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Prebiotic administration normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and IL1-β levels in male mice

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ABSTRACT

The manipulation of the enteric microbiota with specific prebiotics and probiotics, has been shown to reduce the host's inflammatory response, alter brain chemistry, and modulate anxiety behaviour in both rodents and humans. However, the neuro-immune and behavioural effects of prebiotics on sickness behaviour have not been explored. Here, adult male CD1 mice were fed with a specific mix of non-digestible galacto-oligosaccharides (Bimuno®, BGOS) for 3 weeks, before receiving a single injection of lipopolysaccharide (LPS), which induces sickness behaviour and anxiety. Locomotor and marble burying activities were assessed 4 h after LPS injection, and after 24 h, anxiety in the light–dark box was assessed. Cytokine expression, and key components of the serotonergic (5-Hydroxytryptamine, 5-HT) and glutamatergic system were evaluated in the frontal cortex to determine the impact of BGOS administration at a molecular level. BGOS-fed mice were less anxious in the light–dark box compared to controls 24 h after the LPS injection. Elevated cortical IL-1β concentrations in control mice 28 h after LPS were not observed in BGOS-fed animals. This significant BGOS × LPS interaction was also observed for 5HT2A receptors, but not for 5HT1A receptors, 5HT, 5HIAA, NMDA receptor subunits, or other cytokines. The intake of BGOS did not influence LPS-mediated reductions in marble burying behaviour, and its effect on locomotor activity was equivocal. Together, our data show that the prebiotic BGOS has an anxiolytic effect, which may be related to the modulation of cortical IL-1β and 5-HT2A receptor expression. Our data suggest a potential role for prebiotics in the treatment of neuropsychiatric disorders where anxiety and neuroinflammation are prominent clinical features.

1. Introduction

A functional link between the intestinal microbiota and neuronal function in central nervous system has been convincingly established in recent years, which appears to stem notably from alterations in the host immune response (Rhee et al., 2009; Cryan and Dinan, 2012; Burnet and Cowen, 2013; Forsythe and Kunze, 2013). Germ-free mice display a reduction in the ability to clear pathogens (Fritz et al., 2011), an exaggerated hypothalamic–pituitary–adrenal (HPA) reaction to stress (Sudo et al., 2004) and altered anxiety behaviour (Bercik et al., 2010; Neufeld et al., 2011; Diaz-Heijtz et al., 2011). Furthermore, the brains of germ-free animals contain microglia that appear less mature than those of specific pathogen free (SPF) mice and the transcriptome of these microglia also suggests that they are less mature (Erny et al., 2015). By contrast, in a strain dependent manner the administration of specific *Bifidobacteria* and/or *Lactobacilli* probiotic species, induce anxiolytic and antidepressant-like actions in rodents and humans (Messaoudi et al., 2011; Dinan et al., 2013; Savignac et al., 2014) by seemingly altering key neurotrophic molecules or neurotransmitter systems involved in anxiety behaviours (Bercik et al., 2010; Bravo et al., 2011; O'Sullivan et al., 2011). Specific probiotic strains also inhibit stress-induced elevations of plasma corticosterone (Gareau et al., 2007; Bravo et al., 2011; Messaoudi et al., 2011) and a wider range of probiotics have been shown to have anti-inflammatory actions (Konieczna et al., 2012).

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Prebiotics, which are dietary fibres that promote the proliferation of specific intrinsic *Bifidobacteria* and *Lactobacilli*, may also display similar properties as specific products have been shown to modulate the immune system and display anti-inflammatory properties (Hardy et al., 2013; Kanauchi et al., 2013; Vulevic et al., 2013). Importantly, we have recently demonstrated that fructo-oligosaccharides (FOS) and a specific non-digestible galacto-oligosaccharide formulation (Bimuno®, BGOS), elevate the levels of brain-derived neurotrophic factor (BDNF), as well as central N-methyl-D-aspartate receptor (NMDAR) subunit expression (Savignac et al., 2013). This study also demonstrated the prebiotic properties of BGOS, by showing a significant growth of *Bifidobacteria* after its administration. Furthermore, we have demonstrated that BGOS intake reduces the cortisol awakening response, and some emotional processes in healthy volunteers (Schmidt et al., 2015). These findings, therefore, suggest that certain prebiotics, and in particular, BGOS, may have anxiolytic effects, though this, together with potential underlying mechanisms of action, have not been explored.

Specific pro- and prebiotics have been shown to reduce the levels of circulating pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6 or tumour necrosis factor alpha (TNF-α) (Hardy et al., 2013; Vulevic et al., 2013). Elevated levels of these cytokines are associated with certain psychiatric disorders (Rook et al., 2014; Stuart et al., 2015; Wohleb et al., 2015). Thus, these findings suggest that the immuno-modulatory action of certain pro- and prebiotics may be key to attenuating inflammation-related aberrant behaviour. Notably, rodents administered with a single, peripheral dose of lipopolysaccharides (LPS) from Gram-negative bacteria, display 'sickness behaviour' where a reduction of locomotor activity within the first few hours is a principal feature (Cunningham et al., 2009; Biesmans et al., 2013). This effect is followed by longer term depressive-like behaviour and anxiety. At 24 h post injection mice exhibit no overt changes in locomotor behaviour, but do show increased immobility in a forced swim test, as well as decreased sucrose preference and reduced marble burying activity, indicating a depression-like state (Couch et al., 2015). This time point is suitable for studying the impact of prebiotics as it minimises the confounds associated with the acute reductions in locomotor activity. Attenuation of this LPS-induced sickness behaviour is associated with the normalization of exaggerated IL-1, IL-6 or TNF production, which can be achieved through dietary interventions (e.g. calorific restriction prior to LPS injections (MacDonald et al., 2014)). Since exaggerated immune function has been associated with several neuropsychiatric disorders that are linked to altered glutamate and 5-HT neurotransmission (Mitchell and Dinan, 2010; Marsden, 2011; Dantzer, 2012), the ability to use prebiotics to modify neural function by manipulating the gut microbiota, or via their possible direct effect on the host gut mucosa, in a low cost and physiologically safe manner, is an attractive proposition for the development of new therapy.

Here we tested the potential of BGOS to prevent, or attenuate, LPS-induced sickness and anxiety behaviour in mice. To provide further insights into the mechanisms underlying prebiotic action, we also measured the effect of BGOS on the concentration of key cytokines in the frontal cortex and corticosterone in the plasma, and evaluated the impact of the prebiotic on the cortical glutamatergic and serotonergic systems. The frontal cortex was chosen as a region of interest for its susceptibility to inflammatory neuropathology (de Pablos et al., 2006), notably following LPS administration, and for its role in many neuropsychiatric disorders (Shelton et al., 2011; Couch et al., 2013a,b; Pan et al., 2014).

2. Material and methods

2.1. Animals

All experiments were carried out with local ethical approval and a UK Home Office licence granted under the Animals (Scientific Procedures) Act (1986). Male CD1 mice (25–30 g, 6–8-week old, Harlan Orlac, UK), were housed 3 per cage (plexiglas cages 33 × 15 × 13 cm, $L \times W \times H$) and maintained under standard controlled laboratories conditions (12-h light–dark cycle, lights on at 7 a.m., 21 ± 1 °C, humidity 50 ± 5%). The CD1 mouse is outbred and displays a more exaggerated pro-inflammatory cytokine response to LPS compared to other strains such as the C57Bl6 (Nikodemova and Watters, 2011). Male mice were used to maintain consistency with our previous prebiotic studies in male rats (Savignac et al., 2013), and with previous behavioural studies using probiotics (Bravo et al., 2011; Savignac et al., 2014, 2015). Social dominance, which may occur in group-housing and interfere with sickness behaviour (Gibb et al., 2011; Wohleb et al., 2012), was thoroughly monitored by trained experimenter (Savignac et al., 2011) but no overt dominance behaviour was observed in the home-cages throughout the experiment. Mice were fed with standard mouse chow *ad libitum* and allowed 4–5 days habituation to the animal facility before receiving prebiotic treatment.

2.2. BGOS administration

To minimize stress and reduce the likelihood of fatalities associated with the gavage procedure (Damsch et al., 2011), a pilot study was run to test that the administration of BGOS via the drinking water sufficiently elevated faecal *Bifidobacteria*, and to evaluate an appropriate control solution (see below). The supplementation of drinking water with test compounds is a non-invasive approach which has been previously used to deliver other prebiotics (Bindels et al., 2012) and probiotics (Rashid et al., 2014) to rodents. Since the BGOS mix used in the current study consists of 48% w/w galacto-oligosaccharide, 26% lactose, 14% glucose and 12% galactose, a mixture of these sugars in the same proportion in the absence of galacto-oligosaccharides, was also tested. The enzyme used to covert the lactose to galacto-oligosaccharides is removed by means of filtration (Tzortzis et al., 2005), which circumvents the need to correct for any contaminating protein within the BGOS powder.

Pilot Study: Male mice, housed as described above, were provided with either drinking water containing BGOS, (1.3%, w/v, Bimuno®, Clasado, Reading, UK; $n = 6$), BGOS free sugars (BFS, $n = 6$) or normal drinking water ($n = 6$), in addition to their normal chow, for 3 weeks. The BGOS was freshly prepared daily by dissolving 13 g of BGOS powder in 1L of water. The BFS solution was also freshly prepared by dissolving lactose (3.38 g), glucose (1.82 g) and galactose (1.56 g) (each from Sigma–Aldrich, UK) in 1L of water. Clean standard drinking bottles were then filled with an equal quantity of the solution and provided to all cages. Fluid intake was monitored daily by weighing drinking bottles to ensure all animals were drinking an equivalent quantity of fluid. The prebiotic properties of BGOS have been extensively studied, and the product has been consistently shown to selectively increase *Bifidobacteria* and, to some extent, *Lactobacilli* in both humans and animals (Tzortzis et al., 2005; Depeint et al., 2008; Vulevic et al., 2008, 2013; Silk et al., 2009; Savignac et al., 2013). The enumeration of *Bifidobacteria* alone were therefore estimated in our pilot experiment (see below).

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