Brain, Behavior, and Immunity 64 (2017) 33–49

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Named Series: Brain, Behavior, Immunity and the Microbiome

Impact of prebiotics on metabolic and behavioral alterations in a mouse model of metabolic syndrome

Lourdes Fernández de Cossío [a,b], Célia Fourrier [a,b], Julie Sauvant [a,b], Amandine Everard [c], Lucile Capuron [a,b], Patrice D. Cani [c], Sophie Layé [a,b], Nathalie Castanon [a,b,*]

[a] INRA, Nutrition and Integrative Neurobiology, UMR 1286, 33076 Bordeaux, France
[b] Université de Bordeaux, Nutrition and Integrative Neurobiology, UMR 1286, 33076 Bordeaux, France
[c] Université catholique de Louvain, Louvain Drug Research Institute, WELBIO (Walloon Excellence in Life Sciences and Biotechnology), Metabolism and Nutrition Research Group, 1200 Brussels, Belgium

ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form 9 December 2016
Accepted 22 December 2016
Available online 24 December 2016

Keywords:
Metabolic syndrome
Gut microbiota
Prebiotics
db/*db* mice
Hippocampus
Hypothalamus
Inflammation
Cytokines
Anxiety
Spatial memory

ABSTRACT

Mounting evidence shows that the gut microbiota, an important player within the gut-brain communication axis, can affect metabolism, inflammation, brain function and behavior. Interestingly, gut microbiota composition is known to be altered in patients with metabolic syndrome (MetS), who also often display neuropsychiatric symptoms. The use of prebiotics, which beneficially alters the microbiota, may therefore be a promising way to potentially improve physical and mental health in MetS patients.

This hypothesis was tested in a mouse model of MetS, namely the obese and type-2 diabetic *db*/*db* mice, which display emotional and cognitive alterations associated with changes in gut microbiota composition and hippocampal inflammation compared to their lean *db*/+ littermates. We assessed the impact of chronic administration (8 weeks) of prebiotics (oligofructose) on both metabolic (body weight, food intake, glucose homeostasis) and behavioral (increased anxiety-like behavior and impaired spatial memory) alterations characterizing *db*/*db* mice, as well as related neurobiological correlates, with particular attention to neuroinflammatory processes.

Prebiotic administration improved excessive food intake and glycemic dysregulations (glucose tolerance and insulin resistance) in *db*/*db* mice. This was accompanied by an increase of plasma anti-inflammatory cytokine IL-10 levels and hypothalamic mRNA expression of the anorexigenic cytokine IL-1β, whereas unbalanced mRNA expression of hypothalamic orexigenic (NPY) and anorexigenic (CART, POMC) peptides was unchanged. We also detected signs of improved blood-brain-barrier integrity in the hypothalamus of oligofructose-treated *db*/*db* mice (normalized expression of tight junction proteins ZO-1 and occludin). On the contrary, prebiotic administration did not improve behavioral alterations and associated reduction of hippocampal neurogenesis displayed by *db*/*db* mice, despite normalization of increased hippocampal IL-6 mRNA expression. Of note, we found a relationship between the effect of treatment on dentate gyrus neurons and spatial memory. These findings may prove valuable for introducing novel approaches to treat some of the comorbidities associated with MetS.

1. Introduction

The Metabolic Syndrome (MetS) is a widely accepted concept that clusters together several risk factors for Type 2 Diabetes (T2D) and cardiovascular diseases including: overweight/obesity, hypertension, hyperglycemia, and disturbances of lipid/carbohydrate metabolism. Interestingly, low-grade inflammation originating from the adipose tissue and/or gut microbiota environment increasingly appears as another key component of MetS (Cancello and Clément, 2006; Cani et al., 2009; Gregor and Hotamisligil, 2011). In addition, clinical data of patients with MetS and experiments conducted in animal models of obesity/MetS also document an augmented risk for neuropsychiatric disorders (Castanon et al., 2015; Farooqui et al., 2012). Due to the breadth of complications resulting from the MetS, both the quality of life of affected patients is impaired and the costs to health care systems worldwide have increased. This leads therefore to heightened interest in better understanding the physiopathological mechanisms underlying

* Corresponding author at: Nutrition et Neurobiologie Intégrée, INRA, UMR 1286, Bâtiment UFR Pharmacie, 2° tranche, 2° étage, Case courrier 34, Université Victor Ségalen, 146 rue Léo Saignat, 33076 Bordeaux, France.
E-mail address: nathalie.castanon@inra.fr (N. Castanon).

http://dx.doi.org/10.1016/j.bbi.2016.12.022
0889-1591/

MetS-associated complications, with the final goal of improving treatment options.

There is now compelling evidence for the participation of the gut microbiota in the systemic low-grade inflammation associated with obesity and related metabolic disorders (for review see Cani et al., 2012; Nakamura and Omaye, 2012). The gut microbiota is involved in high-fat diet-induced endotoxemia leading to the chronic low-grade inflammation observed in obesity, through impaired gut barrier permeability (Cani et al., 2016). Gut microbial population is also altered in genetically obese and diabetic mice (Geurts et al., 2011; Ley et al., 2006), and transferring the microbial flora from genetically obese mice to germ-free lean animals induces the obese phenotype in the latter (Turnbaugh et al., 2006). More recent evidence further suggests that modulating gut microbiota composition through nutritional interventions may have therapeutic potential in the management of MetS and related co-morbidities (Erejuwa et al., 2014; Petra et al., 2015). Interestingly, these interventions may impact both systemic and brain functions, although the nature of the biological pathways targeted within the so called "gut-brain axis" is still elusive (Cani and Knauf, 2016; Cryan and Dinan, 2012; Moloney et al., 2013). Improvement of metabolic alterations has been shown with the use of probiotics, which are live microorganisms able to confer health benefits to the host when administered in adequate amount (Delzenne et al., 2011). Similarly, prebiotics such as the nondigestible carbohydrate oligofructose, which selectively stimulate the growth of some healthy commensal bacteria, have been shown to improve alterations of glucose homeostasis, plasma lipid profile (Mallappa et al., 2012), and importantly peripheral inflammation associated with MetS and/or obesity (Cani et al., 2007, 2016). Indeed, converging studies strongly suggest that oligofructose's beneficial effects are mediated by the ability of several commensal bacteria to reduce endotoxemia-induced systemic inflammation (Cani et al., 2009; Kootte et al., 2012). Much less is known however about the potential impact on brain inflammation, which is also classically reported in rodent models of MetS/obesity (Dinel et al., 2011, 2014; Guillemot-Legris et al., 2016; Pistell et al., 2010), as well as in other medical conditions sharing systemic inflammation and neuropsychiatric symptoms as common features (Capuron et al., 2016; Capuron and Castanon, 2016).

Accumulating evidence indicates that the gut microbiota can impact the brain (Moloney et al., 2013; Neufeld et al., 2011), with which it communicates through neuronal, hormonal and/or immune pathways (D'Mello et al., 2015; Ochoa-Repáraz and Kasper, 2016). In addition, studies dealing with the pathophysiological consequences of impaired gut-brain communication increasingly report mood and cognitive alterations (Collins et al., 2012; Cryan and Dinan, 2012; Savignac et al., 2016). This new research area has recently drawn much attention, but most of the present knowledge on how microbiota can influence brain function and behavior comes from studies primarily using germ-free mice, which display neuroendocrine, neurobiochemical, and behavioral alterations in basal and/or stressful conditions that can be rescued upon gut colonization with normal microbiota (Bercik et al., 2011; Crumeyrolle-Arias et al., 2014; Neufeld et al., 2011). Similarly, chronic stress exposure has also been reported to induce alterations of both microbiota composition and emotional behavior that can be prevented by prebiotic and/or probiotic treatment (Ait-Belgnaoui et al., 2012; Desbonnet et al., 2010; Gareau, 2014; Tarr et al., 2015). Other compelling data come from studies showing that antimicrobial treatments, which change the microbiota profile in mice, induce neurobiochemical changes, as well as emotional and cognitive alterations (Bercik et al., 2011; Desbonnet et al., 2015; Pyndt Jorgensen et al., 2015), although some contradictory data have also been reported (O'Mahony et al., 2014).

While these studies clearly contribute to better understand the role of the gut-brain axis in those particularly drastic conditions, much less is known regarding MetS-related emotional and cognitive alterations, although recent evidence points to a relationship between high-fat diet-related changes in gut microbiota and behavior (Bruce-Keller et al., 2015; Kang et al., 2014; Magnusson et al., 2015). This issue is however particularly relevant since the MetS emerges as an important risk factor for neuropsychiatric and neurological disorders including depression, anxiety, cognitive impairments, stroke, and Alzheimer's disease (Farooqui et al., 2012). Conversely, these disorders have been shown to significantly aggravate the MetS and related health outcomes, including cardiovascular disease or type 2 diabetes (Capuron et al., 2016; Roberts et al., 2010; Wulsin and Singal, 2003). Understanding the etiology of neuropsychiatric disorders in MetS patients appears therefore as a major public health challenge. Given the recent knowledge on the impact of the gut microbiota on brain and behavior, questions arise as to whether microbiota may play a role in the cognitive and emotional alterations associated with the MetS and whether prebiotics may have potential beneficial effects on such behavioral alterations.

In the present study, we addressed these issues by measuring the consequences of chronic administration of the prebiotic oligofructose on the behavioral alterations displayed by a genetic model of MetS, the *db*/*db* mouse. In addition, we investigated the potential underlying mechanisms by measuring the consequences on metabolism and systemic inflammation, but also on brain systems known to be involved in the control of food intake and behavior. The *db*/*db* model, which displays T2D, obesity, hyperglycemia and insulin-resistance as a consequence of an inactivating mutation in the leptin receptor, is notably suitable in that context since the metabolic alterations associated with this model have been related to changes in microbiota composition (Everard et al., 2014; Geurts et al., 2011). Moreover, previous work from our group further showed that *db*/*db* mice also exhibit emotional and cognitive alterations that are associated with increased inflammation in the hippocampus, a key area in the regulation of mood and cognition (Castanon et al., 2015; Dinel et al., 2011, 2014). By using this model that allows simultaneously assessing the effect of microbiota manipulation on behavioral alterations associated with the MetS, and on their main potential systemic and neurobiological correlates, particularly inflammation, we provided here new and important findings helping to decipher the complexity of the gut brain-axis in the context of the MetS.

2. Materials and methods

2.1. Animals and treatment

All animal care and use were conducted according to the relevant French (Directive 87/148, Ministère de l'Agriculture et de la Pêche) and international (Directive 10/63, European Community) legislation and approved by the Institutional Animal Care and Use Committee (approval ID: 5012047-A). Every effort was made to minimize suffering and the number of animals used. Five week old male *db*/+ (C57BLKS/J-leprdb/+; n = 28) and *db*/*db* mice (C57BLKS/J-leprdb/leprdb; n = 28) from Charles River Laboratories (France) were housed individually under standardized conditions with a 12-h light/dark cycle. Food (Lab Diet 5k52, Charles River, France) and water were available *ad libitum*. Compared to their healthy control *db*/+ mice, *db*/*db* mice are deficient for functional leptin receptor and consequently show typical characteristics of MetS, including severe obesity associated with hyperphagia, altered lipid/carbohydrate metabolism, and several indicators of T2D (polydipsia, polyuria, hyperglycemia, hyperinsulinemia,