Psychoneuroendocrinology (2012) 37, 1885—1895

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats

Afifa Ait-Belgnaoui [a,b], Henri Durand [b], Christel Cartier [a], Gilles Chaumaz [a], Hélène Eutamene [a], Laurent Ferrier [a], Eric Houdeau [a], Jean Fioramonti [a], Lionel Bueno [a], Vassilia Theodorou [a,*]

[a] *Neuro-Gastroentérologie and Nutrition Team, TOXALIM, UMR 1331-INRA/INP/UPS, Toulouse, France*
[b] *Institut Rosell-Lallemand, Blagnac, France*

Received 1 March 2012; received in revised form 28 March 2012; accepted 29 March 2012

KEYWORDS
HPA axis;
Brain-gut interaction;
Intestinal permeability;
Lipopolysaccharide

Summary
Background and aims: Intestinal barrier impairment is incriminated in the pathophysiology of intestinal gut disorders associated with psychiatric comorbidity. Increased intestinal permeability associated with upload of lipopolysaccharides (LPS) translocation induces depressive symptoms. Gut microbiota and probiotics alter behavior and brain neurochemistry. Since *Lactobacillus farciminis* suppresses stress-induced hyperpermeability, we examined whether (i) *L. farciminis* affects the HPA axis stress response, (ii) stress induces changes in LPS translocation and central cytokine expression which may be reversed by *L. farciminis*, (iii) the prevention of ''leaky'' gut and LPS upload are involved in these effects.
Methods: At the end of the following treatments female rats were submitted to a partial restraint stress (PRS) or sham-PRS: (i) oral administration of *L. farciminis* during 2 weeks, (ii) intraperitoneal administration of ML-7 (a specific myosin light chain kinase inhibitor), (iii) antibiotic administration in drinking water during 12 days. After PRS or sham-PRS session, we evaluated LPS levels in portal blood, plasma corticosterone and adrenocorticotropic hormone (ACTH) levels, hypothalamic corticotropin releasing factor (CRF) and pro-inflammatory cytokine mRNA expression, and colonic paracellular permeability (CPP).
Results: PRS increased plasma ACTH and corticosterone; hypothalamic CRF and pro-inflammatory cytokine expression; CPP and portal blood concentration of LPS. *L. farciminis* and ML-7

Abbreviations: ACTH, adrenocorticotrophic hormone; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CRF, corticotropin-releasing hormone; CPP, colonic paracellular permeability; HPA, hypothalamic-pituitary-adrenal axis; IBDi, rritable bowel syndrome; IBS, irritable bowel syndrome; ILi, nterleukin; LPS, lipopolysaccharide; MLCK, myosin light chain kinase; ML-7, specific inhibitor of MLCK; PRS, partial restraint stress; PVN, paraventricular nucleus of hypothalamus; TNF, tumor necrosis factor.

* Corresponding author at: Neuro-Gastroenterology and Nutrition Unit, TOXALIM, UMR 1331-INRA/INP/UPS, 180 chemin de Tournefeuille, BP 3, 31027 Toulouse Cedex 9, France. Tel.: +33 561 28 51 59; fax: +33 561 28 51 45.
E-mail address: vtheodor@toulouse.inra.fr (V. Theodorou).

0306-4530/$ — see front matter
http://dx.doi.org/10.1016/j.psyneuen.2012.03.024

suppressed stress-induced hyperpermeability, endotoxemia and prevented HPA axis stress response and neuroinflammation. Antibiotic reduction of luminal LPS concentration prevented HPA axis stress response and increased hypothalamic expression of pro-inflammatory cytokines.
Conclusion: The attenuation of the HPA axis response to stress by *L. farciminis* depends upon the prevention of intestinal barrier impairment and decrease of circulating LPS levels.

1. Introduction

The intestinal epithelial barrier consisting in a single cell layer delimits the internal milieu from the luminal environment. This delimitation ensures protection from a wide range of factors (pathogens, antigens etc.) entering the lumen resulting to prevention of infection inflammation and alteration of normal body functions. Tight junctions (TJs) between adjacent intestinal epithelial cells are complex and dynamic structures which regulate paracellular transport across the intestinal epithelium (Anderson et al., 1993; Arrieta et al., 2006). Endogenous (humoral and neural signals, inflammatory mediators) and exogenous (dietary, bacterial or viral products) factors are involved in the regulation of TJs integrity and subsequent mucosal immune activation (Turner, 2009). In this respect, the intestinal barrier is particularly relevant with focus on intestinal permeability, immune response and intestinal microbiota. Intestinal epithelial barrier impairment reflected by increased intestinal permeability is reported in various gastrointestinal diseases such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS) (Arrieta et al., 2006; May et al., 1993; Spiller et al., 2000), non-alcoholic steatohepatitis/non-alcoholic fatty liver disease but also autoimmune diseases (Arrieta et al., 2006; de Kort et al., 2011), and depression (Maes et al., 2008). Interestingly, stressful life events are known to exacerbate the risk in the development and/or to facilitate the relapse in IBD and IBS (Longstreth, 2005; Mittermaier et al., 2004), highlighting the contribution of the brain-gut cross talk in digestive diseases associated with anxiety disorders. In animals, acute and chronic stress increases gut paracellular permeability resulting to visceral hypersensitivity (Ait-Belgnaoui et al., 2005), bacterial translocation (Zareie et al., 2006) and exacerbation of experimental colitis (Gue et al., 1997). On the other hand, there is increasing evidence concerning the impact of gut microbiota on the homeostasis of the intestinal barrier. For example, gut bacteria modulate intestinal motility barrier function and visceral perception (Verdu et al., 2004). Further, although in health the intestinal microbiota is characterized by stability and diversity, in IBD or IBS the microbiota has less diversity and its composition is unstable over time (Kassinen et al., 2007; Malinen et al., 2005; Matto et al., 2005). However whether these changes in microbiota under chronic diseases are a cause or a consequence in the underlying pathophysiology remains under debate. More recent striking findings underline the ability of gut microbiota to interact with the central nervous system leading to behavioral and brain neurochemistry changes. Commensal microbiota affects the postnatal development of the HPA stress response in mice (Sudo et al., 2004) and germ-free mice exhibit less anxiety than conventional counterparts. Of particular interest are also findings reporting a central sensing of gastrointestinal infections. Indeed, in mice infection with *Citrobacterium rodentium* (Lyte et al., 2006) and *Campylobacter jejuni* (Goehler et al., 2005) increases anxiety-like behavior. However since the exaggerated HPA stress response observed in germ-free mice was reversed by reconstitution with *Bifidobacterium infantis* (Sudo et al., 2004), one can suggest that the cross-talk between bacteria and brain may be extended over pathogens.

Despite all these data concerning the ability of the intestinal microbiota and probiotics to communicate with the CNS, the pathways and brain areas involved remains poorly understood. Consequently, in this study using an intervention strategy in the gut microbiota (a probiotic strain, *Lactobacillus farciminis* treatment) we aimed to evaluate whether (i) the probiotic treatment affects the HPA axis response to stress; (ii) stress induces changes in lipopolysaccharide (LPS) translocation and central cytokine release which may be reversed by *L. farciminis*; (iii) the prevention of ''leaky'' gut and LPS upload in the mucosa are involved in these effects.

2. Materials and methods

2.1. Animals and bacteria preparation

Female Wistar rats (Janvier SA, Le Genest St Isle, France) weighing 200—225 g and housed individually were kept at a constant temperature (21 ± 1 °C) in a pathogen-free animal facility, and maintained on a 12 h light/dark cycle. Food (UAR pellets, Epinay, France) and water were available *ad libitum*. All protocols were approved by the local institutional animal care and use committee in compliance with the European laws on the protection of animals (86/609/EEC).

L. farciminis (freeze-dried bacteria provided by Institut Rosell-Lallemand, Blagnac, France) were freshly prepared every day in 0.9% NaCl and administrated orally at a concentration of 10^{11} CFU/day/rat.

2.2. Partial restraint stress

Stress sessions were performed at the same time of day (between 10 am and 12 noon) to minimize influence of circadian rhythms. Stress effects were studied using the partial restraint stress (PRS) model which is a mild and non-ulcerogenic stress (Williams et al., 1987). Animals were lightly anesthetized with diethyl-ether and their fore shoulders, upper forelimbs, and thoracic trunk were wrapped in a confining harness of paper tape to restrict, but not to prevent body movements during 2 h. Rats recovered from ethyl-ether within 2—3 min and immediately moved around in their cage, although the restricted mobility of their forelimb prevented grooming behavior. Sham-stress rats (sham-PRS) considered as controls were anesthetized as above, but were not wrapped and were allowed to move freely in their cages.