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Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: Focus on brain indoleamine 2,3-dioxygenase activation

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ABSTRACT

Obesity is associated with a high prevalence of mood symptoms and cognitive dysfunctions that emerges as significant risk factors for important health complications such as cardiovascular diseases and type 2 diabetes. It is therefore important to identify the dynamic of development and the pathophysiological mechanisms underlying these neuropsychiatric symptoms. Obesity is also associated with peripheral low-grade inflammation and increased susceptibility to immune-mediated diseases. Excessive production of proinflammatory cytokines and the resulting activation of the brain tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) have been shown to promote neurobehavioral complications, particularly depression. In that context, questions arise about the impact of diet-induced obesity on the onset of neuropsychiatric alterations and the increased susceptibility to immune-mediated diseases displayed by obese patients, particularly through brain IDO activation.

To answer these questions, we used C57Bl/6 mice exposed to standard diet or western diet (WD; consisting of palatable energy-dense food) since weaning and for 20 weeks. We then measured inflammatory and behavioral responses to a systemic immune challenge with lipopolysaccharide (LPS) in experimental conditions known to alter cognitive and emotional behaviors independently of any motor impairment. We first showed that in absence of LPS, 9 weeks of WD is sufficient to impair spatial recognition memory (in the Y-maze). On the other hand, 18 weeks of WD increased anxiety-like behavior (in the elevated plus-maze), but did not affect depressive-like behavior (in the tail-suspension and forced-swim tests). However, 20 weeks of WD altered LPS-induced depressive-like behavior compared to LPS-treated lean mice and exacerbated hippocampal and hypothalamic proinflammatory cytokine expression and brain IDO activation. Taken together, these results show that WD exposure alters cognition and anxiety in unstimulated conditions and enhances activation of neurobiological mechanisms underlying depression after immune stimulation. They suggest therefore that obesity, and possibly obesity-associated inflammatory priming, may represent a vulnerability state to immune-mediated depressive symptoms.

1. Introduction

Over the past decades, obesity has continuously increased at alarming rates throughout the world fostering the rise in serious obesity-related outcomes, particularly cardiovascular diseases and metabolic alterations such as type 2 diabetes (Malnick and Knobler, 2006). In addition, obesity is often associated with a high prevalence of altered emotional reactivity and cognitive dysfunctions that frequently evolves in neuropsychiatric disorders (Francis and Stevenson, 2013; Luppino et al., 2012; Sellbom and Gunstad, 2012). Moreover, neuropsychiatric symptoms emerge as additional risk factors for obesity-related systemic pathological complications (Fiedorowicz et al., 2008; Scott et al., 2008). It is therefore important to identify the dynamic of development of such emotional and cognitive alterations and the underlying pathophysiological mechanisms in the context of obesity.

Obesity is presently viewed not only as a metabolic disorder but also as an inflammatory disease affecting both innate and acquired immune system (Gregor and Hotamisligil, 2011). Indeed, obese patients often display basal low-grade systemic inflammation elicited

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by both adipose tissue (Cancello and Clement, 2006) and gut microbiota (Cani et al., 2008), and increased susceptibility to immune-mediated diseases (Kanneganti and Dixit, 2012) and to infections (Falagas and Kompoti, 2006; Huttunen and Syrjanen, 2013). Interestingly, clinical studies report positive associations in obese subjects between peripheral inflammatory status and cognitive decline (Sellbom and Gunstad, 2012; Sweat et al., 2008) or mood symptoms (Capuron et al., 2008). Conversely, surgery-induced weight loss is associated with reduced peripheral inflammation (Cancello and Clement, 2006) and significant improvement in emotional status (Capuron et al., 2010; Emery et al., 2007). In addition, studies performed in rodent models of obesity show that inflammation also exist within the brain, particularly in areas involved in mood regulation and memory formation such as the hippocampus (Dinel et al., 2011, 2014) or the cortex (Pistell et al., 2010). Of note, increased cytokine expression in these structures is associated with increased emotional behavior and cognitive impairments.

Both clinical (Capuron and Miller, 2011; Raison et al., 2010) and experimental (Chess et al., 2009; Frenois et al., 2007; Gold et al., 2011; Henry et al., 2009; Moreau et al., 2008; O'Connor et al., 2009a,b,c; Salazar et al., 2012; Walker et al., 2013) studies have shown that dysregulated production and/or brain action of cytokines promote emotional and cognitive complications through selective activation of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), its metabolite kynurenine or the neuroactive derivatives of kynurenine. Moreover, similar association between brain IDO activation and emotional or cognitive impairments also exists in chronic inflammatory conditions such as aging (Capuron and Miller, 2011; Corona et al., 2012; Godbout et al., 2008; Kelley et al., 2013). As severely obese individuals also display increased peripheral IDO activity (Brandacher et al., 2007; Oxenkrug, 2010), peripheral cytokine production and IDO activation may be relevant to the onset of emotional and cognitive alterations in obesity. However, the impact of diet-induced obesity on brain IDO activation and its role in the onset of emotional and cognitive alterations in that context is still largely unknown.

In the present study, we sought to approach this question by studying in mice the emotional, cognitive and inflammatory impact of chronic consumption of western diet (WD; consisting of palatable energy-dense food). In basal conditions, we first followed the development of obesity-associated behavioral alterations by assessing spatial working memory, anxiety-like and depressive like behaviors after 9 and 18 weeks of WD exposure. As WD exposure alone did not affect either inflammatory status or depressive-like behavior, we then used the cytokine inducer lipopolysaccharide (LPS) to evaluate the effect of WD on LPS-induced IDO activation and depressive-like behavior, independently of sickness behavior. Indeed, whereas peripheral inflammatory response to immune challenges has been already studied in obese mice (Amar et al., 2007; Lawrence et al., 2012; Naguib et al., 2004; Pini et al., 2013; Pohl et al., 2013), there is still scarce information regarding LPS-induced depressive-like behavior (Aguilar-Valles et al., 2013; Dinel et al., 2014) and hippocampal inflammatory activation (Dinel et al., 2014) in that context. We show here that in unstimulated conditions chronic WD exposure first impaired spatial memory, then increased anxiety-like behavior. Moreover, it altered LPS-induced depressive-like behavior and exacerbated brain inflammation, including IDO activation.

2. Materials and methods

2.1. Animals and diets

All animal experiments were conducted according to the relevant French (Directive 87/148, Ministère de l'Agriculture et de la Pêche) and international (Directive 2010/63, European Community) legislation. They adhered to protocols approved by the Animal Care and Use Committee from Bordeaux University (approval ID: 5012047-A). Every effort was made to minimize suffering and the number of animal used. Three-week old male C57BL/6J mice were obtained from Charles River (France). On arrival, they were randomly allocated to control group (Standard diet: SD; $n = 24$) or western diet group (WD; $n = 24$). No body weight differences existed between both groups at the beginning of the experiment. Control group was fed with standard chow (A04, Safe, France) that provides 2.9 kcal/g of diet (of which 9.3% from fat). Western diet (WD) group was fed with an in-house prepared palatable energy-dense chow providing 4.0 kcal/g of diet (of which 49% from fat) as previously published (Berraondo et al., 2000). Mice were housed 4 per cage in a controlled environment (normal 12 h light/dark cycle; 22 ± 1 °C), with food and water available *ad libitum*. Body weight was measured once a week over a 20-week period. All mice were individually handled for a few minutes once daily for 1 week before behavioral tests were initiated to minimize stress reactions to manipulation. Mice were 21–23 weeks-old by the time of behavioral assessments.

2.2. Experimental procedures

The present study first aimed at assessing the impact of chronic WD exposure on emotional behaviors (anxiety-like and depressive-like behaviors), as well as spatial working memory performances in unstimulated conditions. Mice were therefore tested in the elevated plus-maze (anxiety-like behavior), the tail suspension test (depressive-like behavior) or the Y-maze (hippocampal-dependent memory test) after 9 and 18 weeks of exposure to either regular chow or WD. Each mouse was randomly exposed to one behavioral test only at each time-point and never tested in the same test twice in order to avoid potential interferences.

One week after completion of these behavioral tests, inflammatory and behavioral responses to a systemic immune challenge with LPS were then measured in order to assess the potential consequences of chronic WD exposure on brain cytokine and IDO activation and related behavioral alterations. Peripheral LPS administration enhances peripheral and brain production of inflammatory cytokines, which are responsible of physiological and behavioral symptoms of sickness (Dantzer et al., 2008). These symptoms progressively wane whereas the expression of depressive-like behaviors remains up to 24 h after treatment (Frenois et al., 2007; Godbout et al., 2008; O'Connor et al., 2009c). Consequently, it is possible to experimentally dissociate emotional behaviors from sickness behavior (particularly motor impairment) by choosing relevant post-treatment time-points. Here, mice fed with SD or WD for 20 weeks were intraperitoneally injected with sterile endotoxin-free saline or freshly prepared LPS (*Escherichia coli*, serotype 0127:B8, Sigma). The dose used (830 µg/kg) was selected on the basis of its ability to induce the full spectrum of sickness response and a reliable increase of brain IDO activity (Frenois et al., 2007; Lestage et al., 2002; O'Connor et al., 2009c). Mice were then divided in two subgroups sacrificed either 2 h (group 1) or 25 h after treatment (group 2). Mice from this last group were respectively tested 23 h and 24 h after treatment for their locomotor activity, in order to verify that they had fully recovered from LPS-induced locomotor impairment, and for their depressive-like behavior. LPS-induced body weight loss was also assessed just before and 3, 6 and 25 h after treatment.

By the time of sacrifice, mice were euthanized by CO_2 inhalation within a few seconds after being picked up from their home cage. Blood samples were immediately collected via cardiac puncture into EDTA (10%)-coated chilled tubes. After centrifugation (10 min, 3000*g*, 4 °C), aliquots of plasma were stored at −80 °C.

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