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Neuro-endocrine and neuro-immune pathways contribute to the pathophysiology of irritable bowel syndrome.

Dervla O'Malley[1,2]

1. **Department of Physiology, University College Cork, Cork, Ireland.**
2. **APC Microbiome Institute, University College Cork, Cork, Ireland.**

Correspondence to:
Dr Dervla O'Malley,
Department of Physiology,
4.103 Western Gateway Building
University College Cork,
Cork, Ireland.
d.omalley@ucc.ie
Tel: +353 21 4205483
Fax: +353 21 4205370

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Abstract

Irritable bowel syndrome (IBS) is a common disorder characterized by recurrent abdominal pain, bloating and disturbed bowel habit, symptoms which impact on the quality of life of sufferers. The pathophysiological changes underlying this multifactorial condition are complex and include increased sensitivity to luminal and mucosal factors which result in altered colonic transit and visceral pain. Moreover, dysfunctional communication in the bidirectional signaling axis between the brain and the gut, which involves efferent and afferent branches of the peripheral nervous system, circulating endocrine hormones and local paracrine and neurocrine factors, including immune and perhaps even microbial signaling molecules have a role to play in this disorder. This mini-review will examine recent advances in our understanding of the pathophysiology of IBS and assess how crosstalk between hormones, immune and microbe-derived factors and their neuromodulatory effects on peripheral nerves may underlie IBS symptomatology.

Introduction

Irritable bowel syndrome (IBS) is a prevalent functional bowel disorder characterized by abnormal regulation of motor and sensory function in the distal gastrointestinal (GI) tract. The pathophysiology of IBS is likely multifactorial and includes heightened sensitivity to luminal and mucosal factors such as enteroendocrine secretions, stress hormones, malabsorbed or maldigested nutrients, bile acids, or alterations in the colonic microbiome. This can result in increased barrier permeability, subsequent immune activation and altered neural regulation of GI secretion and motility. Visceral hypersensitivity and associated abdominal pain, which is one of the most debilitating symptoms of this disorder, is also stimulated by such luminal and mucosal factors ((10) for review).

Underlying the pathophysiology of IBS is dysregulation of the bidirectional brain-gut signaling axis, which comprises efferent and afferent neuronal pathways of the parasympathetic and sympathetic nervous systems, endocrine hormones, including the hypothalamic-pituitary-adrenal (HPA) stress axis hormones and digestive hormones, and immune and microbial signaling molecules. The importance of the central nervous system (CNS) in regulating digestive function and satiety is well established (41) as are the detrimental effects of emotional stress on GI physiology (2). Indeed, the high-comorbidity between centrally-mediated psychiatric disorders such as depression and anxiety with GI dysfunction (18) is further evidence of the importance of the brain-gut signaling axis. However, it is clear that communication between the brain and gut is a bidirectional system and this mini-review will look at recent advances in our understanding of communication between hormones and immune factors originating in the GI tract and how they signal to the peripheral and central nervous systems.

The contribution of endocrine hormones to IBS symptoms

IBS patients present with lower pain thresholds to rectal distension and increased intensity of visceral sensation, observations that are accompanied by enhanced activity in the thalamus, insula and anterior cingulate cortex, which are part of the CNS pain matrix (69). Moreover, this hypersensitivity has been related to mood and emotion in patients, with evidence of high co-morbidity between stress-related disorders, such as depression and anxiety, and IBS. Indeed, stress has a significant role in the initiation, exacerbation and persistence of GI symptoms during symptom flares (76), effects that may be mediated through the stress hormone, corticotrophin-releasing factor (CRF), which is secreted in response to stress, by neurons of the paraventricular nucleus of the hypothalamus.

Clearly, dysregulation of central modulation, or CNS processing, contributes to IBS symptomology, particularly the exacerbating influence of psychological stressors (39). However, perceived pain sensation is initiated through activation of sensory nerves in the periphery (24), which is evident by blocking visceral pain hypersensitivity with rectally-applied analgesics (69), demonstrating the two-way communication system of the brain-gut signaling axis. Colonic nociceptors are stimulated through distension of the colonic lumen but may also be activated through inflammatory mediators. Indeed, the role of immune mediators is now being recognized to play a role in IBS symptoms (24, 36, 61) and activation of the HPA axis is a well-studied response orchestrated by the brain in reaction to infection and inflammation (74).

The neuromodulatory effects of stress peptides in gut function

Given the high co-morbidity of stress-associated mood disorders in IBS patients, it's likely that sensitivity to stress is altered in these individuals. Indeed, CRF, which is secreted in response to perceived stressors and activates the HPA axis, evokes increased secretion of cortisol in IBS patients (18). CRF is key to stress-related alterations in colonic motor and secretory function ((81) for review) and evokes its biological effects through activation of CRF1 and CRF2 receptors, which are expressed in the hypothalamus and other brain regions. Administration of CRF into the central nucleus of the amygdala, which is important in the integration of emotional and sensory information, stimulates local release of norepinephrine and sensitization of visceral nociception, an effect that is inhibited using a CRF1 receptor antagonist (79). Although the effects of CRF in the CNS are crucial to regulation of the GI tract, receptors are also expressed in the colon where they are ideally placed to mediate the effects of stress on gastric emptying, transit and gut motility. In support of the importance of peripheral regulation of GI function are studies demonstrating that stress-induced defecation and visceral hypersensitivity is attenuated by blocking peripheral CRF1 receptor activation (6, 30, 55). However, our studies also noted the potential role of inflammatory molecules in symptoms such as visceral pain and stress-induced defecation and the potential for crosstalk between stress and immune molecules in the manifestation of IBS symptom flares. Indeed, Jizhong *et al,* detected concurrent increases in expression of toll-like receptors and CRF receptors in blood samples from IBS patients (37) and activation of CRF receptors is also linked to increased colonic barrier permeability and inflammation (42). Moreover, stress induces degranulation of mast cells, resulting in the release of pro-inflammatory mediators (40, 57). The efficacy of CRF1 receptor antagonists in animal models (6, 30, 55) is encouraging, however, clinical trials using such antagonists have thus far been disappointing, primarily due to unfavorable pharmacokinetics, tissue accumulation, high protein binding and reactive metabolite formation (31). Moreover, pexacerafont, an orally active, selective

CRFR1 antagonist had no effect on colonic transit in diarrhea-predominant IBS, indicating that CRF 1 receptors may not constitute a useful therapeutic target in humans (80).

GI peptides are key signaling molecules from the gut lumen to the brain

Although food intolerance does not underlie IBS pathophysiology, ingestion of certain food types, particularly carbohydrate and fat-rich meals can result in abdominal pain and bloating. Wheat is thought to be one of the key triggers of IBS symptoms (15) and although celiac disease is no more prevalent in IBS patients than the general population, IBS patients who carry HLA-DQ2 or HLA-DQ8 genotypes, which predispose individuals to celiac disease, display more sensitivity to gluten (84). They are also more likely to benefit from a gluten-free diet than patients without these genotypes (1, 85), a mechanism which appears to be dependent on alterations to the adaptive immune system (85). Additionally, some studies find that diets low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) reportedly show improvements in IBS symptoms (47, 59). However, others report that reducing anxiety using hypnotherapy is just as effective as this restrictive diet (68). This topic remains controversial with one recent meta-analysis supporting the efficacy of a low FODMAP diet in the treatment of functional GI disorders (49), whereas another meta-analysis failed to prove any benefit (70). In mechanistic terms, where a low FODMAP diet was found to be beneficial to IBS patients, immune activation was suppressed and the microbiome was altered with greater numbers of bacteria involved in the consumption of gas (51). Although release of gas by fermentation is normal, the enhanced sensitivity of IBS patients to bowel distension may underlie the debilitating visceral pain they experience following ingestion of a meal rich in FODMAPs.

The cellular and molecular mechanisms underlying the exacerbation of IBS symptoms following a meal are not yet clear. Nerve fibers are thought to terminate before reaching the epithelial layer. Thus, unless the integrity of the mucosal barrier has been lost, nerve fibers cannot directly sense luminal nutrients (25). However, enteroendocrine cells (EECs) detect ingested nutrients, thus acting as electrically-excitable biosensors (13). Indeed, an important physiological response to the arrival of food in the gut lumen is the secretion of digestive and incretin hormones. Moreover, visceral afferent endings are sensitive to gut hormones released on the basolateral side of the mucosal barrier. These include hormones that regulate appetite and energy homeostasis such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), and enteroendrocrine secretions linked to the modulation of motility, absorpto-secretory function and sensation, such as serotonin, substance P and peptide YY (PYY). Thus, EECs could act as a lynchpin in the transmission of luminal signals to the host nervous system. Consistent with this theory, a recent paper by Bohorquez and colleagues provided evidence of direct, physical mechanisms of signaling between EECs and neurons innervating the gastrointestinal tract. The authors provide evidence of PYY- and GLP-1- positive EECs producing a pseudopod-like elongation, termed a neuropod, which synapsed with efferent and afferent nerves (5).

Incretin hormones have neuromodulatory action in the GI tract

The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion and insulin biosynthesis and inhibition of glucagon secretion. In the gut, GLP-1 inhibits gastric emptying and food intake. A clinical trial of a GLP-1 mimetic found that it alleviated some IBS symptoms with anti-spasmodic and pain-relieving properties (32) and may be a useful therapeutic strategy in IBS-C (56). Although, the molecular mechanisms by which GLP-1 achieves this outcome are not completely understood, it is known that GLP-1

increases firing rates in afferent vagal nerves (52). GLP-1-expressing neurons are found in the enteric nervous system but also in brain regions such as the nucleus tractus solitarius and the ventrolateral medulla (43). GLP-1 receptors have also been detected in vagal and dorsal root ganglia and the area postrema and hypothalamus in the CNS (73), revealing that the action of GLP-1 on gut function may be centrally or peripherally orchestrated. Crosstalk between this GI hormone and the HPA stress axis have been revealed through the stimulatory action of GLP-1 on CRF neurons (58), once more hinting that a complex relationship between several physiological systems has been impeding our understanding of IBS. K cells are EECs which secrete an incretin hormone, GIP, which is released in response to luminal carbohydrates to promote pancreatic insulin secretion. Although less has been reported about a potential role for GIP, mucosal biopsies from IBS patients express fewer K cells than healthy controls, which was particularly evident in IBS-D patients (22). As GIP also inhibits gastric acid secretion, a reduction in the number of K cells may lead to unregulated gastric acid secretion, which could contribute to dyspepsia and gastro-esophageal reflux, common symptoms in IBS (71).

Other enteroendocrine secretions in the manifestation of IBS symptoms

Serotonin is primarily synthesized in the gut and exerts its effects through receptors expressed on intrinsic and extrinsic nerves, modulating colonic motility and secretion, and activating extrinsic nerve fibers. Circulating serotonin levels are elevated in diarrhea-predominant IBS patients but decreased in constipation-predominant patients. This has resulted in the use of selective serotonerigc agonists and antagonists to treat specific subtypes of the disorder (11), however these are not without side-effects. EECs also secrete chromogranins and secretogranins, which promote the release of other GI hormones. In IBS, particularly patients with rapid colonic transit, levels of granins are altered, resulting in them

being proposed as an indirect biomarker of colonic secretion or motility (9). Other GI peptides with neuromodulatory effects, PYY (21) and neuropeptide Y (88), are both reduced in IBS biopsies, although the mechanistic consequences of the altered expression has yet to be elucidated.

The GI tract is the largest endocrine organ, secreting more than two dozen different hormones, many of which are altered in the guts of IBS patients. In addition to a hyper-activated stress axis, endocrine signaling and hormonal modulation of nerve signals to the CNS are likely contributors to the manifestation of symptoms in IBS. Nonetheless, evidence is now growing that, although no overt inflammation is evident in the colons of IBS patients, immune activation is an additional consideration in the pathophysiology of the disorder and indeed, interaction between immune and endocrine factors may underlie symptom flares (7) (37).

Neuro-immune interactions contribute to IBS pathophysiology

The neuromodulatory effects of cytokines

The GI tract is unusual in that it is continuously exposed to immunogenic stimuli ingested with food. The immune system must determine if the ingested antigens pose a threat to the host or if they should be tolerated. Thus, the lamina propria, lying below the GI mucosa is home to many immune cells, which are primed to respond to pathogens. In comparison to healthy controls, IBS patients exhibit changes in mucosal immune cell populations, with elevated numbers of mast cells and lymphocytes (29). Degranulation of mast cells causes the release of inflammatory mediators including proteases, prostaglandins, histamine and cytokines which can activate enteric neurons (8), although a recent report suggested that continuous exposure to these mediators may result in desensitization of enteric neurons (64).

Concentrations of pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8 are also elevated in the IBS plasma (53), although not all studies detected this change (12). Cytokines facilitate cell-to-cell signaling in an immune response, but may also participate in modulating intrinsic and afferent nerve activity. Indeed, IL-6 (6, 63), IL-1β (86) and tumor necrosis factor α (72) have direct actions on enteric neuronal activation and we observed that the neurostimulatory effects of IBS plasma on rat submucosal and myenteric neurons is dependent on IL-6, IL-8 and CRF (6, 62). Such cytokines stimulate sensitization of nociceptors, which would increase the excitability of afferent endings innervating the colon. Indeed, IL-1β can sensitize splanchnic afferent nerves to histamine and mesenteric ischemia (28) and also stimulate increased excitability of DRG neurons to mechanical or thermal stimulation (4). Thus, cytokines may be important in initiating and perpetuating visceral hypersensitivity and abdominal pain in IBS.

Vagal Function

Although most dense in the proximal regions of the GI tract, vagal innervation is still significant in the distal colon (25, 83) and vagal afferent neurons are likely to act as the primary interrogators of peripheral colonic signals. Moreover, the vagus nerve, has been shown to modulate the production of pro-inflammatory cytokines by activating the HPA axis, which is anti-inflammatory and the cholinergic anti-inflammatory pathway (65). Vagal tone has been found to be important in cytokine secretion in Crohn's disease with an inverse relationship between vagal tone and plasma levels of TNFα. This wasn't noted in IBS however, and no link was found between IL-6 and vagal tone in either disorder (66), indicating that this mechanism may not be important in IBS pathophysiology.

Barrier Function

IL-6 and other pro-inflammatory cytokines (60) promote breakdown of the mucosal barrier, thus facilitating movement of pathogens across the epithelial barrier and resulting in activation of an immune response. In IBS patients, where plasma IL-6 levels are chronically elevated and the HPA stress axis is hyper-activated (18), a coincident compromise of the mucosal barrier is observed, which could result in sensitization of afferent nerves leading to increased sensitivity to visceral pain (89). Indeed, intervention studies in the stress-sensitive Wistar Kyoto rat model of IBS, which exhibits visceral hypersensitivity, demonstrated that neutralizing IL-6 receptors alleviated visceral pain and altered motility with associated reductions in tight junction proteins. Moreover, co-treatment of animals with the CRF1 receptor antagonist, antalarmin and anti IL-6 receptor antibodies, further alleviated symptoms of stress-induced defecation and visceral pain sensitivity (figure 1, reproduced from original publication (6)).

Microbial signaling to the gut and brain

With the development of germ-free (GF) and antibiotic or pro-biotic treated rodents, we have begun to recognize the importance of the microbiome in gut and whole host homeostasis. The colon is host to over a hundred trillion microbial organisms, most of which are bacterial. These microbes have diverse but important functions which may be beneficial to their host, such as scavenging extra energy through fermentation of non-digestible foods, secreting vitamins and ensuring the normal development of the immune system. Thus, the traditional view of the host-microbiota relationship being symbiotic, where the microbes benefit more than the host, has been re-assessed and the interaction is more akin to a mutualistic relationship, or to take it to the extreme, it has been proposed that microbes may manipulate host physiology and behavior to their own benefit (77). This shift in our appreciation of the relationship between colonic bacteria and the normal development of gastrointestinal,

immune and endocrine physiology and the peripheral and central nervous systems has resulted in a recent surge of studies. Aside from its role in GI function, recent reviews have discussed the importance of gut bacteria in the development of diseases of the CNS, including depression and anxiety (19, 50), Parkinson's disease (23), Alzheimer's disease (33) and Autism spectrum disorder (35). Indeed, a variety of probiotics appear to exhibit psychoactive attributes, producing neuromodulatory factors which can act on host cells (17). The vagus nerve has been implicated as a key component of the signaling axis between the colonic microbiota and the brain (48, 67, 82), however our understanding of the molecular and cellular mechanisms, which facilitate the transmission of a luminally-originating bacterial signal across the gut barrier to the peripheral nervous system, is far from clear.

Microbes and host neurological development.

The importance of gut microbiota in the development of the immune, endocrine and nervous systems, has been established using GF mice. The absence of microbiota in these mice is associated with increased anxiety-like behaviors and alterations in central neurotransmitters, effects which are proposed to be mediated through endocrine mechanisms (14). Elegant electrophysiological studies in GF mice recently demonstrated the importance of the nervous system, as excitability of intrinsic primary afferent neurons, the likely neural starting point of gut-to-brain signaling, was dampened in GF mice (54). Through co-evolution with their hosts, human colonic bacteria have likely developed systems for sensing host-associated signals, including the capacity to identify and respond to human hormones. This enables bacteria to recognize that they are in the vicinity of a suitable host and offers a plausible mechanism of communication between prokaryotes and eukaryotes (26). Moreover, through a mechanism termed 'microbial endocrinology' (44), gut bacteria can also potentially

respond to host signals. Indeed, sensitivity to stress hormones, is reported to underlie the modification of the composition and diversity of the intestinal microbiota (27).

Microbial metabolites and secretory products

Particular strains of *Lactobacillus* and *Bifidobacterium* bacteria can secrete the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (3, 75) or derive it from luminal matter such as monosodium glutamate (3). Other bacterial subtypes have been shown to secrete monoamines such as norepinephrine, dopamine and serotonin (45, 46) and the serotonin precursor, tryptophan (16). Luminal bacteria stimulate synthesis of serotonin in colonic cells, which in turn modulates host GI physiology (87). In addition to these secretory products, major microbial metabolites are short chain fatty acids (SCFAs), including butyrate, acetate and propionate, which are produced during anaerobic fermentation of dietary fiber. Butyrate, has been implicated in microbial-host crosstalk, which may be through specific transporters or receptors or by modulating the immune system and vagus nerve activity (78). Another emerging area of research is the enzymatic modification of bile acids by gut microbes which can modify signals to the host and thereby influence immune homeostasis amongst other functions (38).

Microbes and the host endocrine system

In addition to detecting and responding to ingested nutrients, EECs, which are found embedded in the gut epithelial layer throughout the GI tract, are capable of detecting bacterial by-products. A recent paper by Chimeral and colleagues, demonstrated that GLP-1 secreting L-cells, are capable of sensing indole, a bacterial metabolic product of tryptophan. Indole inhibits voltage-gated K^+ channels in L-cells, resulting in continued depolarization of these

cells, sustained influx of calcium through voltage-gated calcium channels and increased secretion of GLP-1 (13). Additionally, gut bacteria can metabolize prebiotics, which nourish microbes, to form PYY (34), GLP-1, leptin and ghrelin (20).

Visceral afferent endings are sensitive to gut peptides such as GLP-1 and PYY. However, as nerve fibers are thought to terminate before reaching the epithelial layer, nerve fibers are not likely to sense luminal hormones directly (25) However, a neuro-epithelial circuit could act as a conduit for microbe-host signaling with precise, temporal transmission of gut-derived sensory signals and real-time modulatory feedback on EECs (5). Thus, EECs with microbial and GI hormonal biosensing capabilities and neural connections represent a possible means of bridging the gap between microbes and the host.

Conclusions

IBS is a prevalent and debilitating GI disorder, with few effective treatments which target the symptoms rather than the cause. The heterogeneity of this disorder hints at a complex multifactorial pathophysiology and indeed, studies in animal models of IBS and in patients have revealed alterations in endocrine, immune and nerve physiology, with recent evidence that dysfunctional microbiome-gut-brain signaling is also a key player in the manifestation of IBS symptoms (figure 2). In particular, studies implicating gut microbiota in altered CNS physiology and behaviors have resulted in a paradigm shift in our appreciation of the interaction between us and our microbial colonizers. Nonetheless, the molecular and cellular mechanisms employed by the immune, endocrine and neural systems to facilitate communication between gut microbes and the peripheral and central nervous systems are not yet understood and have revealed an emerging avenue of research.

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Figure Legends

Figure 1: IL-6R neutralization and antalarmin alter gastrointestinal dysfunction in WKY rats

A*:* Histogram illustrating the number of fecal pellets excreted by Sprague Dawley (SD, *n* = 7, 7 and 9) and Wistar Kyoto (WKY, *n* = 10, 9 and 11) rats in the open field arena when administered saline, anti-interleukin (IL)-6 receptor antibodies (xIL-6R) or xIL-6R and antalarmin. **B:** Histogram showing the pressure (mmHg) at which SD (*n* = 7, 7 and 9) and WKY (*n* = 10, 9 and 11) rats display pain behaviors in response to colorectal distension when treated with saline, xIL-6R or xIL-6R and antalarmin. **C:** Histogram illustrates the ratios of expression of occludin (n = 5) over the β-actin loading control in mucosal samples from the distal colon of SD and WKY rats treated with saline, xIL-6R and xIL-6R with antalarmin (ant). Asterisks indicate *P < 0.05, **P < 0.01 and ***P < 0.001. Adapted from previously published work (Buckley et al, 2014).

Figure 2: Brain-gut-microbiome signaling

The schematic illustrates potential signaling mechanisms between the luminal microbiota, the intrinsic and extrinsic colonic nerves and the central nervous system with the role of neurocrine, endocrine and immune factors indicated.