Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Immune derived opioidergic inhibition of viscerosensory afferents is decreased in Irritable Bowel Syndrome patients

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ARTICLE INFO

Article history:
Received 1 June 2014
Received in revised form 30 June 2014
Accepted 1 July 2014
Available online xxxx

Keywords:
Irritable Bowel Syndrome
Visceral pain
Opioid
Neuro-immune
Monocyte
Macrophage

ABSTRACT

Alterations in the neuro-immune axis contribute toward viscerosensory nerve sensitivity and symptoms in Irritable Bowel Syndrome (IBS). Inhibitory factors secreted from immune cells inhibit colo-rectal afferents in health, and loss of this inhibition may lead to hypersensitivity and symptoms. We aimed to determine the immune cell type(s) responsible for opioid secretion in humans and whether this is altered in patients with IBS. The β-endorphin content of specific immune cell lineages in peripheral blood and colonic mucosal biopsies were compared between healthy subjects (HS) and IBS patients. Peripheral blood mononuclear cell (PBMC) supernatants from HS and IBS patients were applied to colo-rectal sensory afferent endings in mice with post-inflammatory chronic visceral hypersensitivity (CVH). β-Endorphin was identified predominantly in monocyte/macrophages relative to T or B cells in human PBMC and colonic lamina propria. Monocyte derived β-endorphin levels and colonic macrophage numbers were lower in IBS patients than healthy subjects. PBMC supernatants from healthy subjects had greater inhibitory effects on colo-rectal afferent mechanosensitivity than those from IBS patients. The inhibitory effects of PBMC supernatants were more prominent in CVH mice compared to healthy mice due to an increase in μ-opioid receptor expression in dorsal root ganglia neurons in CVH mice. Monocyte/macrophages are the predominant immune cell type responsible for β-endorphin secretion in humans. IBS patients have lower monocyte derived β-endorphin levels than healthy subjects, causing less inhibition of colonic afferent endings. Consequently, altered immune function contributes toward visceral hypersensitivity in IBS.

Abbreviations: IBS, Irritable Bowel Syndrome; IBS-D, IBS diarrhea predominant; IBS-C, IBS constipation predominant; IBS-A, IBS alternating; PBMC, peripheral blood mononuclear cell; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; MOR, μ-opioid receptor; TNBS, trinitrobenzene sulfonic acid; TRP, Transient Receptor Potential; CVH, chronic visceral hypersensitivity; HS, healthy subjects; LPS, Lipopolysaccharide; PMA, phorbol 12-myristate 13- acetate; DAMGO, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin; qRT-PCR, quantitative RT-PCR; DRG, dorsal root ganglia.

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http://dx.doi.org/10.1016/j.bbi.2014.07.001
0889-1591/

1. Introduction

Irritable Bowel Syndrome (IBS) is a prevalent functional disorder of the gastrointestinal tract estimated to affect more than 10% of the population (Longstreth et al., 2006). IBS patients are defined by symptoms of pain from the lower abdominal region that is associated with altered bowel habit and which occurs in the absence of readily identifiable pathophysiology, a clear differential diagnosis from Inflammatory Bowel Disease (Longstreth et al., 2006). These patients may be further characterized according to bowel habit as diarrhea predominant (IBS-D), constipation predominant (IBS-C), or alternating between these states (IBS-A). The symptom of pain crosses all these subtypes and has the greatest impact on quality of life, but remains the most difficult to treat (Longstreth et al., 2006). Little is known of the mechanisms underlying IBS and it has historically been viewed as a neurological motility disorder involving alterations in the brain–gut axis. However there is increasing evidence that the immune system is altered in these patients, and that these alterations contribute toward symptoms (Hughes et al., 2013b).

Distension of the colo-rectum is sensed by mechanosensitive extrinsic primary sensory afferent nerves, which are best characterized in the mouse (Brierley et al., 2004; Hughes et al., 2009b). Muscular/mucosal afferents respond to fine tactile stimuli and low intensity circular stretch, and in the colo-rectum are unique to the pelvic afferent pathway where they comprise approximately 25% of the total afferent population (Brierley et al., 2004; Feng et al., 2010; Hughes et al., 2009b). These afferent nerves respond linearly to increasing levels of distension and signal into the noxious range. They also express putative nociceptive channels, including members of the Transient Receptor Potential (TRPV1, TRPA1) and Acid Sensing Ion Channel (ASIC3) families, implying they act as intensity encoders and modulate the sensory processing of pain (Brierley et al., 2009; Brookes et al., 2013; Gebhart, 2000; Jones et al., 2005). Immune derived mediators are known to excite viscerosensory nerves and have previously been implicated in the heightened sensitivity to distension of the colo-rectum experienced by IBS patients (Hughes et al., 2009a, 2013b). However, the generation and propagation of action potentials is a dynamic process that not only results from increased excitation, but also loss of inhibition. We recently showed that muscular/mucosal afferent electrophysiological responses to distension were inhibited following incubation with unstimulated peripheral blood mononuclear cell (PBMC) supernatants from healthy subjects in a manner consistent with activation of the μ-opioid receptor (MOR) (Hughes et al., 2009c, 2013a). This inhibition was lost following incubation with supernatants from IBS-C patients and switched to sensitization after incubation with supernatants from IBS-D, an effect we characterized as cytokine driven (Hughes et al., 2009c, 2013a).

Opioids are well known for their analgesic properties, and exert their inhibitory effects by binding to three major receptors; μ, δ and κ. These receptors are $G_{i/o}$ members of the G-protein coupled receptor family, with binding typically decreasing neuronal excitability via inhibition of adenylyl cyclase, activation of potassium channels and inhibition of calcium channels. Immune cells are known to secrete opioids, and T cell derived β-endorphin, a MOR preferring agonist, has previously been shown to be essential for setting the colo-rectal afferent activation threshold to distension in healthy mice (Hughes et al., 2013b; Stein et al., 2003; Verma-Gandhu et al., 2006). However, little is known of how these studies translate to humans, and opioid secretion by immune cells is yet to be directly investigated in IBS.

In rodents rectal administration of trinitrobenzene sulfonic acid (TNBS) induces an acute colitis characterized by transmural damage to the colon wall associated with an influx in neutrophils and accompanied by increased colonic myeloperoxidase concentrations. This colitis is transient, peaking several days after administration and then spontaneously healing such that by 28 days following administration the histology of the colon and myeloperoxidase levels do not differ from untreated animals (Hughes et al., 2009a,b; Krauter et al., 2007; Qin et al., 2011). However, colonic afferent nerves remain sensitized to distension long after the mucosa heals, which models aspects of the chronic visceral hypersensitivity (CVH) experienced by IBS patients (Gschossmann et al., 2004; Hughes et al., 2009a,b). In this model muscular/mucosal afferents are sensitized to mucosal stroking but not circular stretch, and the sensitization observed is relatively modest compared to hypersensitivity displayed by high-threshold afferents (Hughes et al., 2009b). The molecular changes underlying the sensitization of colo-rectal sensory afferents following recovery from inflammation and the contribution by opioid receptors to CVH in this model remain to be determined.

In order to investigate the interaction between the immune system and pain symptoms in IBS patients we aimed to determine the immune cell type(s) responsible for β-endorphin secretion in humans, and whether this is altered in IBS. We also aimed to determine whether the effects that PBMC supernatants from IBS patients had on colo-rectal afferent function in CVH mice differed from those caused by supernatants from healthy subjects (HS).

2. Materials and methods

All experiments were approved by the Royal Adelaide Hospital, Flinders Medical Centre and University of Adelaide Human Ethics Committees, and the Animal Ethics Committees of SA Pathology and University of Adelaide.

2.1. Human subjects

Two cohorts of HS and ROME II categorized IBS patients were recruited from the Department of Gastroenterology and Hepatology at the Royal Adelaide Hospital and Department of Luminal Gastroenterology, Flinders Medical Centre, South Australia. Patients were categorized according to bowel habits as either diarrhea predominant (IBS-D), constipation predominant (IBS-C) or alternating (IBS-A). Patients with more than 3 bowel movements per day and loose stools were categorized as having diarrhea-predominant IBS (IBS-D) and patients with fewer than 3 movements per week and hard, lumpy stools were categorized as constipation predominant IBS (IBS-C). Patients with an alternating bowel pattern or bowel frequencies that did not exceed the earlier described limits were categorized as alternators (IBS-A). HS were recruited by advertisement. Written informed consent was obtained prior to inclusion. A comprehensive diagnostic work up, including colonoscopy and repeated stool testing, did not reveal structural lesions or any evidence for acute infection as the cause of symptoms. Patients were excluded based on concomitant chronic fatigue syndrome, fibromyalgia or a history of analgesic or immunosuppressive medication (nonsteroidal anti-inflammatory drugs, steroids and so forth) within the 3 months prior to sampling, or if results of full blood count, renal and liver function, fibrinogen and C-reactive protein level indicated medical conditions likely to confound the study aims. 5 of the IBS-D patients in cohort 1 were considered post infectious with confirmed acute gastrointestinal infection (3 *Salmonella*, 2 *Campylobacter* enteritis) that preceded the manifestation of symptoms. The time interval between acute infection and

Please cite this article in press as: Hughes, P.A., et al. Immune derived opioidergic inhibition of viscerosensory afferents is decreased in Irritable Bowel Syndrome patients. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.07.001