the antidepressant effects of exercise would be dependent on ApN (Yau et al., 2014). In 2015, we conducted a study showing that some of the EE antidepressant effects were dependent on ApN (Nicolas et al., 2015). These results therefore identify ApN as an important player in the regulation of depressive-like behaviors in mice. However, little is known about the ApN-depending mechanisms implicated in mood control.

Several reports demonstrated anti-inflammatory properties of ApN in periphery (Lara-Castro et al., 2007; Ouchi and Walsh, 2007; Otero et al., 2006; Senolt et al., 2006). Circulating ApN levels were inversely correlated with systemic inflammatory markers, C-reactive protein and IL-6, as well as obesity, type 2 diabetes, and cardiovascular disease (Hotta et al., 2000; Otake et al., 2008). At least some anti-inflammatory properties of ApN could occur through its direct action on monocytes/macrophages. AdipoR1 and AdipoR2 are expressed in mouse and human peripheral monocytes, and in monocyte-derived macrophages (Yamauchi et al., 2003; Chinetti et al., 2004; Pang and Narendran, 2008; Weigert et al., 2008). In these cells, ApN has been shown to modulate the inflammatory activity and to inhibit their transformation into foam cells, a hallmark of atherosclerosis (Kotani et al., 2012; Carvalho et al., 2014; Lara-Castro et al., 2007; Ouchi and Walsh, 2007; Otero et al., 2006). Moreover, ApN induced the expression of the anti-inflammatory cytokine IL-10 in human monocytes and macrophages (Weigert et al., 2008; Wolf et al., 2004; Kumada et al., 2004), while it suppressed the LPS-stimulated release of IL-6 in porcine macrophages (Wulster-Radcliffe et al., 2004). Furthermore, monocytes from patients with type 1 or type 2 diabetes produced less IL-6, IL-8 and CCL2 in response to ApN treatment than those from healthy patients (Weigert et al., 2008; Abke et al., 2006).

Although it was considered homogeneous for many years, it is not known that the "macrophage" population of the CNS includes microglia and CNS-associated mononuclear phagocytes, among which neutrophils, CNS-resident perivascular and meningeal macrophages, choroid plexus macrophages and CNS-infiltrating monocyte-derived macrophages should be distinguished (Campanella et al., 2002; Zhou et al., 2009; Marques et al., 2008; Cazareth et al., 2014). In response to modifications in their environment, microglia and macrophages acquire diverse phenotypes (Franco and Fernandez-Suarez, 2015). "Primed" or "reactive" microglia exhibit increased levels of pro-inflammatory markers including CD80, CD86 (Downer et al., 2010), MHC class II (Frank et al., 2006; Godbout et al., 2005; Henry et al., 2009), and Toll-like receptors (TLRs) Hoogland et al., 2015, especially TLR4, which forms a functional complex with the CD14 surface protein (da Silva Correia et al., 2001). By contrast, IL-10 and IL-4 are two key cytokines promoting anti-inflammatory properties (Mantovani et al., 2004; Strle et al., 2001). Although the anti-inflammatory regulation of peripheral monocytes/macrophages phenotype by ApN is well known, its effects on microglia and CNS-macrophages have not been examined yet.

The aim of this study is to examine how EE modulates the inflammatory status of brains from mice rendered depressive by a chronic corticosterone treatment, to analyze the activation state of microglia and CNS-associated phagocytes according to the depressive state and housing conditions and to explore the contribution of ApN in the regulation of neuroinflammation mediated by EE.

2. Materials and methods

2.1. Animals

Male wt or adiponectin knockout ($ApN^{-/-}$) mice with the same C57BL/6J genetic background were randomly assigned into different treatment groups and housed at 22 °C with a 12-h light–dark cycle (lights on at 07:00) with free access to drink and chow (A04, SAFE). All behavioral testing occurred during the light phase

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