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Editor

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Preface

In 1982, two volumes of the *Handbook of Experimental Pharmacology* edited by Professor Giulio Bertaccini, M.D., addressing Mediators and Drugs in Gastrointestinal Motility I and II were published. In 1993, David R. Brown, Ph.D., edited a volume in the *Handbook of Experimental Pharmacology* on Gastrointestinal Regulatory Peptides. Over 20 years later this latest volume of the *Handbook of Experimental Pharmacology* aims to connect current ideas and concepts about gastrointestinal (GI) disorders with the search for novel therapeutics. Towards this goal, the following chapters will provide a timely state-of-the-art overview of the GI tract in health and disease, current treatment approaches and ongoing developments in drug discovery, and their potential for the better treatment of patients with GI disorders. GI disorders rank among the most prevalent disorders, with the most common including esophageal and swallowing disorders, gastric and peptic ulcer disease, gastroparesis or delayed gastric emptying, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD). Some of these disorders are organic involving pathological damage to the GI tract as seen in IBD when the bowel becomes inflamed and damaged, leading to abdominal pain, diarrhea, and rectal bleeding. Other GI disorders such as IBS are termed "functional" disorders because they lack a structural or biochemically defined cause. Recent estimates suggest that one in four people suffer from a functional bowel disorder and they represent 40% of GI problems seen by physicians. The major symptoms of common GI disorders include recurrent abdominal pain and bloating, heartburn, indigestion/dyspepsia, nausea and vomiting, diarrhea, and constipation. Despite GI disorders placing a growing burden on today's healthcare system, many GI disorders are difficult to diagnose and the symptoms are not effectively managed. In addition, many patients with GI disorders do not benefit from the currently available therapeutics. Novel effective therapeutics are thus urgently needed. Currently, there are a limited number of medications available or approved to treat GI disorders due, in part, to a lack of knowledge of the exact mechanisms underlying GI motility, absorption, secretion, inflammation, and sensation. Although significant gaps in the understanding of GI disorders still exist, new therapies are likely to emerge from current research and development. The immune system in the gut is currently offering a wide variety of therapeutic targets to treat IBD, whereas concepts that have emerged to treat GI dysmotility, abdominal pain and IBS, include the

brain-gut axis linking the nervous system in the GI tract to the CNS. The gut microbiome is currently an area of active research. Moreover, our understanding of the gut microbiota remains in its infancy; however major advances linking the intestinal microbiome to the brain-gut axis are likely over the upcoming years and will offer new therapeutic targets for the development of novel drugs to treat GI disorders.

I am immensely grateful to James Barrett for inviting me to serve as the Editor of this volume on Gastrointestinal Pharmacology in the *Handbook of Experimental Pharmacology* book series. I would like to thank the editorial staff from Springer for all their support. Most importantly, the success of this volume on Gastrointestinal Pharmacology is due to each of my colleagues who generously contributed their expertise and time to preparing such outstanding chapters for this volume of the *Handbook of Experimental Pharmacology*. I am indebted to this team of highly distinguished leaders in the GI field. We hope that this volume of the handbook will serve as an essential reference to investigators and scholars involved in basic and clinical GI research as well as individuals treating patients with GI disorders.

Oklahoma City, Oklahoma, USA Beverley Greenwood-Van Meerveld

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Gastrointestinal Physiology and Function

Beverley Greenwood-Van Meerveld, Anthony C. Johnson, and David Grundy

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Abstract

The gastrointestinal (GI) system is responsible for the digestion and absorption of ingested food and liquids. Due to the complexity of the GI tract and the

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substantial volume of material that could be covered under the scope of GI physiology, this chapter briefly reviews the overall function of the GI tract, and discusses the major factors affecting GI physiology and function, including the intestinal microbiota, chronic stress, inflammation, and aging with a focus on the neural regulation of the GI tract and an emphasis on basic brain-gut interactions that serve to modulate the GI tract. GI diseases refer to diseases of the esophagus, stomach, small intestine, colon, and rectum. The major symptoms of common GI disorders include recurrent abdominal pain and bloating, heartburn, indigestion/dyspepsia, nausea and vomiting, diarrhea, and constipation. GI disorders rank among the most prevalent disorders, with the most common including esophageal and swallowing disorders, gastric and peptic ulcer disease, gastroparesis or delayed gastric emptying, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD). Many GI disorders are difficult to diagnose and their symptoms are not effectively managed. Thus, basic research is required to drive the development of novel therapeutics which are urgently needed. One approach is to enhance our understanding of gut physiology and pathophysiology especially as it relates to gut-brain communications since they have clinical relevance to a number of GI complaints and represent a therapeutic target for the treatment of conditions including inflammatory diseases of the GI tract such as IBD and functional gut disorders such as IBS.

Keywords

Absorption • Barrier function • Central nervous system (CNS) • Colon • Constipation • Diarrhea • Digestion • Enteric nervous system (ENS) • Epithelial barrier • Gut microbiome • Inflammation • Inflammatory bowel disease (IBD) • Intestinal permeability • Irritable bowel syndrome (IBS) • Mucosa • Secretion • Small intestine • Smooth muscle • Stress • Visceral pain

1 Introduction

The overall function of the GI tract is to digest ingested nutrients through complex processes of digestive enzyme secretion and nutrient absorption. Luminal contents move along the GI tract via smooth muscle peristalsis, while smooth muscle segmentation ensures adequate contact time and exposure to the absorptive epithelial mucosal surface. The gut is capable of handling about 9 L of fluid per day, which is mainly absorbed by the small intestine. This fluid movement can occur through paracellular or transcellular routes. The former pathway involves water movements coupled to nutrient absorption via alterations in tight junction expression, while the transcellular route involves the passage of water through apical and basolateral membranes of epithelial cells by passive diffusion, cotransport with ions and nutrients, or through aquaporins. During intestinal absorption the epithelial barrier is specifically designed to protect against the movement of potentially

harmful antigenic, toxic, or infectious material across the GI mucosal surface (Camilleri et al. 2012).

To ensure effective digestion and proper GI tract health requires a complex series of coordinated neural events accomplished by the central nervous system (CNS), the nerve network within the gut itself known as the enteric nervous system (ENS), and a whole host of GI endocrine peptides that target specific cells and tissues that make up the GI tract. Specialized endoderm-derived epithelial cells termed enteroendocrine cells (EECs) form the largest endocrine organ in the body and are widely distributed throughout the GI tract. EECs play a key role in the control of GI function including secretion, motility, and regulation of food intake, postprandial glucose levels, and metabolism (Latorre et al. 2016). The gut also performs important immune functions and a vast array of inflammatory mediators can influence the recruitment of lymphocytes and other immune cells to the gut wall including mast cells, and at the same time modulate the activity of the gut neural networks (O'Malley 2015; Wouters et al. 2016). Additionally, the abundance of microbiota residing in the human intestine estimated at 10^{14} microorganisms plays a pivotal role in the development of the ENS, the overall health not only of the GI tract but also the entire human body via mechanisms that include activation of the immune system, and production of short-chain fatty acids (SCFAs) to promote colon cell health as well as brain-gut interactions (Patterson et al. 2014; Kabouridis and Pachnis 2015; Moloney et al. 2016; Obata and Pachnis 2016; Sandhu et al. 2016).

2 Basic Anatomy of the GI Tract

The human GI tract is composed of multiple different organs and can be divided into the upper and lower GI tract. The upper GI tract refers to the mouth, esophagus, stomach duodenum, jejunum, and ileum, while the colon, rectum, and anus make up the lower GI tract. The esophagus propels ingested food from the pharynx into the stomach via a wave of highly coordinated esophageal peristalsis. Once in the stomach, the food bolus is mixed with gastric acid and digestive enzymes and is broken down to allow digested material, now called chyme, to pass through the pyloric sphincter into the duodenum. Once in the small intestine (duodenum, jejunum, and finally ileum) the digestive process breaks down proteins, fats, and carbohydrates to smaller components to allow for nutrient absorption. Accessory organs that aid in the digestive process include the salivary glands, pancreas, liver, and gallbladder. Once the luminal contents reach the large intestine, the contents are now called feces, and are prepared for expulsion via the rectum and anal canal.

To accomplish the digestive processes in a coordinated manner, the GI tract has a functional anatomy that in general terms is composed of a series of layers including the inner mucosal layer of the GI tract composed of absorptive and secretory epithelial cells. The remaining layers of the GI tract include the submucosal layer containing nerves, lymphatics, and connective tissue; the smooth muscle layer composed of longitudinal and circular smooth muscle; and the

outer serosal layer. Specialized ECCs that are diffusely scattered in the GI mucosa possess the capability of sensing the luminal content and in turn release signaling molecules that enter the circulation to act as classic hormones on distant targets, and act locally in a paracrine fashion on neighboring cells and on distinct neuronal pathways including enteric and extrinsic neurons. Each type of EEC has a characteristic distribution along the GI tract. Among the mediators released, cholecystokinin (CCK) and glucagon-like peptide (GLP-1) play important roles in reflex control of GI function and in regulating food intake. EECs are divided into "*open type*" with a bottleneck shape and an apical prolongation with microvilli facing towards the intestinal lumen or "*closed type*" that are located close to the basal membrane, do not reach the lumen of the gut, and lack microvilli. The open-type EECs directly detect luminal contents through the microvilli reaching the lumen, whereas the closed types are thought to be activated by luminal content indirectly either through neural or humoral pathways (Gribble and Reimann 2016; Latorre et al. 2016). This EEC-sensory neuron connection has thus opened a new exciting prospective on EECs and their role in the communication with ENS and CNS and sheds new light on our understanding of the complexity of the bidirectional communication between the brain and the gut. The therapeutic potential of compounds working via EEC function is high. In support a GLP-1R agonist is used to treat diabetes mellitus type 2, based on its ability to stimulate insulin secretion from pancreatic β-cells (van Raalte et al. 2016). Furthermore, in patients that have a gastric bypass the beneficial role of GLP-1 and $PYY_{3\text{-}36}$ secretion in the reduction of food intake may have additional therapeutic potential to treat obesity (Svane et al. 2016). Drugs acting to alter EEC functions may also participate in the control of depression, anxiety, and visceral hypersensitivity, which are key components of functional GI disorders.

2.1 Basic Functions of the GI Tract: GI Motility

The major functions of the GI tract are motility, secretion, and absorption. Smooth muscle tone and contractility are modulated by interstitial cells of Cajal (ICC), which serve as the pacemaker creating spontaneous electrical slow waves that spread from the ICC to the smooth muscle in the presence of a stimulus such as a neurotransmitter leading to contraction of the GI smooth muscle. The reader is referred to excellent reviews of the topic (Ward and Sanders 2001; Sanders et al. 2016). Small and large intestinal motility is under multiple levels of control including the ENS and CNS, as well as GI hormones and paracrine agents. In general, there are two distinct patterns of small intestinal motility (1) following a meal when the intestinal lumen contains chyme and (2) during the inter-digestive period. During the digestive phase the longitudinal and circular smooth muscle of the GI tract generates coordinated patterns of contractility termed peristalsis and segmentation. Peristalsis occurs in waves of contraction behind and relaxation ahead of the luminal bolus, and travels down the GI tract over short distances. Segmentation is a mixing pattern of contractility that is more irregular and allows

for luminal contents and digestive enzymes to have adequate contact with the absorbing epithelium. During the inter-digestive phase, a complex pattern of motility called the migrating motor complex (MMC) sweeps along the entire small intestine to clear the GI tract of any remaining luminal debris. Large intestinal motility patterns serve to impede aboral movement of luminal contents, which facilitates water absorption. Contractility patterns of the colon are predominantly non-peristaltic and mix the colonic contents back and forth to enhance contact with the absorbing mucosa. A less frequent pattern of colonic motility which occurs 6–8 times/day is the high-amplitude propagating contractions (HAPC) which sweep the colon and often trigger the urge to defecate.

2.2 Basic Functions of the GI Tract: GI Secretion and Absorption

The GI tract secretes up to 9 L of fluid/day containing digestive enzymes, bile, ions, water, and mucus. Important for secretion and absorption of fluids, electrolytes, and solutes are the epithelial cells which differ in structure and function depending on their location in the GI tract. The stomach is a glandular organ. Gastric parietal cells in glands within the gastric body are important for the secretion of gastric acid and intrinsic factor, pepsinogen is secreted by the chief cells also within the gastric body, while hormones (gastrin, histamine, serotonin, and somatostatin) are released from EEC throughout the stomach. Most of the digestive process and intestinal absorption of food and electrolytes occur in the duodenum, jejunum, and ileum. Proteins, fats, and carbohydrates are broken down via the action of digestive enzymes into smaller units in preparation for absorption into the network of capillaries and lymphatic vessels (lacteals) by the small intestinal epithelial cells located on the small intestinal villi. Any remaining material that is not absorbed by the small intestine passes though the ileocecal valve into the colon. The large intestinal mucosa is responsible for the absorption of water, solidification of the colonic contents into feces, and then storage of the feces prior to expulsion.

2.3 Basic Functions of the GI Tract: GI Barrier Function

Solute and particulate matter moves across the intestinal epithelium in a regulated manner either between epithelial cells or across the apical membrane of epithelial cells. Routes of transport across the epithelium include passive permeability (relevant for the passage of larger hydrophilic compounds), transcellular transport of lipophilic and small hydrophilic compounds, transcellular route via aqueous pores of small hydrophilic compounds, and active carrier-mediated absorption of nutrients and electrolytes, and endocytosis, followed by transcytosis and exocytosis of larger peptides, proteins, and particles. Transport between epithelial cells occurs via the tight junction region. Far from being static, tight junctions are continually being monitored and regulated by both intra- and extracellular signals. Several types of proteins contribute to the development of tight junctions including

the claudin family of proteins that form the actual paracellular pore within the tight junction and are associated with other transmembrane proteins called occludins. Zonula occludens (ZO)-1 and other cytoplasmic proteins, such as ZO-2 and ZO-3, attach and serve as junctional complex proteins. Also relevant to the barrier properties are adherens, junctions that are defined as a cell junction where the cytoplasmic face is linked to the actin cytoskeleton. At the basal pole of the intercellular space, desmosomes are formed by interactions between desmoglein, desmocollin, desmoplakin, and keratin filaments (Camilleri et al. 2012; Volynets et al. 2016). An important function of the gut epithelium is its protective role, functioning as a barrier between the external environment and the internal milieu. Several components form the multilayered intestinal barrier that is region specific; in the upper GI tract, gastric acid, pancreatic juice, and production of antimicrobial substances serve as a first line of defense. Next, the microenvironment close to the epithelium consists of the unstirred water layer, glycocalyx, and mucus layer, and below are epithelial cells separated by tight junctions. Under pathophysiological conditions, increases in epithelial permeability allow products to translocate the epithelial barrier including luminal antigens, toxins, and microbial fermentation. These products are capable of activating afferent nerve endings leading to visceral afferent sensitization, which is important since several different human diseases, including IBD, celiac disease (CD), and IBS, have been associated with increases in gut permeability and abnormal barrier function (Camilleri et al. 2012; Ohman et al. 2015).

3 Neural Control of the GI Tract

The neural innervation of the GI tract allows for the movement of contents along the GI tract, secretion of digestive enzymes, and absorption of luminal contents and makes certain that information from GI tract is carefully integrated and that the appropriate reflex responses are generated to ensure that all parts of the digestive system function in concert.

3.1 Enteric Nervous System

Contained within the gut wall is the ENS that is a subdivision of the autonomic nervous system (ANS) and is capable of autonomous activity. The ENS contains many of the transmitters and neuromodulators found in the CNS and is organized into specific circuits that control smooth muscle and mucosal function. These circuits within the ENS allow for the routine mechanisms of digestion to proceed without the involvement of the CNS. The ENS contains sensory neurons, motor neurons, and a complex number of interneurons that enable the information from the GI tract to be carefully integrated. Within the ENS, intrinsic primary afferent neurons are synaptically connected to interneurons that control the activity of

motor neurons regulating the GI musculature and secretomotor neurons innervating secretory cells.

3.2 Extrinsic Innervation of the GI Tract

The gut also has a dense extrinsic afferent innervation that transmits sensory information to the brain and spinal cord and is used as a basis of reflexes through parasympathetic and sympathetic nerves. Sensory information is conveyed from the GI tract to the brain stem and spinal cord via vagal and spinal (splanchnic and pelvic) afferents, respectively. Gut afferents are also involved in nausea and vomiting, feeding, and satiety, and in addition generate sensations particularly under pathophysiological circumstances when the bowel can become hypersensitive leading to visceral pain. Within the GI tract, extrinsic nociceptors can respond to multimodal stimuli, depending on receptor expression, including stretch, pH, bacterial products, substances released from immune cells, and neurotransmitters released from the ENS or ECCs. The nociceptors have nerve endings throughout the layers of the GI tract (mucosal, submucosal, muscular) and their cell bodies are located in the dorsal root ganglion (DRG). The first synapse is in the superficial layers of the dorsal horn of the spinal cord. The nociceptive signal is then transmitted to the spinal cord and pain signals reach the brain via the spinothalamic tract and dorsal column. Within the brain, the signal is then relayed to cortical areas for localization and to limbic areas for the emotional component of the pain response. Output from the cortical and limbic regions in response to the pain of GI origin activates descending inhibitory circuitry within the brain stem that causes release of inhibitory neurotransmitters within the dorsal horn of the spinal cord. Although vagal afferents were not previously thought to be involved in the mediation of visceral pain, evidence suggests a role for vagal transmission of anti/pronociceptive signals, which bypasses the spinal cord.

3.3 Visceral Afferent Sensitization

Peripheral sensitization normally develops rapidly and is relatively short-lived. Visceral afferent sensitization can occur in response to increases in epithelial permeability which allows luminal antigens, toxins, and microbial fermentation products to translocate the epithelial barrier to activate afferent nerve endings. Furthermore, intestinal inflammation and release of immune mediators at the site of injury can produce long-term alterations in the physiology of the afferent terminals. Mediators such as cytokines, prostaglandins, histamine, proteases, and/or low pH at the site of an acute injury activate receptors on the afferent terminals to increase intracellular second messengers causing the release of neurotransmitters, such as substance P, calcitonin gene-related peptide, and/or nitric oxide, which can further sensitize visceral afferents. The second messenger systems (protein kinase A, protein kinase C) also lead to changes in gene expression that induces

neuronal plasticity to alter the expression of receptors (neurokinin receptor 1, tyrosine receptor kinase A, prostaglandin receptor, protease-activated receptors, etc.) that leads to persistent changes in the excitability of the neuron, through modifying the expression or function of ion channels (sodium, calcium, and potassium).

3.4 Central Sensitization

In the presence of maintained injury or inflammation, visceral afferent sensitization can be prolonged by changes in gene expression. These genes may alter the expression of channels, receptors, or mediators in the sensory neuron. They may also modify the amount and pattern of neurotransmitters released by central nerve terminals in the brain and spinal cord. This alters the way that sensory signals are processed within the CNS and contributes to "central sensitization" which involves neuronal remodeling within the dorsal horn of the spinal cord and brain. Such spinal and supraspinal neuroplastic changes likely contribute to chronic pain. Within the dorsal horn of the spinal cord, there are two mechanisms that increase pain signals reaching the brain: (1) increased synaptic transmission and/or (2) decreased descending inhibitory modulation. Thus, a combination of increased excitation and disinhibition can produce a persistent hyperexcitable state in the second-order neuron and chronic nociceptive signaling. The increased synaptic transmission occurs via glutamate to activate a second-order neuron, causing activation of sodium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors followed by the subsequent activation of sodium and calcium-permeable *N*-methyl-D-aspartate (NMDA) receptors. Primary afferents also release algesic mediators, such as substance P, which activates second messenger signaling that initiates remodeling of the second-order neuron by the primary nociceptor leading to changes in the properties of the receptors present in the dendritic structure of the second-order neuron (Woolf and Salter 2000). Decreased descending inhibitory modulation at the level of the spinal interneurons leads to hypersensitivity within the dorsal horn of the spinal cord via the release of neurotransmitters from the primary nociceptive afferent activating presynaptic receptors on the inhibitory interneuron, causing hyperpolarization of the inhibitory interneuron and a decreased release of gamma-aminobutyric acid (GABA) and/or glycine onto the second-order neuron. In the brain a similar mechanism to produce chronic visceral pain can be invoked within the central pain matrix in the brain by which ascending afferent information from the second-order spinal neurons leads to hyperexcitability within the central pain matrix by increasing the sensory signals reaching the tertiary neurons within the thalamus, raphe, or parabrachial nucleus, which then enhances signaling to secondary cortical and limbic structures. Remodeling of integration nuclei (amygdala, ACC/MCC, insula) can make the perception of the noxious stimulus more unpleasant, producing an enhanced negative emotional response (Staud 2012). Imaging studies have demonstrated that in patients with chronic visceral pain, there is increased activation of brain regions that

integrate pain signals and produce negative affect, such as the amygdala and insula, along with a decreased activity in pain inhibitory/positive affect nuclei, such as the prefrontal cortex (PFC) and cingulate. In particular, the amygdala is a key nucleus that integrates noxious visceral signals with anxiety/fear behaviors and hyperactivation could influence not only multiple nuclei in the central pain matrix, but also descending brainstem nuclei that modulate GI function. Additionally, the altered signaling within the central pain matrix disrupts the descending dorsal column inhibitory pathway by modulating the activity in the periaqueductal gray (PAG) and rostroventral medulla (RVM). Recently, a significant amount of attention has been given to a subgroup of EECs secreting 5-HT and their role in visceral perception. 5-HT is a key neurotransmitter in the control of nociceptive responses and mood, with receptors located in both the periphery and CNS. Much evidence suggests that activation of 5-HT receptors, specifically 5-HT_3 and 5-HT_4, sends signals from the gut to the CNS through vagal afferent fibers, which activate multiple pathways including those involved in nociception (Chen et al. 2009; Zhang et al. 2011; Browning 2015).

4 GI Pathophysiology

Proper functioning of the GI tract is essential for supporting life; however disorders of the GI tract are common, unpleasant, and complex affecting the mucosa, musculature, and neural innervation from the esophagus to the colon, and manifested as ulceration, inflammation, obstruction, diarrhea, constipation, and abdominal pain. A growing body of evidence suggests that defects in intestinal barrier function are associated with diseases of the GI tract. Abnormalities in GI function can lead to life-threatening diseases such as IBD or conditions that severely affect quality of life such as gastroesophageal reflux disease (GERD) and IBS. The symptoms of common GI disorders are worsened during periods of stress and negative emotions. Stress has profound effects on the GI tract with both chronic adult stress and early-life stress being capable of modifying central pain circuitry, as well as causing changes in motility and permeability throughout the GI tract. A generally accepted hypothesis is that dysfunction of the bidirectional communication between the brain and the gut in response to chronic stress activates the hypothalamic pituitary (HPA) axis and autonomic nervous system and plays a role in the symptomatology of functional GI disorders such as IBS. Inflammation of the gut mucosal surface has substantial effects on enteric and extrinsic afferent neuronal function though complex changes in neuroimmune interaction. In rodent models, acute colitis alters enteric neuronal function and evidence suggests that these changes are persistent despite recovery from the gut inflammation. Although the effects of an intestinal inflammation on the CNS are less clearly understood, patients with IBD exhibit centrally mediated comorbidities including anxiety, depression, and fatigue, which strongly suggests altered brain function in response to peripheral inflammation perhaps through alterations in central immune-mediated mechanisms. Evidence supporting this concept has been derived from experimental

models of acute colitis in which transient inflammation leads to long-term visceral pain as well as long-term altered expression of CRF in the paraventricular nucleus of the hypothalamus (PVN) (Greenwood-Van Meerveld et al. 2006). Recent evidence points to changes in the gut microbiota playing a key role in GI disorders. Specifically, disorders directly affecting the GI tract such as IBS and CD have been shown to exhibit microbial dysbiosis. Gut inflammation causes marked alterations in the gut microbiota populations and may play a role in gut-brain miscommunication. Another important factor altering the physiology of the GI tract is age. The normal functioning of the gut is compromised as we age, with the elderly often complaining of constipation, hemorrhoids, heartburn, decreased energy, and food allergies. In nonhuman primate models, aging was shown to have profound effects on the intestinal epithelial barrier and the neural control of smooth muscle contractility (Tran and Greenwood-Van Meerveld 2013, 2014). The impact of aging on the intestinal barrier and immune system was recently reviewed (Man et al. 2014). There are also numerous animal studies showing that aging has significant effects on the enteric and extrinsic neural innervation of the GI tract (reviewed by Bitar et al. 2011). Understanding the effects of aging on the gut is of growing and profound importance in light of demographic data demonstrating a steady increase in the aging population.

4.1 Stress and the GI Tract

There is a substantial amount of compelling evidence that psychological and physical stressors play an important role in the onset and modulation of GI disorders. It is a generally accepted hypothesis that dysfunction of the bidirectional communication between the brain and the gut, in part through activation of the principal neuroendocrine stress system, namely the HPA axis, plays a role in the symptomatology of IBS. The HPA axis is activated by stress causing the release of CRF from the paraventricular nucleus of the hypothalamus (PVN) into the hypophyseal portal circulation to bind in the anterior pituitary. Adrenocorticotropic hormone (ACTH) is then released from the pituitary into the systemic circulation to cause the synthesis and release of the glucocorticoid cortisol (*corticosterone in rats*) from the adrenal cortex. Multiple lines of evidence have shown that activation of central mechanism(s) by stress results in colorectal hypersensitivity, and involves descending facilitation from the brain to induce remodeling of colorectal responsiveness via sensitization of spinal dorsal horn neurons. Brainstem regions responsible for the modulation of descending inhibitory pain signals are modulated by both pain and stress. The periaqueductal gray (PAG) receives excitatory signaling from the prefrontal cortex (PFC) and inhibitory signaling from the amygdala. The rostroventral medulla (RVM) receives not only direct nociceptive information from the spinoreticular pathway but also integrated pain and stress signals from the amygdala and PAG. Additionally, the locus coeruleus (LC) and amygdala form a circuit that can potentiate both endocrine and autonomic stress responses. Central structures regulating affective and sensory processes including the amygdala, insula, cingulate, and prefrontal cortex show enhanced activation in IBS patients.

In animal models with visceral hypersensitivity and in IBS patients, imaging studies have shown that limbic regions regulating sensory processing and emotion, including the amygdala, show greater responsiveness in response to visceral stimulation. The amygdala is an important limbic structure involved in the potentiation of the HPA axis with diffuse connections to pain-modulatory networks, and has been implicated to influence visceral sensitivity and may contribute to the aberrant HPA activity observed in IBS patients. The amygdala is sensitive to corticosteroids but in contrast to the hippocampus and prefrontal cortex, the amygdala *facilitates* behavioral, neuroendocrine, and autonomic responses to stress. Thus, this altered balance in stress modulation induced by amygdala hyperactivity may represent an essential aspect of alterations in GI motor function, colonic permeability, and colorectal sensitivity apparent in IBS. In support, elevating amygdala corticosterone in rats by stereotaxically implanting corticosterone micropellets onto the CeA causes a persistent increase in the sensitivity to visceral stimuli as well as induces anxiety-like behavior (Greenwood-Van Meerveld et al. 2001). These findings suggest that in IBS patients exposed to chronic stress increased amygdala activation dysregulates the HPA axis and may be particularly relevant to the etiology and pathophysiology of IBS. Evidence also suggests that remodeling of the epigenome by chronic stress may result in long-term changes in gene expression. Recent studies have also demonstrated the importance of histone acetylation in stress-induced pain of GI origin by showing that direct administration into the brain of a histone deacetylase (HDAC) inhibitor reversed visceral hypersensitivity induced by stress or activation of the amygdala with corticosterone (Tran et al. 2013, 2015). In another study exposure to early-life stress (ELS) was associated with CRF promoter hypomethylation and an increase in CRF transcriptional responses to stress in adulthood suggesting that neonatal stress is capable of causing long-lasting epigenetic changes in the CRF expression within the HPA axis (Chen et al. 2012). During stress and inflammation mast cell mediators such as TNF-α, tryptase [via protease-activated receptor type 2 (PAR-2)], nerve growth factor (NGF), and interleukins may affect paracellular permeability (by altering expression of claudins in the tight junctions) or transcellular uptake route (by increasing macro-pinocytosis), thereby disrupting the barrier to antigens and bacteria. The release of serine proteases from mast cells results in the activation of PAR-2 on epithelial cells; further, activation of PAR-2 has been linked with tight junction disassembly and increased permeability. It is thought that this increase in permeability in response to stress activates and sensitizes sensory nerves within the gut and this barrage of afferent information leads to peripheral and central sensitization to produce visceral hypersensitivity.

4.2 Gut Immune System

The GI tract has a complex innate and adaptive mucosal immune system that is capable of monitoring the luminal content for a diverse array of innocuous antigens including commensal microbiota and food antigens (oral tolerance) versus invasion of the host by potentially toxic pathogens. The immune cells that reside in the intestine mucosa, mesenteric lymph nodes, and Peyer's patches make up the

gut-associated lymphoid tissue (GALT). Cells of the GALT, dendritic cell, macrophages and B-cells make up the antigen-presenting cells and shape the responses of a heterogeneous population of T cells. Such response can be tolerogenic against commensal bacterial antigens or immunogenic against invading pathogens. Together, the cells of the GALT play a role in both innate and adaptive immunity and are pivotal for maintaining immune homeostasis in the gut. The maintenance of a delicate balance between tolerance and immune system activation is key for overall gut health with abnormalities in this equilibrium leading to pathologies of the gut including IBD, CD, and food intolerances. The complexity of the gut immune system is beyond the scope of this chapter; however, the reader is referred to excellent reviews by Mann and Li (2014); Reboldi and Cyster (2016); and Vitale et al. (2016).

4.3 Effect of Aging on the GI Tract

Disorders of the GI tract are common in the elderly; however the precise trait(s) of aging that contribute to the vulnerability of the GI tract are poorly understood. Despite the need to further understand age-associated factors that increase the susceptibility to GI dysfunction, there is a paucity of studies investigating the key factors in aging that affect the GI tract. Thus far studies in rodents have demonstrated that aging alters intestinal smooth muscle contractility (O'Mahony et al. 2002), as well as the neural innervations of the GI tract musculature (Phillips and Powley 2007) and sensory signaling (Keating et al. 2016). Several studies in rodents have also reported an increase in intestinal permeability to macromolecules with age (Hollander and Tarnawski 1985; Katz et al. 1987; Ma et al. 1992; Annaert et al. 2010). Specifically, advancing age was shown to correlate with an enhanced transepithelial permeability of D-mannitol, indicating that there may be an age-associated decline in barrier function (Mullin et al. 2002). Our latest findings showed that a pivotal contributing factor to geriatric vulnerability to GI dysfunction may be increased colonic permeability via age-associated remodeling of intestinal epithelial tight junction proteins. We found that epithelial permeability was greater in colonic biopsies isolated from older baboons (Tran and Greenwood-Van Meerveld 2013). Supporting this observation, we discovered that there is significant tight junction remodeling including a decrease in ZO-1, occludin, and JAM-A proteins, and an increase in claudin-2 expression in old baboon colon compared to young. Upon investigation of the potential mechanisms that may be responsible for age-associated changes in tight junction protein expression, we found an increase in miR-29a, but no observable differences in GLUL expression. We also measured an elevated level of pro-inflammatory cytokines, in the absence of overt inflammation as assessed via routine histology and MPO activity, supporting the claim that inflammatory cytokines modulate intestinal permeability through regulation of tight junction protein expression and trafficking. Advanced age is associated with reduced neurotransmitter content and expression. It has been shown that spinal levels of calcitonin gene-related peptide (CGRP) and substance P (SP) are decreased in aged rats compared to younger animals, as is the expression of dopamine and serotonin receptors. Available data suggests that

advanced age likely has differential effects on subpopulations of neurons in the ENS which demonstrate regional- and species-specific differences. In summary, aging has profound effects on the GI tract and future research is required in relevant animal models to delineate the mechanisms responsible for age-related pathologies of the GI tract.

5 Summary and Conclusion

The GI tract is a complex organ and is responsible for the effective digestion and nutrient absorption. To accomplish this undertaking the GI tract has specialized and region-specific anatomical, histological, and functional diversities that are controlled by a complex interaction between neuronal, hormonal, and paracrine elements. Under pathophysiological conditions, disorders may involve pathological damage to the GI tract as apparent in IBD where the GI tract becomes inflamed and damaged, leading to abdominal pain, diarrhea, and even rectal bleeding. In contrast, other GI disorders, such as IBS, lack a structural or biochemically defined abnormality and are termed "functional" disorders. Currently, there are a limited number of medications available to treat GI disorders in part due to a lack of knowledge of the exact mechanisms underlying the complex physiology of GI motility, absorption, secretion, inflammation, and sensation. Although these significant gaps in the understanding of GI disorders exist today, it is likely that new therapies will emerge from current basic and translational research. Since alterations in the bidirectional communication between the brain and the gut are likely associated with an impairment of gut functions, approaches that have emerged to treat GI dysmotility, abdominal pain, and IBS include mechanisms linking the nervous system in the GI tract to the CNS. Another area of active research is the gut microbiome, and although our understanding of the microbiota within the gut remains in its infancy, major advances linking the intestinal microbiome to the brain-gut axis will likely offer new therapeutic targets for the development of novel drugs to treat GI disorders. Another approach will be to focus on intestinal barrier dysfunction which has been associated with many GI disorders. EEC receptors on the luminal side could also be a potential target of new drugs to activate hormonal and neuronal pathways providing a novel approach to treat diseases such as diabetes and obesity.

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Upper GI Disorders: Pathophysiology and Current Therapeutic Approaches

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Abstract

Symptoms referable to the upper digestive tract are associated with abnormalities of upper gastric neuromuscular function including abnormalities of motility, sensation, and absorption. Of the upper digestive tract, the stomach is of particular importance in its role in symptom generation and is highlighted in this chapter. Gastric symptoms can be associated with alterations in the rates of gastric emptying, impaired accommodation, heightened gastric sensation, or alterations in gastric myoelectrical activity and contractility. Treatment of gastric neuromuscular disorders requires an understanding of pathophysiology of the disorders, the appropriate use and interpretation of diagnostic tests, and the knowledge of effective treatment options. This chapter covers the pathophysiology and current treatment approaches to disorders of the upper gastrointestinal tract, focusing on classic disorders of the stomach, particularly gastroparesis and functional dyspepsia.

Keywords

Dumping syndrome • Functional dyspepsia • Gastroparesis

1 Introduction

Symptoms referable to the upper digestive tract are associated with abnormalities of upper gastric neuromuscular function including abnormalities of motility, sensation, and absorption (Boeckxstaens et al. 2016). The stomach is of particular importance in the upper digestive tract. Gastric symptoms can be associated with alterations in the rates of gastric emptying, impaired accommodation, heightened gastric sensation, or alterations in gastric myoelectrical activity and contractility (Parkman and Jones 2009). Treatment of gastric neuromuscular disorders requires an understanding of pathophysiology, appropriate use and interpretation of diagnostic tests, and treatment options (Parkman et al. 1995; Camilleri et al. 1998). These tests include measures of gastric emptying, contractility, electrical activity, regional gastric motility of the fundus, antrum, pylorus, and tests of sensation and compliance. In addition to these gastric function tests, tests are being developed to help better understand the afferent sensory pathways from the stomach to the central nervous system that mediate gastric sensation. This chapter covers the pathophysiology and current treatment approaches to disorders of the upper gastrointestinal tract, focusing on gastric disorders, particularly the classic gastric disorder of gastroparesis.

2 Normal Gastric Neuromuscular Physiology

Normal gastric physiology is best described in terms of its patterns in the fasting state and the responses of the stomach that occur in response to food intake (the fed response) (Camilleri et al. 1998; Camilleri 2006).

2.1 Fasting Gastric Motility

Fasting gastric contractile patterns are characterized by a cyclic motor phenomenon called the migrating motor complex (MMC) (Camilleri et al. 1998). In healthy people, it occurs approximately once every 90 min in the fasting state, most prominently at night. The fasting state generally starts approximately 4 h after meal ingestion when the stomach has completely emptied a meal. The fasting contractile patterns comprise a period of quiescence (phase I), a period of intermittent pressure activity (phase II), and an activity front, during which the stomach and small intestine contract at their highest frequency (phase III). During the phase III MMC, contraction frequencies reach 3 per minute in the stomach and 12 per minute in the proximal small intestine. This interdigestive contraction wave migrates down the stomach and small intestine and serves to help empty the stomach of indigestible solids and transport them down the small intestine into the colon ("the intestinal housekeeper"). These contractile pressures, especially in the fasting period, are generally recorded in patients with antroduodenal manometry.

2.2 Gastric Responses to Meal Ingestion

2.2.1 Gastric Accommodation

Gastric accommodation is a postprandial, vagally mediated reflex resulting in reduced gastric tone primarily in the proximal stomach that occurs with eating a meal (Cannon and Lieb 1911). Gastric accommodation provides a reservoir for ingested foods without a significant increase in intragastric pressure.

The accommodation reflex has two principal components. *Receptive relaxation* occurs within seconds of eating and is triggered by both oropharyngeal and gastric stimulation. This response involves relaxation of both the lower esophageal sphincter and proximal stomach. *Adaptive relaxation* is a slower process triggered by gastric or duodenal distension and perhaps also modified by specific nutrients (Villanova et al. 1997; Jahnberg et al. 1975). The accommodation reflex is vagally mediated and represents the balance between cholinergic excitatory drive and non-adrenergic non-cholinergic (NANC) inhibitory input. The afferent signal is generated by activation of stretch-sensitive mechanoreceptors in the stomach wall and by activation of osmo- and chemoreceptors in the stomach and duodenum (Feinle et al. 2001). The efferent NANC signal involves nitric oxide (NO) as the principal neurotransmitter (Boeckxstaens et al. 2002; Tack et al. 2002a) and perhaps vasoactive intestinal polypeptide (VIP) (Tonini et al. 2000). Gastric tone is also modulated by sympathetic inputs acting directly through post-junctional α1-adrenoceptors, and indirectly on cholinergic nerve terminals mediated by pre-junctional α2-adrenoceptors (Boeckxstaens et al. 2002; Tack et al. 2002a; Thumshirn et al. 1999a).

The accommodation reflex is most often recorded using either a gastric barostat or imaging methods such as scintigraphic radiolabelling of the gastric mucosa with the use of single-photon emission computed tomography (SPECT). The roles for various nutritional parameters in modifying accommodation such as meal volume and rate of ingestion, caloric density, and macronutrient content require more understanding as this might have important clinical relevance.

2.2.2 Gastric Emptying of a Meal

Normal gastric emptying reflects a coordinated effort between the fundus, antrum, pyloric sphincter, and duodenum (Parkman et al. 1995; Camilleri et al. 1998). Coordination of these fundic-antral-pyloric-duodenal motor events is carefully regulated and governed by gastrointestinal electrical activity through the interstitial cells of Cajal (ICCs) and neural connectivity through enteric nerves and vagal efferent nerves from the central nervous system. Feedback from nutrients and volume in the stomach and small bowel impact the process of gastric emptying and are conveyed through local enteric sensory nerves, vagal afferent nerves, and hormones.

Fundic and antral smooth muscle contractions are primarily cholinergically mediated. Rhythmic antral contractions, classically at 3 cycles/minute, triturate large food particles into an appropriate size for intestinal digestion. The rate of these contractions is governed by the electrical pacemaker area of the stomach and the pacemaker cells, the interstitial cells of Cajal (ICCs).

Pyloric sphincter relaxation, often synchronized with antral contractions, allows smaller food particles and chyme to pass out of the stomach into the duodenum (Camilleri et al. 1998). Pyloric relaxation is mediated through release of inhibitory nerves, especially nitric oxide (NO) and possibly vasoactive intestinal polypeptide (VIP).

Solid and liquid food empty from the stomach at different rates (Camilleri et al. 1998). Liquids empty from the stomach at an exponential rate as their emptying depends primarily on the gastric–duodenal pressure gradient with less importance on pyloric opening. Solids are initially retained selectively within the stomach until particles have been triturated to a size smaller than 2 mm, at which point they can be emptied at a linear rate from the stomach.

2.3 Gastric Sensation

The digestive tract senses ingested meals in various ways (Parkman and Jones 2009). Volume, osmolarity, acidity, and macronutrient composition represent the dominant sensory modalities. Most of this sensory information is acted upon solely by the enteric nervous system to facilitate secretion, absorption, and motility through the gut and never reaches the level of consciousness. Some awareness of digestive sensation such as fullness and satiation helps regulate normal eating behavior. Visceral sensation is transmitted from the digestive tract to the central nervous system primarily via the vagus nerve and spinal afferent system.

Afferent vagal neurons project mainly to the solitary tract nucleus with secondary projections ascending to the thalamus and directly to other brain structures involved in arousal, homeostatic, and emotional behaviors (Sawchenko 1983; Aziz and Thompson 1998). These regions include the hypothalamus, locus coeruleus, amygdala, and periaqueductal gray (PAG). Third-order neurons project from the thalamus to the sensory cortex.

Primary spinal visceral afferent nerves synapse in the dorsal horn of the spinal cord with secondary neurons projecting proximally through the spinoreticular, spinomesencephalic, spinohypothalamic, and spinothalamic tracts (Almeida et al. 2004; Drossman

2004; Jones et al. 2006). Spinoreticular inputs activate reflexive responses to visceral sensation without conscious awareness. The spinothalamic tract projects to the ventral posterior lateral, medial dorsal, and ventral medial posterior nuclei of the sensory thalamus, from which tertiary neurons relay digestive sensory signals to the primary somatosensory cortex (S1 and S2), the cingulate cortex, and the insula, respectively.

3 Gastric Symptoms and Pathophysiology

Symptoms may originate by four types of pathophysiological mechanisms: delayed gastric emptying, impaired accommodation, increased perception (hypersensitivity), or accelerated gastric emptying (Table 1) (Boeckxstaens et al. 2016). This has recently been elegantly reviewed by Azpiroz and colleagues for Rome IV Pathophysiology (Boeckxstaens et al. 2016) and is discussed in this section. The symptoms from gastric dysfunction are limited and the symptoms may be similar in character regardless of the underlying pathophysiological mechanisms involved.

3.1 Delayed Gastric Emptying

Gastric emptying reflects the net output of the stomach, which is regulated by three areas of the stomach: proximal fundus, distal antrum, and pyloric sphincter (Malagelada and Azpiroz 1989). Neural and hormonal pathways from the small intestine also influence gastric emptying. The tonic contraction of the proximal stomach pushes gastric contents distally into the antrum. Impaired tonic contraction of the proximal stomach may result in a delay in the emptying of both solids and liquids. Phasic antral contractions produce the breakdown of solid particles necessary for passage through the pylorus into the small intestine; impaired antral contractions result in the delayed emptying of solids (Camilleri et al. 1998).

Delayed gastric emptying produces symptoms especially from retention of food or chyme in the stomach. The symptoms vary from mild symptoms such as early satiety, epigastric fullness, and nausea to severe manifestations with vomiting of food, often

Table 1 Pathophysiology involved in gastric disorders

Delayed gastric emptying
Gastroparesis
Functional dyspepsia
Impaired accommodation
Functional dyspepsia
Rapid gastric emptying
Dumping syndrome
Cyclic vomiting syndrome
Post fundoplication
Functional dyspepsia
Increased perception/hypersensitivity
Functional dyspepsia

ingested many hours or even days earlier, and nutritional compromise. Absence of fasting gastric phase III MMC activity may result in gastric bezoar formation. Interestingly, nausea and vomiting may occur in some patients during fasting rather than postprandially, especially seen in diabetic patients (Parkman et al. 2016). In some patients, this may lead to inability to eat from symptoms with resultant weight loss. Delayed gastric emptying in the absence of mechanical obstruction is the definition of the disorder gastroparesis (Grover et al. 2012; Janssen et al. 2013). Some patients with functional dyspepsia exhibit a delay of solid emptying.

3.2 Impaired Accommodation

Impaired accommodation of the proximal stomach in response to food ingestion increases gastric wall tension which might activate sensory endings in the gastric wall and produce symptoms. Inappropriate relaxation might be related to impaired enterogastric and antrofundic reflexes that normally modulate the gastric accommodation/emptying process (Azpiroz and Malagelada 1987; Farre and Tack 2013). Impaired fundic accommodation/reduced proximal gastric relaxation in response to a meal can be seen in some patients with functional dyspepsia and has been reported to be associated with early satiety and weight loss (Tack et al. 2001). Impaired accommodation is associated with abnormal intragastric distribution of food in patients with functional dyspepsia, with preferential accumulation in the distal stomach (antrum) (Troncon et al. 1994).

3.3 Increased Gastric Sensitivity

Distending the stomach can produce conscious sensations similar to the symptoms reported by patients with gastric functional disorders. Perception of gastric distension depends on activation of tension rather than elongation or volume receptors in the gastric wall (Distrutti et al. 1999). Some patients with functional dyspepsia exhibit increased perception of gastric distension or hypersensitivity of the stomach (Coffin et al. 1994). Gastric hypersensitivity is more prevalent in patients with predominant epigastric pain (Karamanolis et al. 2006). Gastric hypersensitivity can coexist with impaired gastric accommodation to meal ingestion and delayed gastric emptying.

The cause and mechanism of gastric hypersensitivity are not known. In normal conditions, gastric sensitivity is modulated by several mechanisms. For example, lipids in the small intestine increase perception of gastric distension. This modulatory mechanism is up-regulated in patients with functional dyspepsia, and may contribute to symptoms. Altered perception in a subset of patients with dyspepsia appears to occur as a consequence of an acute, possibly viral, gastroenteritis, which leads to impaired nitrergic nerve function in the proximal stomach (Tack et al. 2002b). Central mechanisms may also play a role. For example, anxiety is negatively correlated with pain and discomfort threshold in hypersensitive functional dyspeptic patients (Van Oudenhove et al. 2007).

3.4 Accelerated Gastric Emptying

In some patients, mainly after partial or complete gastrectomy, rapid gastric emptying is accompanied by vasomotor and gastrointestinal symptoms. This dumping syndrome may be observed after vagotomy, intentional or unintentional at the time of surgery at the gastroesophageal junction. Symptoms typically occur after ingestion of liquids and meals with increased carbohydrates. Dumping symptoms can be subdivided into "early dumping" and "late dumping" symptoms. "Early dumping" occurs in the first hour after meal ingestion and is associated with both abdominal and systemic symptoms due to the rapid passage of hyperosmolar contents into the small bowel leading to a shift of fluids from the intravascular compartment to the gut lumen. This induces intestinal distension and gastrointestinal symptoms like bloating, abdominal pain, and diarrhea (Vecht et al. 1997). Enhanced release of several gastrointestinal hormones, including enteroglucagon, vasoactive intestinal polypeptide, peptide YY, pancreatic polypeptide, and neurotensin, are thought to cause a systemic and splanchnic vasodilation, most likely explaining the vasomotor symptoms. Late dumping occurs 1–2 h postprandially and results from reactive hypoglycemia. Rapid gastric emptying induces high glycemic levels, which lead to increased insulin secretion. Because of the long half-life of insulin, late reactive hypoglycemia may occur.

4 Disorders Associated with Altered Gastric Motility and Function

4.1 Gastroparesis

Gastroparesis is a symptomatic chronic disorder of the stomach characterized by delayed gastric emptying in the absence of mechanical obstruction. Symptoms of gastroparesis are variable and include early satiety, postprandial fullness, nausea, vomiting, abdominal distension, and upper abdominal discomfort (Parkman et al. 2004). Delayed gastric emptying is also common in functional dyspepsia, occurring in approximately 25–40% of patients (Stanghellini et al. 1996; Sarnelli et al. 2003).

The correlation between symptoms and delayed gastric emptying is variable (Horowitz et al. 1989; Koch et al. 1989; Talley et al. 1989). Postprandial fullness, nausea, and vomiting have been reported to predict delayed emptying in patients with functional dyspepsia (Stanghellini et al. 1996; Sarnelli et al. 2003). In patients with diabetes, abdominal fullness and bloating were found to predict delayed gastric emptying (Jones et al. 2001). In some drug trials of prokinetic agents, symptom improvement correlated with acceleration of gastric emptying (Camilleri et al. 1989; Jian et al. 1989); however, in other studies, this relationship has not been demonstrated (Snape et al. 1982; McCallum et al. 2007). In individuals with symptoms of gastroparesis who have normal rates of gastric emptying, other motor, myoelectric, or sensory abnormalities may elicit symptoms (Parkman et al. 1995). Perhaps different pathophysiology may explain different symptoms seen in upper GI disorders (Table 2).

Table 2 Relationship of gastric pathophysiologic alterations and symptoms

Pathophysiology	Associated symptoms
Delayed gastric emptying	Vomiting
	Postprandial fullness
Delayed proximal gastric emptying	Heartburn
	Regurgitation
Impaired proximal gastric accommodation	Early satiety
	Weight loss
Hypersensitivity	Abdominal pain
	Belching
	Weight loss
Gastric dysrhythmias	Nausea

Gastroparesis occurs in many clinical settings; idiopathic, diabetic, and postsurgical etiologies comprise the majority of cases in most series. In one series of 146 patients, gastroparesis was idiopathic in 36%, diabetic in 29%, and postsurgical in 13% of patients (Soykan et al. 1998). Several gastrointestinal and systemic diseases are associated with gastroparesis.

Delayed gastric emptying of solids after gastric surgery or vagal nerve injury is common with patients most commonly experiencing vomiting, weight loss, and bezoar formation. The incidence of postsurgical gastroparesis varies from 5 to 25% (Eagon et al. 1992). The dominant mechanism appears to be vagal injury with resultant loss of both fundal tone and antral peristalsis (Eagon et al. 1992).

4.2 Generalized Disorders of Gastrointestinal Motility

Gastroparesis may occur as a component of a generalized gut dysmotility syndrome. Chronic intestinal pseudo-obstruction is a syndrome with recurrent symptoms suggestive of intestinal obstruction in the absence of mechanical blockage. Radiologic findings of chronic intestinal pseudo-obstruction include luminal dilation with air-fluid levels throughout the small intestine. Chronic intestinal pseudo-obstruction can be caused by a variety of systemic diseases, including scleroderma, amyloidosis, myxedema, long-standing diabetes mellitus, and paraneoplastic complications most commonly seen with small-cell lung carcinoma. However, many cases are idiopathic in nature. The two main forms of chronic intestinal pseudo-obstruction are myopathic and neuropathic. Antroduodenal manometry may assist in differentiating these two forms (Camilleri et al. 1998). In intestinal myopathy, low-amplitude contractions that propagate normally are seen. In intestinal neuropathy, contractions are normal in amplitude but disorganized in morphology, including disruption of phase III activity, bursts of nonpropagating activity during fasting, and failure to convert from the fasting to the postprandial fed motor pattern.

4.3 Dumping Syndrome and Rapid Gastric Emptying

Dumping syndrome is characterized by rapid gastric emptying accompanied by gastrointestinal and vasomotor symptoms. It occurs mainly after partial or complete gastrectomy, vagotomy (often postsurgical but occasionally due to diabetes or dysautonomia), fundoplication, or bariatric surgery (Lee et al. 2007; Tack 2007). Symptoms result from rapid gastric emptying and are characterized into early and late dumping. Early dumping begins shortly after meal ingestion and is characterized by symptoms of epigastric fullness, crampy abdominal pain, nausea and vomiting, diarrhea, sweating, weakness, dizziness, pallor, palpitations, and tachycardia. Late dumping typically begins 90–240 min after a carbohydrate-rich meal and includes symptoms of diaphoresis, tremulousness, tachycardia, light-headedness, weakness, and confusion.

Symptoms of early dumping are explained in part by the rapid passage of hyperosmolar contents into the small bowel, accompanied by a shift of fluids from the intravascular compartment to the lumen (Vecht et al. 1997). This induces intestinal distention and accompanying symptoms. The vasomotor symptoms are due to enhanced release of gastrointestinal hormones resulting in splanchnic vasodilation and vascular pooling along with accompanying fluid shifts. Late dumping is attributed largely to reactive hypoglycemia following transient dumping-induced hyperglycemia with reactive insulin secretion. As the effects of insulin typically persist beyond the transient initial hyperglycemia, reactive glycemia occurs when all sugars have been absorbed.

Dumping syndrome is diagnosed based on clinical symptoms in a patient with predisposing conditions. Gastric emptying studies can demonstrate rapid gastric emptying (Jian et al. 1992). Gastric emptying of liquids focusing on the early phases of emptying may be particularly helpful as gastric emptying of solids is more variably affected. The diagnosis is confirmed by demonstrating hypoglycemia in association with postprandial symptoms.

Rapid gastric emptying of solids has been demonstrated in some patients with functional dyspepsia (Lin et al. 1999; Delgado-Aros et al. 2004) and has also been reported in diabetes, particularly early in the course of type II diabetes (Schwartz et al. 1996). Many of these patients have symptoms indistinguishable from those of gastroparesis. Rapid emptying has been recently observed as an accompanying factor in adult patients with cyclic vomiting syndrome (Namin et al. 2006; Fajardo et al. 2005).

4.4 Functional Gastroduodenal Disorders

According to Rome III and IV criteria, functional dyspepsia is defined as symptoms of bothersome postprandial fullness, early satiation, epigastric pain, and/or epigastric burning with no evidence of structural disease (Tack et al. 2006; Stanghellini et al. 2016). It has been suggested to categorize patients with functional dyspepsia as having pain-predominant symptoms (epigastric pain syndrome) or symptoms related to

the ingestion of a meal such as early satiation and postprandial fullness (postprandial distress syndrome).

There are associations of various dyspeptic symptoms with alterations in gastric emptying, accommodation, and sensitivity (Table 2); these associations are modest but inconsistent (Boeckxstaens et al. 2001, 2002; Tack et al. 2006; Talley et al. 2001). Nevertheless, there is evidence that gastrointestinal motility and sensation are disturbed in at least a subset of patients with functional dyspepsia. Delayed gastric emptying is reported in between 20 and 50% of patients with functional dyspepsia and a meta-analysis of 17 studies demonstrated significantly delayed solid-phase gastric emptying in 40% of patients with functional dyspepsia (Quartero et al. 1998). Several large studies have demonstrated that patients with delayed gastric emptying for solids are more likely to report postprandial fullness, nausea, and vomiting but these associations are not consistently confirmed (Stanghellini et al. 1996; Talley et al. 2001; Perri et al. 1998). Interestingly, in a series reported by Delgado-Anos et al., 17/39 (43%) of patients with functional dyspepsia had initial rapid gastric emptying at 1 h, whereas 16/39 (41%) patients had delayed overall gastric emptying at 4 h (Delgado-Aros et al. 2004). Symptoms did not differentiate those with delayed versus rapid gastric emptying.

There is some overlap between gastroparesis and functional dyspepsia as both symptoms and gastric emptying test results may meet definitions for both in a subset of patients (Parkman et al. 2004; Tack et al. 2006). As a consequence, some patients with mild abdominal pain, nausea, vomiting, and evidence of delayed emptying are considered to have functional dyspepsia by some clinicians and gastroparesis by others. Patients with marked delay in gastric emptying should be diagnosed with gastroparesis not functional dyspepsia. In general, predominant abdominal pain with lesser degrees of nausea is more consistent with a diagnosis of functional dyspepsia, whereas predominant nausea and vomiting with lesser degrees of abdominal pain are more characteristic of gastroparesis.

Using either a gastric barostat or a single-photon emission computed tomography (SPECT), impaired accommodation of the proximal stomach has been reported in up to 40% of patients with functional dyspepsia (Bredenoord et al. 2003; Thumshirn et al. 1999b). While associations have been reported between impaired gastric accommodation and symptoms of early satiety, these are not consistently confirmed (Boeckxstaens et al. 2002; Tack et al. 2001). Similarly, an association between hypersensitivity to gastric distention and symptoms of pain, belching, and weight loss has been reported (Tack et al. 2001; Salet et al. 1998). Again, while heightened sensitivity to gastric distension is commonly observed, symptom associations are not consistently demonstrated (Boeckxstaens et al. 2002; Salet et al. 1998). Additionally, it has been shown that state anxiety is significantly and negatively correlated with discomfort threshold, pain threshold, and compliance (Van Oudenhove et al. 2007). This observation highlights the complex relationships between pain and factors such as psychiatric distress and somatization.

5 Current Therapeutic Approaches to Upper GI Disorders, Particularly Gastroparesis

Management of upper gastrointestinal disorders, particularly gastroparesis, is guided by the goals of correcting fluid, electrolyte, and nutritional deficiencies; identifying and treating the cause of delayed gastric emptying (e.g., diabetes); and suppressing or eliminating symptoms (Camilleri et al. 1998). Care of patients generally relies on dietary modification, medications that stimulate gastric motor activity, and antiemetic drug therapy.

The outcome of patients with gastroparesis has not been well characterized. It is often felt by many clinicians to be a difficult disorder to treat, reflecting not only paucity of medications that are available for this condition, but also the incomplete understanding of the reasons for the symptoms. The outcome of gastroparesis patients was assessed in the NIH Gastroparesis Consortium in patients with either diabetic or idiopathic gastroparesis (Pasricha et al. 2015). Surprisingly, only 28% of 262 patients symptomatically improved at 48 weeks as determined by a decrease in GCSI $\geq$ 1. This illustrates the chronic nature of gastroparesis and that the disease burden remains high. Predictors for improvement included more severe gastroparesis symptoms, more severe delay in gastric emptying, and an initial infectious prodrome. Predictors for a poor improvement included moderate/severe abdominal pain and being overweight.

5.1 Dietary Treatment

Dietary measures entail adjustment to meal composition and frequency (Parkman et al. 1995; Moore et al. 1984). Eating small meals is recommended as patients often have early satiety, that is, feeling full when eating a normal size meal; in addition, larger meals may alter gastric emptying times. Consuming mainly liquids such as soups can be useful as gastric emptying of liquids is often preserved in patients with gastroparesis (Parkman et al. 1995). Avoidance of fats and indigestible fibers is recommended because they delay gastric emptying (Parkman et al. 1995). When small meals are used in the gastroparesis diet, more frequent meals, 3 meals/day plus 2 snack-type meals, are often needed to maintain caloric intake. These dietary recommendations have often been made empirically as to effects on gastric emptying (Moore et al. 1981, 1984). Recently, these have been looked at in respect to symptom generation. A high-fat solid meal significantly increased overall symptoms among individuals with gastroparesis, whereas a low-fat liquid meal had the least effect (Homko et al. 2015). With respect to nausea, low-fat meals were better tolerated than high-fat meals, and liquid meals were better tolerated than solid meals. These data provide support for recommendations that low-fat and increased liquid-content meals are best tolerated in patients with symptomatic gastroparesis. Another study assessed patient tolerances to foods (Wytiaz et al. 2015). Foods provoking symptoms were generally fatty, acidic, spicy, and roughage based. Foods worsening symptoms included orange juice, fried chicken, cabbage, oranges,

sausage, pizza, peppers, onions, tomato juice, lettuce, coffee, salsa, broccoli, bacon, and roast beef. The foods that were generally tolerable were generally bland, sweet, salty, and starchy. Saltine crackers, jello, and graham crackers moderately improved symptoms. Twelve additional foods were tolerated by patients (not provoking symptoms): ginger ale, gluten-free foods, tea, sweet potatoes, pretzels, white fish, clear soup, salmon, potatoes, white rice, popsicles, and applesauce.

Many patients with gastroparesis have diets deficient in calories, vitamins, and minerals. Unfortunately, nutritional consultation is obtained infrequently but this is suggested for dietary therapy and to address nutritional deficiencies (Parkman et al. 2011).

5.2 Glucose Control in Diabetic Patients

Diabetic patients with gastroparesis frequently exhibit labile blood glucose concentrations with prolonged periods of significant hyperglycemia. Hyperglycemia itself can delay gastric emptying. Hyperglycemia can counteract the accelerating effects of prokinetic agents on gastric emptying. Improvement of glucose control increases antral contractility, corrects gastric dysrhythmias, and accelerates emptying. To date, there have been no long-term studies confirming the beneficial effects of maintenance of near euglycemia on gastroparetic symptoms. Nevertheless, the consistent findings of physiologic studies in healthy volunteers and diabetic patients provide a compelling argument to strive for near-normal blood glucose levels in affected diabetic patients. Generally, patients give their mealtime insulin after ingesting the meal, to ensure that the entire anticipated meal is actually consumed and without vomiting.

In a recently reported multicenter pilot study (GLUMIT), continuous subcutaneous insulin infusion with insulin pump therapy with continuous glucose monitoring reduces hypoglycemia in diabetes with gastroparesis (Calles et al. 2015). There were also associated improvements in gastroparesis symptoms and nutrient tolerance benefits which were maintained for the 24-week phase of intensive monitoring and therapy. This pilot study shows the feasibility and potential for dual benefits improving both diabetes control and lowering gastroparesis symptom burdens.

5.3 Prokinetic Agents

Medications with gastric prokinetic properties, which are the mainstay of treatment for gastroparesis, include metoclopramide, erythromycin, and domperidone (McCallum and George 2001). Intravenous agents currently available to treat hospitalized patients include metoclopramide and erythromycin. Several prokinetic agents are being studied for patients with gastroparesis; these include newer 5-HT4 receptor agonists with less cardiac side effects, newer motilin receptor agonists with less tachyphylaxis phenomenon and without antibiotic properties, and newer ghrelin receptor agonists.

5.3.1 Metoclopramide

Metoclopramide, a substituted benzamide structurally related to procainamide, exhibits both prokinetic and antiemetic actions. The drug is a dopamine type 2 receptor antagonist both in the CNS and in the stomach. Metoclopramide also has 5HT-3 receptor antagonist activity that might also provide an antiemetic effect. In addition, it has some 5HT-4 agonist activity releasing acetylcholine from intrinsic myenteric cholinergic neurons that might help enhance gastric emptying. The prokinetic properties of metoclopramide are limited primarily to the stomach. Reglan can cause both acute and chronic CNS side effects in some patients. These side effects should be discussed with the patient prior to treatment and documented in the patient's medical record. In the USA, metoclopramide is approved for diabetic gastroparesis for up to 12-week duration. Patients with gastroparesis have chronic nausea and often need longer periods of treatment. Recently, in Europe, it has been suggested that metoclopramide be used for only several days' duration for acute treatment of chemotherapy-induced vomiting.

5.3.2 Erythromycin

The macrolide antibiotic erythromycin exerts prokinetic effects via action on gastroduodenal receptors for motilin, an endogenous peptide responsible for initiation of the migrating motor complex (MMC) in the upper gut. When administered exogenously, motilin stimulates antral contractility and elicits premature antroduodenal phase III activity. Erythromycin produces effects on gastroduodenal motility similar to motilin.

Clinically, erythromycin has been shown to stimulate gastric emptying in diabetic gastroparesis, idiopathic gastroparesis, and postvagotomy gastroparesis. Erythromycin may be most potent when used intravenously; it is often used to clear the stomach from blood prior to an upper endoscopy for a patient with upper gastrointestinal bleeding. Limited data exist concerning the clinical efficacy of erythromycin in reducing symptoms of gastroparesis. In a systematic review of studies on oral erythromycin with symptom assessment as a clinical end point, improvement was noted in 43% of patients. One study comparing erythromycin and metoclopramide in an open-label, crossover fashion in diabetic gastroparesis found similar efficacy.

Oral administration of erythromycin should be initiated at low doses (e.g., 100–125 mg 3 times daily before meals). Liquid suspension erythromycin may be preferred because it is rapidly and more reliably absorbed. Intravenous erythromycin (100 mg every 8 h) is used for inpatients hospitalized for severe refractory gastroparesis. Side effects of erythromycin at higher doses (500 mg) include nausea, vomiting, and abdominal pain. Because these symptoms may mimic those of gastroparesis, erythromycin may have a narrow therapeutic window in some patients. There is report that erythromycin chronically may be associated with higher mortality from cardiac disease, especially when combined with agents that inhibit cytochrome p-450, such as calcium channel blockers.

5.3.3 Domperidone

The effects of domperidone on the upper gut are similar to those of metoclopramide, including stimulation of antral contractions and promotion of antroduodenal coordination. In addition to prokinetic actions in the stomach, domperidone exhibits antiemetic properties via action on the area postrema, a brainstem region with a porous blood-brain barrier. Domperidone does not readily cross the blood-brain barrier; therefore, it is much less likely to cause extrapyramidal side effects than metoclopramide. Side effects to domperidone include breast lactation, headaches, and palpitations. Domperidone has been associated with prolongation of the cardiac QTc interval.

The FDA has developed a program for physicians who would like to prescribe domperidone for their patients with severe upper GI motility disorders that are refractory to standard therapy to open an Investigational New Drug Application (IND). An IND is a request for FDA authorization to administer an investigational drug to humans. Such authorization would allow the importation, interstate shipment, and administration of the drug even though it is not approved for sale in the USA. Use of this IND mechanism for use of domperidone also will require IRB approval. An EKG and blood work to check potassium and magnesium are obtained prior to starting domperidone; these are repeated after 4–8 weeks of treatment. The patient will need to pay for their domperidone medication since insurance companies do not for this nonapproved treatment.

The benefits and side effects of domperidone to treat symptoms of gastroparesis were recently reported from a large single-center cohort (Schey et al. 2016). In this large single-center study of 125 patients treated with domperidone, side effects necessitating discontinuing treatment occurred in 12%. The most common side effects were headache, tachycardia/palpitations, and diarrhea. The majority of patients (60%) experienced an improvement in symptoms of gastroparesis, particularly postprandial fullness, nausea, vomiting, and stomach fullness.

5.4 Antiemetic Medications

Antiemetic agents are given acutely for symptomatic nausea and vomiting. The principal classes of drugs that have been used for symptomatic treatment of nausea and vomiting are phenothiazines, antihistamines, anticholinergics, dopamine receptor antagonists, and more recently serotonin receptor antagonists. The antiemetic action of phenothiazine compounds appears to be mediated primarily through a central antidopaminergic mechanism in the area postrema of the brain. Commonly used agents include prochlorperazine (Compazine), trimethobenzamide (Tigan), and promethazine (Phenergan).

Serotonin (5-HT-3) receptor antagonists, such as ondansetron (Zofran) and granisetron (Kytril), have been shown to be helpful in treating or preventing chemotherapy-induced nausea and vomiting. The primary site of action of these compounds is probably the chemoreceptor trigger zone, since there is a high density of 5-HT-3 receptors in the area postrema. Zofran is now frequently used for nausea and vomiting of a variety of other etiologies. It is best given on a prn basis due to their expense. Granisetron

transdermal system (GTS) is an appealing delivery system for patients with gastroparesis. In an open-label study, GTS was moderately effective in reducing nausea and/or vomiting in 76% of gastroparesis patients (Midani and Parkman 2016). Side effects can occur such as constipation, skin rash from the patch, and headaches.

Neurokinin receptor antagonists are being used for chemotherapy-induced nausea and vomiting. Aprepitant (Emend) is a recently approved substance P/neurokinin 1 receptor antagonist for chemotherapy-induced nausea and vomiting. In a recent abstract presentation (Pasricha et al. 2016), the effects of the neurokinin-1 receptor antagonist aprepitant on symptoms in patients with gastroparesis (Gp) and related syndromes associated with chronic nausea and vomiting patients. Aprepitant resulted in a greater decline in mean 4-week daily hours of nausea and mean 4-week GCSI score. These data suggest that aprepitant has the potential for safe improvement of a variety of symptoms in gastroparesis and related disorders.

5.5 Refractory Patients with Gastroparesis

Patients with refractory gastroparesis need treatment at a variety of levels directed at nutritional care, prokinetic medications, antiemetic therapies, pain control, glycemic control, and often psychological measures. Surgical and endoscopic approaches are considered in patients in whom drug therapy is ineffective and who cannot meet their nutritional requirements (Camilleri et al. 1998). Surgical treatments include placement of jejunostomy tubes, gastric electrical stimulation, and pyloromyotomy (Camilleri et al. 1998). These options are typically considered only in patients with severe, refractory gastroparesis.

5.5.1 Psychotropic Medications as Symptom Modulators

Tricyclic antidepressants may have significant benefits in suppressing symptoms in some patients with nausea and vomiting as well as patients with abdominal pain. These are not used for their antidepressant effects, but their actions as symptom modulators, acting either peripherally or, most likely, centrally. Doses of tricyclic antidepressants used are lower than used to treat depression. A reasonable starting dose for a tricyclic drug is 10–25 mg at bedtime. If benefit is not observed in several weeks, doses are increased by 10–25 mg increments up to 50–100 mg. Side effects are common with the use of tricyclic antidepressants and can interfere with management and lead to a change in medication in 25% of patients. The secondary amines, nortriptyline and desipramine, may have fewer side effects. The recent NIH gastroparesis consortium study with nortriptyline in idiopathic gastroparesis did not show an effect on overall symptoms of gastroparesis (Parkman et al. 2013). However, there was a suggestion that low nortriptyline doses (10–25 mg) might decrease nausea, whereas higher doses (50–75 mg) might decrease fullness. There are limited data on the use of selective serotonin reuptake inhibitors in gastroparesis or functional dyspepsia.

5.5.2 Pyloric Botulinum Toxin Injection

Gastric emptying is a highly regulated process reflecting the integration of the propulsive forces of proximal fundic tone and distal antral contractions with the functional resistance provided by the pylorus. Manometric studies of patients with diabetic gastroparesis have shown in some patients prolonged periods of increased pyloric tone and phasic contractions, a phenomenon termed pylorospasm. Botulinum toxin is a potent inhibitor of acetylcholine neuromuscular transmission and has been used to treat spastic somatic muscle disorders as well as achalasia. Several studies have tested the effects of endoscopic injection of the pyloric sphincter with botulinum toxin in patients with diabetic and idiopathic gastroparesis (Camilleri et al. 1998). Initial studies were unblinded in small numbers of patients from single centers and have observed mild improvements in gastric emptying and modest reductions in symptoms for several months. Two double-blind studies have been reported; these show an improvement in gastric emptying, but no effect on symptoms compared to placebo. Thus, botulinum toxin injections do not result in sustained improvement in symptoms of gastroparesis.

5.5.3 Gastric Electric Stimulation

Gastric electric stimulation is a treatment for refractory gastroparesis. It involves an implantable neurostimulator that delivers a high-frequency (12 cpm), low-energy signal with short pulses. With this device, stimulating wires are sutured into the gastric muscle along the greater curvature during laparoscopy or laparotomy. These leads are attached to the electric stimulator, which is positioned in a subcutaneous abdominal pouch. Based on the initial studies that have shown symptom benefit especially in patients with diabetic gastroparesis, the gastric electric neurostimulator was granted humanitarian approval from the FDA for the treatment of chronic, refractory nausea and vomiting secondary to idiopathic or diabetic gastroparesis. The rare but worrisome complications of the implantable neurostimulator are infection of the pacemaker site and intestinal blockage from the intra-abdominal wires, which have necessitated device removal in approximately 5% of cases. More recently, a small minority of patients can at times have a shocking sensation. Symptoms of nausea and vomiting can improve with stimulation; however abdominal pain often does not. The symptomatic benefit occurs more often in diabetic gastroparesis than in idiopathic gastroparesis. In a recently reported cohort of 151 patients with refractory gastroparesis treated at a single center, GES improved symptoms in 75% of patients with 43% being at least moderately improved (Heckert et al. 2016). Response in diabetics was better than in nondiabetic patients. Nausea, loss of appetite, and early satiety responded the best.

Further investigation would be helpful to definitively show the effectiveness of gastric stimulation in long-term blinded fashion, which patients are likely to respond, the optimal electrode position, and the optimal stimulation parameters, none of which have been rigorously evaluated to date. Future improvements may include devices that sequentially stimulate the stomach in a peristaltic sequence to promote gastric emptying as well as endoscopically placed gastric electric stimulators.

5.5.4 Other Surgical Treatments for Persistently Refractory Gastroparesis Patients

Other treatments include feeding jejunostomy for nutritional support with a jejunostomy tube that bypasses the affected stomach for feedings. Venting gastrostomy tubes have been tried with success in some patients. Recently, pyloromyotomy has reemerged as a treatment for patients with gastroparesis. This can be performed surgically or more recently endoscopically. Open-label studies report good responses. Gastrojejunostomy has been performed in the past with limited success. Gastric bypass with gastrojejunostomy has been used by several centers to treat gastroparesis. Partial gastrectomy should be used rarely, and only in carefully selected patients. In postsurgical gastroparesis, occasionally completion gastrectomy is performed for persistent gastroparetic symptoms.

6 Concluding Remarks

Upper gastrointestinal function is complex with a variety of disturbances that may impact on symptom generation. Our understanding of the pathophysiology of upper gastrointestinal function in disorders is becoming better with newer modalities assessing motor function, accommodation, and sensation. The relationship of pathophysiology and symptom generation is also becoming better understood. The ability to study the various parameters of gastric function allows for better understanding of the relationships of upper digestive symptoms with alterations in gastric neuromuscular or CNS function. Treatment of upper gastrointestinal disorders is expanding and now often targets specific symptoms. In the future, it is plausible that clinicians will be able to employ selected tests chosen on the basis of clinical variables that will result in implementation of effective treatments leading to improved clinical outcomes.

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Postoperative Ileus: Pathophysiology, Current Therapeutic Approaches

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Contents

Abstract

Postoperative ileus, which develops after each abdominal surgical procedure, is an iatrogenic disorder characterized by a transient inhibition of gastrointestinal motility. Its pathophysiology is complex involving pharmacological (opioids, anesthetics), neural, and immune-mediated mechanisms. The early neural phase, triggered by activation of afferent nerves during the surgical procedure, is short

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lasting compared to the later inflammatory phase. The latter starts after 3–6 h and lasts several days, making it a more interesting target for treatment. Insight into the triggers and immune cells involved is of great importance for the development of new therapeutic strategies. In this chapter, the pathogenesis and the current therapeutic approaches to treat postoperative ileus are discussed.

Keywords
Field effect • Gastrointestinal motility • Inflammatory phase • Macrophages • Mast cells • Neural phase • Pathophysiology • Postoperative ileus

1 Introduction

Each patient undergoing an abdominal surgical procedure, even if minimal invasive techniques are applied, will develop a transient episode of impaired gastrointestinal (GI) motility or postoperative ileus (POI). Although some argue that uncomplicated POI should be considered as a "normal" or "physiological" response of the intestine to a traumatic event and thus should be disregarded, POI clearly has a significant impact on patient morbidity with symptoms such as pain, nausea and vomiting, abdominal distension, absence of defecation, and intolerance to oral feeding (Livingston and Passaro 1990). In clinical practice, a distinction is made between this so-called physiological POI and prolonged POI. The latter is however ill defined and is considered when recovery of bowel function is delayed, ranging from more than 3 to more than 7 days after surgery (Wolthuis et al. 2016). Depending on the definition used, the incidence of prolonged POI after colorectal surgery for example is approximately 10%. Of note, POI is the most common cause of prolonged hospital stay following abdominal surgery with an annual cost estimated as much as $1.5 billion in the USA.

2 Pathophysiology

Transient inhibition of GI motility following abdominal surgery involves the entire GI tract. However not all segments are equally affected. Small intestinal motility is on average disturbed for approximately 24 h and gastric motility between 24 and 48 h, whereas colonic motility is impaired between 48 and 72 h. Before discussing the mechanisms involved in POI, it is important to emphasize that our current insight is based on murine models consisting of standardized manipulation of the small intestine. To date however, no resection of intestine or construction of anastomoses is included in these models. Therefore, these models will most likely study "physiological" POI rather than "prolonged" POI, which may be relevant with respect to translation of preclinical data to clinical practice/therapeutic studies.

The pathophysiology of POI is complex involving pharmacological (opioids, anesthetics), neural, and immune-mediated mechanisms. In the immediate postoperative

period, anesthesia and opioids contribute to POI. Opioids, often used as analgesics following various types of surgery, indeed have a major impact on GI motility by activation of μ-opioid receptors on the myenteric fibers. This leads to inhibition of acetylcholine release from myenteric neurons and reduced GI transit (Holte and Kehlet 2002). Interference with this mechanism by peripheral selective opioid antagonists such as alvimopan has indeed accelerated postoperative recovery of GI motility, but only to a minor extent (Vaughan-Shaw et al. 2012). The latter is mostly explained by the knowledge that other mechanisms play a more important role in the pathogenesis of POI. Indeed, the main cause of POI rather relates to the surgical procedure itself. Two different phases, each with its own dynamics and underlying pathophysiological mechanism (Fig. 1), are now proposed to underlie POI. The first or early phase is neurally mediated and involves neural reflexes activated during and immediately following surgery. In the late 1990s, however, the concept was introduced that manipulation of the intestine triggers the influx of leukocytes in manipulated intestinal segments impairing the contractile properties of the inflamed intestine (Kalff et al. 1998, 1999a, b). This second phase starts 3–4 h after surgery and is responsible for the sustained and thus clinically more relevant inhibition of GI motility. From a clinical perspective, interference with or prevention of this second phase is clearly expected to be most relevant and most effective in the treatment of POI.

2.1 The Neurogenic Phase of POI

Incision of the skin and opening of the peritoneal cavity briefly inhibit GI motility via adrenergic reflexes involving a spinal loop with afferent splanchnic nerves synapsing in the spinal cord activating efferents travelling back to the gut. When intestinal loops are however displaced and manipulated, the nociceptive stimuli become more intense activating additional neural pathways and leading to more prolonged inhibition of motility. The high-threshold supraspinal pathways involved relay to the hypothalamic and pontine-medullary nuclei such as the nucleus tractus solitarius and the paraventricular and supraoptic nucleus of the hypothalamus, and are also adrenergic in nature (Boeckxstaens and de Jonge 2009). In addition, more intense intestinal stimulation activates an inhibitory non-adrenergic vagally mediated pathway contributing to the neural phase of POI (Boeckxstaens et al. 1999). Activation of these pathways, at least by mechanical stimuli, will however cease once the abdomen is closed and thus other factors such as mediators released by tissue damage or subsequent inflammation therefore must come into play explaining the more prolonged nature of POI.

2.2 The Inflammatory Phase of POI

Already in 1978, Bueno et al. described an initial complete but short-lasting inhibition of electrical spiking activity after muscular and peritoneal incision in sheep and dogs, corresponding with the neural phase described above (Bueno et al.

1978). Of major interest was the observation that electrical activity recovered during 3–6 h, but was then inhibited for a second time for a period ranging from 6 to 72 h. These findings were later confirmed in isolated muscle strips from mice revealing two phases of inhibition of muscle contractility separated by a period of recovery (Farro et al. 2016; Kalff et al. 1999a, b). By now, it is generally accepted that this second wave of inhibition results from an immune-mediated cascade of events, also referred to as the inflammatory phase of POI.

Twenty years after the first description of the biphasic nature of POI, Kalff et al. described the temporal association between inflammation of the intestinal *muscularis* externa and the second prolonged phase of POI (Kalff et al. 1999a, b). These investigators elegantly demonstrated that 3–4 h after manipulation of the intestine, mainly neutrophils and monocytes infiltrated the muscular layer, a finding that was associated with impairment of spontaneous and stimulated contractile activity of muscle strips obtained from the inflamed intestine. Most interestingly, animals pretreated with antibodies or antisense oligonucleotides against the intercellular adhesion molecule-1 (ICAM-1) not only prevented the influx of leukocytes, but also preserved normal neuromuscular function of muscle strips providing the proof of concept that inflammation induced by manipulation indeed largely contributes to POI (de Jonge et al. 2005; Kalff et al. 1999a, b; The et al. 2005). Recently, we demonstrated that a first wave of impaired smooth muscle contractility of the manipulated intestine was maximal after 1.5 h, even before the influx of inflammatory cells, which coincided with increased expression of innate pro-inflammatory cytokines and chemokines such as TNFα, IL-1β, and IL-6 (Farro et al. 2016). A second wave of inhibition was paralleled by influx of monocytes and neutrophils entering the *muscularis* from 6 h onwards to peak at 24 h, associated with increased levels of IL-1β and CCL2. Infiltrating inflammatory cells may affect smooth muscle function by releasing pro-inflammatory cytokines and chemokines, nitric oxide (NO), prostaglandins, and other pro-inflammatory components. Cytokine levels and smooth muscle contractility both returned to baseline at day 3, indirectly suggesting that this second wave of impaired smooth muscle function may be mediated by incoming leukocytes. Interestingly however, responses evoked by electrical field stimulation, thus mediated by enteric neurons, remained abnormal until day 10 (Farro et al. 2016). Enteric neurons indeed revealed reduced expression and impaired activity of ChAT and nNOS, both playing a crucial role in intestinal peristalsis. These observations most likely explain why intestinal transit was only fully recovered by day 5, even when smooth muscle function was already normalized 2 days earlier. So based on these data, in addition to the early neural phase, three more phases can be distinguished: an early (until 6 h) and a late phase (starting at 6 h) of smooth muscle inhibition and a long-lasting (up to 10 days) phase of enteric neuron dysfunction (Fig. 1).

2.3 Mechanisms Triggering the Inflammatory Phase

To date, it is generally accepted that the resident population of *muscular* macrophages residing around the myenteric plexus play a central role in POI.

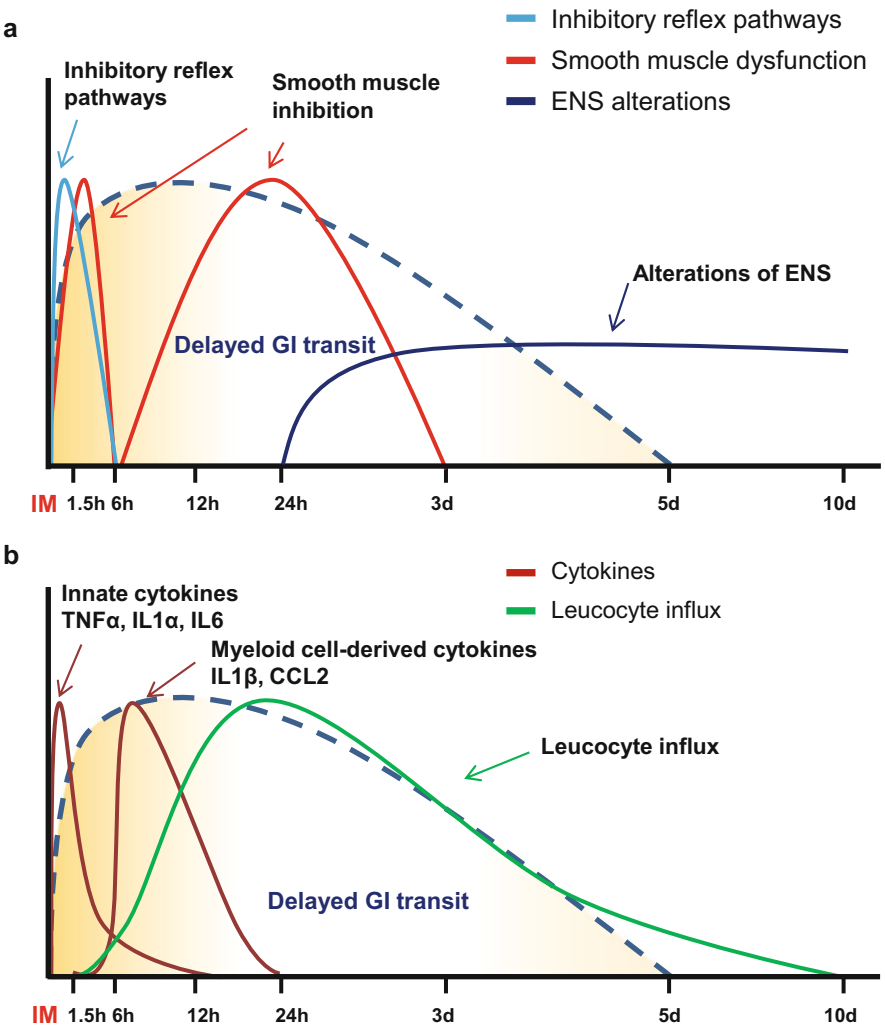

Fig. 1 Schematic representation of the hypothesis proposed regarding the different mechanisms and phases underlying postoperative ileus (POI). Reprinted with permission from Farro et al. (2016)

These macrophages are tolerogenic in nature and are quiescent under normal physiological conditions. Their importance in POI was first demonstrated by Kalff et al. who showed activation of these macrophages by surgical manipulation (Kalff et al. 1999a, b), resulting in activation of transcription factors such as nuclear factor kB (NF-kB), signal transducer and activator of transcription 3 (STAT3) (Wehner et al. 2005), early growth response protein 1 (EGR-1) (Schmidt et al. 2008), and production of pro-inflammatory cytokines and chemokines, integrins, and cell adhesion molecules. As a result, inflammatory cells from the circulation,

mainly neutrophils followed by monocytes, subsequently enter the *muscularis*, further contributing to POI by releasing factors such as NO (Kalff et al. 2000; Turler et al. 2006) and prostaglandins (Kreiss et al. 2003). Conversely, pharmacological or genetic depletion resulted in a decrease of inflammatory mediators and a reduction in the recruitment of leukocytes into the *muscularis* (Bauer and Boeckxstaens 2004; Boeckxstaens and de Jonge 2009).

The mechanisms leading to the activation of the resident macrophages remain however unclear. One potential mechanism may be through activation of damage-associated molecular pattern (DAMP) receptors by molecules released by damaged cells. DAMPs are molecules with no inflammatory capacity per se. They only become pro-inflammatory when released by damaged, stressed, and dying cells into intracellular space (Chen and Nunez 2010). Besides intracellular molecules, also extracellular matrix (ECM) components are danger signals. In fact, degradation of ECM has indeed been demonstrated to contribute to *muscularis* inflammation in POI (Moore et al. 2011). Manipulation of the intestine or even exposure to air leading to dehydration and drop in temperature during surgery may lead to tissue damage and thus release mediators such as ATP, HMGB1, or IL-1α, known to be potent activators of macrophages. IL-1α for example is upregulated maximally 1.5 h after surgery (Farro et al. 2016), while mice pretreated with antibodies against IL-1α and IL-1R$^{-/-}$ mice are protected against POI and show a reduced influx of inflammatory cells (Stoffels et al. 2014), clearly showing that IL-1α and tissue damage may play a crucial role. Although IL-1R is known to be universally expressed (Dinarello 2009), this receptor was shown to be mainly expressed by enteric glia cells, suggesting that enteric glia may be an important source of cytokine production in the very early phase of POI. The degree of tissue damage will clearly depend on the intensity of intestinal manipulation and/or the duration of surgery, and will thus be an important determinant of POI severity (van Bree et al. 2013).

Alternatively, the initial neural phase may be the trigger for the later inflammatory phase. Indeed, intense activation of afferent nerve fibers may trigger neurogenic inflammation via the local release of pro-inflammatory neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP) (Bueno et al. 1997). A role for CGRP released from afferent nerves in response to surgery has been reported already in the early 1990s. In rats, pretreatment of celiac ganglia with capsaicin (eliminating afferent nerves) and a CGRP antagonist (but not an SP antagonist) indeed prevented delayed gastric emptying assessed immediately following surgery (Plourde et al. 1993; Zittel et al. 1994). Of note, activation of IL-1R was proposed to induce the release of CGRP from visceral afferents (Coimbra and Plourde 1996), creating a link between tissue damage (see above) and neurogenic inflammation. Recently, evidence was provided that both intestinal manipulation and capsaicin depleted CGRP from muscular nerve fibers, confirming CGRP release from visceral afferents by intestinal manipulation (Glowka et al. 2015). Of note, both capsaicin and the CGRP antagonist BIBN4096BS reduced *il1β* and *il6* mRNA expression in the *muscularis* externa at 3 h after surgery (Glowka et al. 2015), while CGRP stimulated *il1β* and *il6* mRNA expression in peritoneal macrophages, shown to

express the receptor for CGRP. Taken together, these data indicate that afferent nerves may indeed be involved in the activation of resident *muscular* macrophages and the triggering of the inflammatory cascade. Of interest, the same investigators demonstrated that mast cells were not activated by CGRP. This is somewhat surprising as mast cells have been repeatedly proposed to be a main player in neurogenic inflammation (Bueno et al. 1997) and to be involved in POI both in preclinical models (de Jonge et al. 2004; Snoek et al. 2012) and in humans (Berdun et al. 2015; The et al. 2008, 2009). A recent study, using a specific mast cell-deficient mouse model (Cpa3-Cre), however showed that mast cells, at least in mice, are not involved in POI (Gomez-Pinilla et al. 2014a, b).

Finally, macrophages are potently activated by bacterial cell wall molecules interacting with Toll-like receptors (TLR). Especially as intestinal permeability is transiently increased following intestinal manipulation, bacterial translocation may represent another potential mechanism by which resident macrophages may be stimulated (Schwarz et al. 2002; Snoek et al. 2012). However, as TLR2 and TLR4 knockout mice are not protected against POI, this possibility seems rather unlikely (Stoffels et al. 2014).

2.4 Recovery Phase of POI

Resolution of inflammation is a somewhat neglected phase of the inflammatory process. Mainly a switch of a pro-inflammatory M1 to a tolerogenic M2 microenvironment triggers the resolution phase, a phenomenon mainly mediated by resolvins, a class of polyunsaturated fatty acid-derived proresolving lipid mediators (Serhan 2014). The enzyme involved in the synthesis of resolvins, 12/15-lipoxygenase, is strongly expressed by monocytes infiltrating the postoperative intestinal *muscularis* externa and mediates the production of mainly protectin DX and resolving D2 by these leukocytes. Of interest, pretreatment with protectin DX reduced postoperative influx of neutrophils and improved GI motility. Along the same line, IL-10, mainly expressed by incoming $F4/80^{+}$ monocytes/macrophages, has been proposed to play an important role in the recovery from POI (Stoffels et al. 2009). Mice deficient in IL-10, a cytokine known to possess potent anti-inflammatory properties, had increased expression of numerous pro-inflammatory mRNAs and proteins associated with an increased release of NO and prostanoids. Most importantly, postoperative motility never recovered in these mice, while treatment with recombinant mouse IL-10 reduced neutrophil recruitment and improved POI (Stoffels et al. 2009). We recently showed that also mice lacking monocytes ($CCR2^{-/-}$) recover much slower from POI, indicating that incoming monocytes indeed play an important role in restoring homeostasis and normalizing neuromuscular function (Farro et al. in revision).

2.5 The "Gastrointestinal Field Effect"

POI involves the entire GI tract, and not only the part manipulated during surgery, a phenomenon referred to as the "GI field effect." Two main theories have been proposed to explain this observation: the first includes activation of neural inhibitory pathways by the inflammatory process in the manipulated region (Bauer and Boeckxstaens 2004; Boeckxstaens and de Jonge 2009; de Jonge et al. 2003). Manipulation of the small intestine indeed resulted in inflammation of the manipulated region, not of the stomach or colon, and was associated with c-fos expression (marker of neural activation) in the lumbar spinal cord. Moreover, postoperative neural blockade with hexamethonium and guanethidine normalized gastric emptying without affecting small intestinal transit (de Jonge et al. 2003). This was further corroborated by increased c-fos expression in the spinal cord and brain stem and increased nerve activity of spinal afferent nerves triggered by the intestinal infiltrate (Kreiss et al. 2003; Mueller et al. 2008; van Bree et al. 2013). The second hypothesis proposes the migration of Th1 memory T cells from the inflamed area to the rest of the intestine (Engel et al. 2010; Schwarz et al. 2004). These T cells, under the influence of IL-12 released by dendritic cells, are believed to migrate from surgically manipulated sites through the bloodstream to unmanipulated intestinal areas to induce inflammation and ileus by releasing interferon-γ. Peripheral blood samples indeed reveal increased memory T cells following intestinal manipulation, while prevention of T cell migration with the immunosuppressive agent FTY720 prevented POI (Engel et al. 2010). More recent studies however could not confirm increased levels of IL-12 in the manipulated intestine (Snoek et al. 2012) and could not detect dissemination of inflammation to the large intestine and stomach (van Bree et al. 2013).

3 Current Therapeutic Approaches

Mainly as colonic motility recovers last, first defecation and flatus are often used as primary outcome parameters in clinical trials. These parameters are however nonspecific, mainly as passage of flatus strongly depends on patient reporting, while passage of stool may simply reflect rectal emptying. Using scintigraphic assessment of intestinal transit, we recently showed that the combination of the time to tolerance of solid food and having had defecation best indicates recovery of colonic transit (van Bree et al. 2014), and should preferentially be considered as primary outcome measure in clinical trials evaluating new compounds as treatment of POI.

3.1 Non-pharmacological Strategies

3.1.1 Multimodal Enhanced Recovery After Surgery Programs (Fast-Track Care)

Enhanced recovery after surgery (ERAS) protocols or fast-track programs aim to shorten POI and reduce the rate of perioperative morbidity by a series of general

measures, such as perioperative fluid management, early ambulation and feeding, and optimal analgesia (Kehlet 2011). The LAFA study, a large randomized controlled study evaluating the impact of fast-track care in patients undergoing either laparoscopic or open colectomy, revealed that patients receiving fast-track care already tolerated solid food after 1 day, while those receiving standard care only tolerated solid food at day 3 (laparoscopic) or 4 (open) after surgery (Vlug et al. 2011). Of interest, this clinical improvement was associated with a faster GI transit recovery for fast-track versus standard care (van Bree et al. 2011). Despite the apparent effectiveness of the fast-track approaches, this approach has unfortunately not been fully implemented in the majority of surgical wards. Clearly, programs to enhance its wide implementation should therefore be encouraged.

3.1.2 Laparoscopic Surgery

Minimal invasive surgery using laparoscopy has many potential advantages over conventional open surgery, such as smaller incisions, less pain and inflammation, earlier GI recovery, and shorter hospital stay. Overall, abundant evidence (Basse et al. 2003; Lacy et al. 2002; Milsom et al. 1998), including a recent meta-analysis comprising 4,614 patients with colon cancer (Ohtani et al. 2011), underscores that laparoscopic surgery significantly reduces time until recovery of bowel function (on average 1 day) and duration of hospital stay compared with open colonic resection. In mice, laparoscopic surgery even failed to induce intestinal inflammation and POI compared to standard intestinal manipulation (Gomez-Pinilla et al. 2014a, b).

3.2 Pharmacological Strategies

3.2.1 Prokinetics

Prokinetics are routinely used in clinical practice in the postoperative period to provide symptomatic relief. A recent Cochrane review indicates however that routine administration of these older prokinetics (metoclopramide, cisapride, erythromycin, cholecystokinin, and dopamine antagonists) is not recommended for the prevention of POI (Traut et al. 2008). Clinical studies with metoclopramide for example, a dopamine D_2 receptor antagonist with mixed 5-HT_3 receptor antagonist and 5-HT_4 receptor agonistic properties, are underpowered and reveal controversial results ranging from a reduction in time until first bowel movement and resumption of oral soft diet to no effects (Chan et al. 2005; Seta and Kale-Pradhan 2001).

New 5-HT_4 agents such as prucalopride and mosapride seem more promising. A recent phase II randomized clinical trial, including 110 patients undergoing elective GI surgery, showed a moderate reduction in time to defecation, passage of flatus, and postoperative length of stay in those patients treated with 2 mg of prucalopride (Gong et al. 2016). It should be emphasized that treatment was however only started 24 h after surgery. This may be relevant as we recently showed in our murine model of POI that administration of prucalopride is only effective if administered prior to surgery (Fig. 2) (Gomez-Pinilla et al. 2014c). Only then, prucalopride has anti-inflammatory properties (see below) and prevents POI. Similar preclinical findings were reported with the 5-HT_4 agonist mosapride (Tsuchida et al. 2011). A small clinical study ($n = 30$) evaluated the

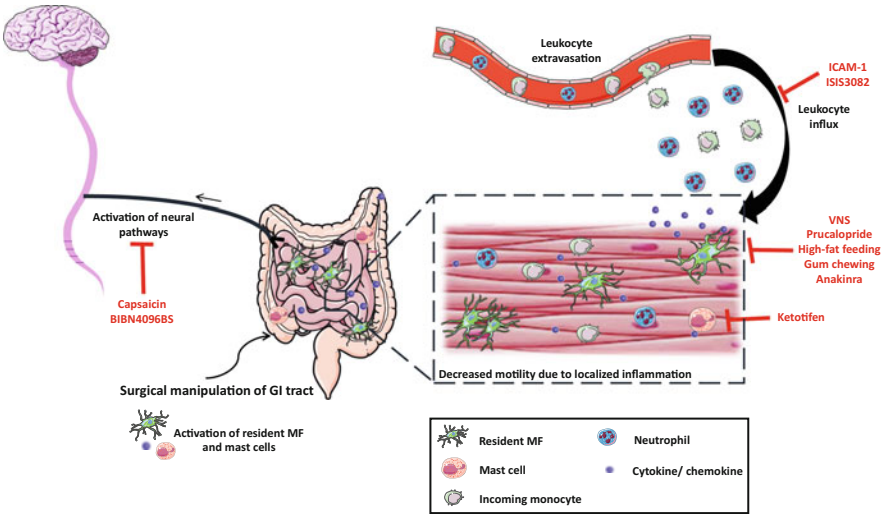

Fig. 2 Schematic representation of the possible therapeutic approaches to prevent postoperative ileus (POI). In the early neuronal phase, GI motility is inhibited via adrenergic and non-adrenergic reflexes activated by skin incision, opening of the peritoneum, and manipulation of the intestines. This neuronal activation can be prevented by capsaicin and BIBN4096BS. In addition, intestinal manipulation activates quiescent resident macrophages and possibly mast cells (at least in humans) present in the intestinal muscularis externa, thereby initiating the inflammatory phase. These activated resident immune cells release cytokines and chemokines, followed by influx of leukocytes, a process that can be inhibited by vagus nerve stimulation, prucalopride, high-fat feeding, and gum chewing, while mast cell activation can be prevented by ketotifen. The influx of leukocytes can be inhibited by ICAM-1 and ISIS3082

effect of 15 mg administered three times a day from the afternoon of postoperative day 1 to the evening of postoperative day 7 and reported a reduction in time to first flatus and defecation. These clinical parameters were associated with an improvement in gastric contractility measured on postoperative day 8 (Toyomasu et al. 2011). Although interesting, these data clearly need confirmation.

3.2.2 Opioid Antagonists

Opioid agonists are often used for postoperative analgesia, and as described above contribute to POI in the early postoperative period mainly by decreasing intestinal motility via stimulation of μ-opioid receptors in the gut. The peripherally acting μ-opioid receptor antagonist alvimopan belongs to a new class of drugs designed to reverse these opioid-induced GI side effects without affecting centrally mediated opioid analgesic effects and thus not compromising pain relief (Becker and Blum 2009). A recent meta-analysis selected five randomized, double-blind, placebo-controlled, phase III trials (Drake and Ward 2016). Administration of alvimopan prior to surgery (6–12 mg) showed beneficial and significantly reduced the time to tolerance of solid food and first defecation. Across all studies, alvimopan was well tolerated and no increased adverse events or serious adverse events were reported compared to

placebo. Methylnaltrexone, another peripherally acting μ-opioid antagonist, was recently studied in two placebo-controlled phase III trials evaluating intravenous (i.v.) administration of 12 and 24 mg. No improvement in time to first bowel movement was observed in a total of 1,048 randomized patients undergoing segmental colectomy (Yu et al. 2011). As both alvimopan and methylnaltrexone intervene with the detrimental effects of opioids administered in the early postoperative period, it remains to be awaited if these compounds will be clinically valuable given the recent strategies to reduce the perioperative use of opioids.

3.2.3 Ghrelin Agonists

Ghrelin is a 28-amino acid peptide mainly produced in the fundus of the stomach and in the pancreas. In view of their powerful prokinetic properties, ghrelin and ghrelin agonists such as ipamorelin and ulimorelin (TZP-101) have been evaluated as potential therapy for POI. To date, two phase IIb studies have been reported on TZP-101 safety and efficacy in POI management. Treatment with 20–600 μg/kg ulimorelin by 30-min i.v. infusion within 1 h of surgical closure and then daily for up to 7 days accelerated the time to first bowel movement and shortened hospital stay (Popescu et al. 2010). In the other phase IIb study, the effect of ulimorelin treatment (480 μg/kg) was tested in 168 patients subjected to major surgery yielding comparable results (Bochicchio et al. 2012). Two subsequent randomized placebo-controlled phase III trials ($n = 332$ and $n = 330$ patients included) however failed to demonstrate a reduction in POI (Shaw et al. 2013). In line, no improvement was found with ipamorelin in 117 patients undergoing colonic resection (Beck et al. 2014), further dampening the enthusiasm to develop this class of compounds as treatment for POI.

3.3 Potential New Treatments

Given that inflammation of the intestine is the main mechanism responsible for POI, one might expect that strategies targeting this process are more effective than those currently available. Conversely, the knowledge that the contractility of the inflamed intestine is significantly compromised may also explain why prokinetics are not as effective as one might anticipate.

Accepting that activation of visceral afferents and the subsequent neurogenic inflammation is one of the first events triggering the inflammatory cascade, compounds interfering with this mechanism may hold promise to treat POI. As indicated earlier, the antagonist of CGRP, BIBN4096BS, is effective in reducing intestinal inflammation and shortens POI in preclinical studies (Fig. 2) (Glowka et al. 2015). BIBN4096BS has been used with success to treat acute attacks of migraine (Olesen et al. 2004). A recent trial evaluated the potential of another CGRP antagonist MK-0974 (telcagepant) for prevention of migraine. Patients received this compound twice daily for a period of 12 weeks. Although the results were promising, the trial was terminated due to hepatotoxicity, indicating that daily use cannot be supported (Ho et al. 2014). To what extent

it can be used safely in the perioperative period for only a few days remains to be investigated.

In view of the important role of IL-1 in POI, IL-1R antagonists may be interesting compounds to study as treatment for POI (Stoffels et al. 2014). Anakinra indeed potently prevented POI in mice (Fig. 2). This compound is studied in immune-mediated diseases such as arthritis (Singh et al. 2016) and sepsis (Shakoory et al. 2016). A promising alternative may be IL-1 receptor accessory protein-Ig/IL-1 receptor-type II-Ig heterodimer which more potently blocks IL-1R than anakinra (Hanawa et al. 2011).

Extravasation of leukocytes into the manipulated intestine results from the upregulation of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1). Pretreatment with the monoclonal antibodies to ICAM-1 (de Jonge et al. 2003; Kalff et al. 1999a, b) or the ICAM-1 antisense ISIS 3082 (The et al. 2005) both reduced manipulation-induced inflammation and POI, suggesting that targeting adhesion molecules may indeed be an interesting approach to prevent POI in humans (Fig. 2). However, no human studies are currently available.

Targeting the immune cells involved may be another interesting approach. Especially as resident macrophages seem to orchestrate the inflammatory process, reducing their activation may prove effective to shorten or prevent POI. Inhibition of p38 mitogen-activated protein kinase (p38 MAPK), one of the intracellular signaling pathways involved in macrophage activation, by semapimod (The et al. 2011; Wehner et al. 2009) or its orally active salt CPSI-2364 (Wehner et al. 2012) has been proven to effectively reduce inflammation and POI in mice and swine. Somewhat related, inhaled carbon monoxide (CO) and administration of CO-releasing molecules possess anti-inflammatory properties through interaction with the p38 MAPK pathway, and other intracellular signaling pathways (Gibbons et al. 2013) have proven effective in POI (De Backer et al. 2009; Moore et al. 2003). The advantage of CO-based therapies would be that it can be administered on an acute basis and potentially be delivered close to the site of action by intraperitoneal injection (Nakao et al. 2006). To date, however, no human data are available.

Groundbreaking work in the field of sepsis has introduced the concept that the vagus nerve possesses anti-inflammatory properties by inhibition of macrophages, referred to as the cholinergic anti-inflammatory pathway (CAIP) (Tracey 2002). Based on the insight that activation of resident macrophages triggers the inflammatory phase of POI, the therapeutic potential of the CAIP in POI has been intensively studied. Electrical stimulation of the vagus nerve (VNS) indeed dampens the activation of intestinal resident macrophages reducing the release of pro-inflammatory cytokines, preventing the influx of leukocytes, and restoring intestinal transit (de Jonge et al. 2005; Matteoli et al. 2014; The et al. 2007). This effect is mediated by vagally mediated stimulation of cholinergic neurons releasing acetylcholine interacting with α7nAChRs on the resident macrophages (Fig. 2). As 5-HT_4 agonists such as prucalopride and mosapride increase the release of acetylcholine from enteric neurons, these agents are proposed to have anti-inflammatory properties by mimicking the effect of VNS (Gomez-Pinilla et al. 2014c; Tsuchida et al. 2011). In mice, prucalopride indeed reduced manipulation-induced intestinal inflammation and improved POI, but only when administered preoperatively

(Gomez-Pinilla et al. 2014c), explaining why clinical trials have been rather disappointing so far. Clinical studies evaluating the effect of electrical stimulation of the abdominal vagus nerve in patients undergoing abdominal surgery are ongoing (Fig. 2) (NCT02524626, NCT02425774).

Not only electrical stimulation can activate the vagus nerve, but also compounds activating motor neurons of the vagus in the brain stem could be effective, as shown for semapimod, indeed mimicking the effect of VNS (The et al. 2011). Interestingly, activation of vagal afferents by high-fat enteral feeding also stimulates the CAIP, an effect mediated by the release of CCK (Fig. 2) (Luyer et al. 2005). In rats, high-fat enteral feeding indeed reduced peritoneal levels of TNF-α and IL-6, reduced the influx of neutrophils, and significantly improved intestinal transit (Lubbers et al. 2009). Of note, also in patients undergoing major rectal surgery for rectal carcinoma, early enteral feeding hastened the time to first defecation and reduced the hospital stay from 16–7 to 13–4 days (Boelens et al. 2014). A multicenter double-blind randomized trial (NCT02175979) is currently evaluating perioperative nutrition on POI and anastomotic leakage in patients ($n = 280$) undergoing colorectal surgery. Sham feeding or gum chewing is believed to have a similar effect by stimulation of the vagus nerve. A recent meta-analysis of randomized trials identified 81 studies evaluating the effect of gum chewing on postoperative recovery of GI function including 9,072 patients (Short et al. 2015). This meta-analysis identified some evidence for the benefit of postoperative gum chewing, but the studies included were rather small and of poor quality, so clearly larger studies of better quality are required (Fig. 2).

Finally, as we know that the resident macrophages are inhibited by acetylcholine and nicotine via binding to α7nAChRs, administration of nicotine, but preferentially specific α7nAChR agonists, could be of interest. In mice, the α7nAChR agonists AR-R17779 prevented POI and reduced leukocyte influx and cytokine upregulation (The et al. 2007) comparable to the effect of VNS. Of note, administration of an equimolar dose of nicotine was not tolerated, most likely as nicotine will act on a variety of nicotine receptors. Although nicotine gum chewing has been proposed as a novel strategy to shorten POI, these data would argue against this approach (Wu et al. 2014).

Mast cells have also been implicated to play a role in POI. The evidence in mice however should be interpreted with care as the mast cell-deficient mice used in these studies also lack interstitial cells of Cajal and are immune compromised (de Jonge et al. 2004; Snoek et al. 2012). Using a cleaner mast cell-deficient mice, the role of mast cells, at least in mice, could not be confirmed (Gomez-Pinilla et al. 2014a, b). Mast cell activation has however been demonstrated in humans (Berdun et al. 2015; The et al. 2008), while a small pilot study using the mast cell stabilizer/histamine 1 receptor antagonist ketotifen improved postoperative gastric emptying (Fig. 2) (The et al. 2009). Although promising, no clinical trials are currently available to further support mast cells as important target to treat POI.

4 Conclusions

Over the past few decades, our insight into the mechanisms leading to POI has significantly increased. Especially the role of inflammation in the transient paralysis of the entire gastrointestinal tract has been appreciated. The introduction of minimal invasive surgery or laparoscopy is another major breakthrough significantly reducing the impact of the surgical procedure and the tissue damage induced, and reducing the need for postoperative opioids, all contributing to faster recovery. Similarly, earlier mobilization and feeding of patients, as in the fast-track care programs, is a significant improvement of patient care shortening hospitalization and reducing costs. Current pharmacological management is however rather disappointing, but the increased knowledge will undoubtedly lead to new and more efficient treatments.

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Constipation: Pathophysiology and Current Therapeutic Approaches

Amol Sharma and Satish Rao

Contents

Abstract

Chronic constipation is a common, persistent condition affecting many patients worldwide, presenting significant economic burden and resulting in substantial healthcare utilization. In addition to infrequent bowel movements, the definition of constipation includes excessive straining, a sense of incomplete evacuation, failed or lengthy attempts to defecate, use of digital manoeuvres for evacuation of stool, abdominal bloating, and hard consistency of stools. After excluding secondary causes of constipation, chronic idiopathic or primary constipation can be classified as functional defecation disorder, slow-transit constipation (STC), and constipation-predominant irritable bowel syndrome (IBS-C). These classifications are not mutually exclusive and significant overlap exists. Initial therapeutic approach to primary

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constipation, regardless of aetiology, consists of diet and lifestyle changes such as encouraging adequate fluid and fibre intake, regular exercise, and dietary modification. Laxatives are the mainstay of pharmacologic treatment for potential long-term therapy in patients who do not respond to lifestyle or dietary modification. After a failed empiric trial of laxatives, diagnostic testing is necessary to understand underlying anorectal and/or colonic pathophysiology. No single test provides a comprehensive assessment for primary constipation; therefore, multiple tests are used to provide complementary information to one another. Dyssynergic defecation, a functional defecation disorder, is an acquired behavioural disorder of defecation present in two-thirds of adult patients, where an inability to coordinate the abdominal, recto-anal, and pelvic floor muscles during attempted defecation exists. Biofeedback therapy is the mainstay treatment for dyssynergic defecation aimed at improving coordination of abdominal and anorectal muscles. A large percentage of patients with dyssynergic defecation also exhibit rectal hyposensitivity and may benefit from the addition of sensory retraining. Our understanding of the pathophysiology of STC is evolving. The advent of high-resolution colonic manometry allows for the improved identification of colonic motor patterns and may provide further insight into pathophysiological mechanisms. In a minority of cases of STC, identification of colonic neuropathy suggests a medically refractory condition, warranting consideration of colectomy. The pathophysiology of IBS-C is poorly understood with multiple etiological factors implicated. Pharmacological advances in the treatment of primary constipation have added therapeutic options to the armamentarium of this disorder. Drug development in the secretagogue, serotonergic prokinetic, and ileal bile acid transporter inhibition pathways has yielded current and future medical treatment options for primary chronic constipation.

Keywords
Chronic constipation • Constipation-predominant irritable bowel syndrome (IBS-C) • Dyssynergic defecation • Slow-transit constipation

1 Introduction

Chronic constipation is a common, persistent condition affecting many patients worldwide. In the United States, the median prevalence of constipation is 16% (Mugie et al. 2011). This figure is consistent with an estimated global pooled prevalence of 14% (Suares and Ford 2011). Older age, female gender, and lower socioeconomic status are consistently identified risk factors associated with constipation (Mugie et al. 2011; Suares and Ford 2011; Higgins and Johanson 2004). Almost half of constipated patients surveyed report symptoms for greater than 5 years (Pare et al. 2001). The presence of chronic constipation is a detriment to quality of life and psychological well-being (Belsey et al. 2010; Rao et al. 2007a). Chronic constipation presents a significant economic burden and results in substantial healthcare utilization for patients. More than US$800 million dollars were spent

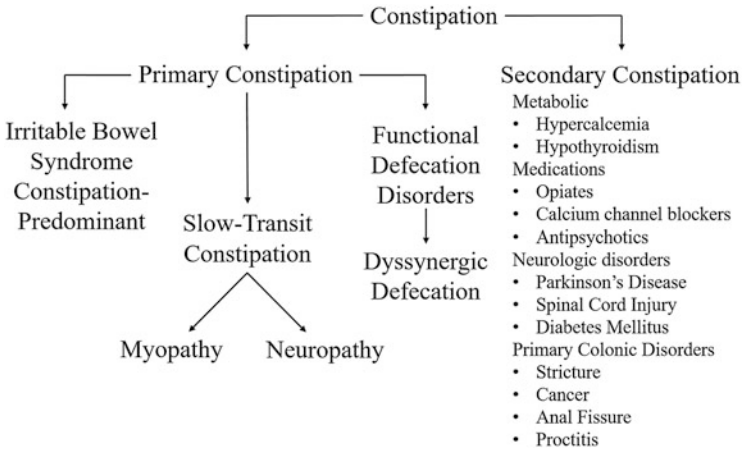

Fig. 1 Aetiologies of primary and secondary constipation

on laxatives by constipated patients in 2007 (Singh et al. 2007). It is estimated that constipation is the primary reason for 2.5 million physician office visits annually (Singh et al. 2007). Furthermore, constipation-related emergency room visits and inpatient admissions are on the rise, especially in a younger cohort of patients (Sethi et al. 2014; Chevalier et al. 2014; Sommers et al. 2015).

The definition of chronic constipation has evolved to encompass more than a decreased number of stools per week. It includes a constellation of symptoms such as excessive straining, a sense of incomplete evacuation, failed or lengthy attempts to defecate, use of digital manoeuvres for evacuation of stool, abdominal bloating, and hard consistency of stools (Herz et al. 1996; Higgins and Johanson 2004; Rao et al. 2004). The broader definition of constipation has translated to a more expansive therapeutic approach.

Constipation is classified into two types: primary and secondary constipation (Fig. 1). Primary constipation is due to disordered regulation of the neuromuscular components within the colon and anorectum as well as disruption in their corresponding ascending and descending pathways in the brain-gut axis. With the help of a symptom-based criteria and diagnostic testing, primary constipation can be further classified into functional defecation disorder, slow-transit constipation (STC), and constipation-predominant irritable bowel syndrome (IBS-C) (Thompson et al. 1999; Bharucha et al. 2006; Longstreth et al. 2006). Functional defecation disorders include dyssynergic defecation and conditions that may obstruct defecation such as rectal prolapse, rectocele, and descending perineum syndrome. Dyssynergic defecation is defined as paradoxical contraction or inadequate relaxation of the pelvic floor muscles during attempted defecation (Rao 2008). STC is defined as inadequate propulsive forces in the colon in the absence of dyssynergic defecation. Multiple underlying factors have been proposed for IBS-C, including genetic, environmental, social, biological, and psychological factors (Crowell et al. 2005). It is worth noting that there is considerable

overlap between dyssynergic defecation, STC, and IBS-C. Greater than half of dyssynergic patients will exhibit delayed colonic transit (Dinning et al. 2011). The opposite also holds true with greater than two-thirds of STC patients having concurrent dyssynergia.

Secondary constipation can result from a multitude of factors such as metabolic disturbances (hypercalcaemia, hypothyroidism), medications (e.g. opiates, calcium channel blockers, antipsychotics), neurologic disorders (parkinsonism, spinal cord lesions, diabetes mellitus), and primary diseases of the colon (stricture, cancer, anal fissure, proctitis) (Bharucha et al. 2013). While a definite association between rectocele and constipation exists, it is unclear if rectocele causes constipation or vice versa.

2 Pathophysiology

Dyssynergia is an acquired behavioural disorder of defecation present in two-thirds of adult patients with difficult defecation, stemming from faulty toilet training, behavioural problems, or parent-child conflicts (Rao et al. 2004). Most patients with dyssynergic defecation suffer from the inability to coordinate the abdominal, recto-anal, and pelvic floor muscles during attempted defecation (Rao et al. 1998a, 2004). Failure of recto-anal coordination consists of either paradoxical anal contraction, inadequate anal relaxation, or impaired rectal/abdominal propulsive forces. Four initial patterns of impaired recto-anal coordination, or dyssynergic defecation, were originally described (Rao 2008). More recently, four additional patterns have been recognized, by further characterizing the location of impaired anal relaxation – puborectalis, external anal sphincter, or both (Rattanakovit et al. 2015). The presence of dyssynergic defecation has been associated with excessive perineal descent (Reiner et al. 2011), rectocele, and solitary rectal ulcer syndrome (Rao et al. 1998b). In some patients, dyssynergic defecation may be more than a locoregional disorder. Approximately 32% of these patients have delayed gastric emptying (Yu et al. 2015) and two-thirds rectal hyposensitivity (Rao et al. 2004), suggesting that dysregulation of brain-gut axis may play a role.

STC is a multifactorial disorder with a strong female prevalence and our understanding of the pathophysiology underlying this condition is evolving. Older studies estimate that up to 90% of STC patients are female (Preston and Lennard-Jones 1986). Colectomy specimens from female STC patients reveal a down-regulation of contractile G-proteins and up-regulation of inhibitory G-proteins correlating to increased progesterone receptors (Xiao et al. 2005). Colectomy specimens from patients with STC also show a pan-colonic decrease in the volume of interstitial cells of Cajal (ICC), intestinal pace-making cells, throughout the colon (Lyford et al. 2002). Underlying pathophysiology in cases of medically refractory STC is best evaluated by colonic manometry (Singh et al. 2013). The absence of two of three normal physiological activities such as high-amplitude contractions (HAPCs), gastro-colonic response, and waking response suggests underlying neuropathy. Only 15% of neuropathic cases will respond to medical and behavioural therapy; therefore, surgical management including colectomy may be considered. However, only one-third of patients with STC have

underlying neuropathy on colonic manometry. In contrast, 64% of STC cases with underlying myopathy will respond to aggressive medical and behavioural therapy. The advent of high-resolution colonic manometry permits detection of retrograde cyclical contractions that accompany HAPCs in response to a test meal in healthy volunteers (Dinning et al. 2014). Further characterization of these colonic motor patterns with this high-definition colonic manometry in STC may provide insight into underlying pathophysiology and impact future treatment. Multiple studies also demonstrate a diminished clinical response to pharmacological stimulation (bisacodyl or neostigmine) or balloon distension in patients with STC (Ravi et al. 2010; Bassotti et al. 1999; Herve et al. 2004), implying dysregulation of autonomic colonic neuromuscular function. Colonic microbiota may also play a role in the pathophysiology of STC. Bacterial fermentation of dietary fibre mainly occurs in the colon, resulting in production of hydrogen gas. The majority of hydrogen gas is disposed via metabolism by colonic microbiota, occurring predominantly via sulphate-reducing bacteria or methanogenic flora (Pimental et al. 2012). Animal studies demonstrate infusion of methane gas results in a delay of gastrointestinal transit and reduced contractility (Pimental et al. 2006). Methanogenic flora is normally only found in up to 35% of the healthy population (Levitt et al. 2006). Both paediatric and adult STC patients (75% of adult STC patients vs. 28% in healthy controls) have an increased prevalence of methanogenic flora (Soares et al. 2005; Attaluri et al. 2010). Interestingly, methanogenic flora was associated with delayed colonic transit but not altered stool consistency.

The pathophysiology of IBS-C is poorly understood, but various factors may play a role such as gut dysbiosis, food intolerances, dysmotility, visceral hypersensitivity, brain-gut interactions, and psychosocial status (Chey et al. 2015). Multiple studies illustrate that the gut microbiota of IBS patients is different from healthy controls, yet, a specific microbial signature for IBS patients remains elusive (Ringel and Carroll 2009; Posserud et al. 2007; Kassinen et al. 2007). Of note, although to a lesser degree than STC patients, normal-transit constipation patients were also noted to have a higher incidence of methogenic flora compared to healthy controls (Attaluri et al. 2010). Up to 10% of patients develop postinfectious IBS after an acute gastroenteritis with *Campylobacter jejuni*, *Escherichia coli* 0157:H7, norovirus, or *Giardia lamblia* (Spiller and Lam 2012). Small intestinal bacterial overgrowth (SIBO) also may be responsible for some cases of IBS, which responds to rifaximin and other antibiotics (Sachdev and Pimental 2012). IBS patients also exhibit intolerance to fructose or fermentable oligo-di-monosaccharides and polyols (FODMAP) (Shepherd et al. 2013). Visceral hypersensitivity has long been accepted as a hallmark of IBS (Mertz et al. 1995). Imaging studies in IBS patients demonstrate an augmented brain response not only to rectal distension but also to the anticipation of pain, suggesting a dysregulation of the brain-gut axis (Mayer and Tillisch 2011). Furthermore, a decreased vagal tone in IBS-C patients suggests autonomic dysfuntion (Coss-Adame and Rao 2014).

After a failed empiric trial of laxatives, diagnostic testing is necessary to understand underlying anorectal and/or colonic pathophysiology. No single test provides a comprehensive assessment for chronic constipation; therefore, multiple tests are used to provide complementary information to one another. Defecography provides useful information regarding anatomical changes in the anorectum, helping detect rectocele, rectal prolapse, rectal intussusception, descending perineum syndrome,

and dyssynergic defecation. Defecography can be performed with fluoroscopy or magnetic resonance; however, it is important for the subject to be in the seated position to assess the physiological state of defecation. Balloon expulsion test is a simple test, using a 4-cm balloon filled with 50 cc of warm water placed in the rectum and timing expulsion of the balloon by the subject (Remes-Troche and Rao 2006). Anorectal manometry (ARM) assesses resting and squeezed anal sphincter tones, recto-anal reflexes, rectal sensation, and pressure during attempted defecation. ARM is an essential test for the diagnosis of dyssynergic defecation, especially in the seated position (Rao et al. 2006). ARM can also detect rectal hyposensitivity (Rao 2008). Newer technologies such as 3D high-definition ARM may reveal distinct abnormalities of the puborectalis and/or anal sphincter function (Lee et al. 2013; Raizada et al. 2011). Overall colonic transit can be assessed by radio-opaque marker test or the more sophisticated wireless motility capsule (WMC) test. On the radio-opaque marker test, delayed colonic transit is defined as greater than five markers present in the colon on an abdominal X-ray 5 days after capsule ingestion (Remes-Troche and Rao 2006). WMC uses pH, temperature, and pressure recordings to assess regional (gastric emptying, small bowel transit, and colonic transit) as well as whole-gut transit. Good agreement exists with both the radio-opaque marker test and WMC (Camilleri et al. 2010); however, both require anorectal testing to rule out co-existing dyssynergic defecation (Ravi et al. 2010; Rao et al. 2005a). In patients with slow-transit constipation refractory to medical therapy, colonic manometry can identify underlying neuropathy that may warrant colectomy (Singh et al. 2013). Hydrogen breath testing may also be useful in patients with chronic constipation suffering from severe gas and bloating to assess for co-existing SIBO or dietary carbohydrate intolerances.

3 Therapeutic Approaches

The therapeutic approach to constipation relies on whether the constipation is a primary or secondary condition and whether it is acute (<3 months) or chronic (>3 months). For acute constipation, it is important to rule out secondary causes, especially colorectal cancer. If faecal impaction is present, enemas, suppositories, large-volume polyethylene glycol solution (PEG), stimulant laxatives, or disimpaction with sedation may be required. In the absence of faecal impaction and other secondary causes, the acute constipation is treated similar to chronic constipation. Treatment of secondary constipation is tailored to addressing the underlying condition. In medication-induced constipation, most often opioid-induced constipation (OIC), the initial step is to withdraw the inciting medication. If opioid withdrawal is ineffective or not possible, pharmaceutical developments have been made to treat OIC. The newest agent for OIC, naloxegol, is an oral peripherally acting mu-opioid receptor antagonist. Naloxegol was shown to be a safe and effective treatment for OIC in a large randomized placebo-controlled study of OIC patients with noncancer pain (Chey et al. 2014). Naloxegol was superior to placebo in patients unresponsive to laxatives, even at lower doses. Another subcutaneously administered peripheral

opioid antagonist, methylnaltrexone, carries a Food and Drug Administration (FDA) warning about severe abdominal pain and bowel perforation. Lubiprostone also has an FDA-approved indication to treat OIC.

3.1 Dietary and Lifestyle Modifications

Initial therapeutic approach to primary chronic constipation (Fig. 2), regardless of aetiology, consists of diet and lifestyle changes such as encouraging adequate fluid and fibre intake, regular exercise, and dietary modification. Fibre is a poorly digested, complex carbohydrate that either acts by bulking stool by drawing fluid into stool residues in the colon as a bulk laxative or undergoes partial fermentation producing short-chain fatty acids, hydrogen, methane, and carbon dioxide (Cummings and Macfarlane 1991). Fibre further accelerates colonic transit, increases biomass, and induces changes in colonic pH and intestinal microbiome, which may, in turn, affect membrane permeability and inflammation (Tomlin and Read 1988; Stephen and Cummings 1980). Fibre may be characterized based on solubility and fermentability. Bloating, abdominal distension, flatulence, and cramping limit the use of insoluble fibres. A systemic review found fair evidence (Level II) with a Grade B recommendation in support of fibre supplementation in mild-to-moderate chronic constipation and

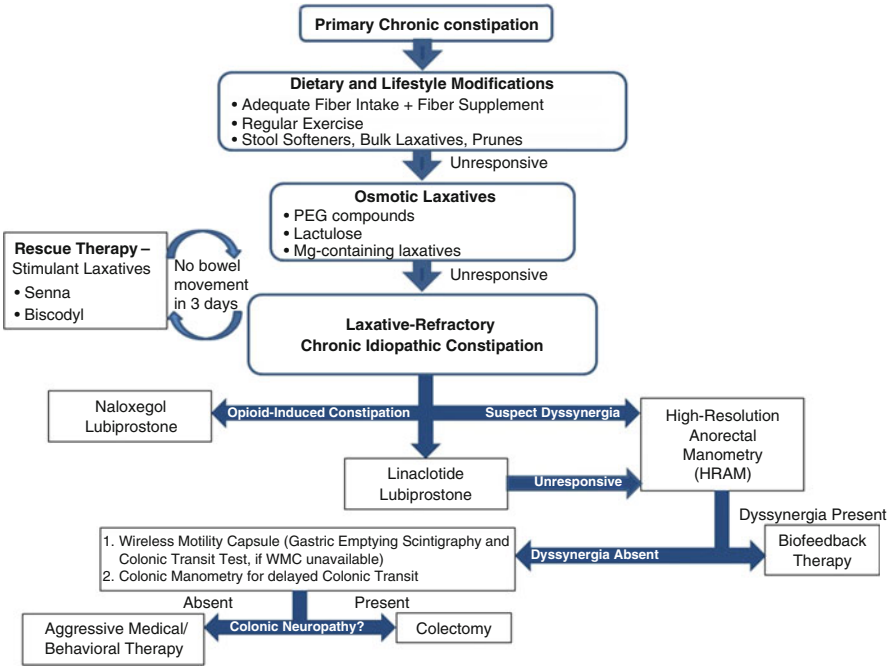

Fig. 2 Therapeutic approach to primary chronic constipation

IBS-C (Rao et al. 2015), particularly mixed fibre supplements with both soluble and insoluble components, such as psyllium (Cheskin et al. 1995; Ashraf et al. 1995; Fenn et al. 1986), prunes (dried plums) (Attaluri et al. 2011), and Suprafiber (Erdogan et al. 2016). FODMAP-restricted diet is effective for symptom management up to 6 weeks in IBS patients. However, the same systemic review found fair evidence (Level II) for chronic constipation and poor evidence (Level III) for IBS-C to support a recommendation for or against the FODMAP-restricted diet (Grade C) (Rao et al. 2015).

3.2 Over-the-Counter Laxatives

Laxatives are the mainstay of pharmacologic treatment for potential long-term therapy in patients who do not respond to lifestyle or dietary modification. Common types of laxatives include stool softeners, osmotic laxatives, and stimulant laxatives. Stool softeners are effective for hard stool consistency and straining during defecation. Osmotic laxatives, such as lactulose, polyethylene glycol (PEG), or magnesium-containing laxatives, draw fluid into the colonic lumen. Stimulant laxatives, such as bisacodyl, sodium picosulfate, and senna, block colonic fluid absorption, induce secretion, and accelerate colonic transit. Osmotic laxatives are better tolerated than stimulant laxatives, causing less abdominal cramping and pain (Chang et al. 2014; Ford and Suares 2011). PEG has been shown to be better than lactulose in improving stool frequency per week, form of stool, relief of abdominal pain, and the need for additional products in a meta-analysis (Lee-Robichaud et al. 2010). Persistent symptoms after at least 6 weeks of osmotic laxatives should be treated with oral stimulant laxatives, such as senna or bisacodyl; however the long-term efficacy of these agents is unknown (Kamm et al. 2011). Stimulant laxatives may also be used as rescue therapy for patients with no bowel movement during treatment of another class of laxatives for 3 days.

3.3 Secretagogues

Secretagogues treat constipation by modulating epithelial ion channels to promote colonic secretion and enhance colonic transit. The two FDA-approved secretagogues readily available for the treatment of chronic constipation and IBS-C include lubiprostone and linaclotide. Lubiprostone is an agonist of type 2 chloride channel (CIC-2), which is located on the apical membrane of the colonic epithelium. Activation of CIC-2 results in indirect activation of $Na^+K^+Cl^-$ co-transport and fluid secretion into the colonic lumen (Bassil et al. 2008). Lubiprostone, a bicyclic fatty acid derivative from prostaglandin-1, also interacts with basolateral prostaglandin E4 receptors to transport cystic fibrosis transmembrane conductance regulator (CFTR) anion channels to the apical membrane, thereby further increasing colonic secretion of chloride and water (Quigley and Neshatian 2016). Three randomized controlled trials (RCT) demonstrate that lubiprostone improves stool frequency, straining effort, stool consistency, constipation severity, and global satisfaction with bowel function (Ford and Suares 2011). Adverse effects include nausea (20%), diarrhoea (10%), and headache (7%) (Lembo

et al. 2011). Lubiprostone is a pregnancy category C drug. Linaclotide, a novel 14-amino acid peptide derived from *E. coli* enterotoxin, is an agonist of apical guanylate cyclase C receptors that increases both intracellular and extracellular cyclic GMP (cGMP). Intracellular cGMP activates CFTR, which increases bicarbonate and chloride colonic secretion (Busby et al. 2010). Extracellular cGMP exhibits nociceptive effects by decreasing firing of afferent nociceptors in response to colonic distension in mice (Castro et al. 2013). This mechanism of action may permit potential treatment of underlying visceral hypersensitivity in IBS-C patients. Two large-scale RCTs show that linaclotide improves stool frequency, bloating, abdominal discomfort, stool consistency, straining, constipation severity, and quality-of-life measures. Adverse effects of linaclotide include dose-dependent diarrhoea, abdominal discomfort, and flatulence (Chey et al. 2012; Rao et al. 2012).

3.4 Serotonergic Prokinetic Agents

Serotonin, or 5-hydroxytryptamine (5-HT), is the most abundant neurotransmitter in the GI tract and produced by enterochromaffin cells. 5-HT receptors are G-protein-coupled receptors that play a key role in visceral sensation and GI motility. Of the 14 known 5-HT receptor subtypes, 5-HT_3 receptors and 5-HT_4 receptors with their role in visceral hypersensitivity and GI motor function, respectively, are key targets for recent advances in drug development for chronic constipation (Crowell et al. 2004). Initial 5-HT agonists, such as cisapride and tegaserod, lacked selective affinity for 5-HT_3 and 5-HT_4 receptors, resulting in cardiac arrhythmias and ischemic events and leading to withdrawal (Quigley 2011; Tack et al. 2012). Prucalopride, a well-absorbed, highly selective 5-HT_4 receptor agonist with half-life of 24–30 h and limited drug-drug interactions has been shown to substantially increase the number of spontaneous bowel movements and improve constipation-related quality of life (Shin et al. 2014). Side effects of prucalopride include headache, abdominal pain, nausea, and diarrhoea; however, to date, no cardiovascular side effects have occurred (Shin et al. 2014). While prucalopride is available in many countries, it is not available in the United States.

3.5 Biofeedback Therapy for Dyssynergic Defecation

Multiple randomized control trials (RCT) have shown biofeedback therapy to be effective in treating dyssynergia when compared to diet and lifestyle modifications, pharmacologic therapy, placebo, or sham biofeedback (Rao et al. 2005b, 2007b; Heymen et al. 1999; Chiarioni et al. 2006). Biofeedback techniques that improve coordination of abdominal and anorectal muscles include diaphragmatic muscle training with simulated defecation, manometric guided pelvic floor retraining, and simulated defecation training. A total of five biofeedback sessions resulted in a durable treatment response for dyssynergia, with benefits lasting for 2 years (Chiarioni et al. 2006). Sixty percent of patients with dyssynergic defecation also had impaired rectal

sensation (Rao et al. 1997; Papachrysostomou and Smith 1994), and the addition of rectal sensory conditioning to biofeedback provides additional therapeutic benefit (Rao et al. 1997, 2005b).

3.6 Surgical Consideration for Chronic Constipation

Patients with persistent chronic constipation unresponsive to medical treatment can be referred to a specialized centre for consideration of colectomy or cecostomy. Further evaluation of gastric emptying, small bowel transit, and colonic transit should be performed prior to surgery. Surgery should be reserved for refractory chronic constipation with severe colonic neuropathy in the absence of gastric and small bowel motility abnormalities. All other patients with chronic constipation should be managed medically, which often requires a multidisciplinary approach for these challenging cases. Cecostomy is generally preferred in institutionalized patients with neurologic lesions, in whom the procedure carries a high success rate, ranging from 40 to 78% (Lees et al. 2004). Colectomy techniques for the treatment of chronic constipation include segmental colectomy, ileorectal anastomosis, ileosigmoid anastomosis, cecorectal anastomosis, ileoanal anastomosis with proctocolectomy, and pouch formation or ileostomy, most of which can be performed laparoscopically (Pemberton et al. 1991). Surgery, in the appropriate patient, has also been shown to be associated with a higher degree of patient satisfaction (Arebi et al. 2011). However, many post-colectomy patients can develop symptoms of gas, bloating, and abdominal distension, a high percentage (65%) of whom have underlying small intestinal bacterial or fungal overgrowth (SIBO/SIFO) (Abdulla et al. 2015). This newly recognized risk factor for SIBO/SIFO is another reason to reserve colectomy only for the appropriate patients.

4 Future Therapies

Plecanatide is a guanylate cyclase C agonist secretagogue similar in structure and pH-dependent action to human uroguanylin. Similar to linaclotide, plecanatide increases intracellular cGMP activating CFTR to increase bicarbonate and chloride colonic secretion (Camilleri 2015). A phase II randomized, double-blind clinical trial demonstrates that 41.9% in the 3 mg plecanatide group and 40.0% in the 9 mg group reached the FDA primary endpoint (an improvement of worst abdominal pain by $\geq$30% and an increase in complete spontaneous bowel movement (CSBM) of $\geq$1 from baseline, both in the same week for $\geq$6 of 12 weeks) compared to placebo in patients with IBS-C (Miner et al. 2014). The most common adverse effect of plecanatide is diarrhoea (Jarmuz et al. 2015).

Velusetrag and naronapride are highly selective 5-HT_4 agonists that have demonstrated the ability to improve all components of regional GI transit, including gastric emptying, small bowel transit, and colonic transit times (Manini et al. 2010; Camilleri et al. 2007). This is advantageous for these newer agents, as a third of gastroparesis patients exhibit global dysmotility and STC (Yu et al. 2015). Both of these agents have

also shown to improve the number of spontaneous bowel movements in chronic idiopathic constipation in phase II clinical trials (Goldberg et al. 2010; Shin et al. 2014). Mosapride, a selective 5-HT_4 agonist and partial 5-HT_3 antagonist, has demonstrated benefit in patients with functional dyspepsia and diabetic gastroparesis in Asian studies (Bang et al. 2015). This agent has also been effective in increasing the frequency of bowel movements in patients with Parkinson's disease-related constipation (Lui et al. 2005).

Inhibitors of the apical ileal bile acid transporter (IBAT) are being developed to treat chronic constipation by inducing choleretic diarrhoea (Camilleri and Gores 2015). IBAT recycles the majority of bile salts into the enterohepatic circulation. However, remaining bile salts are deconjugated by colonic microbiota into secondary bile acids, deoxycholic and lithocholic acid, which increase colonic secretion and motility. Elobixibat, an IBAT inhibitor, has demonstrated accelerated colonic transit, and improved stool consistency and straining in randomized placebo-controlled phase II clinical trials in patients with chronic idiopathic constipation (Simren et al. 2011; Chey et al. 2011). Adverse effects from elobixibat included abdominal cramping.

5 Conclusion

Constipation is a common and complex disorder, symptoms of which encompass more than decreased frequency of bowel movements. Through a detailed history and prospective stool diary, it is important to ascertain the severity and chronicity of symptoms and identify the presence of underlying secondary causes. Chronic idiopathic constipation, refractory to dietary and lifestyle modifications and laxatives, should be evaluated with appropriate testing to understand underlying pathophysiology. Suspicion of dyssynergic defecation on a proper digital rectal examination should be confirmed with anorectal manometry and/or defecography and referred to centres with expertise in manometry-based biofeedback therapy. Colectomy should be reserved for patients with underlying colonic neuropathy identified on colonic manometry. Medications for chronic idiopathic constipation in the pipeline, whether secretagogues, serotonergic agonists, or bile acid transporter inhibitors, seem to be both safe and effective, promising to give clinicians more therapeutic options in their arsenal. It remains to be seen where these newer agents will fit in the treatment algorithm.

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Irritable Bowel Syndrome: Pathophysiology and Current Therapeutic Approaches

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Contents

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Abstract

Irritable bowel syndrome (IBS) is a prevalent condition affecting 10–20% of adults in most countries; IBS results in significant morbidity and health care costs. IBS is a disorder of the brain-gut axis, and recent insights into the pathophysiological mechanisms include altered bile acid metabolism, neurohormonal regulation, immune dysfunction, alterations in the epithelial barrier, and secretory properties of the gut. There remains a significant unmet need for effective treatments, particularly for the pain component of IBS, although the introduction of drugs directed at secretion, motility, and a nonabsorbable antibiotic provides an option for the bowel dysfunction in IBS.

Keywords

Irritable bowel syndrome • Pharmacotherapy

1 Introduction

Irritable bowel syndrome (IBS) is one of the most prevalent gastrointestinal disorders, affecting 11.2% (95% CI, 9.8–12.8%) of people in a study of global prevalence (Lovell and Ford 2012). It results in significant direct and indirect costs and impairment in the quality of life (Leong et al. 2003). IBS is a complex and heterogeneous disorder with different peripheral and central pathophysiological mechanisms responsible for symptoms in subsets of patients (Camilleri 2013). Advances in understanding the pathophysiology of IBS have led to the concept that subsets of patients with more specific mechanisms than a diffuse disorder of the brain-gut axis may lead to a more targeted and individualized approach for treatment of IBS symptoms. Indeed, actionable biomarkers have been described in IBS (Camilleri 2015b; Sood et al. 2014), including colonic transit, low-grade inflammation, and increased bile acid delivery to the colon that is predominantly associated with increased hepatic synthesis of bile acids (Camilleri et al. 2014c). Here we review the pathophysiology of IBS, as it provides the basis for selecting therapy, and the current treatments available for relief of IBS (Table 1).

Table 1 Summary of current treatments for IBS

Therapy	Efficacy	Quality of data	Adverse events	Limitations of data
Dietary therapies and exercise	May be effective	Low	Limited data	Few RCTs, many of crossover design with a small number of participants
Soluble fiber	Effective	Moderate	No increase in adverse events in a meta-analysis of 7 RCTs	Only one trial of high quality
Insoluble fiber	Not effective	Moderate	May be poorly tolerated in some patients, although no increase in adverse events in a meta-analysis of 6 RCTs	Only one trial of high quality
Prebiotics	Unknown efficacy	Low	None reported	Only one RCT, no effect on global symptoms
Probiotics	May be effective	Low	More likely with probiotics in a meta-analysis of 35 RCTs, although the majority were mild	Heterogeneity between studies, and unclear as to which bacterial species or strain is of benefit
Peppermint oil	Effective	Moderate	No increase in adverse events in a meta-analysis of 4 RCTs	Heterogeneity between studies
Herbal therapies	Unknown efficacy	Low	Limited data	Few RCTs, with a small number of participants
Antispasmodic drugs	May be effective	Low	More likely with antispasmodics in a meta-analysis of 22 RCTs, particularly dry mouth, dizziness, and blurred vision	No high-quality trials, heterogeneity between studies, possible publication bias, and only a small number of RCTs assessing each individual antispasmodic
Antidepressants	Effective	Moderate	More likely with antidepressants in a meta-analysis of 17 RCTs, particularly dry mouth and drowsiness	Few high-quality trials, heterogeneity between studies, possible publication bias, and some atypical trials included
Loperamide	Unknown efficacy	Low	Limited data	Few RCTs, with a small number of participants
Eluxadoline	Effective	High	Serious events included acute pancreatitis and sphincter of Oddi	Only a modest benefit over placebo in published RCTs. No benefit over placebo in

(continued)

Table 1 (continued)

Therapy	Efficacy	Quality of data	Adverse events	Limitations of data
			spasm. Nausea and headache commoner with active therapy	terms of abdominal pain
5-HT_3 receptor antagonists	Effective	High	Serious events with alosetron included ischemic colitis and severe constipation. Ramosetron and ondansetron may be safer, although constipation commoner with active therapy	Fewer RCTs of ramosetron and ondansetron. Ondansetron may have no benefit over placebo in terms of abdominal pain
Bile acid sequestrants	Unknown efficacy	Low	Limited data	No published RCTs
Rifaximin	Effective	Moderate	No increase in adverse events in a meta-analysis of 5 RCTs	Only a modest benefit over placebo in published RCTs
Lubiprostone	Effective	Moderate	Nausea commoner with active therapy, occurring in 8% of patients	Only a modest benefit over placebo in published RCTs
Linaclotide	Effective	High	Diarrhea commoner with active therapy, occurring in 20% of patients	None

2 Pathophysiology

In the past, it was generally considered that the predominant pathophysiological mechanisms in IBS were abnormalities intrinsic to the smooth muscle of the gut, visceral hypersensitivity, and central nervous system hypervigilance (Horowitz and Fisher 2001; Lynn and Friedman 1993; Mayer 2008). Brain dysfunction or abnormal interaction of the peripheral and central nervous system has been considered as a potential mechanism causing hypersensitivity in IBS (Mayer 2008; Larsson et al. 2012; Tillisch et al. 2011); however, hypersensitivity (Barbara et al. 2011; Camilleri et al. 2008c) and central dysfunction (Tillisch et al. 2011; Zhou and Verne 2011) are not ubiquitous in patients with IBS. Thus, while persistent nociceptive mechanisms are activated in some patients (Zhou and Verne 2011), IBS is not "all in the head" in all patients. There is a renaissance in IBS: symptoms are not specific to a single etiological mechanism, but are manifestations of several peripheral mechanisms that perturb motor and sensory functions (Fig. 1).

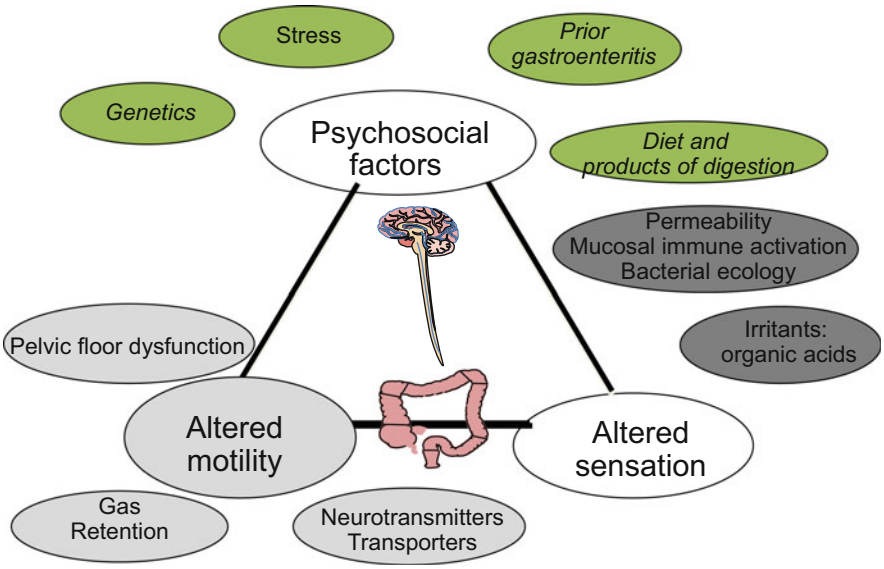

Fig. 1 Pathophysiological mechanisms in irritable bowel syndrome (adapted from Camilleri M. Mechanisms in IBS: something old, something new, something borrowed. Neurogastroenterol Motil 2005;17:311–316 and Camilleri M. Peripheral mechanisms in irritable bowel syndrome. N Engl J Med 2012;367:1626–1635)

3 Altered Brain Responses

The role of the brain in conscious sensation is clearly important in the experience of symptoms in IBS. A large body of research has identified brain regions activated during visceral distension and, more recently, Hong et al. (2016) observed greater engagement of cognitive and emotional brain networks in IBS subjects during contextual threat that may reflect the propensity of IBS subjects to overestimate the likelihood and severity of future abdominal pain. IBS patients have specific abnormalities in attentional network functioning and these deficits may underlie symptom-related anxiety, hypervigilance, and visceral hypersensitivity (Hubbard et al. 2015).

4 Abnormal Colonic Transit and Disorders of Evacuation

Symptoms of patients with constipation-predominant IBS (IBS-C) reflect those of chronic constipation (Halder et al. 2007), and some IBS-C patients can be treated first for constipation. About 25% (range 5–46% in different studies) of patients with IBS-C have slow colonic transit (Bouchoucha et al. 2006; Camilleri et al. 2008c; Tornblom et al. 2012). Treatment with intestinal secretagogues (lubiprostone and

linaclotide) or prokinetic agents (prucalopride) is effective in relieving constipation and associated IBS symptoms such as pain and bloating (Bielefeldt et al. 2016; Camilleri 2012).

There are prominent motor responses in the colon and rectum to feeding. Pain is temporally related to eating in patients with IBS (Ragnarsson and Bodemar 1998), especially in patients with diarrhea-predominant IBS (IBS-D). There are repeated high-amplitude propagated colonic contractions [HAPC (Chey et al. 2001)] that propel colonic content. Patients with IBS-D, compared to healthy controls, also have increased ileocolonic transit (Deiteren et al. 2010), typically induced by meals containing fat and at least 500 kcal (Deiteren et al. 2010). In addition, sensation is also enhanced in the postprandial period. For example, urge, discomfort, and pain scores in response to rectal distension were significantly increased after a 368 kcal meal relative to fasting in patients with IBS. The magnitude of these differences in sensation is small (Ludidi et al. 2012) and is associated with fat, rather than carbohydrate enrichment of the meal (Simren et al. 2007). Overall, the aggravation of symptoms postprandially appears mostly related to colonic motor responses.

In a different series that used different methods of measurement of transit, IBS-D was associated with acceleration of colonic transit in 15–45% of patients (Camilleri et al. 2008c; Tornblom et al. 2012). In addition, several disorders mimic IBS-D or cause accelerated transit and need to be excluded; these include food intolerance or allergy, celiac disease, gluten intolerance without celiac disease, disaccharidase (e.g., lactase, sucrase, isomaltase) deficiencies, microscopic colitis, and idiopathic bile acid (BA) malabsorption (Spiller et al. 2010). Disorders of rectal evacuation, puborectalis spasm, anismus, and the descending perineum syndrome (which typically occurs in older women with prior history of multiple vaginal deliveries) cause constipation, straining, sense of incomplete rectal evacuation, bloating, and left-sided abdominal pain relieved with bowel movement. Thus, these patients have symptoms indistinguishable from those of IBS-C (Prott et al. 2010), and treatment of the evacuation disorder relieves the IBS-C symptoms (Chiarioni et al. 2005). Evacuation disorder should be suspected when constipated patients do not respond to first-line therapies such as fiber and simple (e.g., osmotic) laxatives (Voderholzer et al. 1997).

5 Irritated Bowel: Luminal Factors and Peripheral Mechanisms

Increasingly, the literature documents that there are multiple peripheral mechanisms involved in IBS, with luminal and mucosal factors activating immune, motor, and sensory mechanisms in the small intestine or colon.

5.1 Malabsorbed or Maldigested Nutrients

Malabsorbed sugars, such as lactose, fructose, and sorbitol, may mimic the features of IBS. A Norwegian case-control study suggested that IBS and lactose malabsorption were separate entities (Farup et al. 2004). Fructose and sorbitol malabsorption was observed in patients with IBS from Denmark (Rumessen and Gudmand-Hoyer 1988), but not in patients from the Netherlands (Nelis et al. 1990).

Maldigestion of complex carbohydrates may be more prevalent in IBS patients than actual malabsorption. Fecal short-chain fatty acids (SCFA with <6 carbon atoms) are increased in patients with IBS-D (Treem et al. 1996). SCFAs or medium-chain (6–12 carbon) fatty acids reach the right colon even in healthy people, in whom 2–20% of dietary starch escapes absorption in the small bowel (Stephen et al. 1983), providing substrate for the generation of SCFAs by colonic bacteria in IBS patients. In the presence of borderline absorptive capacity or rapid transit in the small bowel, more substrate may be delivered to the colon, leading to two potential consequences: first, increased SCFA fermentation and second, activation of enteroendocrine and mast cell mechanisms that may also lead to changes of function, leading to IBS symptoms. The latter activation involves the SCFA receptor 2 [called free fatty acid receptor 2 (FFA2) or G-protein-coupled receptor 43 (GPR43)] that is expressed by both enteroendocrine cells (co-localized with chromogranin A) and mucosal mast cells (Karaki et al. 2006). SCFAs have been shown to stimulate colonic transit and motility via intraluminal release of 5-hydroxytryptamine [5-HT (Fukumoto et al. 2003)] from enteroendocrine cells (Mitsui et al. 2005) in rats, and to initiate HAPCs in the colon in dogs (Kamath et al. 1987). The SCFA, propionate, induced transepithelial ion and fluid secretion in guinea pig distal colon mucosal preparations in vitro and increased the expression of FFA2 in enteroendocrine cells (Karaki and Kuwahara 2011).

Fermentable oligosaccharides, disaccharides and monosaccharides, and polyols (FODMAP) are poorly absorbed in the small intestine. FODMAPs lead to production of SCFAs, and induce symptoms of IBS (Shepherd et al. 2008) similar to the mechanisms described above. Conversely, dietary manipulation of FODMAP content may reduce IBS symptoms (Ong et al. 2010), including overall symptoms, and abdominal pain and bloating (Marsh et al. 2016), though other systematic reviews suggest the need for more rigorous studies to determine long-term efficacy (Ford and Vandvik 2015; Rao et al. 2015) and, by inference, the etiopathogenetic role of the FODMAPs in the induction of IBS symptoms. The role of the colonic microbiota in these nutrient-induced symptoms requires further elucidation.

5.2 Gluten Intolerance

The prevalence of celiac disease in patients with IBS (Cash et al. 2011) is similar to that of controls. However, among non-celiac patients with IBS who carry HLA-DQ2 or -8 genotypes (which predispose to celiac disease), there was five times greater likelihood to respond to gluten withdrawal compared to noncarriers (Wahnschaffe et al. 2007). In patients who previously reported intolerance to gluten and response to

gluten withdrawal, a randomized, placebo-controlled trial confirmed that gluten is associated with symptoms of IBS (Biesiekierski et al. 2011). In non-celiac patients with IBS-D, gluten intake was associated with increased stool frequency and bowel permeability, and reduced tight junction protein expression in bowel mucosa (Vazquez-Roque et al. 2013); consistent with the earlier study (Wahnschaffe et al. 2007), the gluten-containing diet had a greater effect on bowel movements per day in HLA-DQ2/8-positive than HLA-DQ2/8-negative patients (Vazquez-Roque et al. 2013). In mice, sensitized to wheat glycoprotein, gliadin treatment increased responses to contractile (carbachol) and secretory (electrical field) stimuli (Verdu 2008), suggesting interaction between immune activation and increased acetylcholine release from the myenteric plexus, muscle hypercontractility, and increased active ion transport.

5.3 Increased Intracolonic Bile Acids (BA)

The ileal mucosa of patients with IBS (compared to controls) shows higher sensitivity to the secretory effects of perfused BAs (Oddsson et al. 1978). However, the effects of BAs result predominantly from effects of bile acids in the colon resulting in diarrhea. Systematic analyses estimated that BA malabsorption, elevated BA synthesis [measured by the fasting concentration of serum 7α-hydroxy-4-cholesten-3-one (C4, a bile acid precursor)], or increased 48-h total fecal BA excretion may account for about 30% of patients with IBS-D (Slattery et al. 2015; Valentin et al. 2015; Wedlake et al. 2009).

The increased intracolonic BAs reported in IBS-D or functional diarrhea result from alterations in the enterohepatic circulation of BAs. Synthesis of BA is regulated homeostatically by hepatocyte feedback inhibition, provided by fibroblast growth factor 19 (FGF19) which is produced by ileal enterocytes. FGF19 is secreted into the portal circulation and binds to fibroblast growth factor receptor 4 (FGFR4) and Klothoβ (KLB) receptor on the hepatocyte cell membrane, inhibiting CYP7A1, a rate-limiting enzyme in BA synthesis.

The identified mechanisms resulting in bile acid diarrhea are the following:

(a) Deficiency in ileal secretion of FGF19—Ileal FGF19 production (reflected in plasma levels of this hormone) is reduced in patients with chronic diarrhea (Walters et al. 2009). There is an inverse relationship between fasting serum FGF19 and serum C4 levels in patients with IBS-D (Odunsi-Shiyanbade et al. 2010; Rao et al. 2010).
(b) Ileal bile acid transporter (IBAT, also termed apical sodium-bile acid transporter, ASBT, or SLC10A2) mutation, resulting in malabsorption of BAs, is extremely rare, even among familial cases of BA malabsorption (Montagnani et al. 2001, 2006).
(c) Genetic variations in BA synthesis or TGR5 (GPBAR1) receptor:
    - Genetic variation in the hepatocyte receptor protein, Klothoβ, to which FGF19 binds (Johnston et al. 2011), is associated with IBS-D and accelerated

colonic transit (Wong et al. 2011). This specific genetic variation in the Klothoβ gene (Arg728Gln) is functionally significant resulting in impaired Klothoβ protein synthesis, thereby preventing FGF19 binding to combined Klothoβ-FGFR4 receptor on the hepatocyte and reducing the FGF19 feedback inhibition of hepatocyte synthesis of BAs, and resulting in increased BAs reaching the bowel and, potentially, accelerating transit and causing diarrhea.

Exome sequencing identified additional variants in *KLB* and *FGFR4* associated with fecal bile acid excretion or colonic transit in IBS-D (Camilleri et al. 2014a). Those variations in *KLB* (rs1015450, downstream) and *FGFR4* [rs434434 (intronic), rs1966265, and rs351855 (non-synonymous)] require further study. In a 633-person cohort, *FGFR4* rs434434 was associated with symptom phenotype and rs1966265 with 24-h colonic transit ($P = 0.066$).

- Genetic variation in G-protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5) is possibly associated with small bowel and colonic transit in health and IBS (Camilleri et al. 2011b, 2014b). The TGR5 receptor is located on myenteric, cholinergic, and nitrergic neurons in colon and proximal small intestine.

5.4 Microbiota

The precise role of the fecal or mucosal microbiome in IBS is unclear; abundance of Firmicutes in the fecal microbiome has been a sole finding (Kassinen et al. 2007; Tana et al. 2010), or combined with a decrease in the abundance of Bacteroidetes (Rajilic-Stojanovic et al. 2011; Salonen et al. 2010). An increase in the ratio of Firmicutes to Bacteroidetes was associated with colonic transit and levels of depression in IBS (Jeffery et al. 2012). Mucosa-associated microbiota show increases in Bacteroides and Clostridia and a reduction in Bifidobacteria in IBS-D (Parkes et al. 2012), and these changes in gut microbiota may potentially influence selection of therapy (Dupont 2014) and may be the mechanism targeted by the approved non-absorbable antibiotic, rifaximin, which appears efficacious in both non-constipated and constipated IBS patients (Pimentel et al. 2011, 2014).

5.4.1 Interaction of Fecal Microbiota and Organic Acids

The fecal microbiome may alter colonic functions through microbial interactions with intraluminal factors, particularly organic acids. There are alterations in the organic acid profiles (such as SCFAs) and the proportion of secondary BAs (deoxycholic and lithocholic acid) compared to primary BAs (cholic and chenodeoxycholic acid) in IBS. For example, there are larger proportions of primary BA in stool of IBS-D patients, possibly because of reduced colonic residence time associated with rapid transit (Duboc et al. 2012; Shin et al. 2013; Tana et al. 2010; Wong et al. 2012). Colonic bacteria deconjugate and dehydroxylate BAs to different extents; however, the primary or secondary BAs with at least 2 α-hydroxyl groups (chenodeoxycholic, cholic, and deoxycholic acid) induce secretion in mammalian intestine or colonic epithelial cell monolayers (Chadwick et al. 1979; Keely et al. 2007). Moreover, chenodeoxycholic acid induces HAPCs in healthy humans (Bampton et al. 2002)

and acceleration of colonic transit in IBS-C (Rao et al. 2010), while deoxycholic acid induces colonic inflammation and hypersensitivity in rats (Traub et al. 2008). The bacterial dehydroxylation of chenodeoxycholic acid to lithocholic acid would theoretically reduce colonic secretion, but lithocholic acid constitutes only ~20% of the total fecal BA (Duboc et al. 2012). Overall, it is still unclear to what extent altered colonic function attributable to microbial differences occurs through changes in BA metabolism. The role of microbiota in the fermentation of complex carbohydrates or disaccharides is discussed above.

Conversely, BAs may modify the microbial content of the colon. For example, in rats, administration of cholic acid resulted in cecal microbiota that reflected the ratio of Firmicutes to Bacteroidetes observed in patients with IBS (Islam et al. 2011).

5.4.2 Interaction of Microbiota and Stress

Stress can lead to long-term changes in the gut microbiota in primates (Bailey and Coe 1999). Stress and the gut microbiota interact in the regulation of behavior (Bravo et al. 2011; Clarke et al. 2013; Desbonnet et al. 2015), as well as visceral nociception (Moloney et al. 2016).

5.5 Enteroendocrine Signals Arising in the Mucosa

The release of several peptides and amines, such as serotonin, from enteroendocrine cells is triggered by luminal factors such as exogenous dietary amines or tastants, or their metabolites (such as SCFAs), and by endogenous chemicals involved in the digestive process such as BAs (Kidd et al. 2008; Peregrin et al. 1999). The potential role of serotonin (5-HT) in IBS is based on its higher circulating levels in IBS-D and lower levels in IBS-C; its generally stimulatory effects on motor, secretory, and sensory functions (Gershon and Tack 2007; Hoffman et al. 2012); and the impact of selective serotonergic agonists and antagonists in the treatment of different IBS phenotypes (Camilleri 2012).

Enteroendocrine cells also release granins such as chromogranins (Cg) and secretogranins (Sg) which are present in secretory vesicles. Activation of nicotinic cholinergic receptors (for example, by acetylcholine released from submucosal nerves) induces granin release, promoting the sorting and release of other peptide hormones from enteroendocrine cells (Montero-Hadjadje et al. 2009). Cg-derived peptides secreted by such cells have antimicrobial properties against bacteria, fungi, and yeasts (Shooshtarizadeh et al. 2010).

Compared with healthy controls, IBS patients, particularly those with faster colonic transit, demonstrated higher levels of fecal CgA, SgII, and SgIII, but lower levels of CgB (Ohman et al. 2012). These findings are nonspecific, since increased fecal granins or Cg cell density in colonic mucosa are observed in other diarrheal diseases, such as lymphocytic colitis (El-Salhy et al. 2011) and celiac disease (Moyana and Shukoor 1991; Pietroletti et al. 1986). The CgA cells also express FFA2 receptors that respond to SCFAs (Camilleri et al. 2009).

5.6 Genetic Factors Impacting Peripheral Mechanisms in IBS

The published literature on potential genetic factors predisposing to IBS is summarized elsewhere (Camilleri and Katzka 2012; Zucchelli et al. 2011). The reported genetic factors of greatest interest predispose to inflammation, bile acid synthesis (discussed above), expression of bioactive neuropeptides, and intestinal secretion through a mutation in the guanylate cyclase-C secretory pathway.

5.6.1 Genetic Susceptibility to Inflammation and IBS Symptoms and Colonic Transit

In a study of 30 susceptibility loci associated with epithelial transport, barrier function, bacterial recognition, autophagy, prostaglandin production, and TH17 lymphocyte differentiation (previously associated with Crohn's disease), there was significant association between *TNFSF15* (rs4263839) and IBS phenotype in separate Swedish and US patients (Zucchelli et al. 2011), and with a UK cohort (Swan et al. 2013). The strongest association in the Swedish-US study was with IBS-C (odds ratio 1.79) and, in the cohort from the UK, genetic variations were protective against having IBS-D. In a meta-analysis comprising 2,894 patients (839 IBS-C, 1073 IBS-D, 502 IBS-M) and 3,138 healthy volunteers with self-reported Caucasian ancestry, the association of the SNP rs4263839 was confirmed with IBS (OR 1.19, 95% CI 1.08–1.31) and IBS-C (OR 1.24, 95% CI 1.08–1.42).

Villani et al. (2010) reported four genes associated with postinfectious IBS in patients in Walkerton, Canada, and these susceptibility loci included TLR9. Colonic transit in patients with IBS was univariately associated with four "inflammation susceptibility" genes that included TLR9, CDH1, and IL6 (Camilleri et al. 2011a).

The association with *KDELR2* identified in a GWAS study of IBS in ~4,000 patients and ~5,000 controls is thought to reflect modification of the effects of bacterial toxins in the gut (Ek et al. 2015).

5.6.2 Genetic Variation in Neurotransmitters or Cytokines

NPSR1, the receptor for neuropeptide S (NPS), is expressed by gastrointestinal (GI) enteroendocrine cells and induces the production of several neuropeptides. The NPS/NPSR1 ligand-receptor complex is involved in inflammation, anxiety, and nociception. Three SNPs of the *NPSR1* gene (rs2609234, rs6972158, and rs1379928) are significantly associated with colonic transit in IBS, and rs1379928 polymorphism was also associated with pain, gas, and urgency sensory ratings (Camilleri et al. 2010). The endocannabinoid, anandamide, is inactivated by the fatty acid amide hydrolase (FAAH). An SNP in *FAAH* gene (C385A) reduces FAAH expression. FAAH CA/AA increases the odds (relative to health) for IBS-D or IBS with alternating bowel function and is significantly associated with accelerated colonic transit in IBS-D (Camilleri et al. 2008b).

The gene controlling the serotonin transporter (SERT or SLC6A4) protein is *5-HTTLPR*. This is associated with IBS phenotype in some ethnicities, but results are inconsistent. The short allele (associated with reduced SLC6A4 function) is associated with higher rectal pain sensory ratings (Camilleri et al. 2008a), increased

activation of regional cerebral blood flow during painful colorectal distensions (Fukudo et al. 2009), as well as reduced response to the 5-HT_3 antagonist, alosetron (Camilleri et al. 2002).

5.6.3 Genetic Mutation in the Guanylate Cycle-C Secretory Pathway

A Norwegian family has been described with dominantly inherited, fully penetrant disease due to a heterozygous base substitution, c.2519G→T, in exon 22 of chromosome 12, GUCY2C. This familial diarrhea is characterized by the onset of symptoms in infancy, chronic, relatively mild diarrhea diagnosed as IBS-D. The functional mutation encodes for the guanylate cyclase-C (GC-C) receptor which induces enterocyte secretion (Fiskerstrand et al. 2012).

5.7 Consequences of Irritation of the Colon

5.7.1 Immune Activation, Minimal Inflammation

Ohman and Simrén (2010) have summarized the evidence in support of inflammation or immune activation in the blood in, at least, some subsets of patients with IBS. The evidence of mast cell and other immune (e.g., cytokine) activation in intestinal or colonic mucosa is less consistently demonstrated (Ohman and Simrén 2010). Inflammation, manifesting as increased T lymphocytes in rectal mucosa in patients with IBS, was associated with increased intestinal permeability (Spiller et al. 2000). These data, in addition to the epidemiological and clinical observations of postinfectious IBS (Halvorson et al. 2006; Spiller and Garsed 2009) and colonic mucosal gene expression profiles demonstrating functional alterations of several components of the host mucosal immune response to microbial pathogens (Aerssens et al. 2008), all support a role of immune activation and altered bowel barrier function in a subgroup of patients with IBS. Genetic susceptibility may predispose to immune activation in a subset of patients with IBS (see above). Immune activation may also be associated with increased mucosal permeability (Matricon et al. 2012).

5.7.2 Increased Mucosal Permeability

Several studies in adults (summarized by Rao et al. 2011) document increased small bowel or colonic mucosal permeability in vivo, in mucosal biopsies in vitro, or in Caco2 monolayers in response to fecal supernatants from patients with IBS. Similarly, children with IBS have evidence of increased proximal gut and colonic permeability and low-grade inflammation (Shulman et al. 2008). Factors associated with increased mucosal permeability and IBS include cow's milk allergy, prior nonspecific infection, atopic disease [rhinoconjunctivitis, rhinitis, eczema (Lillestol et al. 2010; Saps et al. 2008, 2011)], stress, and dietary fat.

- *Inflammation:* The link of increased mucosal permeability with IBS is based on observation that increased permeability enhances mucosal inflammation and activates local reflex mechanisms, stimulating secretion and sensory pathways

that lead to increased visceral sensation (Barbara et al. 2011). There is evidence that there is a distinct subgroup of IBS patients with increased GI permeability and/or increased peripheral blood mononuclear cell production of IL-10. These patients typically have more severe IBS in terms of interference with daily activities and daily IBS symptoms (Shulman et al. 2014).

- *Stress:* Several lines of investigation support a role for stress in increased bowel permeability in humans. First, when the stress hormone, corticotrophin-releasing hormone (CRH), was applied to the serosal side of colonic mucosal biopsies from healthy humans, there was increased transcellular uptake of horseradish peroxidase, an effect that was mediated by mast cells (Wallon et al. 2008). Second, experimental cold pain stress induced increased jejunal permeability in healthy females, but not in males (Alonso et al. 2012). Third, acute psychological stress increased small intestinal permeability in humans; peripheral CRH reproduced the effect of stress and disodium cromoglycate blocked the effect of both stress and CRH, suggesting that mast cells are involved in the effects of stress (Vanuytsel et al. 2014). Fourth, combat-training associated with prolonged, intense, mixed psychological and physical stress induced stress, anxiety and depression, GI symptoms, pro-inflammatory immune activation, and increased intestinal permeability (Li et al. 2013).
- *High-fat diet* results in gut-derived endotoxemia (Pendyala et al. 2012) and may contribute to the immune activation observed in some IBS patients. Animal studies show that emulsified fats increase intestinal permeability, causing endotoxemia and inflammation (Laugerette et al. 2011).
- *Dietary antigens*: An effect of dietary antigens on the intestinal epithelial barrier has been implicated. In a real-time observational study using confocal laser microendoscopy in 36 patients with IBS with suspected food intolerance, Fritscher-Ravens et al. (2014) reported that, within 5 min of exposure to diluted food antigens, gaps formed in the epithelium, intervillous spaces widened, and the number of intraepithelial lymphocytes increased.

5.7.3 Altered Expression of Secretory, Barrier, and Immune Functions

Next-generation sequencing studies have revealed alterations in expression of both small bowel and colonic mucosa in IBS, with most studies to date focused on IBS-D.

Differences in jejunal mucosal expression (at gene and protein levels) and in the distribution of apical junction complex proteins (Martinez et al. 2012, 2013), as well as reduced ZO-1 expression in HLA DQ2/8-positive patients with non-celiac IBS-D (Vazquez-Roque et al. 2012), support the observed alterations in barrier mechanisms seen in colonic mucosa in patients with IBS-D. With FDR correction, the following genes were significantly upregulated ($q < 0.05$) in the participants in the current study of IBS-D relative to controls who had undergone clinically indicated small bowel biopsies (which were histologically normal): INADL, MAGI1, PP2R5C, MAPKAPK5, TLR3, and IL-15 (Camilleri et al. 2016).

In addition, relative to healthy controls, patients with IBS-D had altered mRNA expression in colonic mucosa of factors that could be mapped to a biologically relevant pathway in IBS-D, based on *p*-values with correction for false detection rate. These pathways include changes in gene expression for neurotransmitters (P2RY4 and VIP), ion channels (GUCA2B and PDZD3), cytokines and complement (C4BP4 and CCL20), immune function and stress-related proteins (TNFSF15, IFIT3, HSPA1A, and HSPA1B), mucosal repair, and cell adhesion (TFF, RBP2, and FN1) (Camilleri et al. 2015b).

6 Current Therapeutic Approaches for IBS

Despite the numerous pathophysiological mechanisms that are implicated in IBS, in clinical practice the disorder is considered to have no underlying structural or biochemical explanation. It is unlikely that, in the majority of individuals, there is a single unifying explanation, but rather it is more likely that there are multiple disease processes that lead to pain and diarrhea, or pain and constipation. As a result, and unlike in other organic GI diseases where treatments are often developed based on pathophysiology, IBS treatment is often selected on an individual basis, and is targeted at the predominant or most troublesome symptom experienced by the patient. The implications of this approach are that symptoms of IBS are often chronic, and the natural history of the disorder is usually unaffected by treatment in the longer term, that is, treatment has not yet evolved to disease modification (Ford et al. 2008a). This highlights the need for future high-quality randomized controlled trials (RCTs) of interventions based on the proposed etiologies summarized described above, with the presence of these pathophysiological mechanisms in patients confirmed using biomarkers, such as abnormalities of colonic transit or bile acid metabolism (Camilleri et al. 2014c).

Conventionally, IBS is divided into subtypes according to the predominant stool pattern because this defines treatment options. Over the years there have been numerous well-intentioned attempts to synthesize the available literature, in order to provide evidence-based treatment recommendations for the management of IBS based on these symptom subtypes (Brandt et al. 2009; Chang et al. 2014; Ford et al. 2014a; Spiller et al. 2007). These guidelines are able to make unequivocal conclusions regarding the efficacy of more recently developed drugs for IBS, which are based on large high-quality trials, conducted among patients with IBS recruited according to predominant stool pattern, and using Food and Drug Administration (FDA)-recommended endpoints to judge efficacy. However, for many of the more traditional therapies the inferences made in such guidelines are more contentious because RCTs studying these agents are smaller and of lower quality, and recruit heterogeneous groups of patients with IBS, meaning that efficacy according to stool pattern cannot easily be judged, and the endpoints used were of debatable validity (Camilleri 2015a).

In the remainder of this chapter, we will review the evidence for the efficacy of general lifestyle measures, alternative and herbal therapies, and older therapies for IBS, making suggestions for their optimal use according to IBS subtype, where

individual studies have reported such data, but will report the efficacy of licensed treatments in patients with IBS according to predominant stool pattern.

7 General Lifestyle Measures

7.1 Dietary Modifications

Many patients with IBS believe that their symptoms relate to food sensitivity (Bohn et al. 2013). The mechanism by which food generates symptoms in IBS is unclear, although a change in diet can alter the microbiome rapidly (David et al. 2014), and high levels of insoluble dietary fiber have been implicated in exacerbating IBS symptoms for many years (Francis and Whorwell 1994), despite the fact that patients with IBS are often told to increase their dietary fiber intake. The effect of dietary antigens on intestinal epithelial barrier provides rationale for dietary modifications.

Published trials of dietary interventions in IBS are hampered by problems with small numbers of recruited subjects, and lack of blinding, and many have used a crossover design, leading the authors of a recent systematic review to conclude that, although data are promising, these approaches cannot be recommended strongly until more evidence is accumulated (Moayyedi et al. 2015). Despite this, in the UK, the National Institute of Health and Care Excellence has recommended a low FODMAP diet as a first-line treatment for patients with IBS in primary care (Anonymous. Diagnosis and management of irritable bowel syndrome in adults in primary care: summary of NICE guidance 2015).

7.2 Elimination Diets

Atkinson et al. (2004) conducted an RCT of an elimination diet, based on IgG antibody testing to food items, versus a sham diet, with patients told to avoid the same number of foods, but not those to which they had raised antibodies. The trial recruited 150 patients with any stool pattern subtype of IBS, and excluded celiac disease using a tissue transglutaminase test. The threshold for a positive IgG antibody result was defined as three times the background signal obtained by the same sample against a no-food-allergen-coated control. At 12 weeks, 28% of those allocated to the elimination diet reported that their symptoms were substantially improved, compared with 17% of those assigned to the sham diet ($P = 0.001$), with a number needed to treat (NNT) of 9. Effectiveness of the elimination diet according to stool pattern subtype was not reported. Following reintroduction of eliminated foods, 41.5% of those originally randomized to elimination diet reported substantial worsening of their symptoms, compared with 25% of the sham diet arm ($P = 0.047$). This is in contrast to other studies, which have reported that, among patients with IBS who report dietary triggers, the offending food induces reproducible symptoms in only around a quarter of patients during a double-blind rechallenge (Young et al. 1994).

7.3 Increasing Dietary Fiber Intake

Although there have been misgivings about the use of fiber in IBS, there have been numerous RCTs studying the effect of fiber on symptoms of IBS. In the largest and highest quality of these trials, the authors randomized 275 patients with all subtypes of IBS to 12 weeks of treatment with soluble fiber, in the form of psyllium, insoluble bran fiber, or placebo (Bijkerk et al. 2009). The authors reported that bran was of no benefit, but psyllium was superior to placebo for the treatment of IBS during the first 8 weeks of treatment, with an NNT of 6, although by 12 weeks this benefit was no longer apparent. The reduction in symptom severity with psyllium at 12 weeks was, however, statistically significant (mean reduction 90 points on the IBS symptom severity score versus 40 points, $P = 0.03$). In a subgroup analysis in those with IBS-C, psyllium was of similar benefit. Overall, more patients assigned to bran were unable to tolerate therapy and withdrew, although this difference was not statistically significant, and total adverse event rates were no higher with bran.

In an updated systematic review and meta-analysis (Moayyedi et al. 2014) that identified 14 RCTs, recruiting 906 patients, most of which were of low methodological quality, there was a statistically significant, but modest, benefit of fiber in IBS, with a relative risk (RR) of remaining symptomatic of 0.86 (95% CI 0.80–0.94), and an NNT of 10. This beneficial effect was limited to psyllium, used in seven studies that included 499 patients, with an RR of remaining symptomatic of 0.83 (95% CI 0.73–0.94) and an NNT of 7, with bran having no beneficial effect (RR of remaining symptomatic = 0.90; 95% CI 0.79–1.03). Adverse events were no more common with either psyllium or bran, compared with placebo. The mechanism of action of psyllium is uncertain, and is unlikely to relate to stool bulking alone, as bran has similar effects (Tomlin and Read 1988). It may be that it stems from the effects of psyllium fermentation on gut function, via increased production of short-chain fatty acids, such as butyrate, which provides energy for colonic mucosa cells and may have anti-inflammatory effects (Zimmerman et al. 2012). Another possibility is that psyllium is acting as a prebiotic, altering the intestinal microbiota (Lee et al. 2015), and that this contributes to the beneficial effects on symptoms.

7.4 Low-FODMAP Diet

As discussed earlier FODMAPs, which are present in stone fruits, legumes, lactose-containing foods, and artificial sweeteners, are poorly absorbed and may have osmotic and fermentation effects in the intestine (Shepherd et al. 2008). The osmotic effects have been demonstrated in patients with IBS, as magnetic resonance imaging (MRI) studies show increased small intestinal water content, and small intestinal distension, following administration of FODMAPs (Undseth et al. 2014). Whether this wholly explains their deleterious effects in IBS is unclear. Diets differing in FODMAP content also appear to have effects on the fecal microbiome, with a low-FODMAP diet leading to a reduction in bacterial abundance (Halmos et al. 2015), as well as lower proportions of certain bacteria, including *Bifidobacteria* (Staudacher et al.

2012). The long-term sequelae of the effect of FODMAP restriction on the gut microbiota remain uncertain.

In an Australian RCT using a crossover design (Halmos et al. 2014) that compared a diet low in FODMAPs with a typical Australian diet in 30 patients with IBS of all subtypes, global IBS symptoms, bloating, and pain were all reduced while on the low-FODMAP diet, and the beneficial effects seemed to be greater in those with IBS-D. However, this trial has been criticized as a large proportion of patients were able to identify correctly when they were on the low-FODMAP diet, the endpoint used was a 20 mm difference between treatment arms on a 100 mm visual analogue scale, whose clinical relevance is uncertain, and while on the low-FODMAP diet, patients supplemented their diet with psyllium and starch, meaning that the effects observed may not be attributable only to the low-FODMAP diet (Camilleri and Acosta 2014).

Subsequently, a parallel group RCT has been conducted comparing a low-FODMAP diet with conventional dietary recommendations, which included advice to eat small regular meals, and avoid insoluble fiber, fatty foods, and caffeine (Bohn et al. 2015). In this Swedish study, which comprised 67 patients with all subtypes of IBS, symptom scores were reduced significantly in both arms, compared with baseline, but there was no difference in efficacy between the two interventions, with 50% of those assigned to low-FODMAP diet reporting a $\geq$50% reduction in symptom severity scores, compared with 46% in the traditional dietary advice group.

7.5 Gluten-Free Diet

Some patients with IBS attribute their symptoms to gluten ingestion, despite an absence of immunological, serological, or histological markers of celiac disease, and are often labelled as having non-celiac gluten sensitivity. There is accumulating evidence that a gluten-free diet may be beneficial in a subset of patients with IBS. One small RCT recruited 39 patients with IBS in whom celiac disease had been excluded, and who had responded symptomatically to a gluten-free diet. They were instructed to continue the diet, but were then randomized to receive either gluten-containing, or gluten-free, muffins and bread, which were of identical appearance (Biesiekierski et al. 2011). Overall, after 6 weeks, 68% of those receiving gluten reported inadequate symptom control, versus 40% of those randomized to gluten-free muffins or bread ($P < 0.001$). Pain, bloating, and satisfaction with stool consistency were also significantly improved among those assigned to placebo. It was unclear from this study whether there was an effect of gluten-free diet according to predominant stool pattern.

In another controlled trial in 45 patients with IBS-D who were randomized to gluten-free or normal diet, and who were genotyped for HLA-DQ2 and -DQ8, stool frequency was significantly reduced among those assigned to a gluten-free diet (Vazquez-Roque et al. 2013). This beneficial effect appeared to be more pronounced among those who were HLA-DQ2 or -DQ8 positive. The mechanism of the effect of gluten in IBS is unclear, but in this study small bowel mucosal

permeability was higher among those allocated to a gluten-containing diet, and levels of mRNA encoding tight junction proteins were significantly reduced in mucosal biopsies from these individuals, suggesting that gluten may have effects on epithelial barrier function. However, as wheat contains high levels of the polysaccharide fructans, in addition to gluten, another explanation for the benefit of a gluten-free diet in patients with IBS could be a simultaneous reduction in FODMAP intake. In a recent trial that examined a combined approach of a low-FODMAP diet and a gluten-free diet (Biesiekierski et al. 2013), there was no additive effect of a gluten-free diet, suggesting that reduction in fructans may partly explain the effectiveness of a gluten-free diet in IBS.

7.6 Exercise

Exercise may lead to an improvement in symptom burden in other functional conditions, such as chronic fatigue and fibromyalgia (Richards and Scott 2002; White et al. 2011). However, there are few studies examining this approach in IBS. In a Swedish trial (Johannesson et al. 2011), 102 patients with all subtypes of IBS were randomized to a moderate increase in physical activity over 12 weeks, receiving advice from a physiotherapist, or to maintain their current lifestyle, but with supportive contact from the same physiotherapist. Those in the active intervention arm were told to undertake 20–60 min of moderate to vigorous physical activity on 3–5 days per week, and symptom scores improved significantly over those in the control arm, with a mean reduction of 51, compared with 5 ($P = 0.03$). There was also a trend towards those randomized to an increase in physical activity to report a $\geq$50% reduction in symptom severity scores from baseline (43% versus 26%, $P = 0.07$). The effect of exercise according to IBS subtype was not reported. After 12 weeks those in the control arm were also offered the active intervention, and in a subsequent follow-up of all 39 available individuals who had increased their physical activity, at a median of 5.2 years, 54% of patients had $\geq$50% reduction in symptom severity scores (Johannesson et al. 2015).

8 Alternative and Herbal Therapies

8.1 Prebiotics and Probiotics

Prebiotics are ingredients in food that remain undigested, such as fructo-oligosaccharides or inulin, and which can then stimulate either the growth or the activity of intestinal bacteria, while probiotics are live or attenuated microorganisms that may have effects on the composition of the gut microbiota, but which may also have anti-inflammatory and anti-nociceptive properties (Kamiya et al. 2006; O'Mahony et al. 2005; Verdu et al. 2004, 2006). Both prebiotics and probiotics are intended to have benefits to human health. A recent systematic review and meta-analysis identified no trials of prebiotics in IBS at the time the literature search was conducted (Ford et al. 2014c), although a subsequent RCT of 12 weeks of 6 g partially hydrolyzed guar gum, a

prebiotic fiber, versus placebo has been conducted in 121 patients with IBS of all subtypes (Niv et al. 2016). In this trial, there were significant improvements in bloating scores with the active treatment, but no effect on global symptoms, abdominal pain, or quality of life.

There were 35 trials of probiotics, involving 3,452 patients with IBS, identified by the aforementioned meta-analysis (Ford et al. 2014c). Overall, probiotics appeared to have a beneficial effect on IBS, with an RR of remaining symptomatic of 0.79 (95% CI 0.70–0.89), and an NNT of 7, but there was significant heterogeneity between studies, and only 12 trials were of high methodological quality. Due to the multitude of bacterial species and strains assessed in individual trials, it was difficult to make inferences as to which should be preferred, although there were three RCTs that used *Lactobacillus plantarum* DSM 9843, containing 314 patients, with an RR of symptoms persisting of 0.67 (95% CI 0.51–0.87). In terms of the effect of probiotics on individual symptoms, there appeared to be significant improvements in abdominal pain, bloating, and flatulence, but not urgency. There were insufficient trials recording the effect of probiotics on bowel function for data to be synthesized. Adverse events were significantly more common with probiotics, although the majority of these were mild. As with many of the therapies discussed thus far, effect of probiotics according to predominant stool pattern was not examined by the majority of trials.

8.2 Peppermint Oil

The major constituent of peppermint oil is menthol, which has antispasmodic properties. Menthol inhibits smooth muscle contractility in the GI tract by blocking calcium influx, via L-type calcium channels in the plasma membrane of smooth muscle cells (Amato et al. 2014). In a meta-analysis from 2008, (Ford et al. 2008b) peppermint oil was more effective than placebo in four trials, containing 392 patients with IBS, with a relative risk of remaining symptomatic of 0.43 (95% CI 0.32–0.59), and an NNT of 2.5. However, there was borderline heterogeneity between studies, and none of the trials were of high quality, which may have led to an overestimate of its efficacy. In addition, the effect of peppermint oil according to IBS subtype was not reported. Peppermint oil can worsen gastroesophageal reflux symptoms but a novel formulation, designed for sustained release in the small intestine, may avoid this and is now available for use in the USA. In a 4-week trial of this formulation (Cash et al. 2016), comprising 72 patients with IBS-D or IBS-M, there was a 40% reduction in symptom scores (based on average score of frequency and intensity of eight symptoms: abdominal pain or discomfort, bloating or distension, pain at evacuation, urgency, constipation, diarrhea, passage of mucus or gas, and sense of incomplete evacuation) from baseline with peppermint oil, compared with 24% with placebo ($P = 0.02$).

8.3 Other Herbal Therapies

The efficacy of other herbal therapies in IBS is unclear, as few studies have been conducted. Iberogast, also known as STW-5, is a mixture of extracts of bitter candy tuft, chamomile flower, peppermint leaves, caraway fruit, licorice root, lemon balm leaves, angelica root, celandine herbs, and milk thistle fruit (Madisch et al. 2001). This combination appears to have both antispasmodic and tonic properties on GI smooth muscle (Ammon et al. 2006). In a large, double-blind RCT, containing 208 patients with IBS (Madisch et al. 2004), STW-5 led to a significantly greater improvement in global symptom scores and abdominal pain scores than placebo, but these findings need to be replicated by other investigators. Small trials of ginger capsules (van Tilburg et al. 2014), which may have effects on pain and GI motility, and St. John's wort (*Hypericum perforatum*) (Saito et al. 2010) have produced disappointing results, while any benefit of Chinese herbal medicines in IBS is inconsistent (Bensoussan et al. 1998, 2015; Leung et al. 2006).

9 Antispasmodic Drugs

Antispasmodics have been used in the treatment of IBS for many years, on the basis that a subgroup of patients with IBS have abnormal contractility of GI smooth muscle, and altered GI transit, and that this contributes to pain and disturbances in bowel habit. They have their effects via inhibition of the action of acetylcholine at muscarinic receptors, or via blockade of calcium channels, on GI smooth muscle. A previous meta-analysis identified 22 separate RCTs, studying 12 different antispasmodic drugs, and containing 1,778 patients (Ford et al. 2008b). Overall, as a class, these drugs were more effective than placebo, with a relative risk of remaining symptomatic of 0.68 (95% CI 0.57–0.71), and an NNT of 5. When the efficacy of individual drugs was studied in subgroup analyses, hyoscine (three trials, 426 patients, NNT 3.5), otilonium (four trials, 435 patients, NNT 4.5), cimetropium (three trials 158 patients, NNT 3), and pinaverium (three trials, 188 patients, NNT 3) all appeared to be more effective than placebo. However, there was significant heterogeneity between all 22 studies, none of which were of high methodological quality, and evidence of possible publication bias, and again there was no report of efficacy according to IBS subtype. In addition, side effects were significantly more frequent compared with placebo, the commonest of which were dry mouth, dizziness, and blurred vision. It is also important to point out that most of the drugs studied in the trials included in this meta-analysis are not licensed for the treatment of IBS in the USA.

Since the conduct of this meta-analysis there have been further RCTs of both otilonium and pinaverium. Clavé et al. (2011) randomized 356 patients with all subtypes of IBS to either 40 mg of otilonium or placebo three times daily for 15 weeks. The proportion of patients whose abdominal pain frequency score improved by ≥ 1 point was significantly higher among those randomized to otilonium (69% versus 56%, $P = 0.02$), and this effect was consistent across all IBS subtypes. However, treatment success,

defined as less than two episodes of abdominal pain per week during the last 2 weeks of therapy, was no higher with otilonium, and there was no significant difference in quality of life.

In an RCT of pinaverium conducted in 427 Chinese patients with IBS-D (Zheng et al. 2015), the authors reported that 77.5% of patients receiving pinaverium had either a ≥30% reduction from baseline in abdominal pain or a ≥50% reduction in the number of days with at least one stool with a Bristol stool score ≥6 at week 4, compared with 33.5% with placebo ($P < 0.001$). The proportion of dual responders, those who achieved both of these endpoints at week 4, was also significantly higher with pinaverium (38.1% versus 16.7%, $P<0.001$). This is the only RCT of antispasmodic drugs that utilizes an endpoint that adheres closely to those recommended by the FDA for the assessment of the efficacy of treatments for IBS-D. Although this trial provides an important proof of concept that pinaverium is of benefit in IBS-D, the duration of therapy was only 4 weeks, and these results may not be applicable to non-Chinese patients with IBS.

10 Antidepressants

Coexistent psychological disorders are common among patients with IBS (Henningsen et al. 2003). The presence of depression appears to modify the brain's response to painful stimuli (Schmid et al. 2015), and antidepressants have well-documented beneficial effects on chronic pain disorders (McQuay et al. 1996; Saarto and Wiffen 2007). As a result, there have been numerous RCTs of antidepressants in IBS studying the effects of both tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). An updated systematic review and meta-analysis (Ford et al. 2014b) identified 17 separate trials of antidepressants. Overall, there was a beneficial effect on IBS symptoms, with an RR of remaining symptomatic of 0.67 (95% CI 0.58–0.77), and an NNT of 4. However, only three of the RCTs were of high quality, the majority of trials were conducted in secondary or tertiary care, and there was evidence of heterogeneity between studies and possible publication bias. In addition, two of the studies, which were both conducted in Iran and from the same research group, may have been atypical. In one, which used the SSRI, fluoxetine (Vahedi et al. 2005), the placebo response rate was extremely low at 14%, and, in the other, the authors reported a "complete" response to amitriptyline of 63% (Vahedi et al. 2008), which seems unusually high. All of this suggests that the effectiveness of antidepressants may well have been overestimated in this meta-analysis.

When effectiveness according to type of antidepressant was studied the data were more convincing for TCAs, with an RR of remaining symptomatic of 0.66 (95% CI 0.56–0.79), and an NNT of 4, with no heterogeneity between the 11 studies, than for SSRIs, where the RR of remaining symptomatic and the NNT were similar, but there was significant heterogeneity between the seven trials. There were seven RCTs that reported the effect of antidepressants on abdominal pain, and the RR of abdominal pain persisting was significantly lower (0.62; 95% CI 0.43–0.88).

It is important to point out that side effects were significantly commoner with antidepressants, with the most frequent being drowsiness and dry mouth.

As well as their impact on mood, antidepressants have effects on GI motility, with TCAs having been shown to prolong orocecal and whole-gut transit times, and SSRIs to decrease orocecal transit time (Gorard et al. 1994). It would therefore seem sensible to use TCAs in IBS-D, and SSRIs in IBS-C, but effectiveness according to IBS subtype has only been assessed in two RCTs to date (Vahedi et al. 2005, 2008). The other issue is whether the efficacy of antidepressants stems from the treatment of coexistent depression. Three of the identified studies reported that there was no correlation between improvement in IBS symptoms and depression scores (Tabas et al. 2004; Tack et al. 2006; Vij et al. 1991), and a fourth trial reported that the benefit of the TCA, desipramine, was, if anything, greater in nondepressed individuals (Drossman et al. 2003). However, in an RCT by Ladabaum et al. (2010), which screened potentially eligible participants for depression and excluded them if this was present, there was no benefit of citalopram. The mechanism of action of antidepressants in IBS therefore remains uncertain, although it does appear that amitriptyline reduces activation of pain centers in the anterior cingulate cortex during painful rectal distension in patients with IBS (Morgan et al. 2005), suggesting that its effects on pain processing may be central, although the effects on peripheral mechanisms that may influence sensation (such as colonic compliance and visceral afferent function) have not been adequately studied.

11 Drugs Acting on Opioid Receptors

The use of loperamide, a μ-opioid receptor antagonist, and diphenoxylate, an opioid analgesic, which are considered to be antidiarrheal agents, has been traditional in IBS for many years (Kasich 1961). However, this is based on limited evidence from rigorous RCTs. In one small trial, containing 21 patients with IBS-D, loperamide appeared to be beneficial, in terms of improved stool consistency, pain, and urgency (Lavo et al. 1987). In a second trial conducted among 60 patients said to have IBS (Hovdenak 1987), but of whom only 21 had abdominal pain and disordered bowel habit, there was an improvement in stool frequency and consistency, as well as a reduction in the number of days with pain. A recent position statement for the management of IBS suggested that there was insufficient evidence to recommend the use of loperamide (Ford et al. 2014a), but the drug may be useful in clinical practice in those with debilitating diarrhea or urgency.

Eluxadoline is a novel agent, acting on δ-, κ-, and μ-opioid receptors. In a phase II dose-ranging trial (Dove et al. 2013), comprising 807 patients with IBS-D, 13.8 and 12.0% of patients receiving 200 or 25 mg of eluxadoline twice daily for 12 weeks experienced a $\geq$30% reduction from baseline in abdominal pain and a stool consistency score of 3 or 4 on the Bristol stool scale on 66% of days, compared with only 5% of placebo patients ($P < 0.05$). Adverse event rates were similar in all treatment arms, with the commonest being nausea and headache. However, there were four cases of pancreatitis, three during treatment with eluxadoline, and one 15 days after the patient's last dose of eluxadoline. In two subsequent phase III

RCTs (Lembo et al. 2016b), comprising 2,427 patients, with a dose of either 75 or 100 mg twice daily the drug again demonstrated efficacy, with response rates of 27% in a pooled analysis, versus 17% with placebo ($P < 0.001$), although there was no benefit for abdominal pain. In addition, there were a further five cases of pancreatitis, and eight cases of sphincter of Oddi spasm. The drug is now licensed for the treatment of IBS-D in the USA, but the FDA recommends that patients with a history of biliary obstruction, pancreatitis, severe liver impairment, or severe constipation, and patients who consume more than three alcoholic drinks per day, should not be prescribed eluxadoline (FDA approves two therapies to treat IBS-D, n.d.).

12 5-HT_3 Receptor Antagonists

Serotonin, or 5-HT, is an important neurotransmitter in the brain and the enteric nervous system, with 90% of the body's total store of 5-HT containing within the intestinal enterochromaffin cells (Berger et al. 2009; Gershon et al. 1990). There is evidence to suggest that patients with IBS-D may have reduced 5-HT reuptake, while those with IBS-C have impaired release of 5-HT (Atkinson et al. 2006). Drugs that act on the 5-HT_3 receptor have been known to have effects on GI transit for many years (Prior and Read 1993; Talley et al. 1990), and alosetron, a 5-HT_3 receptor antagonist, was licensed for use in women with IBS-D in the USA. Previous meta-analyses of RCTs have shown that the drug is effective (Andresen et al. 2008; Ford et al. 2009), with an NNT of 8 for relief of abdominal pain, and 4 for improvement in global symptoms. However, the drug was withdrawn because of a reported association with cases of ischemic colitis, and concerns about episodes of severe constipation, requiring hospitalization (Chang et al. 2006). The drug was reintroduced for female patients with IBS-D unresponsive to other therapies, but its use is regulated by an FDA prescribing program.

Ramosetron and ondansetron are 5-HT_3 antagonists that have been in use to treat nausea or vomiting for almost 30 years, and which have well-established safety profiles. Ramosetron has been used in the treatment of IBS-D in both men and women, with response rates of 47–51%, compared with 27–32% with placebo ($P<0.001$) (Fukudo et al. 2016; Matsueda et al. 2008), and is now licensed for use in IBS-D in Japan. In a small crossover RCT of ondansetron (Steadman et al. 1992), containing 14 patients, colonic transit times were prolonged, and stool consistency was improved, although there was no effect on abdominal pain, in contrast to RCTs of alosetron. More recently, in a larger crossover trial (Garsed et al. 2014), comprising 120 patients with IBS-D, ondansetron had a significant effect on stool consistency, with 80% of patients responding when on active drug compared with 41% while on placebo, as well as significant improvements in urgency, frequency of defecation, and bloating, but again no effect on pain. Constipation occurred in 9% of patients while on ondansetron.

13 Bile Acid Sequestrants

Given that up to 25% of patients with IBS-D have evidence of bile acid malabsorption following 23-seleno 25-homotaurocholic acid retention (SeHCAT) scanning (Aziz et al. 2015), the use of bile acid sequestrants, either empirically or based on biochemical evidence of abnormal bile acid metabolism, may be beneficial. However, to date, there are no RCTs of these drugs in IBS. In a single-center open-label trial of 10 days of 1,875 mg twice-daily colesevelam (Camilleri et al. 2015a), which recruited 12 patients with IBS-D and abnormal bile acid kinetics, fecal excretion of bile acids increased; fasting serum C4 increased, suggesting a compensatory increase in hepatic synthesis of bile acids; and there was a reduction in stool consistency on the Bristol stool form scale. In addition, the number of bowel movements per week correlated inversely with the total bile acid sequestered into the stool, providing evidence for sequestration of bile acids being the mechanism for the observed improvement in diarrhea. In another open-label study, Bajor et al. (2015) treated 27 patients with IBS-D and an SeHCAT retention <20% with colestipol at a dose of 1 g twice daily, with the dose titrated. After 8 weeks of treatment, there were significant improvements in IBS symptom severity scores, stool frequency was reduced, and 15 (55.5%) of the 27 patients reported adequate relief of symptoms.

14 Antibiotics

Pimentel et al. (2000) observed that some patients with IBS may have underlying small intestinal bacterial overgrowth (SIBO), detected on hydrogen breath testing, and that treatment of this with open-label antibiotics led to an improvement in symptoms. This led to the conduct of a small RCT of rifaximin, a nonabsorbable antibiotic, in IBS in which global symptoms and bloating improved (Pimentel et al. 2006). In two subsequent phase III randomized placebo-controlled trials (Pimentel et al. 2011), comprising more than 1,200 patients with non-constipated IBS, rifaximin at a dose of 550 mg three times daily for 2 weeks led to significantly higher rates of adequate relief of global IBS symptoms and bloating. However, the benefit over placebo for these endpoints was modest, with therapeutic gains of only 8–11%, equating to an NNT of between 9 and 12.5, although the effect on symptoms persisted out to 10 weeks posttreatment. It is also important to point out that none of the individuals in these larger trials underwent breath testing to confirm the presence of SIBO. In addition, stool consistency, number of bowel movements, and urgency were not improved. Adverse event rates were similar in both treatment arms, and there were no cases of *Clostridium difficile*.

A subsequent meta-analysis of five RCTs of rifaximin (Menees et al. 2012), comprising 1,803 patients, reported similar efficacy with an NNT of 10 for both improvement in global symptoms and bloating, and again rifaximin appeared safe in the pooled data from these trials. A further trial has been conducted (Lembo et al. 2016a), in which 2,579 patients received open-label rifaximin 550 mg three times

daily for 2 weeks. Among the 1,074 patients who responded to treatment and were successfully followed up, 636 (59.2%) had a recurrence of symptoms at a median of 10 weeks (range 6–24 weeks) posttreatment. They were then randomized to up to two repeat courses of rifaximin 550 mg thrice daily for 2 weeks each, separated by 10 weeks, or placebo, in a double-blind manner. Response rates were significantly higher with rifaximin after both the first and the second repeat treatments, but again the magnitude of this difference was modest at 8%. The FDA approved the use of rifaximin for IBS-D patients in 2015, and the treatment course can be repeated in case of recurrence of symptoms, up to two times (FDA approves two therapies to treat IBS-D, n.d.). A small randomized controlled study (Acosta et al. 2016) appraised the potential mechanisms for the beneficial effect on symptoms in non-constipated IBS patients, including colonic transit by scintigraphy, mucosal permeability by lactulose-mannitol excretion, and fecal microbiome, bile acids, and short-chain fatty acids measured on random stool sample. This study showed only acceleration of ascending colon emptying and overall colonic transit at 48 h, which is paradoxical given the indication for this drug in the treatment of IBS-D (Acosta et al. 2016).

15 Intestinal Secretagogues

Lubiprostone, a prostaglandin derivative, acts on CIC-2 chloride channels on the apical membrane of the intestinal enterocyte. This leads to active chloride secretion into the intestinal lumen, with passive movement of sodium ions and water as a result. Thus fluid secretion increases, and GI transit is accelerated and stools become looser. The drug has been studied at a dose of 8 mcg twice daily for 12 weeks in two large phase III trials, containing 1,171 patients with IBS-C (Drossman et al. 2009). In these trials, response to therapy was defined as at least moderate relief of global symptoms for 2 out of the 3 months of therapy. In a pooled analysis from both RCTs, response rates were 17.9% with lubiprostone, compared with 10.1% with placebo ($P = 0.001$). There were also improvements in abdominal pain scores, straining, and stool consistency, although no significant effect on quality of life. Nausea was the commonest side effect, experienced by 8% of patients.

Linaclotide is a minimally absorbed 14-amino acid peptide, which is a guanylate cyclase-C receptor agonist. The resulting increase in intracellular cyclic guanosine monophosphate (cGMP) leads to the secretion of chloride and bicarbonate into the intestinal lumen, via the cystic fibrosis transmembrane regulator and, similar to the action of lubiprostone, water then follows. The increase in cGMP may also have effects on sensory afferent neurons, leading to pain inhibition, an effect noted in the phase III clinical trials of the drug that were conducted in chronic idiopathic constipation (Lembo et al. 2011), where abdominal discomfort and bloating improved significantly. In the two phase III trials conducted in IBS-C (Chey et al. 2012; Rao et al. 2012), which used a dose of 290 mcg once daily, response to therapy was defined as a $\geq$30% decrease in pain, and an increase of $\geq$1% complete spontaneous bowel movement per week. In one RCT (Chey et al. 2012), at

26 weeks, the response rate was 32.4% with linaclotide, compared with 13.2% with placebo ($P<0.001$), equating to an NNT of 5. In the second study (Rao et al. 2012), at 12 weeks the response to therapy was 33.6% with linaclotide, versus 21.0% with placebo ($P<0.001$), with an NNT of 8. In this trial, after 12 weeks patients entered a 4-week randomized withdrawal period, in which those originally assigned to linaclotide were re-randomized to either linaclotide or placebo, and patients originally allocated to placebo were given linaclotide. In this part of the study, those remaining on linaclotide showed a continued benefit; those re-randomized to placebo experienced a deterioration of their symptoms, which returned towards baseline; and symptoms improved in those originally receiving placebo who were given linaclotide. The main adverse event with linaclotide was diarrhea, occurring in almost 20% of participants in both studies.

Both lubiprostone and linaclotide are approved by the FDA for the treatment of IBS-C. The efficacy of plecanatide, another guanylate cyclase agonist (Shailubhai et al. 2011), is being studied in phase II and III trials in IBS, but the results of these are unavailable at the time of writing.

16 Conclusions

Although IBS is traditionally thought of as a functional disorder, with no underlying organic explanation, as our understanding of the potential underlying pathophysiological mechanisms increases, it is becoming evident that there are genuine genetic, microbial, structural, and biochemical abnormalities in a subset of patients. These include abnormal central processing, altered GI motility, immune activation, low-grade inflammatory activity, changes in the diversity of the GI microbiota, altered bile acid metabolism, and aberrant enteroendocrine signaling. An overarching hypothesis of the etiology of IBS would be that, in a genetically susceptible individual, dietary or environmental antigens, microbiota, or a combination of both induce alterations in the function of the GI epithelial barrier, triggering an immunological response, characterized by inflammation, and release of neurotransmitters and cytokines that lead to visceral hypersensitivity, abnormal transit, disordered mood, and central changes in afferent processing.

At the present time, the treatment of IBS remains focused on treating the patient's predominant or most troublesome symptom. Effective therapies exist, and include dietary manipulation, soluble fiber, probiotics, peppermint oil, antispasmodics, antidepressants, eluxadoline, drugs acting on the 5-HT_3 receptor, rifaximin, and intestinal secretagogues. However, the efficacy of most of these is modest, high-quality evidence for some is sparse, and none have been shown to alter the long-term natural history of the disorder. Until therapies are developed that target some of the proposed underlying pathophysiological mechanisms described above, and are tested in high-quality RCTs, the treatment of IBS is likely to remain an inexact science.

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Inflammatory Bowel Disease: Pathophysiology and Current Therapeutic Approaches

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Abstract

Inflammatory bowel diseases, most commonly categorized as Crohn's disease and ulcerative colitis, are immune mediated chronic inflammatory disorders of the gastrointestinal tract. The etiopathogenesis is multifactorial with different environmental, genetic, immune mediated, and gut microbial factors playing important role. The current goals of therapy are to improve clinical symptoms, control inflammation, prevent complications, and improve quality of life. Different therapeutic agents, with their indications, mechanisms of action, and side effects are discussed in this chapter. Anti-integrin therapy, a newer therapeutic class, with its potential beneficial role in both Crohn's disease and ulcerative colitis is also mentioned. In the end, therapeutic algorithms for both diseases are reviewed.

Keywords

Adaptive immunity • Anti integrins • Anti-TNF drugs • Biologics • Corticosteroids • Crohn's disease • Immunomodulators • Inflammation • Inflammatory bowel disease • Innate immunity • Mesalamine • Ulcerative colitis

1 Introduction

Inflammatory bowel diseases (IBD), also called Crohn's disease (CD) and ulcerative colitis (UC), are complex, multifactorial, immune mediated disorders of gastrointestinal tract characterized by chronic relapsing inflammation. Although the exact etiopathogenesis remains unknown, recent studies have implicated different genetic, environmental, and immune mediated factors along with gut microbiome involvement.

Population-based studies have demonstrated genetic factors contributing to the pathogenesis of IBD; an eight to tenfold greater risk of IBD among relatives of UC and CD probands has been described and, most importantly, that there is concordance between identical twins (Cho and Brant 2011). However, the genetic factors account for only a part of disease variance indicating that microbiota and environment may interact with genetic susceptibility. The adaptive immune system has been considered to play the main role in the pathogenesis of IBD. Recent research in immunology has confirmed that the innate immune system maintains great importance in inducing gut inflammation. Recent advances in our understanding of IBD pathogenesis explain important disease mechanisms, including not only the innate and adaptive immunity, but also the complex interactions between different genetic, microbial, and environmental factors (Yi-Zhen and Yong-Yu 2014). Based on our improved understanding in the disease pathogenesis, newer targeted therapies have been discovered with better efficacy and outcomes.

2 Pathophysiology of Inflammatory Bowel Disease

Recent progress in genetic testing and DNA sequencing technology has allowed many genome-wide association studies (GWAS) in IBD resulting in new single nucleotide polymorphisms (SNPs) discovered (Yi-Zhen and Yong-Yu 2014). Nucleotide-binding oligomerization domain containing 2 (NOD2) was the first susceptibility gene for CD discovered in 2001. Dendritic cells from CD patients with susceptibility variants in NOD2 gene are deficient in autophagy induction as well as reduced localization of bacteria in autophagolysosomes (Cooney et al. 2010). Genetic analyses have also reported two other autophagy-related genes, *IRGM* and *ATG16L1*, showing an important role for autophagy in immune responses in IBD. A recent genetic association study, employing genome-wide association data of more than 75,000 IBD patients and controls, has identified 163 susceptibility loci for IBD. Of these 163 loci, 110 confer risk to both IBD subtypes, whereas 30 loci are unique to CD and 23 loci are unique to UC (Liu et al. 2015). However, all identified loci individually contribute only a small percentage of the expected heritability in IBD (Uhlig et al. 2014).

Environmental factors also play an important role in the pathogenesis of IBD. Many environmental factors including smoking, diet, drugs, water pollution, geography, sleep, and stress have been identified as risk factors for IBD. Public health strategies such as environmental sanitation as well as the increasing use of antibiotics have led to changes in the interaction between humans and microbes in the environment. Consequently, improvements in hygiene and health care can alter the composition of the gut microbiota and lead to a state of disequilibrium between protective and pathogenic bacteria (dysbiosis) (Abegunde et al. 2016). Vitamin D has immuno-regulatory properties in several autoimmune diseases via its genomic actions on the vitamin D receptor (VDR) (Cantorna et al. 2004). There is accumulating evidence that vitamin D may play an integral role in the incidence and disease activity in IBD (Ananthakrishnan et al. 2012).

Mucosal immunity, especially the T cell response has also been studied extensively in IBD pathogenesis. Dysfunctions of innate and adaptive immune pathways are considered to contribute to the aberrant intestinal inflammatory response in patients with IBD. The focus on adaptive immune response has ultimately led to the understanding that CD and UC represent two distinct immunological forms of gut inflammation: CD being considered to be driven by a Th1 response and UC being associated with a non-conventional Th2 response. The newly described Th17 cells have also been discovered to be involved in the gut inflammatory response in IBD (Cobrin and Abreu 2005; Targan and Karp 2005). Research studies have recently focused on the innate immune responses, such as epithelial barrier integrity, microbial sensing, autophagy, and unfolded protein response. Recent studies have discovered cells mediating innate immunity and the expression and function of both toll like receptors (TLRs) and NOD proteins are altered significantly in individuals with IBD (Abreu et al. 2005).

3 Current Therapeutics

3.1 Goals of Therapy

The primary focus in the management of IBD is to improve the quality of life and achieve clinical remission. Beyond this, our existing data promotes an emphasis on endoscopic and mucosal remission. Evidence and consensus-based recommendations for determining therapeutic goals for treating to target have been made available (Peyrin-Biroulet et al. 2015). Recommendations for UC treatment target include a clinical and patient-reported remission (defined as resolution of rectal bleeding and diarrhea/altered bowel habit) and endoscopic remission (defined as a Mayo endoscopic subscore of 0–1) with histological remission as an adjunctive goal (Peyrin-Biroulet et al. 2015). For Crohn's disease, a target of clinical remission (defined as resolution of abdominal pain and diarrhea/altered bowel habit) and endoscopic remission (defined as resolution of ulceration at ileocolonoscopy, or resolution of findings of inflammation on cross-sectional imaging in patients who cannot be adequately assessed with ileocolonoscopy) with biomarker remission (i.e., normal C-reactive protein (CRP) and fecal calprotectin) as an adjunctive target (Peyrin-Biroulet et al. 2015). This paradigm shift of achieving deeper endoscopic remission stems from the lack of correlation between clinical symptoms as reported by patients and endoscopic healing. In a systematic review and meta-analysis of over 2,000 patients with active UC, mucosal healing achieved by any medical therapy was associated with long-term clinical remission, avoidance of colectomy, and corticosteroid-free clinical remission (Shah et al. 2016). Similarly, achieving deeper, endoscopic remission correlated with better long-term outcomes, improved quality of life, and fewer surgical operations in Crohn's patients (Colombel et al. 2015).

Monitoring of efficacy and treatment endpoint by colonoscopy and histology should be considered gold standard, however patients and sometimes physicians are hesitant to repeat multiple endoscopic procedures. Alternative options using fecal biomarkers such as fecal calprotectin, fecal lactoferrin can quite closely correlate with disease activity as determined by history and endoscopy in both Crohn's disease and UC (Sipponen et al. 2008a, b; Schoepfer et al. 2010; Vieira et al. 2009).

In certain situations, however, surgery should be a treatment option that must not be overlooked or delayed, especially in cases of fibrostenotic disease. Those with more complex disease such as fistulizing/penetrating disease with intra-abdominal abscesses may warrant multidisciplinary evaluation and treatment with interventional radiologists, dieticians, and colorectal surgeons. Delaying appropriate treatment in these cases is not only harmful in prolonging patient's clinical symptoms but in some cases can worsen or complicate disease.

Table 1 5-ASAs in IBD

5-ASA derivatives	Solubility	Site of release	Pregnancy
Sulfasalazine (sulfapyridine +5-ASA)	Colonic bacteria	Colon	B[a]
Asacol HD	pH ≥7	Distal ileum – colon	C
Delzicol	pH ≥7	Distal ileum – colon	B
Pentasa	Time released	Jejunum – ileum-colon	B
Colazal	Colonic bacteria	Colon	B
Dipentum	Colonic bacteria	Colon	C
Lialda	pH ≥7	Distal ileum – colon	B
Apriso	pH ≥6	Mid to distal ileum – colon	B

[a]Take 2 g/folic/day

3.2 Therapeutic Drugs

Our therapeutic armamentarium for IBD has slowly grown over the past several years. Different therapeutic targets, their indications, benefits, and potential side effects are discussed here.

3.2.1 Aminosalicylates

Oral 5-aminosalicylic acid (5-ASA) preparations are used for the treatment of mild to moderately active ulcerative colitis. Initially developed as therapy for rheumatoid arthritis, clinical improvement in patients with concomitant UC leads to the use of sulfasalazine in IBD. Due to side effects in those with sulfa allergy and nausea, newer forms of 5-ASAs were created specific for its effects on the GI tract. Table 1 lists differences in these compounds. Oral formulations come in various forms, each broken down in different areas of the GI tract (see Table 1) and release its granules and contribute to a topical effect in the GI tract mucosa. Rectal formulations come in suppository or enema form, with suppositories (1 g/day) effective for up to 15–20 cm, and enemas (4 g/day) reaching up to the splenic flexure.

In a Cochrane systematic review of oral 5-ASA for induction of remission in UC, 5-ASAs in a daily dose of 2.4 g was safe and effective for induction therapy for patients with mild to moderate UC (Wang et al. 2016a). Once daily dosing was similar in efficacy and safety as conventionally dosed 5-ASA (Wang et al. 2016a). No differences in efficacy and safety was found among the various 5-ASA formulations but slightly higher number of patients on sulfasalazine (29%) experienced adverse events compared to 15% on 5-ASAs (RR 0.48, 95% CI 0.37–0.63) (Wang et al. 2016a).

In another Cochrane review of 41 studies, 5-ASA was superior to placebo for maintenance therapy (clinical or endoscopic remission) in ulcerative colitis (Wang et al. 2016b). 5-ASA administered once daily is as effective and safe as conventional dosing for maintenance of remission in quiescent ulcerative colitis and no

difference in efficacy or safety was found between the various formulations of 5-ASA although in comparison to sulfasalazine, 5-ASA had a statistically significant therapeutic inferiority. Those with extensive colitis or frequent relapses benefited from a higher dose (4.8 g) of maintenance therapy and were equally safe without any higher incidence of adverse events compared to low dose therapy (Wang et al. 2016b). Common adverse events of 5-ASAs include flatulence, abdominal pain, nausea, diarrhea, headache, dyspepsia, nasopharyngitis, and worsening ulcerative colitis (Wang et al. 2016a, b). Using combination oral and rectal mesalamine therapy provides earlier and more complete relief of distal UC than oral or rectal therapy alone (Safdi et al. 1997; Probert et al. 2014).

5-ASAs are generally safe for pregnancy and all are category B except for Asacol HD due to its coating and olzalazine, both of which are category C. Although all 5-ASAs are excreted through breast milk, they are considered safe to continue throughout lactation. Males on sulfasalazine should discontinue the drug for 6 weeks prior to trying to conceive due to its effects of reversible oligospermia. Pregnant females on sulfasalazine should take at least 2 g of folic acid daily due to the drug's effects on folate metabolism. No dose reduction is required in the geriatric population, but due to its potential for nephrotoxicity, close monitoring of renal function, particularly during induction therapy and at minimum yearly thereafter is warranted in this age group (Gudsoorkar and Abraham 2015).

5-ASA use in CD, on the other hand, is controversial. The topically acting 5-ASA would be unable to treat transmural layers of inflammation characteristic of CD. However, it may benefit those with mild or mucosal Crohn's colitis. The National Cooperative Crohn's Disease Study showed that 5-ASA use in CD induced remission of acute flares in a double-blind randomized control trial in comparison to placebo in patients with Crohn's colitis but did not benefit those with small bowel Crohn's disease (Summers et al. 1979).

3.2.2 Corticosteroids

Corticosteroids have been used for more than 60 years with the first clinical trial in 1954 by Drs. Truelove and Witts demonstrating benefit in ulcerative colitis (Truelove and Witts 1954). The mode of action is thought to stem from its ability to modulate the immune response through interaction with glucocorticoid receptors in the cell nucleus which inhibit expression of adhesion molecules and trafficking of inflammatory cells to the intestine. A meta-analysis by Ford and colleagues confirmed that corticosteroids are effective in both Crohn's disease and ulcerative colitis (Ford et al. 2011). In UC, there is a dose-response effect with oral prednisone between 20 and 60 mg/day, with 60 mg being slightly more effective than 40 mg/day (Kornbluth and Sachar 2010). There is no data on how to taper prednisone, but most recommendations include tapering by 5–10 mg weekly after clinical improvement is achieved (typically by 5–7 days). Because they are quick in onset to remit symptoms, inexpensive, and available in oral formulation, they have become commonly used in IBD patients. However, because of their significant short- and long-term adverse effects (namely diabetes, osteoporosis, risk of opportunistic infections), use should be closely monitored and repeated

courses are limited. While steroids are effective in induction of remission, there is no role for these medications as maintenance therapy.

With the increasing concern over adverse effects related to steroid use, this has led to development of budesonide. With its extensive first pass hepatic metabolism, this drug is delivered locally to the distal ileum and proximal colon. The majority of budesonide is converted by the cytochrome P-450 system in the liver to inactive metabolites, with only 10–15% of the drug reaching the systemic circulation (Greenberg et al. 1994). Therefore, this is ideal for patients with mild to moderate ileal and right-sided colonic CD.

More recently, budesonide has been shown beneficial in UC. For maximum release in the colon, it is coupled with a novel colonic release system (MMX Multi-Matrix System) which allows for delivery of drug to the entire colon (Travis et al. 2014). Budesonide MMX (Uceris) is approved for induction of remission in patients with mild to moderate UC. In a study by Travis et al., Budesonide MMX 9 mg was shown to have a significant increase in the combined clinical and endoscopic remission rate compared with placebo (17.4% vs 4.5%; $p = 0.0047$).

Corticosteroids are also available in a topical formulation. In the USA, they are available as a 100 mg hydrocortisone enema or as a 10% hydrocortisone foam and have been shown to be effective in distal colitis (Cohen et al. 2000). As in oral corticosteroids, these topical agents have not been shown to be effective in maintaining remission.

In hospitalized patients with severe UC, corticosteroids should be administered in the form of IV methylprednisolone (48–60 mg daily) or hydrocortisone (300–400 mg daily). Methylprednisolone is generally preferred due to its decreased mineralocorticoid properties as compared to hydrocortisone (Lichtenstein et al. 2006). Response is typically seen by day 5 of therapy with rates approximating 50% (Jarnerot et al. 1985).

A significant number of patients will experience an adverse effect with one study quoting up to 50% of all patients who have been exposed to corticosteroids (prednisone) (Singleton et al. 1979). Some of the early side effects seen include acne, body edema, sleep and mood disturbance, and glucose intolerance. With prolonged use (typically >12 weeks), some of the more serious adverse effects include cataracts, osteoporosis, osteonecrosis of the femoral head, myopathy, risk of opportunistic infections, and development of diabetes (Lichtenstein et al. 2006). To monitor and prevent complications, recommendations such as calcium and vitamin D supplementation, periodic bone mineral density assessment, annual ophthalmologic examinations (for those on long-term corticosteroids), and monitoring for glucose intolerance are important to consider in the clinical practice.

Corticosteroids, specifically prednisone and budesonide are pregnancy category C. These are considered safe during pregnancy. Some studies have demonstrated a small risk of cleft lip/palate in babies of mothers exposed to steroids in the first trimester of pregnancy (Park-Wyllie et al. 2000). However, there is no evidence demonstrating an increase in major fetal malformations with corticosteroid use (Hosseini-Carroll et al. 2015). High doses of corticosteroids late in pregnancy should be avoided if possible due to the risk of dependence of fetus on

corticosteroids. Corticosteroids are thought to be safe to administer in breast-feeding patients as very low levels are transferred into breast milk (McKenzie et al. 1975).

In the elderly population, studies have demonstrated a higher incidence of steroid dependence. Moreover, more frequent and severe adverse events have been found including longer hospitalizations, osteoporotic-related fractures, altered mental status, and precipitating or exacerbating diabetes mellitus (Akerkar et al. 1997).

3.2.3 Antibiotics

Since microbial dysbiosis has been implicated in its etiology, using antibiotics to treat IBD makes theoretical sense. Several meta-analyses have found antibiotics, the most studied (ciprofloxacin, metronidazole, and rifaximin) to be efficacious for induction of remission and treatment of flares in both UC and CD. From a diverse number of antibiotics tested (anti-tuberculosis therapy, macrolides, fluoroquinolones, 5-nitroimidazoles, and rifaximin) either alone or in combination, a systematic review and meta-analysis of 10 RCTs in active CD of over 1,000 patients were found to be superior to placebo (RR of active CD not in remission = 0.85; 95% confidence interval (CI) = 0.73–0.99, $p = 0.03$) (Khan et al. 2011).

For quiescent CD, 3 RCTs using different antibiotics combinations (all including antimycobacterials) vs. placebo showed a statistically significant effect in favor of antibiotics compared to placebo (RR of relapse = 0.62; 95% CI = 0.46–0.84) (Khan et al. 2011). In perianal fistulizing CD trials, using either ciprofloxacin (500–1,000 mg/day) or metronidazole (1,000–1,500 mg/day) showed a statistically significant effect in reducing fistula drainage (RR = 0.8; 95% CI = 0.66–0.98) used alone or in combination with other therapies (azathioprine, infliximab, adalimumab) (Khan et al. 2011).

In the prevention of post-operative Crohn's disease recurrence, studies using nitroimidazole antibiotics (metronidazole, ornidazole) report a 13% reduction in endoscopic recurrence rates comparing metronidazole to placebo ($p = 0.02$), and reduced clinical recurrence rates at 1 year (4% vs 25%), but the benefit was not sustained at 2 and 3 years (Rutgeerts et al. 1995). Use of ornidazole 1 g/daily started within 1 week of surgery and continued for 1 year showed significantly reduced clinical recurrence rates at 1 year (7.9%) compared to placebo (37.5%; $p = 0.0046$). Endoscopic recurrence at 12 months was also significantly reduced compared to placebo (79% vs 53.6%; $p = 0.037$). However, higher side effects and poor tolerance lead to higher drop-out rates ($p = 0.041$) minimizing their long-term efficacy (Rutgeerts et al. 2005a, b).

Another, less frequently evaluated area where antibiotics can become quite useful is small bowel bacterial overgrowth in Crohn's disease. Use of metronidazole and ciprofloxacin can improve intestinal symptoms such as bloating and abdominal pain in these patients with documentation of normalization of lactulose breath tests (Castiglione et al. 2003).

In active UC, 9 RCTs with 662 patients showed a statistically significant benefit for antibiotics inducing remission (RR of UC not in remission = 0.64; 95% CI = 0.43–0.96) (Khan et al. 2011). Antibiotics have also been found to be useful in treating pouchitis. Patients with IPAA, and chronic refractory pouchitis taking ciprofloxacin 1 g/day and tinidazole 15 mg/kg/d had a greater reduction in the total Pouchitis Disease Activity Index scores and subscores and a greater improvement in quality-of-life scores than those in the mesalamine group ($p \leq 0.03$) with both clinical remission and clinical response in the antibiotic group 87.5% vs. only 50% in the mesalamine group ($p = 0.069$). However, adverse events of peripheral neuropathy and dysgeusia developed in the antibiotic group (Shen et al. 2007). Two other studies found that using rifaximin (either alone or in combination with ciprofloxacin) effectively treated active or chronic, treatment-resistant pouchitis with minimal side effects (Gionchetti et al. 1999; Isaacs et al. 2007).

Although the diverse number of antibiotics tested makes data difficult to interpret for specific medications, overall these results suggest that adjunctive antibacterial therapy is effective for induction of clinical remission improving outcomes in IBD patients. However, systemic side effects can limit long-term antibiotic use.

In regard to antibiotic use in pregnancy, metronidazole is category B, however the CDC recommends against the use of it during the 1st trimester. There have been some studies suggesting increased risk of preterm delivery (Andrews et al. 2003; Klebanoff et al. 2001) but no increased risk of congenital anomalies (Burtin et al. 1995; Caro-Paton et al. 1997). Ciprofloxacin and rifaximin are both category C, and often avoided as these are used mostly as adjunctive therapy.

Special consideration should be given to the elderly with antibiotic use. Although age-dependent dose adjustment is not common, one should keep in mind that a decreased renal excretion of metronidazole and its metabolites occur in the elderly (Lau et al. 1992). Considering the physiologic decline in GFR with age and some studies suggesting increased serum concentrations in the elderly, attention should be paid to the dosage of ciprofloxacin even in the absence of overt renal insufficiency as ciprofloxacin is eliminated renally (LeBel et al. 1986; Bayer et al. 1987). Age greater than 60 years and concurrent steroid use have also been implicated as risk factors for tendinopathy associated with quinolones.

3.2.4 Immunomodulators

The use of immunomodulators, 6-mercaptopurine (6-MP), azathioprine (AZA), and methotrexate (MTX) has been integral in the treatment for IBD as they function as steroid-sparing agents in maintenance of remission. Likewise, their use increased with the advent of biologic therapy since their combined use has been shown to enhance the efficacy of anti-TNF agents as well as decrease immunogenicity (Colombel et al. 2010).

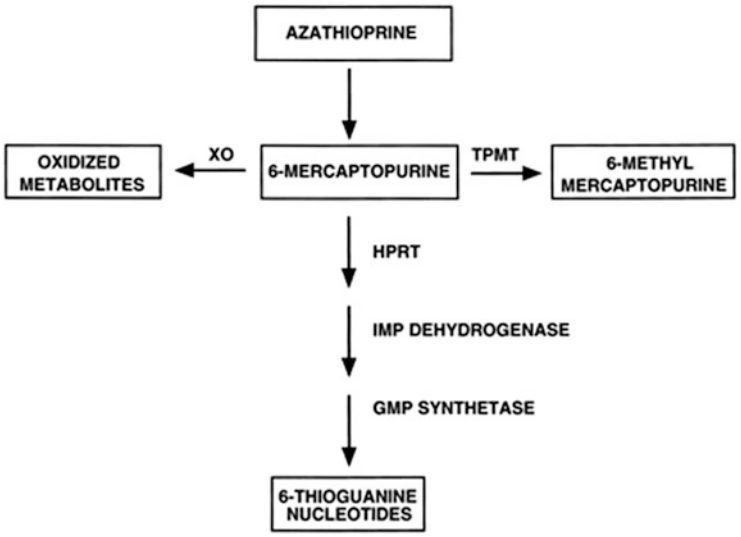

Fig. 1 6-MP/AZA metabolism pathway

6-Mercaptopurine/Azathioprine

The thiopurines, 6-MP and AZA have been shown to maintain remission in both CD and UC. AZA is the prodrug to 6-MP as it is metabolized to 6-MP. Both drugs are thiopurine analogues that work through their active metabolite 6-thioguanine nucleotide (6-TGN) which in turn causes inhibition of DNA and RNA synthesis as well as T-cell apoptosis (see Fig. 1) (Frei et al. 2013). Several studies have suggested the importance of measuring metabolites of 6-MP and AZA as this may be useful in optimizing the dosage. Therapeutic levels of 6-TGN (the active metabolite) should be in range of 235–500 pmol/8 $\times$ 10^8 erythrocytes and higher levels raise concern for leukopenia and bone marrow suppression (Frei et al. 2013). Elevated levels of the inactive metabolite, 6-methyl-MP (6-MMP) $>$ 5,700 pmol/8 $\times$ 10^8 erythrocytes, puts a higher risk for hepatotoxicity. Lastly, if both 6-TGN and 6-MMP levels are low, this would suggest the patient is under dosed or non-compliant with therapy.

Current recommendations include checking TPMT enzyme activity prior to initiation of therapy in order to identify those who have low enzyme activity (homozygous deficient in TPMT) thereby avoiding potential drug toxicity and adverse effects. It has been reported that roughly 0.3% of the population has low to absent enzyme activity, 11% has intermediate levels, and 89% has normal to high levels of activity (Lichtenstein et al. 2006). Both 6-MP and AZA should be avoided in those with low to absent activity. In those with intermediate activity, strict lab monitoring should be done in addition to consideration of starting at lower dose. Based on studies to date, the target dosing for those with normal TPMT activity for AZA is 2.0–3.0 mg/kg and 6-MP is 1.0–1.5 mg/kg. Both drugs typically require

2–3 months prior to seeing clinical benefit as there is a delay in obtaining therapeutic metabolite levels.

6-MP and AZA have not been shown to be effective drugs for induction of remission in either CD or UC. Studies have shown that in CD and UC, both 6-MP and AZA are effective in preventing a relapse based on multiple randomized controlled trials (Frei et al. 2013). Furthermore, Rosenberg et al. found that AZA was superior to placebo in reducing need for steroids in steroid-dependent CD patients (Rosenberg et al. 1975). In prevention of post-operative recurrence of CD, two randomized controlled trials demonstrated superiority of AZA/6-MP over placebo (Frei et al. 2013). Other indications for use of thiopurines include fistulizing CD as well as pouchitis.

Safety and Side Effects

Common side effects include nausea, vomiting, and abdominal pain which typically are mild and improve with either dose reduction and/or with continued use over time. Other nonspecific reactions include fever, rash, and arthralgia which are independent of the dose and may require cessation of drug. One major side effect of either 6-MP or AZA is bone marrow suppression seen in up to 2–5% of patients (Present et al. 1989). Leukopenia is the most common hematologic abnormality but thrombocytopenia and pancytopenia can be seen less commonly. This adverse effect is often dose related and can be managed with reducing dose and/or withdrawal of the drug. In order to avoid this potential drug toxicity, a TPMT enzyme activity is now routinely checked prior to starting 6-MP or AZA. Additionally, routine lab monitoring with a complete blood count (CBC) weekly to biweekly for 4 weeks, then monthly for 3 months, then every 3 months should be continued regardless of TPMT activity. Pancreatitis, which is independent of dose has been reported in 1.3–3.3% of patients treated with AZA or 6-MP for IBD (Present et al. 1989; Haber et al. 1986), typically occurring within a few weeks of starting therapy. The drug must be immediately stopped and not restarted as pancreatitis will generally recur with rechallenge of either drug. Drug induced liver injury has also been reported with 6-MP or AZA such as drug-induced hepatitis, cholestasis, nodular regenerative hyperplasia, and peliosis (Lichtenstein et al. 2006). A range of different infections have been reported such as disseminated cytomegalovirus (CMV) infection, CMV colitis, pneumonia, herpes zoster, and liver abscess to name a few (Lichtenstein et al. 2006). Overall, the risk of adverse events is reported to be from 15% with 10% of patients requiring withdrawal of drug (Lichtenstein et al. 2006). The two most common and important drug interactions with 6-MP or AZA to be aware of: 5-aminosalicylates (higher 6-TGN levels through inhibition of TPMT) and allopurinol (inhibits xanthine oxidase causing 6-MP/AZA toxicity/bone marrow suppression) (Lichtenstein et al. 2006). Lastly, these drugs are associated with an increased risk for non-Hodgkin's lymphoma and nonmelanoma skin cancer (NMSC). A meta-analysis by Kandiel and colleagues demonstrated a fourfold increase of lymphoma in patients with treated with 6-MP/AZA (Kandiel et al. 2005). Long and colleagues studied a cohort of 53,377 IBD patients in order to evaluate the risk of NMSC and found that persistent exposure to thiopurines

(≥365 days) had more than a fourfold risk of NMSC compared to IBD patients not exposed to thiopurines (Long et al. 2010).

Pregnancy and Lactation

The thiopurines are classified FDA pregnancy category D. It was originally given this classification in the 1950s when originally approved for treatment of leukemia (Beaulieu and Kane). The doses used to treat IBD are smaller and as discussed below suggest them to be safer than their pregnancy D classification.

Data from the Pregnancy in Inflammatory Bowel Disease and Neonatal Outcomes (PIANO) registry in 2012 did not find an increase in gestational or fetal anomalies in those women on 6-MP/AZA when compared to other groups (Hosseini-Carroll et al. 2015). In the CESAME cohort of 86 thiopurine-treated pregnancies, no increase in congenital abnormalities was found when compared to 129 IBD controlled pregnant patients (Coelho et al. 2011). Studies to date have demonstrated transfer of very low levels of drug in breast milk during breast-feeding; however, this is thought to be clinically insignificant (Hosseini-Carroll et al. 2015). Therefore, AZA and 6-MP are believed to be safe during breast-feeding. At this time, both 6-MP and AZA are thought to be safe to continue during pregnancy as well as during breast-feeding.

Geriatrics

Efficacy of thiopurines appears to be similar among elderly and younger patients with CD and UC. In the elderly IBD patients, TPMT enzyme activity should be checked to avoid leukopenia and possible infection. Strict lab monitoring should be done in these patients as the elderly are at higher risk for infection. As discussed above, since thiopurines are associated with an increased risk of NMSC, these patients should be counseled on sun-protective measures, use of sunscreen, and periodic dermatological exams. Patients should also be counseled on the increased risk of lymphoma associated with 6-MP/AZA. An important drug interaction to be aware of is allopurinol which is commonly used in elderly for gout. Allopurinol in combination with thiopurines can lead to leukopenia through shunting towards 6-TGN by inhibiting xanthine oxidase. In these patients, the dose of 6-MP/AZA may need to be adjusted (lowered) to factor in use of concomitant allopurinol.

Methotrexate

Methotrexate (MTX) is another immunomodulator that has been widely used, like the thiopurines, in other autoimmune diseases and some cancers long before it was routinely used in IBD. Methotrexate works through inhibition of several enzymes involved in the metabolic pathway of folic acid. With high dose MTX for treatment of cancer, it is thought that many of the cytotoxic and anti-proliferative effects are due to inhibition of the enzyme dihydrofolate reductase, which is important in the synthesis of both purines and pyrimidines. However, low dose MTX used in IBD is postulated to be related to the inhibition of several other folate dependent and independent enzyme pathways which causes the downstream immunomodulatory and anti-inflammatory effects in IBD (van Dieren et al. 2006). Moreover, MTX has

numerous anti-inflammatory effects, including blocking production of interleukin (IL)-1, IL-2, IL-6, and IL-8 (van Dieren et al. 2006).

Feagan and colleagues demonstrated MTX 25 mg intramuscularly weekly for 16 weeks was more likely to induce remission in CD than placebo (39.4% vs. 19.1%, $p = 0.025$) (Feagan et al. 1995). In a 40 week extension of this study, the authors found MTX to maintain remission at a higher rate than placebo (65% vs 39%, $p = 0.04$) when used at a dose of 15 mg weekly (Feagan et al. 2000). In the COMMIT trial, MTX was assessed for its superiority in combination Infliximab over Infliximab monotherapy. Unlike the data from SONIC trial (where combination IFX+ azathioprine was superior to IFX alone), there was no difference in clinical efficacy between the two groups (Feagan et al. 2014a, b). However, the study did demonstrate that patients in the MTX + IFX group had lower rates of immunogenicity and higher trough levels of IFX. This suggests that MTX may be useful in prevention of immunogenicity with biologic agents. Methotrexate taken orally has not been shown to be effective in UC. Two large prospective, placebo-controlled, randomized trials, METEOR and MERIT-UC, are either completed or ongoing evaluating efficacy of subcutaneous dosing of MTX in UC. The results of the METEOR will be published soon and the MERIT-UC study is ongoing. Studies have demonstrated oral dosing has high bioavailability and may be a suitable alternative in some patients. Additionally, subcutaneous injection has become preferred over intramuscular injection.

Side effects of MTX include nausea, vomiting, fatigue, diarrhea, leukopenia, liver fibrosis, hypersensitivity pneumonitis, and teratogenicity (Lichtenstein et al. 2006). Daily folic acid supplementation (1 mg/day) can help to reduce the severity of the side effects. Minor elevations in aminotransferases may occur and are common; however, development of hepatic fibrosis and cirrhosis is infrequent. Periodic monitoring of liver function tests is recommended. Additionally, screening for hepatitis B and hepatitis C is recommended prior to initiation. Moreover, counseling to avoid or minimize alcohol intake with MTX use should be done. Periodic lab monitoring with liver function tests as well as complete blood count is advised along with baseline chest X-ray prior to initiating therapy.

Methotrexate is a pregnancy category X drug signifying that it is an absolute contraindication in pregnancy. It is a folic acid antagonist that has been previously linked with several forms of birth defects affecting fetal organ development. In fact, women should wait to be off this medication for at least 6 months prior to conception (Hosseini-Carroll et al. 2015). Men who take methotrexate are advised to wait 3 months prior to conception due to the effect of oligospermia related to MTX. Lastly, it is not to be used during breast-feeding as it is passed into breast milk.

Methotrexate is used for treatment of CD, but not shown to be efficacious in UC. The efficacy is similar in both the young and elderly; however, its metabolism is affected by renal excretion. The dose may need to be adjusted in those elderly patients with decreased renal function (Greenwald and Brandt 2003). Drug–drug interactions which need to be monitored include: NSAIDs and 5-ASA (inhibit the renal excretion of methotrexate and thus may increase its toxicity), tetracycline

(inhibits methotrexate absorption), penicillin (decreases renal clearance), and methotrexate alters the clearance of theophylline. Folic acid supplementation is important to help prevent or minimize side effects of nausea, fatigue, rash, and stomatitis as well as potential for bone marrow suppression, hepatic fibrosis, and alopecia (Shea et al. 2014; Greenwald and Brandt 2003). Methotrexate has not been shown to increase the risk of lymphoma (Subramaniam et al. 2013).

4 Anti-TNF Agents

Tumor necrosis factor alpha (TNF-a), a ubiquitous cytokine is significantly increased in the setting of inflammation in IBD, along with other conditions such as rheumatoid arthritis, psoriasis, and psoriatic arthritis. Using agents that block these cytokines has significantly changed the landscape of treating IBD. Four anti-TNFs are FDA approved for the treatment of IBD, 3 in CD (infliximab, adalimumab, certolizumab) and 3 in UC (infliximab, adalimumab, golimumab). All except for certolizumab are Immunoglobulin G (IgG) molecules that bind to soluble and bound TNF-alpha. Certolizumab is the Fab fragment of the immunoglobulin (which binds to TNF alpha) attached to a PEG molecule that improves its half-life. Here we evaluate their clinical trial data, of both safety and efficacy in treatment of IBD.

4.1 Infliximab

In Crohn's disease, infliximab 5 mg/kg IV infusion at week 0, 2, and 6 was superior to placebo in achieving clinical remission off steroids based on CDAI scores<150 in 64% compared to 34% placebo at week 12 ($p = 0.03$) (Leman et al. 2006) In the ACCENT I study, of those that achieved clinical remission after induction, 39% maintained clinical remission with infliximab compared to only 21% with placebo ($p = 0.0003$) (Hanauer et al. 2002). Episodic use of infliximab and all other biologics are avoided due to high risk of anti-drug antibody formation as well as lower clinical response and remission rates in comparison to scheduled maintenance therapy (Rutgeerts et al. 2004). In ACCENT II trial, patients with either abdominal or perianal fistulas had higher rates of fistula closure with infliximab than in placebo (36% vs. 19%, $p = 0.009$) (Sands et al. 2004).

Infliximab was studied in the landmark SONIC trial that showed that combination therapy of infliximab plus azathioprine (2.5 mg/kg) for patients with moderate to severe Crohn's disease naïve to biologics was superior in achieving clinical remission at week 26 (57% combination, vs. 44% infliximab monotherapy ($p = 0.02$), and 30% azathioprine monotherapy ($p = 0.001$) (Colombel et al. 2010). Mucosal healing did occur in a higher number of patients on combination therapy with infliximab and azathioprine (44%) compared to 30% on infliximab monotherapy ($p = 0.06$) and only 16.5% in azathioprine monotherapy ($p < 0.006$ vs combination, $p = 0.02$ vs. IFX mono) (Colombel et al. 2010). Although designed differently than the SONIC trial, the COMMIT trial did not show that combination infliximab with methotrexate was more effective than infliximab alone in Crohn's disease patients (Feagan et al. 2014a, b).

In ulcerative colitis, the first anti-TNF trial of UC patients in ACT I and ACT II showed superior induction of remission rates at week 8 as defined by Mayo score of 2 or less and no subscore more than 1 with infliximab at 38.8% ($p < 0.001$) compared to 14.9% with placebo (Rutgeerts et al. 2005a, b). Mucosal healing defined by absolute Mayo endoscopy subscore of 0 or 1 was also achieved in higher number of patients on infliximab at 62% ($p < 0.001$) compared to only 34% on placebo. For maintenance, these trials again showed superior sustained clinical remission with infliximab in both ACT I and II 23% ($p = 0.001$) and 14% ($p < 0.001$), respectively, compared to placebo 8% at week 30. At week 54, 20% of patients on infliximab maintained clinical remission compared to only 6.6% on placebo ($p = 0.002$). Similar to the SONIC trial, the SUCCESS trial also showed that combination therapy with infliximab 5 mg/kg and azathioprine 2.5 mg/kg daily achieved higher rates of corticosteroid free clinical remission (40% compared to only 22% with infliximab monotherapy ($p = 0.017$), and 24% azathioprine monotherapy ($p = 0.032$) for UC patients (Panaccione et al. 2014). For those with severe colitis, infliximab was found not inferior to cyclosporine as a rescue treatment to avoid colectomy (Laharie et al. 2012).

4.2 Adalimumab

For Crohn's disease, the CLASSIC I trial showed that adalimumab induction therapy at 160 mg SQ at week 0, 80 mg SQ at week 2 induced clinical remission (CDAI score <150) in 36% compared to only 12% in placebo at week 4 ($p = 0.001$); (Hanauer et al. 2006). The CHARM trial showed that adalimumab 40 mg SQ every other week maintained clinical remission of 40% and 36% at weeks 26 and 56, respectively, compared to only 17% and 12% in those on placebo (both $p < 0.001$) (Colombel et al. 2007a, b). The study also showed that adalimumab was superior in complete closure of fistulas in 33% at week 56, compared to only 13% of patients on placebo ($p = 0.016$) (Colombel et al. 2007a, b). In the EXTEND trial, mucosal healing (defined as the absence of mucosal ulcerations) was achieved of the ileocolonic mucosa at week 12 and 52 in 27% and 24% of patients on adalimumab maintenance therapy compared to only 13% and 0% on placebo ($p = 0.056$ and $p < 0.001$), respectively (Rutgeerts et al. 2012). Retrospective studies showed a superior effect of preventing disease flares in the first 6 months of use with combination adalimumab plus thiopurine therapy (86% vs 64% compared to adalimumab monotherapy $p = 0.02$) and higher rates of clinical remission ($p = 0.046$) (Reenaers et al. 2012; Ishida et al. 2013). Adalimumab was also useful to gain remission in patients that had previously lost response to or unable to tolerate infliximab compared to placebo (21% vs. 7% $p < 0.001$) at week 4 in the GAIN trial (Sandborn et al. 2007a, b).

In ulcerative colitis, the ULTRA I and II trials showed adalimumab induction at 160 mg at week 0 and 80 mg SQ at week 2 had higher week 8 clinical remission rates based on Mayo scoring (see infliximab above) at 18.5% vs. 9.2% on placebo ($p = 0.031$), and 16.5% vs. 9.3% placebo ($p < 0.019$), respectively (Reinisch et al.

2011). With maintenance dosing of 40 mg SQ every other week, 17% of patients on adalimumab achieved clinical remission at week 52 compared to only 8.5% on placebo ($p = 0.004$). Mucosal healing was also higher 41% (week 8) and 25% (week 52) with adalimumab maintenance therapy compared to placebo 31.5% (week 8) and 15% (week 52) (both $p < 0.05$), respectively. No clinical trial is available for adalimumab as rescue therapy to avoid colectomy (Sandborn et al. 2012).

4.3 Certolizumab

Certolizumab at 400 mg SQ induction at week 0, 2, and 4 in patients with moderate to severe Crohn's disease showed 37% clinical response rate based on CDAI score decrease >100 compared to only 26% with placebo ($p = 0.04$) in the PRECISE I trial (Sandborn et al. 2007b, 2010). In the PRECISE II trial, maintenance therapy with certolizumab at 400 mg SQ dosing every 4 weeks was superior 48% compared to placebo 29% ($p < 0.001$) at week 26 in maintaining remission (Schreiber et al. 2007). The MUSIC trial also showed that certolizumab contributed to mucosal healing as defined by CDEIS score <6) at week 54 in 27% of patients (Hebuterne et al. 2012). Fistula closure was also higher in patients on certolizumab maintenance in 36% of patients compared to only 17% on placebo ($p = 0.038$) (Schreiber et al. 2011). In those patients who lost response to infliximab, the WELCOME study showed that switching to certolizumab 62% of patients achieved clinical response at week 6 after induction of certolizumab, and maintained clinical response after 26 weeks in 40% on certolizumab (Sandborn et al. 2010).

4.4 Golimumab

For moderate to severe UC, 18% of patients receiving induction with golimumab at 200 mg SQ week 0, and 100 mg SQ week 2 in the PURSUIT-SC trial achieved clinical remission at week 6 compared to only 6.4% on placebo ($p < 0.001$) (Sandborn et al. 2014a, b). The PURSUIT-M trial showed that sustained clinical remission at week 54 was 28% in the 100 mg SQ every 4 week maintenance dosing compared to only 15.6% with placebo ($p = 0.004$). Mucosal healing was achieved in 42% at both weeks 30 and 54 compared to only 27% on placebo ($p = 0.011$ and $p = 0.002$), respectively (Sandborn et al. 2014a, b).

No head to head trials have been done to date in either Crohn's disease or UC between the anti-TNFs. Although some clinical judgment can be made based on efficacy based on the trial data and our own clinical experience, a prospective trial would be needed to truly evaluate differences between these agents.

4.4.1 Safety and Side Effects

Safety of anti-TNFs is of concern to patients. However, close monitoring for possible side effects and discontinuing therapy immediately if they occur can minimize morbidity. In comparison to corticosteroids, for example, anti-TNF for

long-term use is far safer. Most often, patients fail to realize the benefits of these therapies in comparison to its side effects and an algorithm that evaluates the prognostic factors of patients with IBD can be used to reassure the patient that the benefits of these medications most often far outweigh its possible side effects.

One of the main concerns of these biologic agents is the occurrence of opportunistic infections. Due to the risk of reactivation of tuberculosis and hepatitis B, all patients prior to starting anti-TNF therapy should be evaluated for latent or active TB and active hepatitis B infection. If negative, vaccination to hepatitis B should be given ideally prior to initiating therapy. Other fungal infections have been reported and those on anti-TNF therapy with fevers of unknown origin should be evaluated for these less commonly thought of infections. Patients may also develop antidrug antibodies to these agents and can present with a variety of side effects, most commonly infusion or injection site reactions. Neurological side effects have been reported including multiple sclerosis – plaque like lesions in the brain that subsides with discontinuation of therapy. Although used to treat psoriasis, anti-TNFs can bring about a paradoxical worsening of psoriasis in some patients. More concerning is pustular psoriasis that occurs in the palms of the hands and soles of the feet and can be quite debilitating. These patients should discontinue anti-TNF therapy immediately. Anti-TNFs may also exacerbate CHF and those with significant cardiac history should be cleared by cardiology prior to starting therapy. The potential for lymphoma and other cancer risks may deter many patients from starting anti-TNF therapy. However, the absolute risks of these are small and perhaps not as high as previously thought and should be weighed against the risk of small bowel and colon cancer with untreated disease. One must also note that most of these drugs are usually given in addition to other drugs like mesalamine, azathioprine, and corticosteroids and thus, drug interactions and cumulative toxicities should be taken into consideration.

4.4.2 Pregnancy and Lactation

All anti-TNFs are category B in pregnancy. The negligible prevalence of pregnancy complications of congenital abnormalities, preterm delivery, low birth weight, and misscarriages with anti-TNF exposure suggests that these medications can be used well throughout the third trimester, when there is active transplacental transfer of IgG molecules (El et al. 2010). Data from the PIANO registry of over 1,200 mothers receiving anti-TNF therapy did not reveal any increased risk of infections to their infants (Mahadevan et al. 2012). However, live virus vaccines should be avoided in infants unless there is documentation of undetectable serum levels. Certolizumab, on the other hand, is unique in that it lacks the Fc portion of the antibody which is required for active transfer to the fetus, thus very limited levels reach the fetus ($<4\%$ of the mother) (Mahadevan et al. 2013). Due to this, infants born to mothers on certolizumab can take the live virus rotavirus vaccine safely. Breast-feeding is considered safe (category B) for all anti-TNF therapies as even minimal breast milk output does not implicate absorption to the newborn as the immunoglobulins are degraded by the acid in the infant's stomach to render them useless (Ben-Horin et al. 2010, 2011).

4.4.3 Geriatrics

Anti-TNF use should proceed with even closer monitoring in the elderly as those older than 65 years had higher incidence of severe infections 11% and 10% total mortality compared to 2.6% and 1% incidence, respectively, in the younger patients (Cottone et al. 2011). Risk of severe adverse events was also 3 times higher in this population compared to BD patients <65 years old on anti-TNF therapy (Lobatón et al. 2015). Older age has been shown to be a statistically significant predictor of suboptimal early response to anti-TNF therapy (Lobatón et al. 2015; Ferrante et al. 2007), and age greater than 60 years was a significant risk factor for discontinuation of this treatment (Desai et al. 2013a, b).

5 Anti-Integrin Agents

Both UC and CD involve persistent recruitment of leukocytes into gut tissue, coupled with dysregulated activation of immune cell function. The *a*4 integrins are key molecules which mediate infiltration of the GI tract by memory T lymphocytes binding to mucosal address in cell adhesion molecule 1 (MAdCAM-1) on endothelial cells, and blockade of this interaction has shown to decrease inflammation in IBD. Therefore, the *a*4 integrins are an ideal therapeutic target for IBD (Xavier and Podolsky 2007; Soler et al. 2009).

There are a number of patients who fail either immunomodulators or anti-TNF agents either because of (1) primary non-response; (2) loss of response; (3) or intolerance. The anti-integrin agents have been approved for both primary biologic therapy and for those who failed anti-TNF therapy.

5.1 Natalizumab

Natalizumab is a humanized IgG4 monoclonal antibody that acts by blocking the adhesion and subsequent migration of leukocytes into the gut by binding *a*4integrin. It has also been shown to be effective in the treatment of multiple sclerosis – another chronic inflammatory disease as it antagonizes both the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins which explains its efficacy in the central nervous system and the GI tract, respectively (Soler et al. 2009).

In the ENACT-1 and ENACT-2 studies, natalizumab was evaluated as both induction and maintenance therapy for patients with moderate to severe CD. In the first trial, 905 patients were randomized to receive 300 mg of natalizumab or placebo at weeks 0, 4, and 8. Primary outcome was response at week 10, defined by a decrease in the Crohn's Disease Activity Index (CDAI) score of at least 70 points. In the second trial, 339 patients who had a response to natalizumab in the first trial were then randomized to receive 300 mg of natalizumab or placebo every 4 weeks through week 56. Primary outcome was a sustained response through week 36. A secondary outcome in both trials was disease remission (a CDAI <150). In the induction trial, both groups, natalizumab and placebo, had similar rates of response (56% and 49%; $p = 0.05$) and remission (37% and 30%, respectively;

$p = 0.12$) at 10 weeks. Continuing natalizumab into the second (maintenance) trial resulted in higher rates of sustained response (61% vs 28%, $p < 0.001$) and remission (44% vs. 26%, $p = 0.003$) through week 36 than switching to placebo (Sandborn et al. 2005).

The ENCORE trial, a global multi-center study, further evaluated the efficacy of natalizumab as induction therapy in patients with CD. Patients with moderate to severe CD and active inflammation, characterized by elevated CRP, were randomized to receive natalizumab 300 mg or placebo intravenously at weeks 0, 4, and 8. Induction of response was assessed at week 8 sustained through week 12. Response occurred in 48% of natalizumab-treated patients and 32% of patients receiving placebo ($p < 0.001$). Sustained remission occurred in 26% of natalizumab-treated patients and 16% of patients receiving placebo ($p = 0.002$) (Targan et al. 2007).

5.1.1 Safety and Side Effects

In the ENCORE trial, the most commonly reported adverse effects included headache, nausea, abdominal pain, nasopharyngitis, dizziness, fatigue, and exacerbation of Crohn's disease (Targan et al. 2007). Studies of natalizumab to date have not demonstrated an increased rate of lymphoma, cancers, autoimmune diseases, or cardiovascular events (Targan et al. 2007).

Natalizumab was initially approved in the USA in December 2004 for relapsing multiple sclerosis. A few months later in February 2005, two cases of progressive multifocal leukoencephalopathy (PML) were reported among natalizumab-treated patients with multiple sclerosis. Shortly thereafter, in an open-label extension study from ENCORE-2, a patient treated with natalizumab died from PML, associated with the JC virus, a human polyomavirus. As a result of these three cases of PML, natalizumab was voluntarily withdrawn from the market in 2005. In 2006, it was reintroduced, under watchful surveillance by the FDA along with the TOUCH (Tysabri Outreach: Unified Commitment to Health) Prescribing Program. Three risk factors have been identified for PML in natalizumab-treated patients: longer duration of natalizumab therapy, especially beyond 2 years; prior treatment with an immunosuppressant; and the presence of anti-JCV antibodies (Lichtenstein et al. 2012). Based on available date, the estimated incidence for PML is as follows:

For JCV positive patients who receive natalizumab for up to 24 months, the risk of developing PML is relatively low: <1 in 1,000 with no prior immunosuppressant (IS) use; 2 in 1,000 with prior IS use. For JCV positive patients who receive natalizumab for 25–48 months, the risk of developing PML is higher: 4 in 1,000 with no prior IS use; and 11 in 1,000 for patients with prior IS use. When deciding to use natalizumab, it is important to understand that natalizumab should only be used in patients who had an inadequate response or were unable to tolerate conventional therapies (such as anti-TNF agents) (Lichtenstein et al. 2012).

5.1.2 Pregnancy and Lactation

Natalizumab is categorized as FDA pregnancy Class C, which carries an unknown risk. There are limited safety data on the use of natalizumab during pregnancy and

lactation. To date, there is one study in the literature examining natalizumab exposure in pregnancy. In this small study, the risk of congenital malformations was not found to be increased in 164 pregnancies in patients with CD or multiple sclerosis on natalizumab during the first trimester (Nazareth et al. 2008). This same study examined eight men who were exposed to natalizumab at the time of conception, with no evidence of birth defects. There is no data on breast-feeding.

5.1.3 Geriatrics

Clinical studies of natalizumab did not include adequate numbers of patients aged 65 years and older to determine whether they respond differently than younger patients.

5.2 Vedolizumab

Vedolizumab is an IgG1 humanized monoclonal antibody which was approved by the FDA in May 2014 for the treatment of UC and CD. It works to specifically bind to α4β7 integrin, blocking its interaction with mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which is mainly expressed on gut endothelial cells. By inhibiting binding, vedolizumab blocks the migration of a subset of memory gut-homing T lymphocytes into inflamed intestinal tissue. The gut specificity is an important aspect of this treatment as it should not affect lymphocyte trafficking to other sites, such as the central nervous system (CNS), and consequently should limit both systemic and CNS toxicity (progressive multifocal leukoencephalopathy linked to the JC virus) as seen with natalizumab (Soler et al. 2009). Three clinical trials have shown its efficacy in both moderate to severe UC and CD.

In the Gemini 1 trial, two randomized, double-blind, placebo-controlled trials were conducted for induction and maintenance using vedolizumab in moderate to severe ulcerative colitis. In the induction trial, 374 patients received vedolizumab (dose of 300 mg) or Placebo at weeks 0 and 2, and 521 patients received open-label vedolizumab at weeks 0 and 2. Disease activity was then assessed at week 6. In the maintenance trial, those patients from either group who had a response at week 6 were then randomized to (1) Continue receiving vedolizumab every 8 weeks (2) Continue receiving vedolizumab every 4 weeks or (3) Switch to Placebo for up to 52 weeks. A response was defined as reduction in the Mayo Clinic score (range from 0 to 12 with 12 being severely active disease) of at least 3 points and a decrease of at least 30% from baseline, with an accompanying decrease in the rectal bleeding subscore of at least 1 point or an absolute rectal bleeding subscore of 0 or 1. Response rates at week 6 were 47.1% in the vedolizumab group as compared to 25.5% in the placebo group ($p < 0.001$). At week 52, 41.8% of patients in the Q8 week Vedolizumab group and 44.8% of patients in the vedolizumab Q4 weeks were in clinical remission (Mayo Clinic score ≤ 2 and no subscore >1), as compared with 15.9% of patients who switched to placebo ($p < 0.001$) (Feagan et al. 2013).

In the Gemini 2 trial, vedolizumab was studied in patients with moderate to severe Crohn's disease for both induction and maintenance of remission. In the induction trial, 368 patients were randomized to receive vedolizumab or placebo at weeks 0 and 2, and 747 patients received open-label vedolizumab at weeks 0 and 2. Disease activity was then assessed at week 6. In the maintenance trial, 461 patients who responded to vedolizumab were then randomized to placebo or vedolizumab every 8 weeks or every 4 weeks for 52 weeks. At week 6, 14.5% of the patients who received vedolizumab and 6.8% who received placebo were in clinical remission (CDAI of $\leq$150) ($p = 0.02$). A total of 31.4% and 25.7% of the patients, respectively, had a CDAI-100 response ($\geq$100-point decrease in the CDAI score) ($p = 0.23$). Of those patients who responded to induction therapy, 39.0% of the vedolizumab every 8 weeks and 36.4% of the vedolizumab every 4 weeks were in clinical remission as compared with 21.6% assigned to placebo ($p < 0.001$ and $p = 0.004$ for the two vedolizumab groups, respectively, vs. placebo) at 52 weeks (Sandborn et al. 2013).

In the Gemini 3 trial, vedolizumab was specifically evaluated in those patients with moderate to severe Crohn's disease and previous anti-TNF failure. Previous anti-TNF failure was defined as an inadequate response to, loss of response to, or intolerance to any of the anti-TNF agents. 15.2% of the patients who received vedolizumab and 12.1% of those who received placebo were in remission at week 6 ($p = 0.433$). At week 10, 26.6% of the vedolizumab group compared to 12.1% of the place group were in remission ($p = 0.001$). This study found that the actual benefit was not detectable until week 10 in treatment with vedolizumab in those patients with Crohn's disease who were previously treated with anti-TNF therapy (Sands et al.).

5.2.1 Safety and Side Effects

Some of the less severe adverse effects encountered with vedolizumab use include nasopharyngitis, arthralgia, headache, pyrexia, nausea, abdominal pain, upper respiratory infection, and fatigue. A meta-analysis by Wang and colleagues demonstrated that vedolizumab was not associated with increased rates of any serious adverse effects (RR: 1.21; 95% CI: 1.00–1.46), serious infections (RR: 1.17; 95% CI: 0.51–2.69) and other adverse events including progressive multi-focal leukoencephalopathy (PML), death, and cancer (Wang et al. 2014. Of note, no cases of PML were found in the approximately 3,000 patients exposed for a median time of 18 months of whom 80% had prior exposure to immunosuppressant (Wang et al. 2014). Due to the gut selectivity of this drug, there was a concern that this agent may increase risk of gastrointestinal infections. This was not seen in either the UC or CD trial. Certainly, longer term observational data is needed to further understand the full safety profile of this drug.

5.2.2 Pregnancy and Lactation

Vedolizumab is FDA pregnancy category B indicating no risk to fetus is expected. Because vedolizumab is an IgG monoclonal antibody, its placental transfer is similar to the other IgG drugs (infliximab, adalimumab) with linear increase as

pregnancy progresses. Moreover, the largest amount of drug is transferred during the third trimester. There are no controlled studies with vedolizumab in pregnant women and data is currently being collected through an observational pregnancy registry. A recent observational study by Dubinsky and colleagues demonstrated that among 16 vedolizumab exposed pregnancies, there were 9 live births, 2 spontaneous abortions, 2 elective terminations, and 3 undocumented outcomes at the last follow -up visit (Dubinsky et al. 2015). At this time, it is unknown if vedolizumab is present in human milk. Current recommendations include to exercise caution when administering vedolizumab to a nursing woman.

5.2.3 Geriatrics

Studies have shown that younger age at onset of disease is a risk factor for more aggressive disease phenotype and surgery. Additionally, there is concern that the anti-TNF agents may cause serious adverse events in older patients (Desai et al. 2013a, b). As mentioned above, Vedolizumab has been shown to have a favorable safety profile. Likewise, sub-group analysis from Gemini 1 trial, which enrolled pts. from 18 to 80 years, has shown that Vedolizumab is effective and safe among elderly patients (Feagan et al. 2013). A recent position paper recommends Vedolizumab as the preferred agent for patients with higher risk of opportunistic infections such as the elderly population (Armuzzi et al. 2016).

6 Current Therapeutic Algorithm for UC

Patients should be risk stratified into low risk and high risk for colectomy. Those patients at low risk for colectomy include limited anatomic extent and mild endoscopic disease. Patients at high risk for colectomy include extensive colitis, deep ulcers on colonoscopy, age <40, high CRP and ESR, steroid requiring disease, history of hospitalizations, and infections such as C diff and/or CMV (Kornbluth et al. 2010). Below are guidelines recently published from the Ulcerative Care pathway by Dassopoulos and colleagues (Dassopoulos et al. 2015).

6.1 Induction and Maintenance Therapy for Low Risk Patients (Outpatient)

Induction therapies for low risk patients in the outpatient setting include oral mesalamine and topical mesalamine therapies as well as oral steroid (budesonide or prednisone) and topical steroids. In those patients who are responders, recommendations include oral and/or topical mesalamine therapy as maintenance therapy after slowly tapering steroids off.

6.2 Induction and Maintenance Therapy for High Risk Patients (Outpatient)

In those patients who do not respond to the aforementioned medications or relapse with steroid taper, these patients would follow the treatment algorithm reserved for high risk patients. The options for induction therapy include steroids along with either of the thiopurines, anti-TNF therapy +/− thiopurine, vedolizumab +/− thiopurine. For those patients who respond to any of these therapies, the maintenance therapy would be to continue either the thiopurine or biologic +/− thiopurine as steroids are tapered.

6.3 If No Remission or Relapse with Above, See Below for Outpatient Therapy Options

For those patients who fail to achieve remission with thiopurine therapy, then 6-TGN levels should be checked to assess if patient is therapeutic or not. If subtherapeutic, then dose can be increased and if therapeutic, patient should be switched to a biologic agent (either anti-TNF or vedolizumab). If patients lose response to anti-TNF therapy, again drug levels can be checked to assess for therapeutic concentration and development of antibodies. If subtherapeutic anti-TNF level and no antibody detected, then dose can be increased or interval decreased as well as consideration of addition of an immunomodulator. If subtherapeutic anti-TNF level and antibody detected, then switch to a different anti-TNF agent can be made. Lastly, if therapeutic anti-TNF level is found, then therapy should be changed to vedolizumab with or without an immunomodulator. If patient has lost response to vedolizumab, then dose may be increased to every 4 weeks or change to anti-TNF therapy with or without immunomodulator should be considered.

6.4 Induction and Maintenance Therapy for High Risk Inpatients

For patients who are hospitalized and at high risk for colectomy, induction therapies include IV steroids, IV infliximab, or IV cyclosporine. If patients respond to IV steroids, then decision can be made to start maintenance therapy with either thiopurine, anti-TNF therapy +/− thiopurine, vedolizumab +/− thiopurine. In patients who are IV steroid refractory, options include infliximab, cyclosporine, or colectomy. In those patients who respond to Infliximab, maintenance therapy would include to continue infliximab +/− thiopurine. In those patients who fail infliximab, recommendations are for colectomy. For those patients who respond to IV cyclosporine, decision must then be made to switch to thiopurine or anti-TNF therapy +/− thiopurine or vedolizumab +/− thiopurine for maintenance. Lastly, in those patients who fail IV cyclosporine, recommendations are for colectomy.

7 Current Therapeutic Algorithm for CD

Due to increasing availability of different therapeutic agents, the medical management of Crohn's disease has rapidly evolved. Early stratification of high risk patients with poor prognosis and aggressive management can avoid disease complications. With therapeutic drug monitoring, optimizing dosing and medications to the individual patient can increase efficacy of drugs and improve their clinical outcome.

In the management algorithm, a newly diagnosed patient should first be risk stratified based on disease location, severity of endoscopic and histologic activity, disease phenotype of inflammatory, penetrating or stricturing activity as well as any other prognostic markers of genetic mutations such as NOD2 or serologic antibodies. Classifying patients at low risk (mild disease), moderate risk (moderate disease), or high risk (severe disease) should guide the provider in appropriate medical therapy. Once treatment is initiated, then treatment target should be based not only on clinical remission but also on endoscopic and ideally if possible, histologic remission.

Low risk (*mild disease*) *at presentation*: >30 years age at diagnosis; limited anatomic involvement; no perianal or severe rectal disease; superficial ulcers (Sandborn et al. 2014a, b).

If mild/superficial colonic Crohn's disease, consider 5-ASA therapy and step up as needed.
If mild but small bowel or deeper inflammation, start with immunomodulators therapy with azathioprine 2.5 mg/kg/day or 6-mercaptopurine 1.5 mg/kg/day if TPMT normal, or methotrexate 25 mg SQ weekly with 1 mg folic acid daily.
Consider bridging with budesonide EC with ileo-colonic disease, or budesonide MMX if colonic involvement.

Moderate risk (*moderate disease*) *at presentation*:

Consider induction with steroid therapy with either budesonide or prednisone and use of immunomodulator therapy at treatment dose (see dosing above – low risk section).
Consider monotherapy with anti-TNF therapy or with low dose immunomodulator therapy (azathioprine 50–100 mg or 50 mg 6MP; or MTX 15 mg PO weekly) to prevent anti-drug antibody formation.
Consider monotherapy with anti-integrin therapy or with low dose immunomodulator therapy.

High risk (*severe disease*): Age less than 30 years at diagnosis; extensive anatomic involvement, perianal and or severe rectal disease; deep ulcerations, prior surgical resection, stricturing and/or penetrating behavior (Sandborn et al. 2014a, b).

Consider combination therapy with anti-TNF and full dose immunomodulators (AZA/6MP or MTX).
Consider combination therapy with anti-integrin therapy and full dose immunomodulators therapy.

For those that have either primary nonresponse or secondary loss of response to medications, therapeutic drug monitoring can help in management: For patients on azathioprine or 6-MP subtherapeutic thiopurine metabolite levels of 6-thioguanine suggests either nonadherence to therapy or need for higher dosing. Those that develop secondary loss of response due to subtherapeutic levels of anti-TNF medication can benefit from increased dose or frequency of the drug. Those with subtherapeutic or no anti-TNF levels with high antidrug antibodies will benefit most from switching to another anti-TNF agent. Those with low titers of antidrug antibodies may benefit from addition of immunomodulators to reduce and remove antibodies and increase drug levels. Alternatively, switching to another anti-TNF agent could be beneficial. Those with therapeutic drug levels and no antidrug antibodies should be evaluated for other causes of symptoms such as IBS, small bowel bacterial overgrowth, bile acid induced diarrhea, *C. difficile* or CMV infection, and structural disease such as fibrostenosis requiring surgery. If active disease is confirmed and other etiologies of symptoms are ruled out, these patients benefit most by switching out of class to a different mechanism of action agent such as an anti-integrin.

Similarly in the post-operative setting, risk re-stratifying patients can prevent further complications from Crohn's disease:

Low risk of disease recurrence patients: Patients with short strictures less than 10 cm, greater than 10 years of lead time to first surgery and no history of smoking: can be monitored with disease reassessment every 6–12 months with colonoscopy.
Those with Rutgeerts scores of i0 can be followed without medical therapy. Those with Rutgeerts score of i1 should be considered for immunomodulator therapy. However, those with significant recurrence (scores of i2–i4) should start anti-TNF therapy. In a meta-analysis of 9 controlled trials, anti-TNF therapy was more effective at preventing ($n = 6$) endoscopic recurrence (odds ratio 0.05; 95% confidence interval 0.02–0.13, $p < 0.0001$; NNT = 1.9), at preventing ($n = 5$) clinical recurrence (odds ratio 0.10; 95% confidence interval 0.05–0.21, $p < 0.0001$; NNT = 2.4), and treating endoscopic postoperative recurrence ($n = 2$; odds ratio 16.64; 95% confidence interval 2.51–110.27, $p < 0.004$; NNT = 2.3), than control treatment with thiopurines or mesalamine (Carla-Moreau et al. 2015).
Moderate risk patients, those with longer strictures or inflammatory stenosis, and time to index surgery less than 10 years, should receive 3 months of metronidazole along with either immunomodulator treatment (6-MP, AZA) or even consider anti-TNF therapy. Post-operative colonoscopy is recommended within 6–12 months of surgery.

High risk patients are those with greater than two surgeries, penetrating/perforating disease, complicated peri- and postoperative course, and/or those that continue to smoke should start anti-TNF therapy within 4 weeks of surgery and smoking cessation should be highly encouraged and emphasized. Repeat colonoscopy at 6 months post-operatively should be done to reassess disease activity and optimize medical therapy as needed.

8 Conclusion

Crohn's disease and ulcerative colitis are complex, multifactorial, immune mediated inflammatory disorders of the gastrointestinal tract. More than 150 genes have been identified to be involved in the pathogenesis of IBD. Different environmental factors, such as smoking, diet, pollution, stress and sleep have also been discovered. Defects in the immune system and bacterial pathogens in the intestinal tract also play an important role in the chronic inflammatory process. Considering a complex interplay of these different factors, it is difficult to discover a curative therapy for these illnesses. The current therapeutic goals are to achieve clinical remission along with mucosal healing, avoidance of complications such as surgeries and to improve the quality of life. Different drug classes such as aminosalicylates, antibiotics, immunomodulators and biologics are being used as treatment for CD and UC. Recent studies have shown that early aggressive therapy with combination of different agents such as immunomodulators and biologics yield to better outcomes in terms of steroid free clinical remission and mucosal healing. However, aggressive immunosuppression can lead to increased risks of cancers and infections and careful selection of these therapeutic agents and risk stratification are important. Different therapeutic algorithms as laid out above in the chapter would help clinicians to choose optimal and most effective therapy.

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Gastrointestinal Pharmacology

Miguel Saps and Adrian Miranda

Contents

Abstract

There is little evidence for most of the medications currently used to treat functional abdominal pain disorders (FAPDs) in children. Not only are there very few clinical trials, but also most have significant variability in the methods used and outcomes measured. Thus, the decision on the most appropriate pharmacological treatment is frequently based on adult studies or empirical data. In children, peppermint oil, trimebutine, and drotaverine have shown significant benefit compared with placebo,

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each of them in a single randomized clinical trial. A small study found that cyproheptadine was beneficial in the treatment of FAPDs in children. There are conflicting data regarding amitriptyline. While one small study found a significant benefit in quality of life compared with placebo, a large multicenter study found no benefit compared with placebo. The antidepressant, citalopram, failed to meet the primary outcomes in intention-to-treat and per-protocol analysis. Rifaximin has been shown to be efficacious in the treatment of adults with IBS. Those findings differ from studies in children where no benefit was found compared to placebo. To date, there are no placebo-controlled trials published on the use of linaclotide or lubiprostone in children. Alpha 2 delta ligands such as gabapentin and pregabalin are sometimes used in the care of this group of children, but no clinical trials are available in children with FAPDs. Similarly, novel drugs that have been approved for the care of irritable bowel with diarrhea in adults such as eluxadoline have yet to be studied in children. Conclusions: Little data support the use of most medications commonly used to treat FAPDs in children. More randomized, placebo-controlled studies are needed to assess the efficacy of pharmacological interventions in the treatment of FAPDs in children.

Keywords
Children • Clinical trials • Functional abdominal pain disorders • Irritable bowel syndrome • Treatment

1 Pharmacotherapy for FAPDs in Children

1.1 Definition

The recently published Rome IV criteria (Hyams et al. 2016) defines functional abdominal disorders (FAPDs) as a group of functional gastrointestinal disorders (FGIDs) with pain as the driving symptom. This group of disorders includes four diagnoses: irritable bowel syndrome (IBS), functional dyspepsia, functional abdominal pain-not otherwise specified (FAP-NOS), and abdominal migraine. The diagnosis of functional dyspepsia is now subdivided into two different groups: postprandial distress syndrome that applies to patients who have postprandial fullness or early satiation, and epigastric pain syndrome that applies to those patients who have pain or burning localized to the epigastrium without improvement of their symptoms with bowel movements. The Rome IV criteria have also subcategorized IBS according to their predominant bowel pattern as IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), IBS with mixed bowel habits, and IBS unsubtyped.

1.2 Epidemiology

FAPDS and defecation disorders are the two most common groups of FGIDs. School studies worldwide have shown a prevalence of FAPDs of 8–14% using the Rome III criteria (Devanarayana et al. 2011; Gulewitsch et al. 2013; Jativa et al. 2016; Lu et al. 2016; Saps et al. 2014; Saps et al. 2009; Udoh et al. 2016). A survey interviewing a large sample of mothers of the US general population found that more than 13% of children and adolescents met diagnosis for at least one FAPD according to the Rome III criteria (Lewis et al. 2016). IBS is the most common FAPDs with most of the studies worldwide showing that 4–7% of all school children qualify by this diagnosis (Devanarayana et al. 2011; Gulewitsch et al. 2013; Jativa et al. 2016; Lu et al. 2016; Saps et al. 2014; Saps et al. 2009; Udoh et al. 2016). Studies conducted in high-school students in China found a much higher prevalence (Dong et al. 2005; Zhou et al. 2010a). The reason/s for these differences in prevalence remains unknown. FAPDs may manifest alone or overlap with other functional (Friesen et al. 2016) or organic diseases. Three studies have shown that FAPDs frequently overlap with inflammatory bowel disease (Diederen et al. 2016; Watson et al. 2016; Zimmerman et al. 2013), with one of the studies showing that up to 24% of children with an inflammatory bowel disease in remission met the criteria for an FAPD (Watson et al. 2016). This can add a level of complexity to the treatment of organic disorders and worsen quality of life and disability in some children.

1.3 Impact and Prognosis

Children with FAPDs have higher anxiety (Janssens et al. 2015) and disability scores (Otu-Nyarko et al. 2015) and worse quality of life (Rippel et al. 2012; Warschburger et al. 2014) than their peers. FAPDs are the most common reason for referral to pediatric gastroenterology in the USA (Rouster et al. 2016) accounting for 52% of all consultations in children and adolescents. The healthcare costs associated with this group of children are enormous (Dhroove et al. 2010; Hoekman et al. 2015). A recent study shows that the cost of inpatient care for children with FGIDs has tripled in the past 12 years (Park et al. 2015). Still, outcomes of medical treatment of children with FAPDs continue to be less than optimal with studies showing poor results in 30–60% of children (Croffie et al. 2000; Lisman-van Leeuwen et al. 2013). A systematic review showed that 29% of children that sought medical care for FAPDs had abdominal complaints after 5 years (Gieteling et al. 2008). Among children with FAPDs, those with symptoms of IBS seem to have a less favorable prognosis compared with children with functional abdominal pain or functional dyspepsia (Czyzewski et al. 2016; Gieteling et al. 2008).

1.4 Pharmacological Treatments

Currently, no drugs have been approved by the FDA for the treatment of any FAPD in children or adolescents. There is a dearth of clinical trials for FAPDs in this age

group. A comprehensive review of pediatric studies published in 2015 found only seven randomized clinical trials for the treatment of FAPDs in children (Saps et al. 2015). Another systematic review that investigated the efficacy and safety of pharmacologic agents in children with FAPDs found only six randomized controlled trials that met criteria for inclusion (Korterink et al. 2015). Both reviews found that most of these drug studies had methodological flaws that precluded the authors from drawing meaningful conclusions regarding efficacy. In contrast, several trials and multiple meta-analyses have been conducted in adults with IBS and multiple drugs have been approved for the treatment of IBS in adult patients. Currently, there are three FDA-approved drugs for the treatment of IBS-D in adult patients. Alosetron is a 5-HT3 receptor antagonist that was initially approved for the treatment of IBS-D. The drug was transiently withdrawn from the market and later reinstituted for the treatment of women with severe and refractory IBS-D. Two new drugs have been recently approved for the treatment of IBS-D by the FDA. These include eluxadoline, a mixed mu-opioid kappa receptor agonist and delta receptor antagonist, and rifaximin, a gut-specific antibiotic derived from rifampin. Two drugs have been approved in the USA for the treatment of adults with IBS-C: linaclotide, a peptide guanylate cyclase C-agonist, and lubiprostone, a ClC-2 chloride channel activator.

The high prevalence of FAPDs in children, their impact on quality of life, and the poor treatment outcomes call for well-designed, randomized controlled trials investigating the efficacy of currently used drugs and the development of novel safe and effective therapies for this population. One of the challenges in evaluating the therapeutic benefit of any drug for chronic abdominal pain in children and adults has been the high placebo response rates that can range from 20 to 50% in some studies (Pourmoghaddas et al. 2014; Saps et al. 2009). The decision to use a specific therapy often depends on a significant therapeutic gain over placebo that can sometimes be achieved successfully with more natural or alternative therapies. This is important in children because of ongoing neuroplasticity and development that can be influenced by centrally acting drugs. In all cases, the long-term risks of medications in children are unable to be assessed in adult trials and need to be considered prior to selecting a therapy in the clinical setting. For that reason, in children with FAPDs without significant disability, the first line should always be a therapy with the lowest side-effect profile.

In this chapter we review the pediatric literature on the commonly used drugs for the care of children with FAPDs, highlighting some of the strengths and limitations of the published studies and the gaps in the literature when those exist. While the focus will be on treatments for which data in children exist, in some instances, only adult studies have been done. In those cases, the drug will be reviewed only if there is anecdotal evidence of any benefit in children that is based on expert opinion.

1.5 Antispasmodics

There are several classes of antispasmodics: smooth muscle relaxants, antimuscarinic agents/anticholinergics drugs, selective calcium channel blockers, and peripheral

opiate agonists. Antispasmodics are commonly used as first line in the treatment of children with IBS and FAP-NOS. There are no meta-analyses and only few randomized clinical studies have been conducted in children investigating the efficacy of this group of drugs in children and adolescents. Thus, the use of these drugs is mostly based on anecdotal experience and adult data. There are no data on the efficacy of two of the most commonly used antispasmodics in children in the USA (hyoscyamine and dicyclomine). Both myorelaxants have not proven efficacy in reducing abdominal pain in adult patients with IBS (Chang et al. 2006). An evidence-based systematic review on drug treatments for IBS by the American College of Gastroenterology (ACG) IBS task force (Brandt et al. 2009) found evidence for the efficacy of antispasmodics as a class for the treatment of IBS. It was suggested that hyoscine, cimetropium, pinaverium, and peppermint oil (which has antispasmodic properties) could be effective for the short-term relief of abdominal pain and discomfort in adults with IBS (ACG recommendation Grade 2C). There was no evidence available to assess the efficacy of antispasmodics for long-term treatment. A meta-analysis by Ford et al. on 12 antispasmodics found that in addition to cimetropium, pinaverium, and hyoscine, otilonium was also effective in the treatment of IBS (Ford et al. 2008). A Cochrane meta-analysis found that antispasmodics were effective for the treatment of IBS in adult patients (Ruepert et al. 2011). Fifty-eight percent of patients receiving antispasmodics improved compared to 46% of patients in the placebo group (RR 1.32; 95% CI 1.12 to 1.55; $P < 0.001$; NNT = 7). The meta-analysis found that otilonium, cimetropium, dicycloverine, hyoscine, pinaverium, and trimebutine were more effective than placebo in improving abdominal pain and global symptoms of IBS in adults.

Another meta-analysis found that that after excluding low-quality trials, octylonium bromide was efficacious in improving global IBS symptoms (OR: 2.1; 95% CI:1.8–2.9), although those results were based on only two studies (Lesbros-Pantoflickova et al. 2004).

1.5.1 Mebeverine

Mebeverine is a beta-phenylethylamine derivative that acts as an intestinal smooth muscle relaxant with antiperistaltic effects. Although its mechanism of action is not completely understood, studies have shown that it blocks voltage-operated sodium channels and inhibits intracellular calcium accumulation (Den Hertog and Van den Akker 1987).

A randomized, double-blind, placebo-controlled trial on 115 children 6–18 years of age diagnosed with FAP according to the Rome III Criteria showed no statistically significant effect of mebeverine over placebo in intention-to-treat or per-protocol analysis (Pourmoghaddas et al. 2014). Subjects were randomized to receive mebeverine (135 mg, twice daily) or placebo for 4 weeks. Treatment response rate in the mebeverine and placebo groups based on ITT analysis was 40.6 and 30.3% at week 4 ($P = 0.469$] and 54.2 and 41.0% at week 12 ($P = 0.416$), respectively. Three out of 44 subjects discontinued the use of mebeverine due to side effects (drowsiness, nervousness, and nausea). Subjects in the mebeverine group experienced significantly more dry mouth than subjects in the placebo group (43.1% versus 23.2%, $P = 0.047$).

Studies in adults showed similar results to pediatric studies. The meta-analysis by Ford found it unclear whether mebeverine was more effective than placebo in

the treatment of IBS in adults (Ford 2012). A meta-analysis of eight trials (555 adult patients) (Darvish-Damavandi et al. 2010) that were randomized to receive mebeverine or placebo found that it was not beneficial in achieving clinical improvement of abdominal pain [relative risk (RR) 1.13, 95% CI: 0.59–2.16, $P = 0.7056$] or relief of abdominal pain (RR 1.33, 95% CI: 0.92–1.93, $P = 0.129$) compared to placebo.

1.5.2 Trimebutine

Trimebutine maleate is an opioid agonist that acts on the peripheral delta, mu, kappa, and delta opiate receptors; modulates the release of peptides such as vasoactive intestinal peptide, gastrin, and glucagon; and induces the release of motilin (Delvaux and Wingate 1997). Thus, trimebutine has prokinetic as well as antinociceptive effects. As a prokinetic, trimebutine accelerates gastric emptying, induces premature phase III-like motor complexes of the intestinal migrating motor complex, and increases postprandial colonic motor activity (Shannon et al. 1989). Although motilin release could be at least partially responsible for the increase in phase III intestinal motor activity, a study has shown that in humans, this effect could be at least partially mediated by its agonistic effects in opiate receptors as the injection of naloxone suppresses trimebutine-induced intestinal complexes without a clear effect on the interdigestive contractile activity of the colon (Valori et al. 1987). In animals, trimebutine lowers reflexes induced by the distension of the gut that may result in modulation of visceral sensitivity (Delvaux and Wingate 1997).

A study on 78 children with IBS found a significant benefit of trimebutine maleate compared with a non-medicated group (Karabulut et al. 2013). Ninety-five percent of children receiving trimebutine achieved clinical recovery (adequate relief) compared with 20.5% who had spontaneous recovery ($P < 0.0001$). The study did not report side effects on any of the groups. In adults with IBS, the efficacy of trimebutine had mixed results. Two systematic reviews comparing trimebutine with placebo in adults with IBS showed improvement in abdominal pain (Lesbros-Pantoflickova et al. 2004; Martínez-Vázquez et al. 2012). However, the results were statistically significant in one of the reviews (81) and not significant in the other one (Martínez-Vázquez et al. 2012). A randomized clinical trial conducted in a group of patients with IBS that included some adolescents (15–60 years of age) compared the effect of mebeverine and trimebutine and found no statistically significant difference in effect between both drugs (Rahman et al. 2014). The authors reported that no patients in the study had worsening of symptoms or side effects. No studies comparing both drugs have been conducted in children or adolescents alone.

1.5.3 Peppermint Oil (Menthae Piperitae Aetheroleum)

Peppermint oil is an essential oil isolated via steam distillation from the fresh leaves of peppermint (*Mentha piperita* L.) that has been used to treat abdominal ailments since antiquity (Ulbricht et al. 2010). Peppermint oil and l-menthol, the active ingredient of peppermint oil, have multiple mechanisms of action that makes them attractive to be used in the care of FAPDs. Peppermint oil is a smooth muscle calcium channel blocker (Grigoleit and Grigoleit 2005; Hawthorn et al. 1988).

Studies on the gastrointestinal effect of peppermint oil have shown a decrease in the number and amplitude of contractions of the migrating motor complex with an increase in length of phase I and II and shorter phase III (Micklefield et al. 2000). Oral administration of peppermint oil has also been shown to prolong orocaecal transit time (mean time: placebo 65 min, peppermint oil 85 min) while having no effect on gastric emptying time (Goerg and Spilker 2003). In addition, studies have shown a relaxing effect on the gallbladder with inhibition of gallbladder emptying (Goerg and Spilker 2003). The effect of peppermint oil varies with enteric coating. Enteric coated preparations have an effect on gastroduodenal motility during the second MMC after its administration while non-enteric coated have more limited (possibly due to dilution and decrease in concentration of the substrate) but faster effect (Micklefield et al. 2000). Peppermint oil may also have a neuronal site of action since it directly inhibits the function of human 5-HT3 receptors. It is likely that some of the actions of menthol are related to the modulation of 5-HT3 receptor function (Ashoor et al. 2013). In rats, 5-HT3-induced smooth muscle ileum contractions are suppressed by menthol (Heimes et al. 2011). It has been proposed that peppermint oil and menthol antiemetic effect (Tate 1997) could be at least partly explained by their action on the 5-HT(3) receptor ion-channel complex (Heimes et al. 2011). Orally administered menthol is effectively absorbed by the gastrointestinal mucosa. Menthol undergoes extensive enterohepatic recirculation, and it is incompletely metabolized to menthol glucuronide. Although the effects of peppermint oil are thought to be mostly due to local action, there is some evidence of a possible systemic effect. Menthol is rapidly absorbed into the brain, and therefore, functional modulation of 5-HT3 receptors could explain some of the effects noted (Pan et al. 2012). A study has also shown that menthol has direct effects on the spinal cord, decreasing pain hypersensitivity (Pan et al. 2012). Menthol blocks voltage-gated Na+ and Ca2+ channels decreasing neuronal excitability that contributes to menthol-induced central analgesia (Enna and McCarson 2006). It has been suggested that menthol could centrally activate γ-aminobutyric acid (GABA) type A receptors that could explain some of its antinociceptive properties (Enna and McCarson 2006). A study in children showed peak plasma concentrations (Tmax) varying between 2.5 and 8 h post-dose. The residence of the drug in the intestine in children and adults and the length of time to its central effect may vary with the peppermint oil formulation (Kline et al. 2001). An additional factor of variability could be related with the metabolism of menthol and its clearance from plasma. The clearance of menthol is associated with cytochrome P450 (CYP)2A6 activity, the major enzyme involved in the hydroxylation of menthols by liver microsomes in humans (Miyazawa et al. 2011). The activity of P450 (CYP)2A6 is not dependent on age, sex, race, or weight. There is little data on the efficacy of the use of peppermint oil in children with FAPDs. A 2-week, placebo-controlled, multicenter trial conducted in 42 children with IBS showed a significant benefit of enteric coated pH-dependent release of peppermint oil (75%) compared with controls (43%) (Kline et al. 2001). The study did not report any side effects. A meta-analysis of nine studies conducted in adults with IBS showed that peppermint oil was significantly superior to placebo in global improvement of IBS symptoms and improvement in abdominal pain (Khanna

et al. 2014). Patients receiving peppermint oil were significantly more likely to report adverse events; however the events were mild and transient. Heartburn was the most commonly reported adverse. Aromatherapy with peppermint has also been used to reduce postoperative nausea. A study comparing aromatherapy with alcohol, placebo, and peppermint found that aromatherapy with peppermint was as effective as placebo (Anderson and Gross 2004).

1.6 Antibiotics

Rifaximin is a semisynthetic poorly absorbed antimicrobial derivative of rifamycin. Rifaximin formulation contains an extra pyrido-imidazole ring to reduce systemic absorption that is less than 1% after oral administration (Huang and DuPont 2005). Rifaximin elicits its antimicrobial properties by binding to the beta-subunit of the bacterial DNA-dependent RNA polymerase and thus inhibiting bacterial RNA synthesis (Ojetti et al. 2009). It has a wide local antimicrobial effect against aerobic and anaerobic Gram-positive and Gram-negative bacteria (Ojetti et al. 2009). Rifaximin's effect in restoring the balance of the gut microflora has led to its use in several organic and functional gastrointestinal conditions such as traveler's diarrhea (Alajbegovic et al. 2012), hepatic encephalopathy, inflammatory bowel disease, colonic diverticular disease, and small intestine bacterial overgrowth (Ojetti et al. 2009).

Rifaximin was approved by the FDA in 2015 for the treatment of adults with IBS-D but has not received approval for use in children. The adult approval includes an initial use at a dose of 550 mg three times daily for 14 days and retreatment on a 14-day course up to two times in patients who experience recurrent symptoms.

The mechanism of action of rifaximin in the treatment of IBS-D is not completely understood. Quantitative and qualitative alterations in the gut flora had been found in some patients with IBS (Kerckhoffs et al. 2009; Posserud et al. 2007; Pyleris et al. 2012). Based on these data it would have been reasonable to suggest that the sole mechanism of action of rifaximin is due to its antimicrobial effect. However, clinical trials on patients with IBS-D have shown persistent symptom improvement $\geq$12 weeks after one course of treatment (2 weeks) with rifaximin. This suggests that the mechanism of action may not be limited to its immediate bactericidal effect and that the lasting effects that result from the treatment with rifaximin affect an underlying cause/s of IBS. Multiple mechanisms of action have been proposed including rifaximin playing a role in modulating the gut microenvironment by improving dysbiosis and promoting the colonization of the gut with beneficial bacterial species (DuPont 2016a, b), inducing changes in the production and metabolism of intestinal bacteria, reducing bacterial products that negatively affect the host, and modulating the inflammatory responses of the host to bacterial products (Pimentel 2016).

The approval of rifaximin in adult patients was based on multiple clinical trials including two major studies (TARGET 1 and TARGET 2) (Pimentel et al. 2011) conducted in adult patients with IBS without constipation. In both studies the subjects received rifaximin 550 mg or placebo, three times daily for 2 weeks, and were followed for another 10 weeks. Significantly more patients in the rifaximin

group compared to the placebo group reported adequate relief of IBS symptoms during the first 4 weeks after treatment (TARGET 1: 40.8% vs. 31.2%, $P = 0.01$; TARGET 2 40.6% vs. 32.2%, $P = 0.03$). More patients in the rifaximin group also reported improvement in bloating, abdominal pain, and stool consistency. There was a similar incidence of adverse events in both groups. The TARGET 3 study investigated the effects of retreatment and showed the benefit of rifaximin (Lembo et al. 2014) after a first and second repeat treatment. An important consideration when using antimicrobials to treat FAPDs is the possible selection of resistant bacteria, but this appears to be unusual. The TARGET 3 trial did not find evidence of pathogenic gastrointestinal bacterial growth or overall bacterial overgrowth after repeated treatment (Lembo et al. 2014).

Two clinical trials have investigated the effect of rifaximin in children with IBS (Collins and Lin 2011; Scarpellini et al. 2013). A double-blind, placebo-controlled trial conducted in 75 children aged 8–18 years with chronic abdominal pain found no significant difference in symptom improvement between both groups (Collins and Lin 2011). The study included a mixed group of diagnosis including functional dyspepsia, functional abdominal pain, and IBS. This was considered acceptable since, regardless of symptoms, 91% of children with chronic abdominal pain had abnormal lactulose breath hydrogen (LBT) (Collins and Lin 2010). Children in the study were randomized in a 2:1, double-blind fashion to receive 550 mg of rifaximin or placebo three times per day (same dose as TARGET adult trials) for 10 days. Participants had LBT and questionnaires at baseline and 2 weeks after treatment. Over 90% of children in both groups tested positive for LBT (criteria used to diagnose small bowel bacterial overgrowth) at baseline. The authors hypothesized that the negative results of the study were due to the low frequency of successful treatment of small bowel bacterial overgrowth after treatment with only 20% of children treated with rifaximin having normalized LBT after treatment. Only one child withdrew from the study after 1 day of receiving rifaximin due to abdominal pain. An open trial by Scarpellini et al. (2013) was conducted in 50 children with IBS. All children underwent an LBT test at the beginning of the study. Children who tested positive in the LBT received rifaximin 200 mg three times daily for 7 days, while the rest remained untreated. Among the 33 children who tested positive for small bowel bacterial overgrowth, the LBT normalized in 64% of the children after rifaximin treatment. Children who normalized the LBT had a significant improvement in intensity of abdominal pain, bloating, and flatulence compared with their baseline symptoms. Children who did not normalize the LBT after rifaximin treatment had no significant difference in intensity of gastrointestinal symptoms from baseline. Only two adverse events were reported and both were related to mild constipation. Together, the interpretation of the results of both pediatric trials deserves consideration. The results of the open trial seem to be in line with the explanation given by Collins et al. to explain their negative study (few children achieved a negative LBT with treatment) as most children who normalized the LBT in the Scarpellini et al.'s (2013) study had improvement of their gastrointestinal symptoms while those who did not normalize their LBT had no improvement. However, certain aspects of these studies remain unexplained. The dose of rifaximin in the placebo-

controlled study was much higher than in the open-label study (1650 mg versus 600 mg daily) and still only 20% of children versus 64% of children normalized their LBT. The comparison of the results of the pediatric and adult studies raises the questions on whether the pathophysiology of IBS and the effect of treatment are different in adults and children. The TARGET studies found a beneficial effect of rifaximin without the requisite of having a positive LBT (inclusion criteria was only IBS without constipation) while the results of the pediatric open-label study (Lembo et al. 2014) suggest that a negative LBT and thus the absence of small bowel bacterial overgrowth would be necessary to achieve a positive effect of the treatment. However, an alternative explanation is that the designs of the pediatric and adult studies are different and thus do not allow comparisons since the adult studies were limited to patients with IBS, while the randomized clinical trial in children included a mixed sample of children with various FAPDs (15% functional dyspepsia, 55% IBS, and 31% functional abdominal pain).

1.7 Cyproheptadine

Cyproheptadine is an antagonist of histamine (H-1), serotonin (5-HT2A, 5-HT2B, and 5-HT2C), and calcium channels (Yamamoto et al. 2006). In addition to its extraintestinal uses including allergy, migraines, and neuroleptic induced akathisia, it is commonly used in pediatric gastroenterology to treat abdominal pain (Johnson et al. 2014; Madani et al. 2016; Weiss et al. 1995). Only one randomized, placebo-controlled trial has been published assessing the efficacy of cyproheptadine in children abdominal pain. This study included 29 children with functional abdominal pain who were randomized to cyproheptadine or placebo. Cyproheptadine was found to be significantly better than placebo (Sadeghian et al. 2008). Children (4–12 years) in the study were randomized to cyproheptadine 0.25–0.5 mg/kg or placebo daily in two divided doses for 2 weeks and their outcomes were assessed at the end of the first and second weeks. Pain resolved in 20% of cases and improved in 66.6% of cases. In the placebo group, there was no change in 50% of cases, improvement in 35.7% of cases, and worsening pain in 14.3% of cases. There was also improvement in both the patients' self-report and the parents' impression in the cyproheptadine group compared with the placebo group. Two children had side effects including increased appetite or airway problems. Among other pain-predominant conditions, a case series and a retrospective study reported benefit of cyproheptadine in the treatment of abdominal migraine (Lundberg 1975; Worawattanakul et al. 1999). Worawattanakul et al. reported that among 12 children who received treatment for abdominal migraine with cyproheptadine at a dose of 0.25–0.50 mg/kg daily, 83% had an excellent or a fair response (Worawattanakul et al. 1999). Most of the patients in this chart review received propranolol as first-line drug and had a similar rate of improvement (83%); however 75% of children that received propranolol were categorized as excellent improvement compared to only 33% of those in the cyproheptadine group. Parents reported side effects from cyproheptadine in only two children (one patient had drowsiness and another one had increased appetite and excessive weight gain). Another retrospective review (Madani et al. 2016) on the use of cyproheptadine

reported improvement in 72% (13/18) of children with abdominal migraine, 75% (6/8) of children with cyclic vomiting syndrome, and 100% (10/10) of children with IBS. Rodriguez et al. conducted a retrospective, open-label study on the use of cyproheptadine in children with dyspeptic symptoms (Rodriguez et al. 2013). The study that was conducted on a sample of 80 children with refractory upper gastrointestinal symptoms (retching post-fundoplication and abdominal pain nausea, vomiting and early satiety) found a beneficial response to therapy in 55% of cases. This is the only pediatric study that has reported the effect of cyproheptadine in a sample of patients with retching after a Nissen fundoplication. The response rate in this group of children was (12/14) 86%. The authors attribute the benefit of cyproheptadine in patients with fundoplication to its effect in improving fundic accommodation and/or gastric hypersensitivity to distention. There were side effects in 30% of children (somnolence 16%, irritability/behavioral changes 6%, increased appetite and weight gain 5%, abdominal pain 2.5%). Despite the authors showing a tendency to a lower response in patients with functional dyspepsia, this medication is frequently used for this purpose. One of the most common indications is for the treatment of functional nausea in children; however due to the fact that this diagnosis was only recently implemented in children by the Rome IV Criteria (Hyams et al. 2016), there have been no prospective studies specifically designed to assess its effect in this group of children. One of the most common side effects of cyproheptadine is increased appetite; thus the drug is often used as an appetite stimulant in children with nonorganic failure to thrive.

1.8 Prosecretory Drugs

1.8.1 Linaclotide

Linaclotide is a 14-amino-acid synthetic peptide agonist of guanylate cyclase C (GC-C) that is currently approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of chronic constipation and IBS-C in adult patients (Kang et al. 2015). Linaclotide is homologous to the bacterial heat-stable enterotoxins which causes global endemic diarrhea (Bharucha and Waldman 2010). Linaclotide pH-independently binds with high affinity to GC-C that is selectively expressed in mucosa cells from the duodenum to the rectum (Lucas et al. 2000). GC-C catalyzes the conversion of GTP to cGMP leading to the accumulation of intracellular cyclic guanosine-3′,5′-monophosphate (cGMP) (Bryant et al. 2010; Busby et al. 2010) and stimulation of signaling cascades through downstream effectors (Lucas et al. 2000). Linaclotide has a concentration-dependent dual mechanism of action increasing intestinal fluid secretions and accelerating intestinal transit in addition to reducing visceral hypersensitivity. Linaclotide is stable in simulated gastric fluid (pH 1) and resistant to hydrolysis by pepsin (Busby et al. 2010). Linaclotide appears to be well tolerated (Bharucha and Waldman 2010; Lee and Wald 2011) and acts locally in the GI tract. Since it is poorly absorbed it has minimal systemic exposure, reducing the risk of systemic adverse effects. The most common side effect of linaclotide is diarrhea that is inherent to its mechanism of action. Linaclotide is approved for IBS-C at a 290 μg dose and for chronic constipation at a 145 μg dose (Layer and Stanghellini

2014). There are no published studies on the efficacy and safety of linaclotide in children or adolescents.

1.8.2 Lubiprostone

Lubiprostone belongs to the prostone group, a group of drugs derived from a metabolite of prostaglandin E1. Lubiprostone is an orally administered synthetic bicyclic fatty acid secretagogue agent that acts at the apical membrane at the luminal side of the intestinal epithelial cells. It is a selective type 2 chloride channel activator (ClC-2) that elicits its effect in the small intestine and colon through the CFTR-dependent pathway (Bijvelds et al. 2009) promoting efflux of chloride and water secretion (Raschi and De Ponti 2014). This leads to luminal distension and secondary peristalsis that accelerates small bowel and colonic transit (Saad and Chey 2008). Although it was initially thought that the lubiprostone effect could be independent of CFTR, animal studies show that in the absence of CFTR activity, lubiprostone does not induce Cl secretion in the ileum (Bijvelds et al. 2009). Prostones, unlike prostaglandins, do not stimulate smooth muscle contraction, and in fact lubiprostone inhibits neuronally mediated contractions of colonic circular muscle (Camilleri et al. 2006). Thus, in healthy subjects, lubiprostone does not directly increase colonic motor function and has no apparent effect on sensation (Sweetser et al. 2009). Lubiprostone also has an effect on the stomach. It increases fasting, but not postprandial, gastric volume and delays gastric emptying of a solid meal. It has, however, been shown to decrease fullness 30 min after a satiating meal (Camilleri et al. 2006).

Lubiprostone is rapidly metabolized by carbonyl reductase and its metabolism is independent of the hepatic cytochrome P450 system. Lubiprostone has been approved by the US FDA for the treatment of chronic idiopathic constipation (24 μg twice daily), irritable bowel syndrome with constipation (IBS-C) (8 μg twice daily), and opioid-induced constipation in adults. The most common side effects are transient nausea (approximately 20% of patients), diarrhea, and headaches (Camilleri et al. 2006; Johanson 2002, 2003, 2004). Lubiprostone appears to be safe in long-term studies. An open-label study that included 127 children assessed its effects on stool consistency and number of bowel movements (Hyman et al. 2014). In this study, children received lubiprostone for 4 weeks at doses of 12 μg once daily, 12 μg twice daily, or 24 μg twice daily based on their age and weight. The study showed a significant increase in the mean number of bowel movements compared with baseline (3.1 vs. 1.5/week). The most common adverse events were nausea (18.5%), vomiting (12.1%), and diarrhea (8.1%). No randomized placebo-controlled studies have been published in children.

1.9 Antidepressants

Adult and pediatric patients with FAPDS have visceral hypersensitivity and perceive higher pain intensity (Castilloux et al. 2008; Di Lorenzo et al. 2001; Nozu and Kudaira 2009; Van Ginkel et al. 2001; Zhou et al. 2010b). It has been demonstrated that psychological stress, including poor sleep, can cause changes in intestinal motility and hypersensitivity in IBS patients compared to healthy controls (Dickhaus et al. 2003; Patel et al. 2016; Rao et al. 1998). Several studies have investigated

the effectiveness of antidepressants in patients with FAPDs. Some of the most commonly used antidepressants to treat FAPDs are tricyclic antidepressants (TCAs) that include amitriptyline, imipramine, and desipramine. Selective serotonin reuptake inhibitors (SSRIs) have also been studied, including citalopram, fluoxetine, and paroxetine. However, the clinical efficacy of antidepressants for FAPDs is still controversial.

1.9.1 Tricyclic Antidepressants (TCAs)

To date, the exact mechanisms for the antinociceptive effects of TCAs remain unknown. Most studies that have investigated the effects of antidepressants on IBS symptoms do not include patients with clinical psychological comorbidities, making it very difficult to determine whether the presence of these comorbidities influences the effects of the treatment. In other words, whether symptom improvement with antidepressant therapy occurs through treatment of the psychological condition or primarily by affecting pain pathways is not well known. It is important to consider that the doses of TCAs used to treat chronic pain are much lower than those used to treat depression. Overall, TCAs are likely to have several targets and mechanisms responsible for the analgesic effect. This group of drugs has been used to treat patients with FAPDS and other chronic pain conditions such as migraine headaches, fibromyalgia, and neuropathic pain (Moore et al. 2015; Powers et al. 2013; Rico-Villademoros et al. 2015). In IBS patients, amitriptyline reduces visceral sensitivity to rectal balloon distention and acute stress-induced visceral hypersensitivity (Thoua et al. 2009). One potential mechanism is related to its effect on voltage-gated sodium channels. Amitriptyline inhibits currents in these channels and decreases their expression in animals (Liang et al. 2014). The antinociceptive effects may also be related to (1) effects on serotonergic and adenosine pathways, (2) effects on the endogenous pain modulation system known as diffuse noxious inhibitory control (DNIC) or potentiation of endogenous opioids, or (3) modulation and antagonism of the NMDA receptor (Eisenach and Gebhart 1995; Gray et al. 1998; Liu et al. 2013).

A recent meta-analysis from adult studies demonstrated that TCAs (amitriptyline, imipramine, desipramine, doxepin, trimipramine) significantly improve clinical symptoms of IBS compared to placebo (Rahimi et al. 2009). One of the most commonly used TCAs is amitriptyline. A meta-analysis of four large randomized, placebo-controlled trials of amitriptyline for the treatment of IBS in adults showed a fourfold clinical improvement in those undergoing treatment compared to placebo (Chao and Zhang 2013).

In children with FAPDS, the benefits of TCAs are more controversial since data are conflicting. In a large multicenter trial involving 90 children with chronic abdominal pain (including functional dyspepsia, functional abdominal pain, and IBS), low-dose amitriptyline was no better than placebo. There was a 53% improvement in satisfactory relief of symptoms and satisfaction with treatment in children who received amitriptyline and 50% in those receiving placebo. In this study, the large placebo response may have explained the negative results (Saps et al. 2009). Interestingly, in that study, amitriptyline did lower anxiety scores at the end of the 4-week trial compared to placebo. A smaller randomized trial of amitriptyline in 33 adolescent females with IBS showed improvement in quality of life at 4 and 8 weeks (Bahar et al. 2008). This study was difficult to interpret due to incomplete

information regarding abdominal pain scores. More importantly, patients in the placebo group responded with worsening pain, which questions the validity of the study.

Because amitriptyline has been associated with a modest QTc prolongation, a screening EKG is recommended prior to initiating treatment (Castro et al. 2013). However, there are no clear guidelines on the need for ECG prior or after treatment in children receiving low doses of amitriptyline. It should be noted that antidepressants have an FDA black box warning due to increased risk of suicidal thoughts and ideation that has been linked to this class of medication (Olfson et al. 2008). The prevalence of major psychiatric conditions in children reporting chronic abdominal pain is low and there are currently no published reports of increased suicidal behavior in nondepressed children receiving low dose of amitriptyline for the treatment of FAPDs.

1.9.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

More than 90% of the body's serotonin is located in the gastrointestinal tract. Because serotonin and its receptors play a critical role in gastrointestinal function and motility, SSRIs represent an attractive therapeutic option. Further, a number of adult and pediatric patients who seek treatment for FGIDs have underlying psychiatric comorbidities such as anxiety or depression (Sibelli et al. 2016; Walker and Greene 1989). Despite not having FDA approval for the treatment of FGIDs, SSRIs are believed to have some effect on pain perception and are commonly used in clinical practice. Several studies in adults have yielded conflicting results in regard to their effectiveness. A recent study evaluated the effects of intravenous citalopram in 23 adult IBS patients (Tack et al. 2006). This randomized, placebo-controlled study measured IBS symptom severity as the primary outcome. Overall, citalopram resulted in a significant improvement in the number of days of abdominal pain over placebo. Those in the citalopram group also had improvement in bloating severity and overall well-being after 6 weeks. The generalizability of this small study was questioned mainly due to the use of intravenous citalopram that is not practical. A placebo-controlled trial and a meta-analysis that included four studies in adults with IBS demonstrated that citalopram was not superior to placebo in reducing abdominal pain (Ladabaum et al. 2010; Ruepert et al. 2011). A separate study randomized adult IBS patients to one of the three treatment arms that included imipramine, citalopram, or placebo (Talley et al. 2008). Patients were adequately matched based on IBS type and the primary outcome was the percentage of responders who reported "adequate relief" of IBS symptoms. There was no difference in relief of IBS symptoms between groups or in the secondary outcome defined as the number of subjects who experienced $\geq$50% of weeks of adequate relief. A recent randomized, placebo-controlled trial in children with functional abdominal pain showed that citalopram was not superior to placebo on per-protocol and intention-to-treat analysis (Roohafza et al. 2014). This study included 86 patients that were randomized to receive citalopram 20 mg/day or placebo for 4 weeks. Treatment response was defined as $\geq$2 point reduction in the Faces Pain Scale (6-point). At 4 weeks, 55.8% of patients in the citalopram group showed improvement in pain compared to 39.5% in the placebo group ($P = 0.097$). Changes in depression, anxiety, or somatization scores were no

different between groups. These results were different than what was found in a small ($n = 25$), open-label study of citalopram in children with recurrent abdominal pain. This study found that 21/24 (84%) of subjects responded to citalopram based on a Clinical Global Impression Scale score of ≤ 2. Abdominal pain, anxiety, depression, and functional impairment also improved over the 12 weeks of treatment (Arkwright et al. 2016).

Fluoxetine, a SSRI, has also been evaluated for the treatment of adults with IBS in two clinical trials. In one study, abdominal pain scores, global symptom relief, and psychological symptoms were assessed before and after 6 weeks of treatment with either fluoxetine 20 mg or placebo (Kuiken et al. 2003). This study also assessed changes in rectal sensitivity using a barostat and pain perception scales. The authors found no difference in the threshold for discomfort/pain compared to placebo. Also, there was no difference between fluoxetine and placebo in the proportion of patients reporting significant abdominal pain, bloating, flatulence, urgency, and global relief of IBS symptoms. In a separate randomized, controlled, double-blind study, fluoxetine was compared to placebo (Vahedi et al. 2005). The study duration was 12 weeks and included adult patients with IBS-C. Fluoxetine significantly improved abdominal discomfort, bloating, stool consistency, and frequency after 4 weeks of treatment compared to placebo.

Two small clinical trials have investigated the effects of paroxetine in adults with IBS. One of the studies investigated whether a high-fiber diet alone or in combination with paroxetine or placebo was more effective (Tabas et al. 2004). The trial first investigated the effects of a high-fiber diet alone (Group 1) followed by a randomized, double-blind trial of diet with paroxetine or placebo (Group 2). Improvement in overall well-being in at least 30% of study participants using a 5-point Likert scale was the primary objective. Improvement in abdominal pain, and abdominal bloating, was also recorded. Those that received paroxetine experienced a greater improvement in overall well-being (63.3%) compared to placebo (26.3%). There were no significant differences between treatment groups in regard to abdominal pain, bloating, or social functioning. Results should be interpreted with caution since improvement in overall well-being could reflect the effects of the antidepressant in mood and not necessarily on IBS symptoms. A separate double-blind, randomized, placebo-controlled investigated the efficacy of paroxetine in patients with IBS over a 12-week period (Masand et al. 2009). The primary outcome measured was composite pain scores. There was no difference in the composite pain scores between paroxetine or placebo group. At the end of treatment, the authors reported a significant difference in the clinical global impression-improvement score with 65.9% of subjects in the treatment group having a score of 1 or 2 versus 16.7% in the placebo group ($P < 0.01$). Only 72 patients were included in this trial.

1.10 Alpha 2 Delta Ligands (Gabapentin and Pregabalin)

Two compounds, gabapentin and pregabalin, that bind to the α2δ subunits of the voltage-gated calcium channels have been used to treat neuropathic pain, including

diabetic neuropathy and post-herpetic neuralgia (Markman et al. 2016; Taylor 2009; Tzellos et al. 2010). Pregabalin is currently approved for the treatment of fibromyalgia and generalized anxiety disorders (GAD). These drugs are structurally related to gamma-aminobutyric acid (GABA) but not functionally active at the GABA or benzodiazepine receptor sites and do not have any effects on GABA synthesis or degradation. To date, few studies have examined the distribution of the α2δ subunit outside the central nervous system. While the α2δ subunit has been found in areas of the brain involved in pain processing, such as amygdala, hippocampus, anterior cingulate cortex, insula, and spinal cord (Taylor and Garrido 2008), only limited studies have investigated the expression in the gastrointestinal tract (Taylor and Garrido 2008). Overall, the exact mechanisms that could influence symptoms associated with FAPDs are not well understood.

There are numerous studies in animals demonstrating the anti-hyperalgesic effects of gabapentin and pregabalin. These include models of inflammatory and neuropathic pain (Diop et al. 2002; Feng et al. 2003; Field et al. 1997; Million et al. 2007; Ohashi-Doi et al. 2010; Ravnefjord et al. 2008; Stepanovic-Petrovic et al. 2008). Pregabalin has been shown to influence nociceptive processing by altering the firing of spinal neurons (You et al. 2009). Because the effect on spinal neurons was not present in spinalized animals, the authors of that study suggested a supraspinal site of action of pregabalin. Another effect of α2δ subunit ligands involves altering the release of noradrenalin, glutamate, substance P, calcitonin gene-related peptide (CGRP), and serotonin (Brawek et al. 2009; Dooley et al. 2000; Fehrenbacher et al. 2003; Patel et al. 2000). The increase in serotonin seen with these compounds may explain its anxiolytic effects (Dixit and Bhargava 2002). In patients with generalized anxiety disorder, pregabalin has been shown to improve not only anxiety, but also gastrointestinal symptoms (Stein et al. 2009). No study has examined the efficacy of α2δ subunit ligands on clinical symptoms of IBS in adults or children. However, two studies have investigated the effects on visceral sensitivity in adults with IBS. In one study, 40 patients with diarrhea-predominant IBS underwent a baseline barostat study and then were subsequently randomized to receive placebo or gabapentin. After 5 days, the barostat study was repeated. Gabapentin increased the threshold for bloating, discomfort, and pain and placebo did not, suggesting a reduction in rectal sensory thresholds. Rectal compliance also significantly increased after gabapentin, but not after placebo (Lee et al. 2005). A separate study assessed the effect of pregabalin in 26 IBS patients with rectal hypersensitivity. Rectal sensitivity was assessed through the barostat technique. In this study, pregabalin increased the sensory threshold for pain, desire to defecate, and first sensation (Houghton et al. 2007). Overall, there are limited studies on the antinociceptive effects of pregabalin in adults with FAPDs and no studies in children.

1.11 Peripheral Opioid Receptor Acting Drugs

1.11.1 Loperamide

This μ-opioid receptor agonist has been used to treat diarrhea and has been evaluated for the treatment of IBS-D (Cann et al. 1984; Efskind et al. 1996). While there are no studies in children with IBS, the existing studies in adults using this drug are limited by significant methodological flaws. In general, however, it is accepted that loperamide improves stool consistency, form, and urgency in the IBS-D population, but has marginal effects on abdominal pain. Similar to other opioid receptor agonists that target the gastrointestinal tract, loperamide has very little penetration of the blood–brain barrier, limiting the potential for CNS side effects and dependence.

1.11.2 Eluxadoline

A peripherally acting mixed μ- and κ-opioid receptor agonist and δ-opioid receptor antagonist has been approved for the treatment of IBS-D in adult patients. Opioid receptors in the enteric circuitry play an important role in regulating gastrointestinal motility, secretion, and visceral sensation. This drug has very low oral bioavailability and is primarily excreted through the gut unchanged (Davenport et al. 2015; Fujita et al. 2014). It is thought that the δ-opioid receptor activity of eluxadoline may lessen the constipating effects of unopposed μ-opioid receptor activation and improve the analgesic effects.

There are no pediatric studies on eluxadoline at this time. Two large, randomized, placebo-controlled trials in adults showed that eluxadoline improves abdominal pain and IBS symptoms. In a phase 2 study, patients that received eluxadoline at 100 or 200 mg twice daily reported a decrease in symptoms of IBS with diarrhea (Dove et al. 2013). In this study, 807 patients received eluxadoline (5, 25, 100, or 200 mg) or placebo twice daily for 12 weeks. The primary endpoint measured was a reduction in daily pain score of $\geq$30% from baseline and at least 2 points on 0–10 scale, as well as stool consistency of 3 or 4 on the Bristol Stool Scale for at least 66% of daily stooling diary entries. At the 200 mg dose, 13.8% of subjects met the primary endpoint compared to 5.7% in the placebo ($P < 0.05$). There was also improvement in frequency of bowel movements, urgency, quality of life, adequate relief assessment, and global symptoms at doses of 100 and 200 mg ($P < 0.05$). In the phase 3 trial, a total of 2428 patients were enrolled in two trials lasting 26 weeks (IBS-3002 trial) and 52 weeks (IBS-3001 trial) (Dove et al. 2013). The primary endpoint included a composite response of patients that recorded a reduction of $\geq$30% from baseline on $\geq$50% of the days and improvement in stool consistency on the same day for at least 50% of the days. Patients that received 100 mg of eluxadoline twice daily showed reduced symptoms of IBS-D and sustained efficacy over 6 months compared to placebo. At this dose, 25.1% of patients (IBS-3001 trial) reached the primary endpoint compared to 17.1% in the placebo group ($P = 0.004$). In the IBS-3002 trial, 29.6% of subjects at the 100 mg twice daily dosing reached the primary endpoint compared to 19% in the placebo group ($P < 0.001$).

1.11.3 Future Directions

To date, most studies have been conducted in adults and only a limited number of trials have investigated the benefit of pharmacotherapy in children with FAPDs (Table 1). A recent meta-analysis concluded that most of those studies were single center, had small sample sizes, and were heterogeneous in terms of methods and endpoints (Saps et al. 2015). The study also concluded that there is an urgent need for well-designed clinical trials using validated outcome measures. In view of the need to develop meaningful and standardized methods and measures that will guide future recommendations for the treatment of FAPDs in children the Rome Foundation recently formed a subcommittee on clinical trials to develop guidelines for the design of clinical trials in children with IBS (Table 2) (Saps et al. 2016). These guidelines provide recommendations on methodological design, clinical outcomes, and minimum length of trials; inclusion and exclusion criteria; and recommended workup.

Table 1 Randomized clinical trials on functional abdominal pain disorders in children and adolescents

Study	Centers (*N*)	Active component	Participants	Diagnosis	Study design and primary outcome measures	Results (drug vs. placebo) and side effects
Kline et al. (2001)	3	Peppermint oil (pH-dependent enteric coated capsule) 187 mg capsule 1 t.i.d. <45 kg; 2 capsules t.i.d. >45 kg	Children and adolescents 8–17 years ($n = 42$)	IBS	Randomized double-blind placebo control, parallel trial	8 Weeks
					Severity of abdominal pain and change in symptoms	More children in peppermint oil group reported improvement of symptoms
						Peppermint oil-75% versus 43%; $P < 0.002$ placebo
						No adverse effects reported
See et al. (2001)	1	Famotidine 0.5 mg/kg/dose 2 times daily (max. 40 mg/d)	Children and adolescents 5–18 years ($n = 25$)	Functional dyspepsia	Randomized double-blind placebo control, crossover trial	3 Weeks and crossover 3 weeks
					Abdominal pain intensity, global dyspepsia score	No significant differences in abdominal pain intensity
						Significant improvement of global dyspepsia score
						66.7% versus 15.4% ($P = 0.015$)

(continued)

Table 1 (continued)

Study	Centers (*N*)	Active component	Participants	Diagnosis	Study design and primary outcome measures	Results (drug vs. placebo) and side effects
Bahar et al. (2008)	1	Amitriptyline 25 mg	Adolescents, 12–18 years ($n = 33$)	IBS	Randomized double-blind placebo control, parallel trial	8 Weeks
					Quality of life	Significantly greater
						Improvements 6, 10, 13 weeks ($P = 0.019$, 0.004, and 0.013) No adverse effects reported
Sadeghian et al. (2008)	1	Cyproheptadine: 0.25–0.5 mg/kg/d (max. 12 mg/d children 2–6 years, max. 16 mg/d children 7–14 years)	Children and adolescents 4–16 years ($n = 29$)	Functional abdominal pain	Randomized double-blind placebo control. Frequency and intensity of abdominal pain	2 Weeks
						Significant improvement/ resolution
						Abdominal pain frequency ($P = 0.002$); pain intensity ($P = 0.001$)
						Side effects: increased appetite and hypoactive airway
Saps et al. (2009)	6	Amitriptyline 10 mg <35 kg, 25 mg >35 kg	Children and adolescents 8–17 years ($n = 90$)	IBS, functional dyspepsia, functional abdominal pain	Randomized double-blind placebo control, parallel trial	4 Weeks
					Satisfaction with treatment, sensation of relief	No significant improvement compared with placebo
						No difference in side effects

Collins and Lin (2011)	1	Rifaximin 550 mg 3 times daily	Children and adolescents 8–18 years ($n = 75$)	Functional dyspepsia, functional abdominal pain, IBS	Randomized double-blind placebo control	10 Days
					2:1, double-blind trial. Intensity of gastrointestinal symptoms	No significant difference in symptom improvement
						Side effects: constipation
Karabulut et al. (2013)	1	Trimebutine 3 mg/kg/day, 3 doses daily	Children and adolescents 4–18 years ($n = 78$)	IBS	Randomized double-blind NO placebo control	3 Weeks
					Clinical recovery	Significant improvement 94.9% vs. 20.5% non-medicated group ($P < 0.0001$)
						No difference in side effects
Pourmoghaddas et al. (2014)	1	Mebeverine 135 mg, twice daily	Children and adolescents 6–18 years ($n = 115$)	Functional abdominal pain	Randomized double-blind placebo control	4 Weeks
					Pain intensity	No significant improvement Mebeverine group: dry mouth significantly higher than placebo group
Roohafza et al. (2014)	1	Citalopram 20 mg once a day	Children and adolescents 6–18 years ($n = 86$)	Functional abdominal pain	Randomized, placebo-controlled study	4 Weeks
					Pain intensity	No significant improvement in pain
						No difference in side effects

Table 2 Primary endpoints, entry, and responder definition criteria for clinical trials on pediatric IBS[a]

Indication	Primary endpoints	Entry criteria	Responder definition
IBS-C	Abdominal pain intensity AND stool consistency	Abdominal pain intensity	Abdominal pain intensity (dual criteria)
		Weekly average of worst abdominal pain in past 24 h ≥3.0 on a 0–10-point scale or ≥30 mm in 100 mm visual analog scale[b]	≥30% improvement in abdominal pain
		AND	AND improvement ≥reliable change index (RCI) at the last week of trial compared with baseline
		Stool consistency	AND
		Bowel movements during run-in period with average consistency <3 on the Bristol Stool Form Scale: type 1 (very hard) or type 2 (hard)	Stool consistency
			Improvement in (≥1 Bristol Stool Form Scale – to a higher number) average consistency at the last week of trial compared with baseline
IBS-D	Abdominal pain intensity AND stool consistency	Abdominal pain intensity	Abdominal pain intensity
		Weekly average of worst abdominal pain in past 24 h ≥3.0 on a 0–10-point scale or ≥30 mm in 100 mm visual analog scale[b]	Dual criteria
		AND	≥30% improvement in abdominal pain
		Stool consistency	AND improvement ≥reliable change index (RCI) at the last week of trial compared with baseline
		Bowel movements during run-in period with average consistency >5 on the Bristol Stool Form Scale: type 6 (loose), 7 (very loose)	AND
			Stool consistency
			Improvement in (≥1 Bristol Stool Form Scale – to a lower number) average consistency at the last week of trial compared with baseline

[a]Use only abdominal pain endpoints in trials on FAP-NOS
[b]Or cutoff equivalent to moderate pain if using a different pain scale

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Sex-Related Differences in GI Disorders

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Abstract

Epidemiological studies indicate sex-related differences among functional gastrointestinal disorders (FGIDs) wherein females are more likely to receive a diagnosis than their male counterparts. However, the mechanism by which females exhibit an increased vulnerability for development of these pathophysiologies remains largely unknown, and therapeutic treatments are limited. The current chapter focuses on clinical research outlining our current knowledge of factors that contribute to the female predominance among FGID patients such as the menstrual cycle and sex hormones. In addition, we will discuss progress in preclinical research, including animal models, which serve as valuable tools for the investigation of the development and long term manifestation of symptoms observed within the patient population. Although much progress has been made, additional longitudinal studies in both clinical and preclinical research are necessary to identify more specific mechanisms underlying sex-related differences in FGIDs as well as targets for improved therapeutic approaches.

Keywords

Animal models • Functional gastrointestinal disorders • Gender • Irritable bowel syndrome • Sex differences • Sex hormones • Stress

1 Introduction

Sex differences have been reported in some gastrointestinal (GI) disorders but has been a particular area of focus in functional GI disorders (FGIDs), which has now more recently been defined as disorders of gut–brain interactions (Drossman 2016). One of the most common of these disorders is irritable bowel syndrome (IBS), which is characterized by recurrent abdominal pain or discomfort associated with a change in bowel habits (Mearin et al. 2016). A meta-analysis of 80 studies that included 260,960 subjects identified a prevalence rate of 11.2% (95% CI, 9–8 to 12.8%) (Lovell and Ford 2012b). Most but not all FGIDs have a female predominance. Sex and gender differences in FGID patients have been studied to a limited extent, but most of the research has been in IBS. Additionally, in an effort to better understand sex-related differences in FGIDs such as IBS, preclinical science has developed rodent models mirroring facets of human experiences and subsequent alterations in pain. Thus, this review will focus on IBS and will cover sex and gender differences in epidemiology, pathophysiology, and treatment as well as preclinical studies which support or enhance our understanding of the patient population (Fig. 1).

2 Epidemiology

Women are more likely to report symptoms of FGIDs. With respect to esophageal disorders, globus sensation and rumination syndrome are reported in females more than males (Chial et al. 2003; Drossman et al. 1993). Functional chest pain of

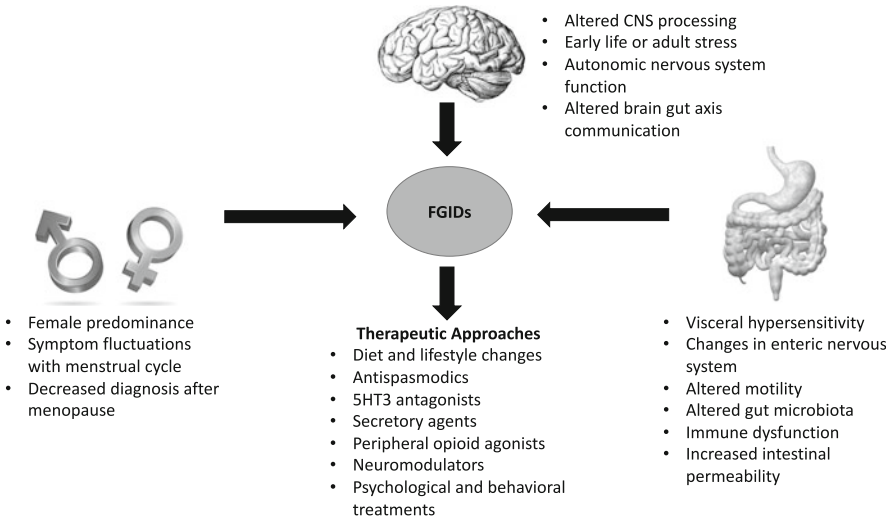

Fig. 1 Development of FGIDs such as IBS is influenced by complex interactions between central, peripheral, and sex-related factors. 5HT3-serotonin receptor 3, CIC2-chloride channel 2, GCC-guanylate cyclase

esophageal origin, which includes those with non-cardiac chest pain, has a female predominance in the tertiary care referral population (Cormier et al. 1988). Other esophageal disorders and symptoms, including GERD, heartburn, and dysphagia are not associated with gender differences (Houghton et al. 2016). Although there are no gender differences in the prevalence of functional dyspepsia, female gender was found to be a significant predictor of functional dyspepsia compared with organic causes of dyspepsia (Stanghellini et al. 1996). In addition, women with functional dyspepsia appear to have delayed gastric emptying, lower tolerance to the gastric water loading test, greater symptom burden, and worse health related quality of life than men.

Traditionally, IBS has been regarded as a female predominant condition with a female-to-male ratio of 2–2.5:1 in those who seek health care. Female gender is also a risk factor for post-infection IBS (Spiller and Garsed 2009). However, the female predominance is less apparent in the general population, which suggests that women with IBS are more likely to seek healthcare for their symptoms (Adeyemo et al. 2010; Camilleri and Choi 1997; Drossman et al. 2002; Müller-Lissner et al. 2001; Talley 1999; Thompson 2000). Lovell and Ford (2012a) conducted a meta-analysis of 56 studies containing 188,229 subjects with IBS and found that the odds ratio (OR) for IBS in women, compared with men, was 1.67 (95% CI: 1.53–1.82). However, the prevalence of IBS was not significantly higher in women than men in studies conducted in the South Asian, South American, or African populations. There likely is an interaction between gender and cross-cultural differences which impacts these findings. The IBS with constipation subtype was more common in women than men (OR: 2.38; 95% CI: 1.45–3.92) and the IBS with diarrhea subtype

was less common in women (OR: 0.45; 95% CI: 0.32–0.65) than men. Adeyemo and colleagues (Adeyemo et al. 2010) performed a systematic review and meta-analysis of the literature to evaluate gender differences in individual IBS symptoms. They evaluated 22 studies that included nine conducted in the general population, 12 in an IBS only sample, and 1 in both the general population and IBS patients. Women in the general population were more likely to report abdominal pain and pain-related IBS diagnostic symptoms, but this gender difference did not exist in IBS. In both the general population and IBS patients, women were more likely to report predominantly constipation-related symptoms including abdominal distension, bloating, straining, and hard stools than men. In contrast, men with IBS were significantly more likely to report the diarrhea-related symptoms of loose/watery stools and increased stool frequency than women with IBS.

The recently established Rome IV diagnostic criteria was based on normative data in the US general population. The Rome IV diagnostic criteria for IBS differs from Rome III criteria in that the presence of abdominal pain and/or discomfort occurring for at least 3 days per month in Rome III was changed to at least 1 day of abdominal pain per week in Rome IV (Mearin et al. 2016). In a community survey of almost 6,000 subjects across 3 countries, (USA, UK, and Canada), the prevalence of IBS based on Rome IV vs. Rome III criteria was 5.8% vs. 11.1%. Similar to Rome III, women were more likely to have IBS by Rome IV than men (7.1% vs. 4.1%; OR 1.87, $p < 0.0001$) (Palsson et al. 2016).

Other FGIDs which have a greater female-to-male ratio include chronic constipation, including outlet type of constipation (Siproudhis et al. 2006), functional abdominal bloating and distension, functional abdominal pain syndrome, and functional anorectal pain. However, functional diarrhea is more common in men than women (Houghton et al. 2016).

3 Role of Female Sex Hormones on IBS Symptoms

3.1 Effect of Menstrual Cycle on IBS Symptoms

A systematic review of 13 studies that assessed the effect of menstrual cycle on IBS symptoms found that all but three studies found that, on average, 40–60% of women reported increased GI symptoms at the time of menses compared to other phases of the menstrual cycle. These symptoms included loose stools, bloating, abdominal pain, stool frequency, and other changes in bowel habit with increased diarrhea more common at the time of menses than constipation (Adeyemo et al. 2010). One study that measured menstrual cycle phase using ovulation kits which is the more accurate method found that while menstrual cycle effects on symptoms were similar in healthy women and IBS women, symptom severity was greater in women with IBS. There is an amplification of symptoms during the late luteal and early menses phases coincident with falling estrogen and progesterone levels (Heitkemper et al. 2003).

3.2 Effect of Menopausal Status on IBS Symptoms

The effect of menopausal status, when ovarian hormone levels decline, on IBS symptoms has not been well studied. In menopause, the incidence of IBS decreases but constipation and somatic discomfort syndromes increase in menopause (Gonenne et al. 2006; Houghton et al. 2016). In women with IBS, nausea has been reported more frequently by premenopausal women than postmenopausal women (Gonenne et al. 2006; Lee et al. 2001). Healthy women have been shown to experience more gaseousness and excessive flatulence than postmenopausal women.

3.3 Effect of Hormone Replacement Therapy on IBS Symptoms

Two studies investigated the effect of hormone replacement therapy (HRT) on IBS symptoms in women over the age of 50 who were presumably postmenopausal (Gonenne et al. 2006; Ruigomez et al. 2003). In one study, women who use HRT are more likely to develop IBS than women who do not, although the prevalence and severity of IBS symptoms were similar between these two groups (Ruigomez et al. 2003). The other study was conducted in postmenopausal healthy women who were more likely to have looser stools and greater ease of passage if they took estradiol or progesterone therapy alone for 7 days compared to those on placebo (Gonenne et al. 2006).

In premenopausal women, two studies evaluated the effect of hormone supplementation on IBS symptoms. One study assessed the effect of oral contraceptive pills (OCP) in women with IBS which was associated with lower abdominal pain severity compared to non-OCP users, although this difference did not meet statistical significance after correcting for multiple comparisons (Heitkemper et al. 2003). The second study was a placebo-controlled trial that evaluated the effect of a gonadotropin releasing hormone (GnRH) agonist, which stimulates the release of follicular stimulating hormone and luteinizing hormone, which suppress the secretion of ovarian hormones. Compared to placebo, the GnRH agonist improved the severity of IBS symptoms (Heitkemper et al. 2003; Palomba et al. 2005).

4 Pathophysiology of IBS

IBS is a multifactorial condition with a complex pathophysiology (Fig. 1). As with the other disorders of gut–brain interactions as defined in Rome IV, IBS is classified by symptoms related to any combination of motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and altered CNS processing (Drossman 2016). A unifying theory in the pathogenesis of IBS and other FGIDs is that there is a dysregulation in the complex interplay among multiple domains, including the gut lumen, intestinal mucosa, enteric nervous system, and the CNS that results in enhanced sensation and altered motility.

4.1 Visceral Perception

Increased visceral perception is due to increased peripheral sensitivity of visceral afferent pathways (peripheral sensitization) or to central amplification of visceral afferent input (central sensitization) (Mayer 2000). In multiple experimental studies, enhanced visceral perception in IBS has been demonstrated as decreased pain or discomfort thresholds, increased sensory ratings, and increased viscerosomatic referral areas with rectal and/or colonic balloon distension using a computerized distension device (barostat) (Mertz et al. 1995; Munakata et al. 1997). Studies have demonstrated either increased or similar visceral perception in women with IBS compared to men with IBS and healthy women (Chang et al. 2006; Houghton et al. 2016). Multiple factors can influence visceral sensitivity including menstrual cycle phase, stress, bowel habit subtype, and psychosocial factors which may explain differing results.

4.2 Motility

In general, men appear to have more rapid gastric emptying and intestinal transit compared to women (Houghton et al. 2016). Ambulatory colonic manometry over 24 h performed in healthy men and women demonstrated lower pressure activity, particularly in the transverse and descending colon than healthy men (Rao et al. 2001). Slower colonic transit in women likely contributes to the female predominance in chronic constipation.

4.3 Autonomic Nervous System Function

Sex differences in the autonomic nervous system (ANS) function have been found in IBS. Heart rate variability is a measure of cardioautonomic tone as it assesses cardiosympathetic and vagal balance. Men with IBS had greater cardiosympathetic and lower vagal tone at baseline and in response to rectosigmoid balloon distension. Skin conductance, a measure of sympathetic nervous system function, was also higher in men with IBS compared to women with IBS (Tillisch et al. 2005). In another study, cardiosympathetic/vagal balance was also higher in men with IBS than women with IBS during flexible sigmoidoscopy (Cheng et al. 2013). These studies support alterations in ANS responses in IBS that are similar to that seen in response to stress.

4.4 Morphologic and Functional Brain Imaging

A number of studies have demonstrated alterations in brain activation patterns to visceral balloon distension in IBS. In addition, sex differences in IBS have been reported. Men show greater activation of the corticolimbic inhibition system (e.g., dorsolateral prefrontal cortex, insula), while women show greater activation in limbic and paralimbic regions (e.g., amygdala, ventromedial prefrontal cortex) (Berman et al. 2006; Naliboff et al. 2003). Sex differences were also found in the

effective connectivity of functional neural networks in IBS at rest. Men with IBS showed normal feedback inhibition within a network involved in limbic activity. Women with IBS had greater connectivity between the amygdala and infragenual anterior cingulate cortex (ACC) activation suggestive of a greater responsiveness of a network regulating amygdala reactivity to emotionally salient stimuli (Mayer et al. 2007). Another study showed sex differences in gray matter density and volume with greater cortical thickness in the subgenual ACC in men with IBS compared to women with IBS (Hong et al. 2013). These studies demonstrate morphologic and functional brain differences in men and women with IBS that could help explain differences in how men and women respond to visceral stimuli as well as the increased vulnerability of IBS in women.

4.5 Stress

FGIDs, particularly IBS, are stress-sensitive disorders. Multiple studies have shown that early life stress (ELS) is associated with IBS. ELS includes sexual, emotional, and physical abuse and general trauma experienced in childhood. Bradford et al. (2012) found that women with IBS reported more ELS experiences than men with IBS. A recent study evaluated the interaction of sex and IBS on hypothalamic-pituitary adrenal (HPA) axis response to hormone challenge (Videlock et al. 2016). Men with IBS had a greater cortisol response to adrenocorticotropin hormone (ACTH) administration vs. healthy men, while women with IBS had a blunted response compared to men. IBS was also associated with decreased glucocorticoid receptor (GR) expression on peripheral blood mononuclear cells (PBMC). GR expression levels negatively correlated pituitary (ACTH) response to corticotropin-releasing hormone, suggesting that PBMC GR expression is a peripheral marker of central HPA axis function and could support impaired negative feedback as a mechanism of HPA axis dysregulation in IBS. Interestingly, these findings were mainly seen in men with IBS (Videlock et al. 2016). A history of ELS was associated with a greater cortisol response to hormone challenge (Videlock et al. 2016) as well as to a visceral stressor, a flexible sigmoidoscopy (Videlock et al. 2009). The effect of ELS on cortisol response to a visceral stressor was seen in men but not women. Taken together, these findings provide evidence that IBS is associated with a dysregulated HPA axis and that there are divergent responses in men and women with IBS.

4.6 Mucosal Immune Function

IBS has been associated with alterations in cytokine profiles although there is more apparent in blood levels than mucosal levels (Chang et al. 2012; Ohman et al. 2009). Colonic mucosal levels of the anti-inflammatory cytokine IL-10 were found to be lower in women with IBS compared to healthy women but no differences were seen within men (Chang et al. 2012). Women with IBS have also been shown to have a greater number of colonic mucosal mast cells and a lower number of CD+3 and CD+8 T cells (Cremon et al. 2009). These findings suggest that mucosal immune activation is sex dependent in IBS (Houghton et al. 2016).

5 Animal Models

In preclinical research, sex-related differences in visceral pain responsiveness have been primarily studied in rodent models of ELS. ELS, characterized by childhood neglect, physical abuse, and/or sexual abuse, affects approximately 40% of the population before adolescence and has been correlated with increased instances of FGIDs in females (Bradford et al. 2012; Costello et al. 2002; Dong et al. 2004; Finkelhor et al. 2005). Although animal models cannot completely mirror the complexities of the human ELS experience, they are important tools for isolating specific variables and expanding our understanding of how environmental influences may lead to sex-related differences in FGIDs. The following section outlines several important models of ELS and their key findings regarding visceral pain, the hallmark symptom of IBS.

The rodent model of maternal separation (MS) utilizes removal of pups from mother and nest, most commonly for 3 h/day on postnatal (PN) day 2–14 (Plotsky and Meaney 1993) in order to mimic childhood neglect and abuse. MS results in alterations in maternal care, including altered licking and grooming behaviors and arched back nursing (Plotsky and Meaney 1993). Variations in neonatal separation time and duration have resulted in conflicting reports regarding sex-related differences in visceral sensitivity in adulthood (Coutinho et al. 2002; Prusator and Greenwood-Van Meerveld 2016; Rosztóczy et al. 2003). However, a 3-h/day separation from PN days 2–14 has been shown to elicit male specific visceral hypersensitivity, as evidenced by an increased visceromotor response (VMR) to colorectal distension (CRD) in adult male Long Evans rats (Prusator and Greenwood-Van Meerveld 2016). In this particular study, adult female animals previously exposed to the same paradigm exhibited no alterations in pain responsiveness. The limited nesting (LN) model of ELS emulates areas of poverty or lower socioeconomic standing where neglect and abuse are more common than in the general population (Gilles et al. 1996; Ivy et al. 2008). From PN day 2–9, the dam and pups are placed on a wire cage bottom with only a single paper towel for nesting material, leading to disruptions in normal maternal care without removal of pups from the dam. Neonatal exposure to LN has been shown to produce increased visceral sensitivity in adult male Long Evans and Sprague Dawley rats, as quantified by increased VMR to CRD (Prusator and Greenwood-Van Meerveld 2014, 2016). The LN and MS models of ELS are considered to be more psychological in nature as they rely on environmental manipulations that stress the dam and result in alterations in maternal care. These changes in maternal interaction have been shown to have widespread influence on adult pathophysiologies (Francis and Meaney 1999; Liu et al. 1997; Weaver et al. 2004).

A third model of ELS, the odor-attachment learning (OAL) paradigm, relies on classical conditioning utilizing paired or unpaired odor/shock presentations to mirror an attachment to an abusive caregiver (Sullivan et al. 2000). In adulthood, pairing of an odor with an unpleasant stimulus elicits an aversion, however, during the early postnatal days rat pups experience both a sensitive and a hyporesponsive period that allow this pairing to result in a learned attachment. Pups exhibit

heightened olfactory sensitivity in order to find the dam in the cage for care and nursing. This behavior coincides with a dampened ability to initiate a stress response, ensuring that pups do not learn an aversion to the dam when she is stepping on or moving them around the cage or nest (Sullivan and Wilson 1994). Therefore, the OAL model exploits these developmental advantages to mimic patterned interactions between pup and dam and creates an attachment to the conditioned odor in response to paired odor/shock presentations. In addition, OAL employs an unpaired odor/shock presentation, and an odor only presentation as a control. In adulthood, female rats previously presented with the unpaired stimulus exhibit an increased VMR to CRD (Prusator and Greenwood-Van Meerveld 2016), an effect that has been shown to be estrogen dependent (Chaloner and Greenwood-Van Meerveld 2013). Thus, when considering the patient population, the OAL model of ELS has far reaching translational relevance. The OAL model not only parallels the female predominance found in IBS patients, but also allows for investigation of vulnerability and resilience factors linked to the context of the stress experienced. Overall, rodent models provide a tool for exploration of potential therapeutics aimed at improving or eliminating the symptoms observed in IBS patients.

6 Estrous Cycling and Female Hormones in Animal Models

In parallel with patient studies, sex differences in pain responsiveness have been well characterized in rodent models as exemplified by cyclical changes in pain sensitivity observed across the menstrual cycle in adult female rats (Iwasaki-Sekino et al. 2009; Ji et al. 2008, 2012; Kayser et al. 1996; Ralya and McCarson 2014; Vendruscolo et al. 2004). In response to luminal distension, exaggerations in visceral hypersensitivity were observed in adult female rats during proestrus, when estrogen and progesterone are high, compared to diestrus or metestrus when hormone levels are significantly decreased (Ji et al. 2008; Sapsed-Byrne et al. 1996). Fluctuations in female hormones have been shown to influence the development of visceral pain in response to a pharmacological stimulus, and administration of female hormones alone can induce visceral hypersensitivity in the absence of additional manipulation (Gustafsson and Greenwood-Van Meerveld 2011; Myers et al. 2011). Additionally, estrogen can alter expression of pain-related genes within the central nervous system as well as at the level of the spinal cord such as glucocorticoid receptor, corticotropin-releasing factor, NMDA receptor (Tang et al. 2008), metabotropic glutamate receptor 2 (Cao et al. 2015), and GluN2B subunit activity (Ji et al. 2015; Miller et al. 2004; Uht et al. 1997; Vamvakopoulos and Chrousos 1993). Taken together, current preclinical research indicates a critical and multifaceted role for cycling female hormones in the development of aberrant pain responsiveness and sex-related differences in FGIDs such as IBS.

7 Brain Gut Axis and Animal Models of IBS

IBS is thought to involve a dysfunctional interaction between the brain and the gut, and preclinical models have been used to investigate abnormalities in the brain gut axis as they relate to heightened visceral pain. Brain imaging in rodents exposed to ELS revealed abnormal activation of stress related nuclei including the amygdala in adulthood in response to pain (Holschneider et al. 2016), and direct manipulation of the amygdala by addition of corticosterone has been shown to elicit visceral hypersensitivity in adult male rodents (Myers and Greenwood-Van Meerveld 2010). Furthermore, adult stress and ELS have been shown to induce abnormalities in the expression of glucocorticoid receptor (GR) as well as corticotropin-releasing factor (CRF), key modulators of stress and pain, in adult rodents exhibiting visceral hypersensitivity (Francis et al. 1999; Johnson et al. 2015; Ladd et al. 2004, 2005; Prusator and Greenwood-Van Meerveld 2017; Tran and Greenwood-Van Meerveld 2012). Stress induced visceral hypersensitivity can be inhibited by treatment with CRF-1 antagonists (Bradesi et al. 2009; Gilet et al. 2014), and targeted gene knockdown using oligodeoxynucleotides (Johnson and Greenwood-Van Meerveld 2015; Prusator and Greenwood-Van Meerveld 2017).

In conjunction with alterations within the central nervous system, many preclinical models of IBS exhibit alterations in immune function. Mucosal mast cells are directly innervated and considered one of the most important mediators of brain–gut interactions (O'Mahony et al. 2011). Degranulation of mast cells is considered a potential mechanism for the development of visceral hypersensitivity. Following exposure to MS, increases in mucosal mast cell number have been observed at 4 weeks post-MS and these elevations are maintained into adulthood (Barreau et al. 2004a, b). A second study indicated that exposure to a second stressor in adulthood could also induce elevations in mast cell numbers (van den Wijngaard et al. 2009). Histamine-1 receptor (H1R) antagonists such as fexofenadine have shown the ability to stabilize mast cells, and thus reduce visceral hypersensitivity in maternally separated rats (Stanisor et al. 2013). Additional investigation of sex-related differences in preclinical models of IBS is necessary to determine what bias may exist for mechanisms which leave females more vulnerable to development of FGIDs as a result of altered brain gut axis function.

8 Treatment Response in IBS

The study subjects in most pharmacologic and non-pharmacologic treatment trials in IBS are predominantly women, which makes it more difficult to assess sex and gender differences. Alosetron, a $5HT_3$ antagonist, which has been shown to be efficacious in the treatment of IBS with diarrhea in a number of studies (Ford et al. 2009) was approved by the Food and Drug Administration (FDA) only in women because a significantly beneficial effect was demonstrated in women but not men. However, a subsequent study in men with IBS with diarrhea only showed that alosetron was also efficacious in men (Chang et al. 2005). Lubiprostone, a ClC-2

activator, appeared to be more efficacious in women with IBS with constipation than men, although this might have been due to the small number of men in the clinical trial (Lunsford and Harris 2010). Thus, lubiprostone was only approved for the treatment of IBS with constipation in women. However, it was shown to be efficacious for the treatment of chronic idiopathic constipation in both men and women (Johanson and Ueno 2007). Linaclotide, a guanylate cyclase C receptor agonist, was found to be efficacious in both men and women with IBS with constipation (Chey et al. 2012) and chronic idiopathic constipation (Lembo et al. 2011). Psychologic and behavioral treatment trials show small or no gender differences in response rates (Houghton et al. 2016).

9 Conclusions

Sex and gender differences have been demonstrated in FGIDs, particularly in IBS and chronic constipation. These differences include a greater prevalence of IBS, including post-infection IBS, and constipation symptoms in women compared to men. Cultural factors and gender role contribute to these differences. Sex differences in pathophysiologic mechanisms in IBS include enhanced visceral perception, slower gastrointestinal motility, and greater immune activation in women than men. Men with IBS have a greater sympathetic activation and HPA axis response than women with IBS. Brain imaging studies demonstrate sex differences in morphologic and functional changes within IBS. Female sex hormones likely contribute to these sex differences although they have not been studied in a comprehensive manner. Future studies are needed to determine if other pathophysiologic mechanisms such as increased intestinal permeability and gut dysbiosis differ in men and women and if they play a role in FGID pathophysiology. There is some evidence of differences in treatment response in men and women with IBS, although there are relatively few men enrolled in clinical trials. Greater attention needs to be placed on recruiting similar numbers of men and women in human studies and studying the role of female sex hormones, menstrual cycle phase, and menopausal status in FGID studies. The reasons for a greater female predominance in IBS and other FGIDs are still not well understood.

As indicated previously, animal models have significantly enhanced our ability to understand the physiology and pathophysiology of FGIDs such as IBS. These rodent models allow researchers to recapitulate specific symptoms of the human situation, and provide for easy inclusion of both male and female study subjects. However, FGIDs are complex multifactorial disorders involving complicated interactions between central, peripheral, and sex-related factors (Fig. 1). Although animal models are useful tools for isolating specific variables that may contribute to disease states, these investigations can be limited by biological, psychological, and sociological variables that cannot be completely mimicked in a laboratory setting. In addition, mechanisms unearthed by preclinical studies may not be directly translatable to the patient population, as there are inherent differences in physiology that serve as limiting factors. For example, it is clear that female cycling hormones

have profound effects in preclinical models, however, the direct translational relevance of these studies is not yet well-defined. Continued progress will depend heavily on longitudinal studies in both clinical and preclinical research to identify more specific triggers for the onset of symptoms and the sexually dimorphic mechanisms underlying disorders such as IBS.

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Abnormal Barrier Function in Gastrointestinal Disorders

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Contents

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Abstract

There is increasing concern in identifying the mechanisms underlying the intimate control of the intestinal barrier, as deregulation of its function is strongly associated with digestive (organic and functional) and a number of non-digestive (schizophrenia, diabetes, sepsis, among others) disorders. The intestinal barrier is a complex and effective defensive functional system that operates to limit luminal antigen access to the internal milieu while maintaining nutrient and electrolyte absorption. Intestinal permeability to substances is mainly determined by the physicochemical properties of the barrier, with the epithelium, mucosal immunity, and neural activity playing a major role. In functional gastrointestinal disorders (FGIDs), the absence of structural or biochemical abnormalities that explain chronic symptoms is probably close to its end, as recent research is providing evidence of structural gut alterations, at least in certain subsets, mainly in functional dyspepsia (FD) and irritable bowel syndrome (IBS). These alterations are associated with increased permeability, which seems to reflect mucosal inflammation and neural activation. The participation of each anatomical and functional component of barrier function in homeostasis and intestinal dysfunction is described, with a special focus on FGIDs.

Keywords

Barrier function • Enteric nervous system • Epithelial integrity • Intestinal permeability • Paracellular transport • Tight junctions • Transport routes

1 Introduction

The gastrointestinal (GI) mucosa represents the largest body surface exposed to the external environment and is, quantitatively, the most important route of entry for pathogens. An effective defensive mechanism that limits luminal antigen access to the internal milieu while maintaining nutrient absorption is, therefore, essential for human health. To successfully accomplish this complex task, the intestinal mucosa establishes a dynamic semipermeable barrier which supports active and passive transport of substances and excludes the entry of potentially harmful substances, a process tightly regulated by neurohormonal and immune constituents. The intestinal barrier consists of a number of anatomical and functional elements (cellular and extracellular) distributed across the tissue layers, which tightly interact to maintain homeostasis. Functionality of this intestinal barrier can be assessed by measuring flux rates of certain molecules across the intestinal wall (by in vivo or ex vivo techniques), allowing the indirect quantification of intestinal permeability.

Although not validated as a clinical parameter, assessment of intestinal permeability is of clinical relevance as it enables the comparison between pathological and healthy conditions and also allows monitoring the effect of therapeutic interventions on barrier function. In fact, clinical studies have demonstrated that intestinal barrier function can be modulated by a number of factors, including diet, stress, bacteria, and drug consumption (Bischoff et al. 2014), and that persistent changes in intestinal permeability, if uncontrolled, can turn into disease. Notably, increased permeability is associated with digestive (celiac disease, inflammatory bowel disease, irritable bowel syndrome, and food allergy) and non-digestive conditions (schizophrenia, diabetes, and sepsis) (Pascual et al. 2001; Shen and Turner 2006) evidencing the relevance of the mucosal barrier to host health. However, whether barrier dysfunction is cause or consequence of disease and the underlying mechanisms remain to be precisely determined. This review describes the components of the intestinal barrier and its role in maintaining the functionality of such a defensive system in health and the most common GI disorders.

2 The Intestinal Barrier

2.1 Anatomical Distribution of Barrier Components

The intestinal barrier is a complex defensive structure with physicochemical properties and is composed of cellular and extracellular elements, distributed in two main intestinal compartments. These elements are characterized by increased level of specificity from the external to the internal milieu:

– *Luminal compartment*: The harsh environment at the luminal compartment represents the first defensive mechanism, as low pH and gastric, pancreatic, and biliary secretions, which contain active enzymes, exert a toxic action on most microorganisms. Luminal microbiota also restricts colonization by pathogens by changing pH and releasing antimicrobial substances that allow interspecies communication, also favoring the amount of beneficial organisms for the host (Neish 2009). The microbiota is considered a host barrier element, which closely interacts with intestinal epithelial cells and the underlying immune and nervous systems, encompassing the bacteria–gut–brain axis (De Palma et al. 2014). The viscous mucus layer, epithelial turnover, water secretion, and peristalsis also limit host exposure to harmful substances, the former also containing secretory immunoglobulin A (sIgA) and antimicrobial products such as lysozyme, phospholipids, negatively charged mucins and trefoil factor family peptides, cathelicidins, ribonucleases, and defensins (Bevins and Salzman 2011). The viscous non-stirred mucus layer (glycocalyx), adhered to the epithelial lining, acts as an efficient barrier and also protects the epithelium from luminal shear stress and digestive enzymes, and participates in epithelial renewal and differentiation, as well as in the maintenance of oral tolerance, thus limiting intestinal antigen immunogenicity via tolerogenic signaling (Shan et al. 2013).

– *Tisular compartment*: The intestinal epithelium physically separates the internal milieu from the external environment. It is composed of a single cell layer of specialized epithelial cells, the majority of which are enterocytes (80%), multifunctional cells that develop barrier, digestive, metabolic, and immune functions. Other epithelial cells are specialized in production and secretion of mucus (goblet cells), defensins (Paneth cells), and hormones and neuropeptides (enterochromaffin cells), and in antigen uptake from the bowel lumen, the latter located on the surface of lymphoid aggregates (M cells) (van der Flier and Clevers 2009). In order to assure an effective sealing, cells are connected through cell adhesion proteins. Thus, intestinal epithelial cells form a continuous polarized monolayer, which maintains an electrolyte gradient between the internal and external habitat, key for maintenance of the mucosal barrier. Adjacent to the epithelium, the connective tissue harbors a repertoire of immune cells, neurons, blood vessels, and fibroblasts. Fibroblasts are essential cells of stroma and maintain the extracellular matrix, primarily by secreting collagen and metalloproteinases, and also promote epithelial proliferation (Goke et al. 1998), actively contributing to the maintenance of the mucosal barrier. The gut-associated lymphoid system (GALT) is an essential component of the intestinal barrier and is distributed in lymphoid structures, mainly lymphoid follicles, Peyer's patches, and mesenteric lymph nodes, where immune response is initiated, and scattered effector cells through the epithelium and the lamina propria of the intestinal mucosa (Brandtzaeg et al. 2008). Moreover, an integrated network of neural cells from both the central and the enteric nervous systems (CNS, ENS) coordinate digestive and barrier functions by establishing a tight communication with intestinal cells in all anatomical layers of the gut. Neural mediators from enteric neurons modulate epithelial barrier integrity by directly stimulating epithelial cells or by the intermediate action of enteric glia or immune cells.

2.2 Innervation of the Gastrointestinal Tract

The GI is innervated by both the parasympathetic and sympathetic systems through afferent (sensitive pathway) and efferent fibers (motor pathway).

– *Sensitive pathways*: Sensory information from the GI to the brain is conveyed by both spinal and vagal sensory afferents. The spinal afferents are specialized to detect mainly nociceptive stimuli (pain), while vagal afferents transmit non-painful (physiological) information to the brain. GI symptoms are produced generally in two ways: through stimulation of chemosensitive nociceptors present in spinal afferent nerves that innervate the lamina propria or through stimulation of mechanosensitive nociceptors present in smooth muscle (activated through repeated deformation or distension of the intestinal wall) (Brierley and Linden 2014). This implies that changes in intestinal motility, the luminal content or the milieu in the lamina propria (presence of inflammatory mediators), can signal through afferents to the CNS, which potentially translate into GI symptoms.

– *Motor pathways*: The efferent innervation of the GI tract follows a gradient. The upper part is predominantly innervated by the parasympathetic and the distal part (mainly distal colon and rectum) by the sympathetic nervous system. Nevertheless, the efferent pathways do not innervate the smooth muscle directly to regulate intestinal motility. The GI tract possesses an intrinsic innervation carried by the ENS that is distributed in two different plexi: (a) the myenteric plexus or Auerbach's plexus located between the circular and longitudinal smooth muscle layer and (b) the submucous plexus also named Meissner's plexus located in the submucosa. The first is responsible for the different motility patterns of the GI tract and the second mainly regulates the secretion in the intestine. Although the ENS receives substantial innervation from the autonomic nervous system (sympathetic and parasympathetic nervous system), it operates independently.

2.2.1 Neural Signaling in the Enteric Nervous System

The ganglia of the myenteric plexus possess inhibitory and excitatory motor neurons that finely regulate intestinal motility. In the entire human GI tract, the neurotransmitters released by the excitatory motor neurons are acetylcholine and substance P and the inhibitory neurotransmitters are nitric oxide (NO) and adenosine triphosphate (ATP) (Farre et al. 2006; Gallego et al. 2008; Opazo et al. 2011). The contribution of each neurotransmission depends on the segment of gut evaluated. Concerning the inhibitory neurotransmission, it seems that NO is the main neurotransmitter in the upper (esophagus and stomach) and ATP in the lower part (small intestine and colon). Although in most of the textbooks, vasoactive intestinal polypeptide (VIP) is always mentioned as a co-transmitter together with NO, only one study in human gastric fundus may support this wrong and long-lasting assumption (Tonini et al. 2000). The intestinal intrinsic primary afferent neurons (IPAN) are the only afferent neurons described with cell bodies outside the central nervous system; they are located in the submucous and myenteric plexus. After its activation, they can transmit the signal to second-order neurons (interneurons and motor neurons), modulating intestinal motility and secretion. IPAN activation by luminal or mechanical stimulation of the mucosa is indirect by the release of serotonin from enterochromaffin cells in the epithelium (Furness et al. 1998).

The fine communication between the lumen and the intrinsic and extrinsic nervous system allows a precise and coordinated control of intestinal motility and secretion. Such regulation is very important to maintain the distribution of the mucous layer and a good clearance of intestinal pathogens in case of infection.

2.3 Intestinal Immune System

The strategical location of the GALT, at the mucosal interface, allows surveillance and innate and adaptive effector functions of immunocytes, addressed at maintaining gut homeostasis. Epithelial cells cooperate in this immune defense, as they express receptors, process and present antigens, and release mediators that facilitate bidirectional communication with the immune system.

2.3.1 Innate Immunity

It stands as the first line of immunological defense against potentially harmful substances in the gut mucosa and is characterized by the lack of immunological memory. The main cellular constituents of the innate response are enterocytes, goblet and Paneth cells, as well as subepithelial neutrophils, dendritic cells, macrophages, eosinophils, and mast cells. This response is triggered by pathogen-recognition receptors (PRRs), which serve as sensors of pathogen-associated molecular patterns (PAMPs) and of damage-associated molecular patterns (DAMPs) that are produced by cells in response to injury or stress (Santaolalla and Abreu 2012). Different types of PRRs mediate innate responses, such as toll-like receptors (TLRs), nucleotide-binding domain leucine-rich repeat-containing receptors (NLRs, which include nucleotide-binding oligomerization domain protein-1 (NOD1) and NOD2), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), C-type lectins (CTLs), and cytosolic DNA sensors (Fukata and Arditi 2013). PRRs activate an inflammatory response characterized by NF-κB activation; subsequent chemokine and cytokine production, which recruit and activate immune cells; and release of antimicrobial molecules such as defensins and cathelicidins (Avila 2016). Its role is essential to restrain pathogen load and also to maintain barrier function, as PRR signaling has been shown to be involved in epithelial cell proliferation, maintenance of tight junctions, and immunoglobulin (Ig) production (Hooper and Macpherson 2010; Santaolalla and Abreu 2012). In response to pathogens, mucosal macrophages, eosinophils, and mast cells release toxic and inflammatory mediators such as nitrogen radicals, histamine, and TNF-α, pyrogen which drives epithelial barrier dysfunction by disruption of intercellular adhesion proteins and increase in chloride and water secretion. Recruited neutrophils phagocytose and kill microorganisms by toxic enzymes such as lysozyme and peroxidase. However, this response is not always enough to avoid antigen penetration; therefore immune adaptive mechanisms are then activated.

2.3.2 Adaptive Immunity

It is triggered by antigen exposure, characterized by immunological memory (long-term protection) and is essential for the development of oral tolerance. Adaptive responses are mediated by lymphocytes, dendritic cells, mast cells, and macrophages, by mechanisms that include antigen processing and presentation; production of regulatory and effector molecules such as chemokines, cytokines, and Igs; and activation of cytotoxic processes against antigen invasion. These immune cells are critical for host defense, but are also major drivers of immune-mediated gut disorders. The two main T cell populations are localized in the intraepithelial compartment (preferentially CD8 lymphocytes) and below the basal membrane within lymphoid aggregates and throughout the lamina propria (mainly CD4 lymphocytes). Intraepithelial lymphocytes (IELs) represent a unique cell population as they contain cell subsets based on αβ/γδ T-cell receptor (TCR) chains and αβ/αα CD8 co-receptor phenotype with specific activity. IELs participate in pro-inflammatory and regulatory functions. They release TNF-α and IFN-γ in response to infection, which promote inflammation and barrier dysfunction and also produce keratinocyte growth factor, TGF-β1 and TGF-β3, and prothymosin β4, factors with direct and indirect functions in maintaining epithelial

integrity (Cheroutre et al. 2011). Luminal antigens are mainly transported across the epithelium through M cells covering the follicle-associated epithelium of Peyer's patches, or by the neonatal Fc receptor for IgG, and are delivered to dendritic cells. Dendritic cells can also capture luminal antigens by forming tight junction-like structures with intestinal epithelial cells and determine whether to develop tolerogenic or immunoreactive responses towards luminal antigens (Coombes and Powrie 2008), a crucial step in homeostasis maintenance. Activation of naïve CD4 T leads to different subsets based on the cytokine milieu: Th1, Th2, Th17, Th9, Th22, regulatory T (Treg) cells, and follicular helper T (Tfh) cells (O'Shea and Paul 2010). Responses by Th1 cells are primarily driven by TNF-α and IFN-γ, which facilitate neutrophil and macrophage recruitment, antigen recognition, and phagocytosis. Th2 cells produce IL-4, IL-5, and IL-13, which activate Ig production, facilitate macrophage activation, and also promote mucus secretion. Activated Th17 cells produce IL-17, a cytokine with potent pro-inflammatory function, and also IL-22, which is specifically secreted by Th22 cells, and exerts a key defensive role against enteropathogenic bacteria (Basu et al. 2012). Th9 cells produce IL-9, which regulates barrier function by modulating tight junction (TJ) protein expression (Gerlach et al. 2015). Treg cells are key to immune regulation and T cell tolerance. They are induced by TGF-β and produce TGF-β and IL-10, mediators that inhibit dendritic cell and macrophage activation and Th1 proliferation. Moreover, TGF-β also contributes to IgA production, further promoting the integrity of the mucosal barrier. The gut mucosa produces the largest amount of IgA in the body, which mainly binds to pathogenic and commensal microbial components and dietary antigens, exerting a fundamental role in shaping intestinal microbiota while preventing unnecessary stimulation of the immune system. IgA production and B-cell clone variety are dynamically adapted to microbial composition, as an increase in the complexity of gut microbiota leads to enhanced diversity of the IgA pool (Lindner et al. 2015). IgA can be produced by T cell-dependent (in germinal centers of Peyer's patches and lymph nodes) and T cell-independent (in mucosal lamina propria) mechanisms, the latter involving the participation of epithelial cells, dendritic cells, and soluble factors such as a proliferation-inducing ligand (APRIL), B-cell-activating factor (BAFF), IL-6, and IL-10 (Cerutti 2008).

The immune response in the gut is tightly regulated by the nervous system. Immune cells express receptors for neurohormones and neurotransmitters and release a variety of mediators that interact with nerve terminals. Proximity between immunocytes and nerves facilitates a rapid response between both systems, and allows the modulation of the inflammatory response and pain signaling. This interaction occurs at different levels: the peripheral nervous system promotes inflammatory responses at local sites; the sympathetic (adrenergic) and parasympathetic (cholinergic) systems inhibit inflammation through innervation of immune organs; and the hypothalamic–pituitary–adrenal (HPA) axis modulates the inflammatory response through corticoid secretion at the systemic level (Sternberg 2006). In response to antigen invasion, mediators released by immune cells activate neural pathways, which amplify immune responses to clear antigens and promote systemic and neuroendocrine responses to minimize the inflammatory response and return to a steady state (Sternberg 2006). Alteration in the control of these routes might predispose to excess of response with

prolonged inflammation and tissue damage, leading, in the gut mucosa, to uncontrolled defensive mechanisms towards the antigenic load and pathology.

3 The Intestinal Epithelium

The epithelial cell monolayer is sealed by means of cell-to-cell adhesion proteins that generate polarity, allow cell communication, and maintain the physical integrity of the epithelium. The most topical of the intercellular junctions, the TJs, defines the boundary between the apical and the basolateral plasma membrane domains and regulates paracellular permeability to small water-soluble molecules (Turner 2009). Next, adherens junctions and desmosomes, a type of anchoring junctions, connect actin filaments and intermediate filaments of adjacent cells. Gap junctions form channels that facilitate small molecules and ions to pass through adjacent cells, contributing to cell coupling and maintenance of epithelial homeostasis. Finally, hemidesmosomes also connect intermediate filaments and attach the basal surface of epithelial cells to the underlying basal lamina. The integrity of the intestinal epithelium mostly relies on the maintenance of cell junctions and the correct cell assembly. In fact, alterations in these structural and regulatory proteins have been linked to barrier dysfunction and intestinal disease (Koch and Nusrat 2009; Turner 2009). Due to their fundamental role in maintaining polarity and in limiting antigen translocation, TJs have been the objective of intensive research. Besides its barrier function, TJ also participates in epithelial cell proliferation and differentiation, and is associated with the development of human cancer (Martin and Jiang 2009).

3.1 Molecular Structure of Tight Junctions

TJs are protein complexes constituted by the transmembrane proteins claudins (CLDNs), occludin (OCLN), junctional adhesion molecule (JAM), and tricellulin. Their intracellular domains interact with the scaffold intracellular proteins zonula occludens (ZO-1, ZO-2, ZO-3), which anchor them to the actin cytoskeleton. CLDNs are sealing proteins which constitute the primary factor determining permeability at the TJ, as they control water and ion passage through the paracellular space by structures similar to ion channels (Hartsock and Nelson 2008). The pore-forming claudins CLDN-2 and CLDN-10b are selective for cations, and CLDN-7, CLDN-10a, CLDN-13, CLDN-15, CLDN-16, and CLDN-17 are selective for anions. Their upregulation increases the permeability to ions. In contrast, CLDN-1, CLDN-3, CLDN-4, CLDN-5, CLDN-6, CLDN-8, CLDN-9, CLDN-11, and CLDN-14 are predominantly a barrier-forming TJ protein (selective for anions) and CLDN-7 (selective for cations) (Gunzel and Yu 2013). Then, it is the downregulation of these CLDNs that increases the permeability to ions. Another transmembrane TJ protein is OCLN, which is not essential for TJ formation, as barriers can develop in the absence of OCLN (Saitou et al. 1998), but seems to have a regulatory role in TJ integrity. Phosphorylation of OCN at Tyr residues has been associated with loss of

interaction between OCLN and ZO proteins, and the disruption of the TJ (Kale et al. 2003). JAM proteins belong to a large family of transmembrane immunoglobulin superfamily molecules, and participate in the regulation of permeability and cell migration through the regulation of ß1 integrin (Severson et al. 2009). Tricellulin is mainly localized in tricellular TJ, the meeting point of three cells, and also in bicellular TJ. This protein complex is essential for barrier formation and modulates its function by limiting macromolecule pass and regulating ion permeability (Krug et al. 2009a).

3.2 Transport Routes Across the Intestinal Epithelium

Luminal products cross the intestinal epithelium between intercellular spaces (paracellular route) or through the plasma membrane of the epithelial cell (transcellular route), depending on its size, hydrophobicity, and other chemical characteristics (Fig. 1). Ions and small hydrophilic and lipophilic compounds use the transcellular route. Ion, water, and larger hydrophilic compounds from approximately around 400 Da and up to 10–20 kDa can pass between the epithelial cells using the paracellular route precisely regulated by TJ proteins. Nutrients as sugars, amino acids, and vitamins use transporters to cross the epithelium and require energy (active transport). Larger peptides, proteins, and bacteria or large bacterial products are endocytosed into vesicles

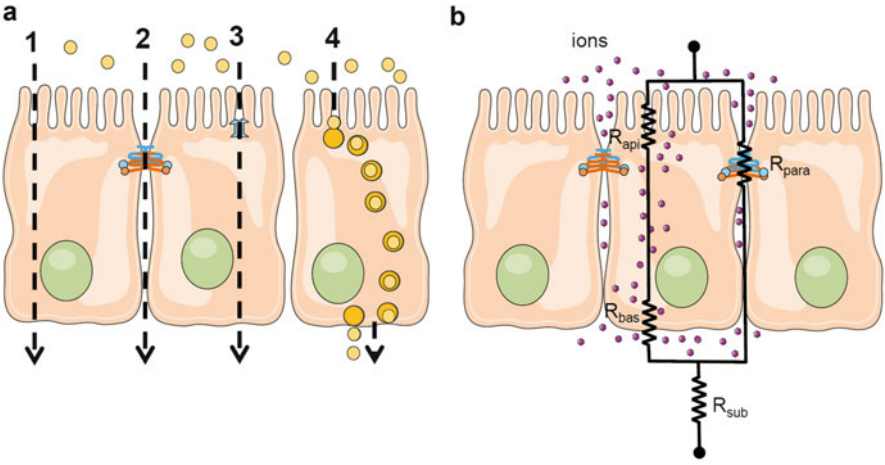

Fig. 1 Transport routes in the intestinal epithelium. (**a**) Transcellular transport of ions is controlled by transporters in the apical and basolateral surfaces (1). Ion, water, and larger hydrophilic compounds use the paracellular route regulated by TJ proteins (2). Sugars, amino acids, and vitamins use active transport (3). Large molecules and bacteria are endocytosed into vesicles (4). (**b**) Equivalent electrical circuit model discriminating between the apical (R_{api}), the basolateral (R_{bas}), the paracellular (R_{para}), and the subepithelial (R_{sub}) resistances in a simple epithelium. Notice that the transepithelial electric resistance or conductance measured in the regular Ussing chamber techniques is the sum of all these individual resistances. *TJ* tight junctions, *R* resistance

and transported through the cells via transcytosis and posterior exocytosis. Alterations in the paracellular pathway and in the uptake of large molecules as peptides, food antigens, and even bacteria are believed to be relevant in the pathogenesis of gastrointestinal disorders.

Altered paracellular permeability has been postulated to be critical for the migration of bacteria and bacterial products such as endotoxins across the gut wall. Based on the molecular weight, LPS can potentially cross the epithelium through the paracellular pathway as has been shown in different studies (Watson et al. 2005). The N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), one of the major intestinal microflora-derived chemotactic peptides (MW 437 Da), is also able to cross the epithelium through the paracellular pathway (Tanaka et al. 2015). Nevertheless, bacteria and large particles are too big to cross the epithelium by using the paracellular pathway. Although uptake of bacteria is a more common phenomenon in the Peyer's patches and M cells, it is also occurring in the regular epithelium (Keita et al. 2008). The brush border of M cells is poorly organized with short irregular microvilli (Bye et al. 1984) allowing the internalization of material from the intestinal lumen that is then transported to the underlying lymphoid tissue. The mechanisms by which M cells can take up bacteria and large molecules differ depending on the nature of this material. (1) Large particles and bacteria induce phagocytosis, which is associated with a rearrangement of the actin cytoskeleton and the formation of pseudopod-like structures. (2) Viruses and other adherent particles are taken up via clathrin-mediated endocytosis, whereas (3) non-adherent material is internalized by fluid-phase endocytosis (pinocytosis). Independently of the mechanism, once the material is internalized, it is quickly followed by the transport of endocytic vesicles to the endosomal compartment and then to the basolateral membrane by exocytosis (Keita and Soderholm 2010). Recently, the uptake of enteric bacteria by the enterocytes has been shown to depend on the phosphorylation of the myosin light chain (MLC) (Wu et al. 2014). Then, the perijunctional actomyosin ring contraction produces a change in the configuration of the microvilli called "brush border fanning" that facilitates the contact of the bacteria with the enterocyte. Remarkably, this mechanism of bacterial internalization is modulated by low concentrations of IFN-γ, a cytokine that also increases intestinal permeability to large molecules (Watson et al. 2005).

3.2.1 Assessment of Permeability at the Paracellular Pathway

Evidences for the role of TJ as a key barrier component have been historically based on observation from two very different fields, electron microscopy and electrophysiology. The junctional complexes between two epithelial cells were extensively described for the first time in 1976 in the guinea pig epithelium of the gall bladder by using transmission electron microscopy (Farquhar and Palade 1963). The epithelial tight junction is a dynamic and permeant barrier that in physiological conditions is also permeable to ions and plays a role in processes as water absorption and secretion as has been described initially by Hans Ussing in the frog skin more than 50 years ago (Lindemann 2001). More recently, knowledge has been accumulating on the complexity, selectivity, and dynamic character of intestinal epithelial barrier. Most studies on paracellular permeability have found the presence of at least two populations of pores regulated by TJs: (a) a high-capacity charge-selective

pore, permeable to small ions and small uncharged molecules, and (b) a much larger low-capacity pore (sometimes referred to as the "leak" pathway) that is permeable to large ions and molecules regardless of charge. The former is regulated mainly by CLDNs, and the latter by OCLN and TJ proteins of the ZO family. The leak pathway is a fast manner of regulating the barrier, in contrast to the slower regulation of the pore pathway, because it involves changes at the transcriptional level. The permeability of the pore and leak pathways can be measured by using several functional complementary methods with increasing complexity. Transepithelial electrical resistance (TEER or R_t) measures the net flux of all ions (cations and anions) across the epithelium and reflects the contribution of the paracellular resistance (R_{para}) that reflects the resistance of the TJs, the transcellular resistance (R_{trans}) that reflects the resistance to ions of the apical and basolateral membranes and finally the subepithelial resistance (R_{sub}). The use of more complex techniques based on impedance spectroscopy can discriminate between the R_{para} and the R_{trans} ($R_{epi} = R_{para} + R_{trans}$) and R_{sub} (Krug et al. 2009b; Zeissig et al. 2007) (Fig. 2). Unfortunately, these complex impedance spectroscopy techniques are only available for few research groups and TEER measurements are mainly used. TEER is evaluated with the Ussing chamber technique by applying a current in the epithelium, by measuring the generated potential difference, and by using Ohm's law to calculate the resistance of the epithelium to the current flow. In other words, TEER is the resistance that the epithelium offers to ions. This electrical current is mainly driven by Cl^- and Na^+, which are the most common ions in physiological solutions. An increased permeability of the pore and the leak pathway reduces TEER. The permeability of the leak pathway can be assessed by measuring the flux of large molecules across the epithelium. Commonly used molecules include EDTA, mannitol, sucrose, inulin, and polyethylene glycols (PEG) or dextran molecules of variable sizes (from 4 up to 20 kDa).

4 Modulation of Epithelial Permeability

The correct interplay between all components of the barrier function determines the level of permeability at the intestinal epithelium. This function is regulated by different factors, mainly through activation of immune function and neural activity, and through changes in plasma membrane composition. Given the association between mucosal inflammation and barrier dysfunction, the immune system stands as a key player in the progression from increased permeability to disease state. In fact, immune mediators regulate both transcellular and paracellular pathways; among them TNF-α, IFN-γ, and IL-13 have received considerable interest. TNF-α induces the phosphorylation of the MLC, leading to the contraction of the actin-myosin ring located in the most apical part of the enterocyte, favoring the redistribution of OCLN, and ZO-1, as is shown in Fig. 2. Functionally, this is translated into a reduced TEER, an increased flux of large molecules, and the caveolin-mediated endocytosis of OCLN and ZO-1 through an MLC kinase-dependent mechanism as showed by the use of inhibitors of these enzymes (Han et al. 2016; Schwarz et al. 2007; Weber et al. 2010). IL-1β also affects the leak pathway as is demonstrated by

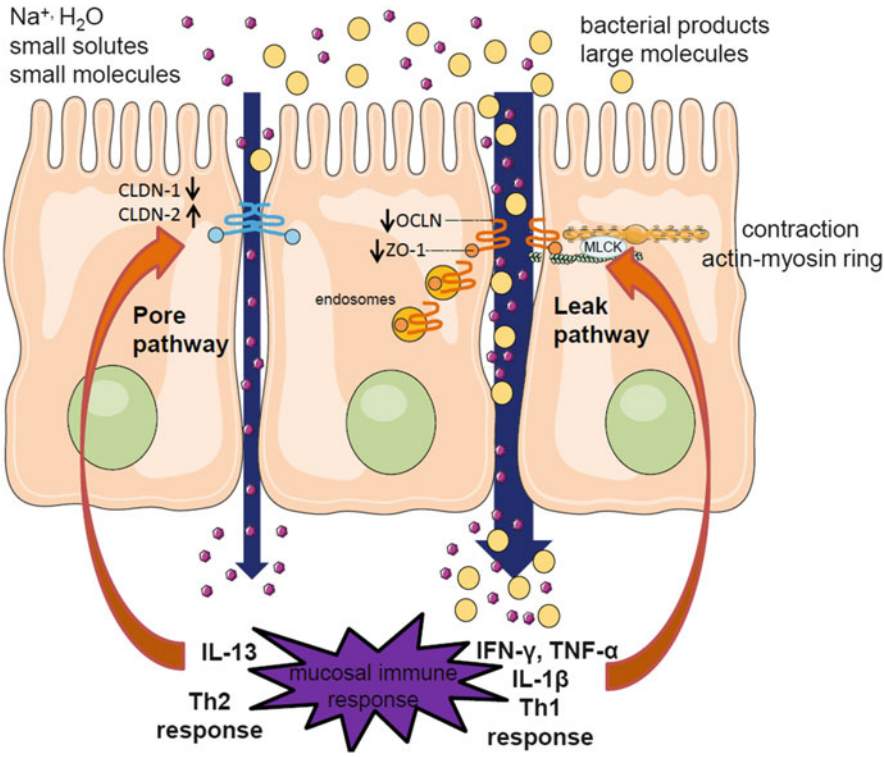

Fig. 2 Paracellular transport in the intestinal epithelium. The tight junction is composed for at least two main functionally pathways: a pore pathway that allows passage of small ions and uncharged molecules (charge-selective) and a leak pathway that allows flux of larger ions and molecules irrespective of charge. The pore pathway is mainly regulated by CLDNs and sensitive to IL-13, a cytokine of the Th2 response. In contrast, the leak pathway is mainly regulated by OCLN and ZO-1, involves the contraction of the actin-myosin ring by the MLCK, and is regulated by Th1 cytokines. *CLDN* claudin, *ZO-1* zonula occludens, *MLCK* myosin light-chain kinase

the upregulation of MLCK and quantified by a reduction on TEER and an increase in the passage of the paracellular marker inulin (Al-Sadi et al. 2008). In contrast to the Th1 cytokines, the Th2 cytokine IL-13 involves an MLCK-independent mechanism by increasing the ion conductance (reduces TEER) but not modifying the paracellular flux of large molecules (Weber et al. 2010). The pore and the leak pathway do not work always independently and some interactions were already described (Weber et al. 2010). We recently described in a rat model of spontaneous increased permeability (Vanuytsel et al. 2014b) that the pore pathway is early altered (at 50 days) due to the upregulation of CLDN-2. Later, at 90 days, the pore pathway is disrupted and the passage of molecules of 20 kDa is increased. Then, at 110 days, an increase in mast cells and eosinophils was evidenced and associated with an impaired nitrergic function at 220 days. Whether a similar sequence occurs in patients with GI disorders remains to be elucidated.

4.1 Stress

Clinical and preclinical studies have demonstrated that stress, both acute and chronic, exerts an effect on barrier function. Remarkably, the high prevalence of psychiatric comorbidities in patients with GI disorders (Singh et al. 2012) highlights the relevance of stress in the etiopathogenesis of GI dysfunction. The stress response is driven by the HPA axis and the sympathetic nervous system through neurohormonal mediators released at both central and peripheral sites. This response, which includes endocrine and behavioral changes, and increase in motility and water secretion in the GI tract, is mainly mediated by corticotropin-releasing factor (CRF) (Rodino-Janeiro et al. 2015). A number of resident cells, including immune cells, nerves, and enterochromaffin cells, release CRF, which interacts with its receptors (CRF-1, CRF-2), broadly expressed within the GI tract. Mast cells are key players of the gut-brain axis and facilitate neuroimmune interaction in the GI mucosa, and express CRF and neural receptors. Stress-induced increase in paracellular permeability seems to be mainly mediated by mucosal mast cells through activation of CRF-1 and the release of nerve growth factor (NGF) (Barreau et al. 2007; Wallon et al. 2008). Mast cell proteases and chemokines, released upon stress and during inflammation, also contribute to epithelial dysfunction. In fact, oral pretreatment with a mast cell stabilizer, disodium cromoglycate, blocked the increase in intestinal permeability by both acute stress and CRF injection in healthy humans (Vanuytsel et al. 2014a).

4.2 Microbiota

As a component of the intestinal barrier, the microbiome plays key roles in the development of epithelial integrity, mucosal immunity, and food and drug metabolism, factors that reciprocally modulate microbiota composition. An increasing number of studies implicate gut microbiota in brain function and behavior, and growing evidences point at barrier function as the linking node in the brain–gut–microbiota axis (Kelly et al. 2015). Microbial compounds have a positive or negative impact on barrier function by directly acting on epithelial cells or through activation of immune or neural responses. Metabolites produced by some bacteria, such as short-chain fatty acids, increase epithelial integrity (Morris et al. 2016), and other molecules, such as acetate, avoid pathogen invasion (Fukuda et al. 2011). On the contrary, some pathogens target TJ proteins as receptors for their attachment, which provides them a gateway to the underlying tissue (Guttman and Finlay 2009), while others interact with PRRs on epithelial cells, eliciting defensive responses. Pathogens that invade the mucosa by counteracting first-line host defensive mechanisms (mucus layer, epithelial turnover, innate immunity) (Kim et al. 2010) activate inflammatory responses, whose mediators have a deleterious effect on epithelial integrity.

4.3 Diet

Besides providing energy, some dietary components exert a role in maintaining barrier function by either promoting epithelial integrity or acting as immunomodulatory molecules. The specific mechanism on barrier function of most nutrients has been identified in in vitro and preclinical assays; therefore, care should be taken when translating to human metabolism. Glutamine preserves intestinal permeability by regulating TJ proteins (Wang et al. 2016) and a decrease in its availability may result in deterioration of barrier function. Vitamins such as vitamin D and A have also proved positive effects on barrier integrity and immune function (Bono et al. 2016). Polyphenols such as quercetin and curcumin also facilitate TJ protein assembly and show anti-inflammatory functions (Sergent et al. 2010; Suzuki and Hara 2009), both beneficial for maintaining barrier integrity. Alcohol consumption, on the contrary, has deleterious effects on intestinal barrier. It alters mucus composition and phosphorylation status of OCDN and ZO-1, promotes neutrophil infiltration, and inhibits T cell responses (Hammer et al. 2015). Consumption of processed food is associated with increased intestinal permeability. High-fat diet increases oxidative stress and disrupts gap junctions, which facilitates endotoxemia, inflammation, and tumorigenesis (Park et al. 2016). Moreover, certain food components elicit specific immune responses, as is the case of gliadin and allergens, which generate mucosal inflammation and significantly increase intestinal permeability.

5 Barrier Defects in Gastrointestinal Disorders

Functional gastrointestinal disorders (FGIDs) are chronic conditions in which GI symptoms arise in the absence of structural or biochemical abnormalities. However, recent research, with the use of technological advances and a more unified criterion for selection of patients, is becoming to challenge this concept by providing evidence of structural gut alterations in certain subsets, mainly in functional dyspepsia (FD) and irritable bowel syndrome (IBS) patients. Intestinal barrier dysfunction is a common finding in most FGIDs, for which alterations in certain components of the barrier have been identified.

5.1 Gastroesophageal Reflux Disease

The stratified esophageal epithelium is very tight, and also possesses the same cell-to-cell adhesion complexes as the simple epithelium that covers the rest of the GI tract. Nevertheless, less is known about the expression, distribution, and relevance of these complexes in the esophageal epithelium. Gastroesophageal reflux disease (GERD) is defined as a condition which develops when the stomach contents reach the esophagus and cause troublesome symptoms and/or complications (Vakil et al. 2006). Typical symptoms associated with reflux disease are heartburn, a burning sensation in the retrosternal area, and regurgitation, a perception of the refluxate

into the mouth or hypopharynx. GERD is a common disorder with increasing prevalence around the world affecting 10–15% of the adult population. Erosions are present in the distal esophagus only in 30% of the patients; the rest are considered to suffer from non-erosive reflux disease (NERD). Patients with abnormal acid exposure with or without symptom-reflux association are the ones considered as true NERD. Patients with normal endoscopy and biopsies but with a positive symptom-reflux association are considered as having reflux hypersensitivity and the ones with a negative symptom-reflux association are considered as patients with functional heartburn (Aziz et al. 2016). The presence of an impaired epithelial integrity is very obvious in patients with erosions but those subjects have also a functional impaired barrier function in non-eroded areas (Jovov et al. 2011). The presence of an impaired barrier function in patients without erosions is not well studied and contradictory findings have been reported. Although it seems clear that epithelial integrity is impaired in vivo, by measuring impedance baseline, published in vitro experiments in Ussing chambers do no confirm these findings. This discrepancy may be due to inclusion criteria and group heterogeneity, and some studies do not discriminate between true NERD, hypersensitive esophagus, and functional heartburn patients. The cell-to-cell adhesion proteins involved in the impaired epithelium present in some of the GERD patients need to be further studied. The adherens junction protein E-cadherin is cleaved in non-eroded areas of GERD (Jovov et al. 2011). Some tight junction proteins as CLDN-1 (Monkemuller et al. 2012) and the desmosomal proteins as plakoglobin, desmoglein-1, desmoglein-2, and desmoglein-3 (Wex et al. 2012) are upregulated in GERD patients with and without erosions. Whether an upregulation of cell-to-cell adhesion proteins may be responsible for the altered barrier function present in some patients and its role in GERD pathogenesis need to be further elucidated.

5.2 Functional Dyspepsia

FD is defined as the presence of symptoms thought to originate in the gastroduodenal region, in the absence of any organic, systemic, or metabolic disease that readily explains the complaints. The cardinal symptoms are early satiation, postprandial fullness, epigastric pain, and epigastric burning and are mainly triggered or exacerbated after a meal (Farre et al. 2013). FD is particularly common, affecting up to 15–20% of the population, and significantly decreases quality of life (Castillo et al. 2004). The FD pathogenesis is poorly understood and can involve alterations in motility and sensitivity of both the stomach and the duodenum (Vanheel and Farre 2013). More recently, we found that FD patients have increased number of mast cells and eosinophils in the duodenal mucosa, providing support of inflammatory mechanisms in its pathogenesis. We also described an impaired duodenal integrity to ions but also to molecules of 4 kDa. This functional alteration is associated by the downregulation of TJ proteins ZO-1, OCLN, and p-OCLN; the adherens junction proteins E-cadherin and β-catenin; and the desmosomal protein DSG-2 (Vanheel

et al. 2014). The changes in TJ protein expression in these patients indicate the main implication of the leak pathway.

5.3 Irritable Bowel Syndrome

IBS is characterized by recurrent abdominal pain on average at least 1 day a week in the last 3 months associated with two or more of the following: related to defecation; associated with a change in frequency of stool; and associated with a change in stool form (consistency). Symptoms must have started at least 6 months ago, according to the Rome IV criteria (Drossman 2016). IBS is a highly common FGID of unknown origin, with a worldwide prevalence of up to 20% in the general population (Lovell and Ford 2012). Based on bowel habit, IBS can be subtyped into constipation predominant (IBS-C), diarrhea predominant (IBS-D), mixed (IBS-M), and unsubtyped (IBS-U). Altered intestinal permeability has been demonstrated in all IBS subtypes in different studies (Camilleri et al. 2012) by in vivo (oral probe excretion assays) and ex vivo (biopsies mounted in Ussing chambers) methodology. Alteration in barrier components (distorted TJ patter, low-grade mucosal inflammation) in these patients is well documented; however, no pathognomonic biomarker has been validated yet. Increased permeability has been linked to downregulation of ZO-1 and proteasome-mediated OCDN degradation in the colonic mucosa of IBS patients (Coeffier et al. 2010; Piche et al. 2009). In the jejunum, downregulation and redistribution of ZO-1 and OCLN are associated with mast cell activation and symptom severity (Martinez et al. 2012, 2013), supporting the hypothesis that clinical manifestations of IBS may rely on mast cell-related impairment of epithelial TJ function. Recently, increased B cell and plasma cell activation, with IgG production in the jejunal mucosa, has been described (Vicario et al. 2015) and may represent activated acquired immunity towards permeability to luminal antigens.

6 Therapeutic Interventions to Modulate Barrier Function

FGIDs are a clinical problem of substantial magnitude for the health care system due to the high prevalence, the chronic nature of symptoms, and the absence of satisfactory therapy. Several pathophysiological mechanisms have been suggested but the presence of an impaired permeability seems a common pattern. Whether an altered barrier function precedes the local immune activation already observed in these patients and whether this is crucial in symptom generation need to be elucidated. The complex pathogenesis of these disorders impedes an effective therapeutic approach; however targeting the barrier function stands as a reasonable strategy. From a therapeutic point of view, this strategy identifies two main groups of agents: direct modulators of epithelial integrity and compounds that target local immune activation.

6.1 Direct Effect on the Barrier Function

6.1.1 Glutamine

Glutamine is a nonessential amino acid that is an important energy source for enterocytes and colonocytes. Glutamine preserves protein synthesis and paracellular permeability to ions and dextran molecules of 4 kDa in Caco-2 cells submitted to luminal fasting (Le Bacquer et al. 2003). It also reduces macromolecular permeability (40 kDa) (Kouznetsova et al. 1999) and its deprivation facilitates TNF-α-induced bacterial translocation without affecting TEER (Clark et al. 2003). These and other in vitro studies suggest that glutamine supplementation restores the increased paracellular permeability to ions and small molecules by interfering with the leak pathway (Beutheu et al. 2013). Although studies in preclinical animal models characterized by an impaired barrier function show a consistent effect of glutamine in preventing all alterations, studies in patients with impaired barrier function have provided scarce and contradictory results (Den Hond et al. 1999; Noyer et al. 1998; Peng et al. 2004). This discrepancy can be due to the fact that glutamine has to be administered when the barrier function is not yet altered, as close as possible prior to the injury or for a longer time. Recently, a randomized placebo-controlled trial in IBS-D showed that oral glutamine supplementation (10 g three times daily for 8 weeks) improves gastrointestinal symptoms and restores intestinal permeability compared to placebo, suggesting that glutamine may be a useful therapeutic agent to restore impaired barrier function in these patients (Basra et al. 2013).

6.1.2 MLCK Inhibitors

As mentioned before, the leak pathway and the bacterial translocation through the enterocyte are controlled by the MLCK. Then, MLCK inhibitors could be potential drugs to treat or prevent alterations in the barrier function. For the moment, these type of inhibitors are only tested in preclinical animal models with a promising effect. Inhibition of MLCK-dependent cytoskeleton contraction by ML-7 prevents the LPS-induced paracellular permeability, bacterial translocation, and hyperalgesia (Moriez et al. 2005). Similarly, the TJ blocker 2,4,6 triaminopyrimidine (TAP) infused intracolonically or i.p. administration of ML-7 suppressed the stress-induced increase in colonic paracellular permeability and sensitivity to colonic distension (Ait-Belgnaoui et al. 2005). There are evidences that similar drugs as ML-9 have an effect on human tissue in vivo (Feighery et al. 2008). The beneficial effects on barrier function demonstrated by specific MLCK inhibitors could be a new therapeutic option in GI disorders.

6.1.3 Zonulin Peptide Inhibitors

Zonulin was identified as a physiological regulator of paracellular intestinal permeability. When the intestinal epithelium (isolated small intestine or epithelial cells) of several animal species including nonhuman primates is exposed to affinity-purified zonulin, TEER is reversibly reduced. Gluten and commensal and pathogenic bacteria have been described as luminal factors that release zonulin from the enterocytes. Then, zonulin binds to the epidermal growth factor receptor (EGFR) and protease-activated receptor 2 (PAR-2) of the enterocyte initiating a complex

signaling pathway that results in TJ disarrangement and increased intestinal permeability (Fasano 2011). In vivo, the zonulin peptide inhibitor larazotide acetate (synonyms FZI/0 and AT1001) normalized the permeability defect in IL-10 knockout mice and prevented subsequent colitis (Arrieta et al. 2009). Moreover, larazotide restored the increased conductance and HRP flux in gluten-sensitive transgenic mice (Gopalakrishnan et al. 2012). A recent randomized control trial in celiac disease patients showed that despite the beneficial effect on symptoms of this first-in-class oral peptide that prevents TJ opening (Leffler et al. 2015), the intestinal permeability evaluated by the lactulose/mannitol test is not reduced (Kelly et al. 2013; Leffler et al. 2012). The use of zonulin peptide inhibitors for treating GI disorders needs to be further studied.

6.2 Effect on Immune Activation

The association between mucosal inflammation and increased epithelial permeability reasonably suggests the use of anti-inflammatory therapy as a strategy to restore barrier dysfunction. However, despite significant inflammatory markers (yet low, as compared with inflammatory GI disease), a common inflammatory pattern in terms of cell infiltration and mediators release has not been determined as pathognomonic in FGIDs. Research has identified mast cells, eosinophils, and T cells as leaders of this feature; however most therapeutic assays have been designed to target mast cell activation in FGIDs, especially in IBS. Oral treatment with mesalazine, an anti-inflammatory drug, was effective in reducing mucosal mast cell and T lymphocyte infiltration (Corinaldesi et al. 2009) and ketotifen, a histamine H1-receptor antagonist and a mast cell stabilizer, reduced visceral perception in IBS patients (Klooker et al. 2010), drugs not assayed in FD, despite evidences of mast cell infiltration and activation (Vanheel et al. 2015). Another mast cell stabilizer, disodium cromoglycate, has offered promising results in reducing mast cell activation and also improving GI symptoms in IBS (Lobo et al. 2015) and FD (Friesen et al. 2006), although the underlying mechanism of barrier improvement remains to be determined (Mereu et al. 2015). Preclinical research supports the use of CRF antagonists (Million et al. 2013) and endocannabinoids and non-cannabinoid biolipids (Fichna et al. 2014) to control main GI symptoms by mechanisms that include neural modulation and mast cell inhibition, but clinical studies are necessary to validate its use.

7 Summary

The intestinal barrier exerts a fundamental role in homeostasis maintenance as it represents the largest surface exposed to the external environment. The regulation of this defensive system is very complex and relies on tight communication between its structural and functional constituents. Increased intestinal permeability is a common finding in FGIDs, and may be the result of altered structural elements, immune activity,

and neural response. Understanding the molecular mechanisms involved in the deregulation of the cell-to-cell adhesion proteins, especially TJ, and response to luminal pathogens is crucial to better design effective therapeutic strategies in these disorders. However, single interventions do not offer satisfactory results in most FGIDs and the synergistic effect of several compounds, acting at different levels of the barrier function (epithelial integrity restoration, reestablishment of physiological inflammation) may better target disrupted mechanisms and result in a more beneficial outcome.

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Conflict of Interest

The authors disclose no conflicts of interest and no financial arrangements with any company whose product figures in the submitted manuscript.

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Irritable Bowel Syndrome and Stress-Related Psychiatric Co-morbidities: Focus on Early Life Stress

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Contents

Abstract

Irritable bowel syndrome is a functional gastrointestinal disorder, with stress playing a major role in onset and exacerbation of symptoms such as abdominal pain and altered bowel movements. Stress-related disorders including anxiety and depression often precede the development of irritable bowel syndrome and vice versa. Stressor exposure during early life has the potential to increase an individual's susceptibility to both irritable bowel syndrome and psychiatric

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disease indicating that there may be a common origin for these disorders. Moreover, adverse early life events significantly impact upon many of the communication pathways within the brain-gut-microbiota axis, which allows bidirectional interaction between the central nervous system and the gastrointestinal tract. This axis is proposed to be perturbed in irritable bowel syndrome and studies now indicate that dysfunction of this axis is also seen in psychiatric disease. Here we review the co-morbidity of irritable bowel syndrome and psychiatric disease with their common origin in mind in relation to the impact of early life stress on the developing brain-gut-microbiota axis. We also discuss the therapeutic potential of targeting this axis in these diseases.

Keywords
Brain-gut-microbiota axis • Depression • Early life stress • Irritable bowel syndrome • Psychiatric disease

1 Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder (FGID) affecting about 20% of people worldwide (Lovell and Ford 2012). IBS patients suffer from recurrent abdominal pain associated with altered bowel habits without obvious demonstrable structural abnormalities (Drossman 1994). IBS is currently diagnosed based on symptoms outlined by the Rome IV criteria (Palsson et al. 2016). While symptoms range from extremely debilitating to mild or moderate this disorder has a significant impact on the quality of life of the patient which is comparable to other disorders such as diabetes mellitus and hepatitis (Enck et al. 2016). IBS is often co-morbid with other somatic disorders such as pain syndromes, overactive bladder and migraine (Enck et al. 2016) as well as psychiatric disease such as anxiety and depression (Sibelli et al. 2016).

IBS is a complex and multifaceted disorder which has been proposed as a systems disease involving not only individual systems including the nervous, endocrine, immune, digestive, microbiota and the environment but also the interactions of these systems (See Fig. 1) (Mayer et al. 2015b). Multiple systems within the periphery and the brain interact bi-directionally within the brain-gut axis and these systems form loops that can reinforce the dysfunction seen in IBS (Mayer et al. 2015b).

Although highly prevalent, patients with IBS do not always receive optimal treatment and no comprehensive disease model has emerged that would guide the development of novel and effective therapies. This is due to the range of functional alterations as well as the noted complexity of these system-level interactions. IBS-related biological abnormalities occur at several levels of the brain-gut-microbiota axis: gut epithelium and barrier (Bischoff et al. 2014; Hyland et al. 2014), neuroendocrine system (Mawe and Hoffman 2013), immune system (Dinan et al. 2006), brain structure and function (Mayer et al. 2015a), stress response (Dinan et al.

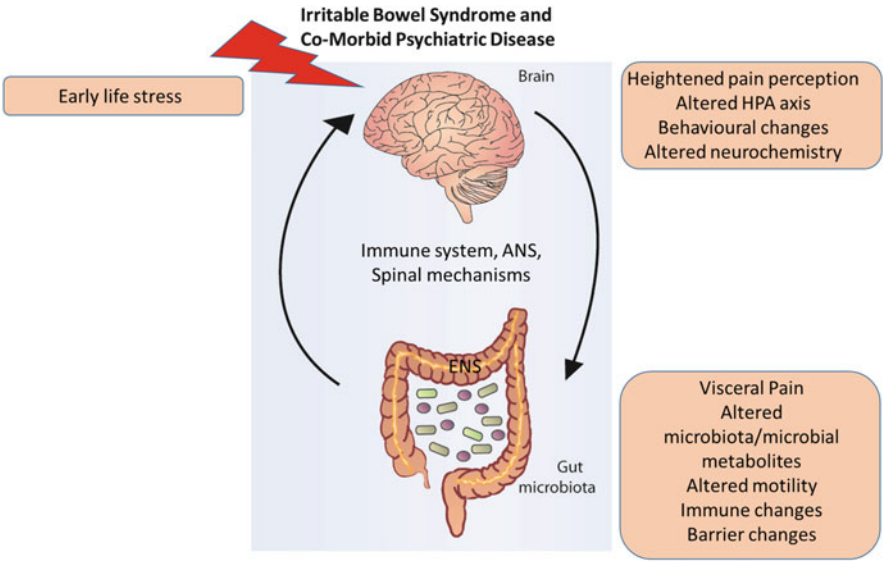

Fig. 1 Co-morbid irritable bowel syndrome and psychiatric disease-common origins. Irritable bowel syndrome (IBS) and psychiatric disease are often co-morbid. Both disorders have early life origins. The brain and the gut show reciprocal interactions in health and disease. Early life adversity induces epigenetic changes in this signalling pathway and leads to altered brain outputs via the central nervous system to affect behaviour, autonomic nervous system (ANS) and hypothalamic pituitary adrenal (HPA) axis. In the gastrointestinal tract changes include increase visceral pain sensation, altered gut microbiota, altered motility and barrier function. The changes that occur are reinforced by the altered communication between the individual systems

2006), affective domains (Elsenbruch 2011), cognition (Kennedy et al. 2014a), pain modulation (Major et al. 2016) and the gut microbiota (Mayer et al. 2014b).

Familial studies of IBS highlight that having a first degree relative with bowel issues or abdominal pain is associated with increased reporting of IBS symptoms (Locke et al. 2000). Parental attitudes play a role in the development of IBS as well as non-gastrointestinal disorders as children of parents with IBS tend to have more health care visits and are more prone to have gastrointestinal (GI) and non-GI symptoms (Kanazawa et al. 2004; Levy et al. 2004). The fact that IBS and related symptoms run in families is not only due to "learned illness" behaviour as twin studies in Australia (Morris-Yates et al. 1998) and the USA (Levy et al. 2001) have shown concordance rates for IBS between monozygotic twins to be higher than that between dizygotic twins. Polymorphisms such as for the serotonin transporter (SERT) (Yeo et al. 2004) and interleukin-10 (Gonsalkorale et al. 2003) are associated with an increased risk of IBS.

Risk factors for developing IBS include being female, previous infection, early life stress and stress-related disease (Enck et al. 2016). There is a female predominance with IBS (Drossman et al. 1993) potentially due to differences in gender

patterns of symptom reporting and health care utilisation. Early life stress increases the risk of developing IBS and this type of stress is associated with dysfunction of many of the systems that are abnormal in this disorder.

2 Co-morbid Stress-Related Psychiatric Disease and IBS

Research into the co-morbidity of stress-related psychiatric disorders and IBS has been continuing for over 40 years (Liss et al. 1973; Hausteiner-Wiehle and Henningsen 2014). Depression and anxiety are among the most disabling and prevalent psychiatric conditions (Kessler et al. 2008). These disorders are often co-morbid with a variety of other systemic and somatic illnesses, ranging from cardiovascular disease (Xiang and An 2015) to alterations in the GI tract (Dinan and Cryan 2013). According to previous studies, nearly 50–60% of IBS patients experience major psychosocial problems (Levy et al. 2006). Along with depressive symptoms IBS patients can also feel exhausted, have sleeping problems, and a loss of appetite and anxiety is often manifested as worrying, rumination and panic attacks (Hausteiner-Wiehle and Henningsen 2014).

There has been an abundance of reports implicating stress in the onset or exacerbation of symptoms of IBS (Fukudo 2013). It has been reported that following acute gastroenteritis, prior anxiety and depression might be risk factors for the subsequent development of post-infectious IBS (Marshall et al. 2010). In addition, high anxiety and depression scores have been reported in a post-infectious IBS population following initial infection (Schwille-Kiuntke et al. 2011; Lee et al. 2015).

Individual responses to stress vary, a phenomenon thought to be based both on genetic and epigenetic mechanisms (Dinan et al. 2010). The area of stress susceptibility and stress resilience is of interest across all areas of psychiatry (Charney 2004; Haglund et al. 2007) and also in the context of co-morbidities such as IBS (Drossman et al. 2000).

In a preclinical setting, animal models of IBS are predominantly stress-based models (Moloney et al. 2015) aimed at elucidating biomarkers of this complex biopsychosocial disorder (Tanaka et al. 2011). Animal models such as the Wistar Kyoto rat and the maternal separation model both show co-morbid IBS and stress-related central nervous system (CNS) alterations (Felice et al. 2015).

Physical and psychological stressors have also been shown to increase the perception of symptoms of IBS including visceral pain, which is referred to as visceral hypersensitivity (Théodorou 2013). Stess also has a significant impact on gut motility (Venkova et al. 2010), intestinal barrier function (Hyland et al. 2014) and the gut microbiota (Clarke et al. 2014) all of which are implicated in IBS (Kennedy et al. 2014b). Furthermore, psychological (i.e. hypnotherapy), non-pharmacological and pharmacological interventions that mediate stress perception have been demonstrated to ameliorate IBS symptoms (Simren et al. 2004; Cryan

and Dinan 2012). It is also well documented that early life stress is a predisposing factor for psychiatric disease (Lupien et al. 2009) highlighting the overlapping origins of IBS and stress-related psychiatric disease.

3 Early Life Stress

Stress at different stages throughout life, and especially early in life, can have deleterious effects on both psychological wellbeing and gastrointestinal function of the host. Early life represents a critical neurodevelopmental window during which an organism is particularly vulnerable to stressful insults. Adverse events can take many different forms including physical trauma, loss of a parent and abuse (physical/sexual) all of which have been associated with an increased risk of developing FGIDs later in life (Barreau et al. 2007; Chitkara et al. 2008) and facilitating the development of depression and anxiety disorders (Plotsky et al. 2005).

During early life, the hypothalamic-pituitary-adrenal (HPA) axis, the main stress axis, is characterised by reduced responsiveness to stress, defined as the stress hyporesponsive period (SHRP), which is hypothesised to protect the developing brain from elevated glucocorticoids (Sapolsky and Meaney 1986; Levine 1994; Gunnar and Cheatham 2003). However, adverse early life events can produce long-lasting epigenetic changes in the HPA axis resulting in impaired glucocorticoid negative feedback and an increased susceptibility to stress-related disorders in adulthood (Francis et al. 1996; Liu et al. 1997; Weaver et al. 2004).

Early life adverse events are associated with alterations in the bidirectional communication within the brain-gut-microbiota axis (Clarke et al. 2014). A growing body of literature, albeit predominantly still at the preclinical stage, shows that perturbation of this axis results in alterations in the stress response and behaviour and has been proposed to be involved in several CNS diseases such as anxiety (Davis et al. 2016; Pirbaglou et al. 2016), depression (Dash et al. 2015; Kelly et al. 2016b; Slyepchenko et al. 2017), autism (O'Mahony et al. 2015a, b; Inoue et al. 2016), Alzheimer's disease (Pistollato et al. 2016), stroke/brain injury (Winek et al. 2016), Parkinson's disease (Dinan and Cryan 2016; Sampson et al. 2016; Scheperjans 2016) as well as IBS (Kennedy et al. 2014b).

4 The Brain-Gut-Microbiota Axis in IBS and Psychiatric Disease

The brain-gut-microbiota axis is the bidirectional communication network between the CNS and the GI tract which involves neural and metabolic pathways, immune and endocrine mechanisms (Cryan and O'Mahony 2011; Bercik et al. 2012; De Palma et al. 2014b; Dinan et al. 2015). Under normal physiological conditions this axis is responsible for the modulation of digestive processes (i.e. motility, secretion), immune function, perception and emotional response to visceral stimuli (Mayer et al. 2006a, b). The gut microbiota within each of our GI tracts is individual

specific and is composed of mainly bacteria but also viruses, archaea and protozoa, and is approximately the same number as the number of human cells in the human body (Sender et al. 2016).

The high co-morbidity between stress-related psychiatric symptoms such as anxiety with GI disorders including IBS and inflammatory bowel disease (Bonaz and Bernstein 2013; Dinan and Cryan 2013) is further evidence of the impact of this axis.

Whilst there is a large body of clinical and preclinical data highlighting the alterations at several interacting levels of the brain-gut-microbiota axis in IBS (Kennedy et al. 2014b) the concept of an altered brain-gut-microbiota axis in psychiatric disease is gaining traction (Slyepchenko et al. 2017). For example, depression in a patient population was shown to be associated with decreased gut microbiota richness and diversity (Kelly et al. 2016b). Faecal microbiota transplantation from depressed patients to microbiota-depleted rats induced changes in behaviour as well as physiological features characteristic of depression (Kelly et al. 2016b). This suggests altered signalling originating from the gut microbiota may play a causal role in the development of the cardinal features of depression.

Many studies focus on the brain to gut direction of this axis as an origin for FGID such as IBS, with anxiety and depression being significant predictors of developing IBS (Koloski et al. 2012). Furthermore, evidence from preclinical (O'Mahony et al. 2009; Prusator et al. 2016) and clinical studies (Dickhaus et al. 2003) highlights the influence of brain to gut pathways. A prospective study following 1,002 cases and controls randomly selected from the Australian electoral roll showed that among people free of FGID, higher levels of anxiety but not depression were predictive of developing FGIDs 12 years later (Koloski et al. 2012). Moreover, among those with an FGID that did not have anxiety or depression at baseline were more likely to develop depression and anxiety than healthy controls at the 12-year follow-up. This indicates that gut to brain signalling is also extremely significant with regards to development of co-morbid FGIDs such as IBS and psychiatric disease with two thirds of individuals with FGIDs preceding a mood disorder and one third with a mood disorder preceding an FGID (Koloski et al. 2016). Therefore, it has been demonstrated that the brain to gut pathway is exists in a subset of these co-morbid patients but, in a major subgroup, gut manifestations appear to occur prior to psychological distress, implicating a dominant gut to brain pathway. A number of gut to brain signalling pathways may be disturbed, from gut bacteria, to alterations in innate immunity, to an abnormal serotonergic system (Dinan and Cryan 2013; Keightley et al. 2015). While these patient sets appear phenotypically similar, those with a predominant gut to brain pathway may respond to gut directed interventions that modulate locally disturbed pathways, such as prebiotics, probiotics or non-absorbable antibiotics. Alternatively, those with a predominant brain to gut pathway may best respond to centrally directed therapy, which may explain resistance to interventions in some patients.

Whilst the source of the disease, be it CNS or gut, is important, the axis between the two is dysfunctional in both IBS and psychiatric disease. Moreover, these

disorders have overlapping aetiologies with respect to the involvement of early life stress in the manifestation of IBS and psychiatric disease.

5 Developing Systems Within the Brain-Gut-Microbiota Axis

The brain-gut-microbiota axis, including the immune system, neural connections such as the vagus, the intestinal barrier, the stress axis and the microbiota itself develop in parallel with the neonatal CNS. The concomitant development of the CNS and the communication pathways of the brain-gut-microbiota axis during early life means there is potential for interaction between the systems in both normal and pathological situations to potentially lead to disorders such as IBS and psychiatric disease.

Development of the human brain continues into postnatal life and even adolescence (Marin 2013). While the framework of subcortical–cortical connectivity is established before midgestation and the intracortical circuits that integrate information across functional domains also form before birth, their growth and modification extends into puberty (Levitt 2003). Due to the dynamic nature of brain development during early life a vulnerability exists that allows both genetic and environmental perturbations to influence adaptive changes in neuronal circuits. Historically the neonatal blood–brain barrier was considered immature and leaky. Although it is now known that this barrier is fully functional at birth, the delicacy of the developing cerebral vasculature indicates that the neonatal brain is more vulnerable to circulating substances than during adulthood (Saunders et al. 2012). This is particularly important in the context of the variety of new molecules which the infant brain is exposed to postnatally and at various nutritional and developmental milestones (MacFabe 2012).

Humans babies are born with an immune system that is immature and is established from birth onwards (Holsapple et al. 2003). As the immune system develops there is an induction of a pathogen-specific response with establishment of an immunological tolerance to dietary constituents and commensal microbes (Rautava and Isolauri 2002). The GI tract contains the largest immunological tissue in the body and colonisation studies have demonstrated microbial stimulation is required for maturation of lymphoid structures (Smith et al. 2007). Colonisation of the gut also greatly influences adaptive immunity development, altering abundance, phenotype and function of T and B cells (El Aidy et al. 2013b; Williams et al. 2006; Gaboriau-Routhiau et al. 2009; El Aidy et al. 2012).

The vagus nerve provides a direct two-way neural connection between the brain and the gut providing both motor and sensory innervation for several essential functions such as sensation of pain, sphincter operation and peristalsis. Vagotomy studies highlight the importance of the vagus in relaying signals from the microbiota to the brain (Bravo et al. 2011). While axons from cell bodies in ganglia in the brainstem find their way to their enteric targets during development in utero, efferent terminals increase in number and density in the early postnatal period (Ratcliffe et al. 2011) with sensory axons maturing later in postnatal life (Ratcliffe

et al. 2011). Given the importance of normal vagal function it is conceivable that disorders of the brain-gut-microbiota axis such as IBS may be rooted in developmental abnormalities of enteric vagal innervation (Ratcliffe et al. 2011).

The HPA axis provides hormonal communication within the brain-gut-microbiota axis (Dinan et al. 2006; Dinan and Cryan 2013). The appropriate development of this axis determines the ability of an individual to cope and adapt to stressors and as mentioned above the hyporesponsive period exists in early life. There is also evidence that this SHRP exists in human children (Gunnar et al. 2003) and is thought to extend throughout childhood. It appears that if this SHRP is maintained then the HPA axis develops normally and the child responds appropriately to stressors as an adolescent and adult. In contrast if this period is interrupted with stressful situations that are substantial enough to mount a stress response the axis can develop to over-activate in stressful situations (Raabe and Spengler 2013) such as in disorders such as IBS.

Microorganisms begin to largely colonise the infant GI tract at birth. Studies have shown that colonisation may occur before birth (El Aidy et al. 2013a) but the major establishment takes place in early life with a stable community by the age of 3 years in humans. Colonisation patterns are dependent on a number of factors including genetics, maternal health, mode of delivery, feeding regime and exposure to medication or disease (O'Mahony et al. 2015a). This early colonisation period is particularly important as host–microbe interactions are forged and can determine the health of an individual and risk of disease.

The enteric nervous system (ENS) provides the intrinsic innervation of the bowel (Burns and Pachnis 2009) and is derived from vagal and sacral neural crest cells which invade, proliferate and migrate within the wall of the GI tract (Lake and Heuckeroth 2013). While the ENS of the newborn is mature enough to permit oral feeding, major development occurs in the postnatal period (Gershon 2012). This allows the events of early life such as growth, dietary changes as well as bacterial colonisation to influence the ENS development. Thus, it is plausible that epigenetic alterations in the enteric microenvironment during the development of the ENS can induce lasting changes on this nervous system (Gershon 2012) and lead to disease.

The intestinal barrier is the host's first line of defence against luminal pathogens and toxins, but must permit tolerance towards the commensal microbiota and environmental antigens while allowing passage of water, electrolytes and nutrients (Bischoff et al. 2014; Hayes et al. 2015). This intestinal barrier is physiologically and morphologically immature at birth and upon bacterial colonisation, it undergoes changes in order to cope with microbial interactions and reach a homeostatic state that maintains gut function and provides protection to the host, whilst avoiding aberrant inflammation. Barrier changes induced by colonisation have been studied using germ-free animal models, and have centred on the impact of commensal microbiota on immune parameters of barrier maturation (El Aidy et al. 2012). Since impaired barrier defences and permeability regulation are associated with a number of GI and psychological disorders, determining if barrier dysfunction originates from early life microbiota-induced alterations may further elucidate disease pathogenesis.

6 Impact of Early Life Stress on the B-G-M Axis-Implications for IBS and Co-morbid Psychiatric Disease

Several animal models of IBS have been developed including early life colon irritation both physical and chemical (Al-Chaer et al. 2000; Moloney et al. 2015), adult stress-induced hypersensitivity to colorectal distension (CRD) (Gue et al. 1997; Moloney et al. 2015) as well as post-infectious models (McLean et al. 1997; Barreau et al. 2007). While these are good animal models they really only reproduce one or some of the aspects of IBS of the syndrome and tend to lack complete validity. Given the early life psychological stress element of IBS, models such as maternal separation, which was developed to model neglect and abuse (O'Mahony et al. 2011), the limited nesting model, developed to reproduce abuse as a result of an impoverished environment, and the odour attachment learning model which emulates attachment to an abusive caregiver (Prusator and Greenwood-Van Meerveld 2016) have been used. Of these the maternal separation model really displays a robust and reproducible phenotype most similar to the IBS patient with significant alterations throughout the entire brain-gut-microbiota axis. Adding to the validity and usefulness of the separation model is the fact that it models the co-morbidity observed between IBS and psychiatric disorders. This co-morbidity suggests potential common neurobiological pathways involved in the aetiology such as early life stress. Moreover, the overlapping pathophysiologies in systems such as those within the brain-gut-microbiota axis also point towards a shared origin.

6.1 Early Life Stress and the CNS

6.1.1 Brain Neurotransmitter Systems and Impact of Early Life Stress

Altered functioning of monoamine neurotransmitter systems such as serotonin (5-HT) and noradrenaline within the central nervous system are seen in stress-related disorders such as psychiatric disease (Hirschfeld 2000) and IBS (O'Mahony et al. 2008). Serotonin is an essential neurotransmitter and in the brain it is involved in the emotional response (Brummelte et al. 2016) as well as descending pain modulation (Gershon 1999; Kim and Camilleri 2000), highlighting its role in the modulation of the brain-gut-microbiota axis (O'Mahony et al. 2015b). It is thought that 5-HT can act as a growth regulator in selective developmental events (Bonnin and Levitt 2011) as it is capable of inducing effects on target cells and organs during both the prenatal and postnatal periods (Nasyrova et al. 2009). Given that stress can influence the 5-HT system this of course has far reaching implications.

Most studies assessing the impact of early life stress on 5-HT are preclinical studies with reductions in the expression of SERT being observed in response to early life stress in non-human primate and rodent models (Lee et al. 2007; Kinnally et al. 2010). Expression patterns of the 5-HT transporter may contribute to the risk for adverse psychological outcomes following early life stress with higher SERT CpG methylation exacerbating the effects of early life stress in rhesus monkeys (Kinnally et al. 2010).

Decreased maternal care is also considered an early life stress and induces changes to 5-HT metabolism in the hippocampus (Henriques et al. 2014). Furthermore, maternal separation induces alterations in the central 5-HT system (Daniels et al. 2004; Sung et al. 2010), with increases in 5-HT in the frontal cortex, and increased expression of 5-HT receptors in the cortex and hippocampus (Vazquez et al. 2000; Matthews et al. 2001). Increased numbers of activated serotonergic neurons in the spinal cord and raphe nucleus both being associated with the modulation of pain are seen in adult rats following early life stress (Ren et al. 2007). This corresponds to the clinical presentation of IBS where we have shown that patients have an enhanced serotonergic response to a 5-HT_{1A} receptor agonist (O'Mahony et al. 2008). Moreover, antidepressants targeting central monoaminergic systems have shown efficacy in IBS patients (Xie et al. 2015) with tricyclic antidepressants showing greater benefit than selective serotonin inhibitors (Xie et al. 2015). Duloxetine, administered to co-morbid IBS-Major Depressive Disorder (MDD) patients in an open-label, 12-week trial led to significant improvement in both Gastrointestinal Symptoms Rating Scale and Montgomery-Åsberg Depression Rating Scale scores (Lewis-Fernandez et al. 2016). Abdominal pain severity decreased by 56% and both IBS and MDD symptoms improved gradually (Lewis-Fernandez et al. 2016).

Other monoamines altered by early life stress include noradrenaline which was found to be decreased in the cingulate cortex, an area associated with the expression of fear and anxiety (Arborelius and Eklund 2007). Also, glutamate is both a critical signal in the guidance of brain development and a major mediator of emotional, reward-related and cognitive behaviours in the adult brain (Lovinger et al. 2003). There is increasing evidence that early life emotional trauma produces long-lasting changes in glutamatergic neurotransmission with differences in the expression of hippocampal AMPA and NMDA glutamate receptors in offspring (Pickering et al. 2006; Ryan et al. 2009). This may also be linked to cognitive deficits found in rats subjected to postnatal separation (Huang et al. 2001; Pryce et al. 2003). Altered cognitive function, which was related to cortisol levels and independent of psychiatric co-morbidity was seen in IBS patients (Kennedy et al. 2014a).

Other changes in central pathways following early life stress include a decreased brain derived neurotrophic factor (BDNF) expression and hypermethylation of the BDNF exons IV and IX in the prefrontal cortex on postnatal data (PND8) with associated reduction of BDNF mRNA which persisted into adulthood in rats exposed to an abusive mother during the first week of life (Roth et al. 2009). Changes in BDNF expression are associated with exacerbation of visceral hypersensitivity in rats (Winston et al. 2014) as well as antidepressants (Nuernberg et al. 2016).

6.1.2 Activation Patterns/Structure Within the CNS Induced by Early Life Stress

Brain imaging techniques are important tools for delineating circuits that are dysfunctional in disorders of the brain-gut-microbiota axis (Mayer et al. 2006a, b;

Johnson et al. 2010). Early life stress has been associated with regional thinning of the subgenual cingulate cortex, a brain area implicated in the development of disorders of mood, and often co-morbid disorders, such as IBS (Gupta et al. 2016). Female IBS patients showed a decrease in thickness of this region and overall thinning in this area was associated with early life stress scores as well as the minor interleukin-1β allele indicating that regional neuroinflammation associated with stress has been suggested as a possible mechanism underlying these neuroplastic changes (Gupta et al. 2016). Central areas involved in the processing of the affective component of pain in IBS such as the pregenual anterior cingulate cortex and the orbital frontal cortex showed an increase in grey matter in IBS patients, which was abolished once data was corrected for anxiety and depression in these patients (Elsenbruch 2011). These findings further confirm the involvement of emotional systems in the processing of visceral pain.

Early life stress in rodents has been shown to induce differences in central activation patterns compared to controls (Gibney et al. 2009; Zhang et al. 2009). Quantification of c-fos (immediate early gene) can be employed to give an indication of regions activated both basally and following a stressful experience (Singewald 2007). The central areas most often notably differentially activated are associated with emotion and anxiety and include the anterior cingulate cortex, the medial amygdala and hippocampus (Troakes and Ingram 2009; Johnson et al. 2010) and pain modulation such as laminae I and II of the lumbarsacral spinal cord and the periaquaductal gray (Chung et al. 2007). Basally separated rats often display no difference in central activation compared to controls. Following noxious distension of the colon increased expression of central c-fos indicated that maternal separation sensitised the cingulate cortex and upregulated the activity of the ascending pathway at spinal level as well as the thalamo-cortico-amygdala pathway (Chung et al. 2007). The upregulation and sensitisation of these pathways may be responsible for the development of visceral hypersensitivity in IBS and an altered stress response seen in psychiatric disease.

6.2 Early Life Stress and HPA Axis Development

The HPA axis is the main stress axis and our ability to cope with stress and hassles in life is dependent on the activity of this axis (Scott and Dinan 1998). Altered functioning of this axis has been linked for many years to stress-related psychiatric disease (O'Keane et al. 2012) and is now well accepted to be involved in the onset and exacerbation of IBS symptoms (Kennedy et al. 2014c). Glucocorticoids, the endpoint of HPA axis activation, are required for normal brain development (Meyer 1983). Both suppressed and raised glucocorticoid levels impair brain development and functioning (Lupien et al. 2009). As mentioned the SHRP exists in both rodents and humans and is proposed to function as a protective mechanism for the developing CNS from high levels of circulating glucocorticoids (Levine 1994). During this time complex interactions occur between mother and pup/infant with nursing and grooming influencing neurodevelopment and behaviour in the offspring

(Ivy et al. 2008). Initially shown in rats, consistent low levels of maternal care act as a stressor to influence the development of the HPA axis, stress response and anxiety (Liu et al. 1997). This effect has since been shown across different species including mice, hamsters and non-human primates (Bale 2015). Moreover, human studies indicate that the quality of care received by a baby or toddler in early life is associated with the development of stress-related problems in later life (Struber et al. 2014). Parent–child interactions as well as the psychological wellbeing of the mother have a major impact on the development of the child's ability to cope with stress later in life as well as the development of psychiatric or behavioural disorders (Lupien et al. 2009).

Animal studies of early life stress directly show that despite the SHRP, stressors, such as maternal separation are capable of stimulating the HPA axis during this period as seen by increased adrenocorticotrophic hormone and glucocorticoids (Levine and Wiener 1988). The impact of separation of rat pups from their mother is seen throughout the CNS with increased density of corticotrophin releasing hormone binding sites (Anisman et al. 1998) and increased basal and stress-induced levels of the stress hormones, corticosterone and adrenocorticotrophic hormone (ACTH) (Walker et al. 1991; Suchecki et al. 1993, 1995; van Oers et al. 1998). This indicates a reduced sensitivity to glucocorticoid negative feedback (Sapolsky et al. 1990; Biagini et al. 1998). Maternal separation has also been shown to induce elevated ACTH levels in response to a stressor (Daniels et al. 2004), alter CRF binding sites in several brain areas (Vazquez et al. 2006), as well as affecting glucocorticoid and mineralocorticoid receptor expression (Wilber and Wellman 2009).

Other postnatal stressors that are powerful enough to mount a HPA axis response include modifying/altered maternal behaviour and administration of synthetic glucocorticoids (Lupien et al. 2009). Rodent studies have provided much of the basis for the implication that early life stress has a detrimental effect on HPA axis functioning in adulthood. The extrapolation to humans is not straightforward as the rodent brain is far less developed than the human brain at birth as mentioned above. Nonetheless situations such as low socioeconomic status, maltreatment and war can be considered as severe early life stress in humans do affect the development an appropriate stress response (Lupien et al. 2009).

6.3 Changes Within the Gastrointestinal Tract Related to Adverse Events in Early Life

IBS is associated with altered function and physiology of the gastrointestinal tract and usually without any obvious structural changes. Nonetheless these patients suffer from altered bowel motility with diarrhoea or constipation predominant symptoms as well as changes in mucus production and barrier function (Hyland et al. 2014). Psychiatric diseases such as depression are also associated with alterations within the gut including permeability of the gut wall possibly due to an altered gut microbiota (Kennedy 2014; Macedo et al. 2017).

The largest concentration of 5-HT in the body exists in the gut and here it is involved in motility, sensation and secretion. Hence alterations to the serotonergic system have diverse impacts on the gastrointestinal function. Increases in mucosal 5-HT-containing enterochromaffin cells have been seen in the gut of IBS subjects (Dunlop et al. 2005). Early life stress in rodents induces a significant increase in the numbers of colonic enterochromaffin cells (Bian et al. 2010) as well as increasing 5-HT content in the colon following CRD when compared to control rats (Ren et al. 2007). Despite increased levels of 5-HT, and potentially as a compensatory response to elevated 5-HT content, rats subjected to early life stress also display increased expression of colonic SERT relative to control animals (Bian et al. 2010). This increase in colonic 5-HT has implications for altered motility and sensation in IBS patients (Gershon 1999; Gareau et al. 2007). Polymorphisms in the SERT gene are known to result from early life stress and predispose to disorders such as IBS (Jin et al. 2016) and depression (Homberg and van den Hove 2012).

Increased macromolecular permeability noted in separated rats compared to non-separated controls appears to be mediated largely by cholinergic pathways (Gareau et al. 2007). Separated rat pups displayed an elevated horseradish peroxidase flux in the colon, which was inhibited by muscarinic and nicotinic receptor antagonists (Gareau et al. 2007) indicating that this neurotransmitter is also involved in the dysfunctional gut to brain signalling. Moreover, increased gut permeability is linked to bacterial translocation (Barreau et al. 2004) which leads to increased systemic inflammation and potentially sickness or depressive behaviours.

Maternal separation induces time dependant changes in the development of ENS density and plasticity (Barreau et al. 2008). Notable is the increased immunoreactivity of the pan-neuronal marker PGP 9.5 in colonic sections from adult separated animals which is absent 4 weeks after the stress, in contrast increased synaptogenesis occurs 4 weeks post-separation and normalises in adulthood (Barreau et al. 2008). Despite increased nerve density and synaptogenesis occurring in stressed animals no concomitant increase in the expression of the sensory neurotransmitter Calcitonin gene related peptide was observed in the colon of these animals (Barreau et al. 2008).

The elaboration of the mucosal immune system is also contingent on the host microbiota with the colonising and residential bacteria imprinting and instructing the mucosal immune system throughout the life of the host (Di Mauro et al. 2013). Gut associated lymphoid tissue, which modulates tolerance versus luminal antigens and prevents the transit of potentially harmful antigens across the intestinal barrier, is dependent on the microbiota for development (Bauer et al. 2006). The immature immune system of the newborn has a Th2 bias which shifts to a Th1 response with bacterial colonisation (Di Mauro et al. 2013). Different lactic acid producing bacteria such as *Bifidobacterium* and *Lactobacillus* have been shown to promote a Th1 shift (Tsai et al. 2012) indicating that qualitative differences in composition may affect immunological homeostasis. Administration of broad-spectrum antibiotics, frequently used in paediatric practices, has been shown to reduce the biodiversity of faecal microbiota and delay the colonisation by certain *Bifidobacterium* and *Lactobacillus* strains (Bennet et al. 2002). Also, early life antibiotic exposure is associated with

allergic disease (Marra et al. 2006; Murk et al. 2011) and inflammatory bowel disease (Shaw et al. 2010).

6.4 Stressful Events in Early Life and the Gut Microbiota

The gut microbiota has emerged as an important factor that potentially contributes to the pathophysiology of IBS (De Palma et al. 2014a; Hyland et al. 2014) yet there is conflicting evidence regarding the organisation and function of the gut microbiota in both adult and paediatric patients (Simren et al. 2013; Mayer et al. 2014b). Several studies have reported decreased proportions of the genera *Lactobacillus* and *Bifidobacterium* with increased ratios of Firmicutes to Bacteroidetes at the phylum level (Rajilic-Stojanovic et al. 2011; Nagel et al. 2016) [for review, see Rajilic-Stojanovic et al. (2015)]. The causal role for microbiota in IBS symptoms has yet to be fully established and may be due to changes in motility and secretion as these are frequent in IBS.

Stress itself, predominantly mediated through HPA axis, has long been known to influence the composition of the gut microbiota (Tannock and Savage 1974). However, this influence is now more widely acknowledged and we have shown that stress in early life alters the gut bacterial content of the adult rat (O'Mahony et al. 2009). Hence, early life stress capable of activating the HPA axis can potentially impact on the developing microbiota in the neonatal period and vice versa, ultimately leading to dysbiosis and an inappropriate stress response. Few studies in humans have addressed this, for ethical reasons, but Bailey and Coe (1999) investigated the stability of the indigenous microflora in maternally separated rhesus monkeys (Bailey and Coe 1999). There was a significant decrease in faecal bacteria, in particular *Lactobacilli*, 3 days after separation, which correlated with stress-indicative behaviours and susceptibility to opportunistic bacterial infection. Interestingly, another study which investigated the effect of early life stress on the intestinal microbiota on PND15 and PND35 (Garcia-Rodenas et al. 2006) observed an effect of separation on bacterial species at the later date only. Total aerobic and anaerobic bacteria were increased in the small intestine and in the colon of the separated animals, with Enterococci and Bacteroides being the species that were affected the most. Long-lasting effects of the neonatal stress in the luminal environment and/or the mucosal immune system may be responsible for these results. For instance, stress has been reported to decrease gastric acid (Lenz and Druge 1990) and increase bicarbonate (Lenz and Forquignon 1990) secretion in rats, thereby resulting in higher pH of the digesta and lowering the capacity of the upper part of the intestine to avoid penetration of acid-sensitive microorganisms. A complete gut microbiota appears to be necessary for the impact of maternal separation to occur as germ-free mice (without any bacteria) did not respond to this early life stress (De Palma et al. 2015). Furthermore, the separated signature was transferrable via the microbiota.

A pioneering study carried out by Sudo et al. (2004) has shown that normal development of the HPA axis is contingent on the presence of bacteria during a specific postnatal period. Germ-free mice exposed to restraint stress exhibited a significantly higher level of ACTH and corticosterone than conventionally colonised

mice (Sudo et al. 2004). Colonisation of these mice with *B. infantis* during early life prevented the exaggerated response to the stressor (Sudo et al. 2004). Taken together, these studies indicate that the stress system and the gut microbiota are capable of influencing each other during early life.

6.5 Pain Pathways and Influence of Early Life Stress

Given the intertwined relationship of stress and pain it is not surprising that alterations in both pathways resulting from early life stress continue to aberrant interactions in adulthood. The early neonatal period is a time of intense plasticity for both somatic and visceral sensory systems. This neuronal plasticity often contributes to adaptive or maladaptive function, and possibly to the development of chronic pain. The nociceptive neuronal circuits form during embryonic and postnatal times when painful stimuli are absent or limited. During this critical period, particularly before the maturation of the descending inhibitory systems (Boucher et al. 1998), pain can lead to prolonged structural and functional alterations in nociceptive pathways that can be maintained in adult life (Barreau et al. 2007).

A subset of IBS patients suffer from increased sensation from the GI tract and IBS is often co-morbid with other pain syndromes such as migraine, bladder pain and somatic pain (Enck et al. 2016). Moreover, psychiatric disorders such as depression and anixety often present co-morbidly with increased visceral pain (Mayer et al. 2014a). However, the reason and causality for this relationship is unclear; it is not known if stress-related disorders predispose to the development of visceral hypersensitivity or if altered emotional states arise in response to visceral hypersensitivity.

In IBS abnormal central processing of nociceptive signals in the brain-gut-microbiota axis plays an important role in the pathogenesis of visceral hypersensitivity (Tillisch and Mayer 2005). The processing of visceral nociceptive signals involves an integrated network of various structures, which include sensory cortex, thalamus, hypothalamus and midbrain. These centres modulate processing of the nociceptive signals at the spinal cord level through descending pathways (Gebhart 2004). CRD is used to measure visceral hypersensitivity and readouts include threshold pressure as well as the number of visually identifiable pain behaviours (Al-Chaer et al. 2000). We and others have shown that rats exposed to adverse early life events suffer from stress-induced visceral hyperalgesia and increased colonic motility (O'Mahony et al. 2009; Gosselin et al. 2010).

Dysfunction of the descending pain modulatory system has been implicated in stress-induced visceral hyperalgesia associated with early life stress (Chung et al. 2009; Sengupta 2009). An enhanced neuronal sensitivity to noxious visceral stimuli in the central brain nuclei has also been demonstrated (Chung et al. 2007; Zhang et al. 2009). These nuclei, including the cingulate cortex, the thalamus and the amygdala, play important roles in the central pain matrix and are involved in generating emotional and autonomic responses (Mayer 2000; Jones et al. 2006).

The amygdala, in particular, plays a central role in the emotional affective component of pain (Phelps and LeDoux 2005; Neugebauer 2007). The increased excitability in response to visceral stimuli in the spinal cord reflects changes in plasticity in descending pain circuitry (Chung et al. 2007). An increased level of c-fos-like immunoreactive nuclei have been found predominantly in the dorsal horn following CRD, with significantly more being present in separated animals (Ren et al. 2007). This suggests that early life stress leads to hyper-activation of neurons in the dorsal horn during CRD in response to descending influences originating in the brain stem (Traub et al. 1996). More recently we have shown that CRD induces a hyper-responsive c-fos activation in the amygdala and prefrontal cortex of rats following early life stress compared to controls (Gibney et al. 2009). Furthermore, stress in early life also results in a decrease in the levels of the glial glutamate transporter EAAT1 in the spinal cord (Gosselin et al. 2010) whilst the EAAT activator riluzole reversed the associated visceral hypersensitivity (Gosselin et al. 2010). These data provide a novel strategy for treating early life stress-induced pain. Given that riluzole has shown antidepressant potential in clinical trials (Sanacora et al. 2007; Machado-Vieira et al. 2009; Mathew et al. 2010) it is tempting to speculate that it may be an effective treatment option for multiple manifestations of early life stress.

Infantile colic is common and is defined as excessive crying in healthy, thriving infants (Milidou et al. 2014). An underlying organic cause for colic is found in less than 5% of these infants. While the aetiology of colic is not fully understood several mechanisms have been proposed such as psychological (Brand et al. 2011) and GI factors (Savino et al. 2011; Barnes and Yeh 2015). Colic increases the risk of developing migraine, recurrent abdominal pain, allergic disease and psychological disorders later in life (Savino et al. 2005; Napthali et al. 2015) as it possibility acts as an early life stress on the infant and on child–parent interactions. Furthermore, recurrent abdominal pain in childhood often precedes IBS in adults (Walker et al. 1998) indicating that colic in babies, recurrent abdominal pain in childhood and then IBS adults may be the same syndrome at different developmental stages.

7 Therapeutics and Future Implications

Early life stress in its various guises, while initially silent in its clinical impact, can manifest in adulthood across the symptom spectrum associated with IBS. Conversely, while the impact on the gut microbiome may be detectable initially, such traces can fade before the symptoms emerge. We have demonstrated this previously using vancomycin to disrupt the assembly of the gut microbiota which ultimately recovered to leave a residual impression on visceral hypersensitivity during adulthood (O'Mahony et al. 2014). As discussed above, the sequence of events can potentially arise due to the overlapping developmental trajectories of the CNS and gut microbiota during critical developmental time windows and may also generalise to alternative stressful insults. To further complicate matters, some of the stressors discussed earlier can manifest in adulthood via both gut microbiota and symptomatic

alterations (O'Mahony et al. 2009). This scenario presents both challenges and opportunities in efforts to detect and counteract these adverse consequences. Nevertheless, targeting the gut microbiota can now be envisaged as a means to both prevent and treat at least certain aspects of IBS as well as the co-morbid psychiatric features.

While the optimal time for intervention may thus be early life, the most efficient and effective option remains unclear. Reseeding the gut microbiome of caesarean section delivered infants with vaginal swabs looks promising (Dominguez-Bello et al. 2016) but it remains unproven and requires further study before widespread implementation. Improving the quality of the maternally transmitted microbiome may also prove effective (Jacka et al. 2013) while a number of other logical preventative and restorative measures have been proposed to reduce the impact of a compromised gut microbiota development (Mueller et al. 2015). Although many of these recommendations are desirable from a general healthcare perspective, much work remains to verify the benefits of these approaches for the later emergence of IBS and to determine if they can also be applied to situations where the stressors experienced are not directly related to the gut microbiota.

Evidence continues to accrue that the consumption of certain probiotic strains during adulthood might be useful for specific symptom domains in IBS (Clarke et al. 2012). Recently, the concept of psychobiotics has met with both positive and negative results depending on the strain used (Kelly et al. 2016a). Thus in healthy control subjects, *B. longum* 1714 has been shown to reduce both the subjective stress experience and the physiological cortisol output from an acute stress (Allen et al. 2016). Interestingly, this candidate psychobiotic also had a beneficial impact in a cognitive assessment of visuospatial memory, a feature which has previously been shown to be slightly impaired in IBS (Kennedy et al. 2014a).

Given that diet plays a major role in sculpting the gut microbiota, it is also interesting to consider whether this might represent a viable possibility to improve IBS symptoms. However, we should note that one of the dietary strategies shown to be of benefit (FODMAP diet) actually reduces bifidobacteria component of the gut microbiome (Staudacher and Whelan 2016).

As our appreciation of the impact of early life stress on the developing brain-gut-microbiota axis signalling grows, the potential for effective and earlier interventions for disease prevention is a tangible prospect. Translating this potential is undoubtedly a challenge but also an extremely worthwhile multidisciplinary venture.

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Neuroimmune Modulation of Gut Function

Terez Shea-Donohue and Joseph F. Urban, Jr.

Contents

Abstract

Neuroimmune communications are facilitated by the production of neurotransmitters by immune cells and the generation of immune mediators by immune cells, which form a functional entity called the "neuroimmune synapse." There are several

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mechanisms that further facilitate neuroimmune interactions including the anatomic proximity between immune cells and nerves, the expression of receptors for neurotransmitters on immune cells and for immune mediators on nerves, and the receptor-mediated activation of intracellular signaling pathways that modulate nerve and immune phenotype and function. The bidirectional communication between nerves and immune cells is implicated in allostasis, a process that describes the continuous adaptation to an ever-changing environment. Neuroimmune interactions are amplified during inflammation by the influx of activated immune cells that significantly alter the microenvironment. In this context, the types of neurotransmitters released by activated neurons or immune cells can exert pro- or anti-inflammatory effects. Dysregulation of the enteric nervous system control of gastrointestinal functions, such as epithelial permeability and secretion as well as smooth muscle contractility, also contribute to the chronicity of inflammation. Persistent active inflammation in the gut leads to neuroimmune plasticity, which is a structural and functional remodeling in both the neural and immune systems. The importance of neuroimmune interactions has made them an emerging target in the development of novel therapies for GI pathologies.

Keywords
Innate lymphoid cell • Macrophage • Mast cell • Neuroimmune plasticity • Neuroimmune synapse • T cell • Vagal cholinergic reflex

There is considerable interest in the mechanisms and pathways involved in the neuroimmune regulation of gut function. The number of cell types and possible interactions is staggering and there are a number of recent reviews detailing various aspects of these interactions (de Jonge 2013; Di Giovangiulio et al. 2015a; Olofsson et al. 2012), many of which focus on the emerging recognition of the microbiota in maintenance of homeostasis and of dysbiosis in gastrointestinal (GI) pathologies (Reading and Kasper 2011; Thaiss et al. 2016). There are several important factors to consider in any discussion of the neuroimmune regulation of function: (1) the microenvironment in determining the nature and phenotype of the resident and infiltrating immune cells; (2) the effect of acute versus chronic changes in the immune microenvironment on neuroimmune plasticity; and (3) the contribution of acute and chronic dysfunction of neuroimmune interactions to alterations in function associated with GI pathologies.

1 Overview of the Autonomic Nervous System (ANS)

The ANS is comprised of two separate systems, the sympathetic nervous system, in which the primary neurotransmitter is norepinephrine (NE), and the parasympathetic nervous system, in which the primary neurotransmitter is acetylcholine (ACh). The afferent-dominated parasympathetic and sympathetic nervous systems have little direct access to structural cells in the gut and the enteric nervous system,

embedded in the wall of the gut, acts to coordinate local and central inputs to alter gut function. There is abundant anatomical evidence for the neural modulation of immune function including the close approximation of nerves to immune cells, the innervation of the gut-associated lymphoid tissue (GALT), and the presence of receptors for immune mediators on nerves and for neurotransmitters on immune cells. Neuroimmune interactions play a major role in the GI response to stress as well as to inflammation from infection or disease resulting in acute and chronic changes in the GI function that contribute to disease symptoms.

In the sympathetic nervous system, sympathetic postganglionic fibers innervate both enteric nervous system (ENS) plexuses and the GALT and release NE as well as ATP, neuropeptide Y, and nitric oxide (Pongratz and Straub 2014). In general, simulation of the sympathetic nervous system inhibits gut motility, and reduces epithelial secretion and blood flow to the gut. It should be noted that hematopoiesis is regulated also by the sympathetic nervous system (Ordovas-Montanes et al. 2015). Pro-inflammatory effects are mediated by α2-adrenergic receptors (Bai et al. 2009) while anti-inflammatory effects are mediated by β2-adrenergic receptors (Vasina et al. 2008). Although stimulation of the sympathetic nervous system is considered to be pro-inflammatory, it is now clear that the outcome depends significantly on the context of the microenvironment.

The parasympathetic nervous system input to the GI tract includes the vagus, which supplies the stomach through the proximal colon, and the fibers originating in the sacral region. Preganglionic fibers from these areas synapse in the ENS while postganglionic fibers are in the ENS. Stimulation of the parasympathetic system increases epithelial secretion and promotes motility coordinating both excitatory and inhibitory nerves. The parasympathetic nerves are considered to exert anti-inflammatory effects.

There is a hierarchy of control of the gut in the autonomic nervous system. Sensory afferents transduce signals from the intestinal lumen to the ENS with activation of autonomic nerves (ANS) and the central nervous system (CNS). The fact that the bulk of the vagal fibers and a majority of the spinal fibers are afferents emphasizes the importance of luminal stimuli in orchestrating gut function. Physiological stimuli lead to activation of parasympathetic afferents (primarily vagal), while supraphysiological and pathological signals are carried by the sympathetic (spinal) afferents. The result is local and/or centrally coordinated changes in effector functions such as gut motility or secretion. Although a majority of neuroimmune interactions may be mediated by the ENS, there is considerable interest in the role of the parasympathetic and sympathetic nerves in GI health and disease.

2 The Basis of Neuroimmune Interactions

The ability of peripheral nerves to modulate immune responses has been recognized for more than 30 years. The GI tract contains a large population of resident immune cells that are important for development of normal intestinal morphology and

function, the maintenance of homeostasis, and the response to deleterious luminal stimuli or pathogens. There is a growing appreciation that, like the nervous system of the gut, the immune system is also hierarchical (Iwasaki and Medzhitov 2015). Similar to the nervous system, immune cells transduce specific pathogen-derived signals into effector functions which involve non-hematopoietic cells such as epithelial cells and resident/innate immune cells. The products of these cells are cytokines associated with the "innate" immune response that can in turn activate a higher level of adaptive immune cells that release cytokines (Iwasaki and Medzhitov 2015). As neurotransmitters are the means of communication in the nervous system, cytokines perform a similar role for the immune system. These effects can be short- or long-term depending on the level of engagement in both the neuro- and immune compartments.

The main function of the immune system is the ability to distinguish between self and non-self as a determinant of non-danger and danger signals. In the classical paradigm, innate immunity has a limited ability to produce antigen-specific effectors, while the adaptive immune system can elaborate effectors over the life span of the host. Innate immune cells include national killer cells, innate lymphoid cells (ILC), and γδT cells and innate responses are considered to be nonspecific, hardwired, and rapidly mobilized defense mechanisms against pathogens (Shih et al. 2016). The primary adaptive immune response is slower than the innate to react, but leads to long-term highly specific recognition of foreign (non-self) antigens. Innate mechanisms are usually sufficient for protecting the host, but an important feature of the adaptive response is the ability of effector lymphocytes (T cells) to develop immunological memory to eliminate pathogens more quickly upon a subsequent exposure.

There are a number of cells, however, that cross the boundaries of innate and adaptive immune responses (Artis and Spits 2015) including macrophages, mast cells, and the newly described ILC. In addition, the surface epithelial cells that line the GI tract are considered an integral part of the immune system as they form the first line of defense in the gut. Despite the large array of pathogens, the cells involved in innate immunity recognize conserved features of pathogens through pattern recognition receptors (PRR), which include the membrane-associated toll-like receptors (TLR). Thus, the division of immunity into innate versus adaptive may be an artificial concept as both innate and adaptive immune processes are involved in maintaining homeostasis (Bedoui et al. 2016). Blurring the lines between innate and adaptive immunity may provide a more relevant platform to assess the contribution of immune cells to GI pathologies and their associated changes in gut function that trigger symptoms.

The concept of a hierarchical arrangement of both neural and immune products is important in appreciating the bidirectional communication between immune cells and nerves. Both the GI immune system and the ENS evolved in the presence of pathogens. In this manner, the location of PRR on epithelial cells and resident immune cells allows them to function as sensors (Iwasaki and Medzhitov 2015). In the presence of enteric pathogens, these sensors provide information regarding the nature, location, and virulence/infectivity of the pathogen. The first level of activation is

release of cytokines and chemokines from PRR-expressing cells. The importance of epithelial cells should be emphasized also as these cells express PRR and elaborate chemokines and cytokines that bind to receptors on the resident subepithelial immune cells (ILC, mast cells, and macrophages) and induce an influx of immune cells to the affected area. Mediators released from these resident and recruited cells bind to naïve lymphocytes that develop the appropriate response to the pathogen and initiate a polarized immune response at this level. Cytokines have effector function on both hematopoietic and non-hematopoietic cells. Their receptors can be linked to second messenger systems that control cell function or to transcription factors like STATs that result in gene transcription.

In this hierarchy, there is a cost associated with the activation of host immunity with a greater metabolic demand associated with recruitment of immune cells to the affected area (Iwasaki and Medzhitov 2015; Chang and Pearce 2016a). In addition, there is evidence that innate immune cells recognize endogenous substances such as fatty acids via TLR (Shi et al. 2006) and regulate function and differentiation in ILC during nutritional stress (Wilhelm et al. 2016). Of interest is that 99% of multicellular organisms lack an adaptive response (Cooper and Alder 2006), suggesting that the adaptive immune response may be confined to species that can handle the metabolic demands of this response.

3 Bidirectional Communication Between Nerves and Immune Cells

There are several mechanisms by which immune cells and their products modulate the function of non-hematopoietic and other immune cells. The observation that immune cells approximate structural cells provides a context for initiating neuroimmune interactions (Stead 1992). The anatomical connection between these cell types was expanded to include a functional correlate termed the neuroimmune synapse (Tournier and Hellmann 2003) (Fig. 1). This concept was applied initially to enteric nerves and T and B cells in the adaptive immune system, but currently encompasses a number of immune cells including macrophages (Dustin 2012). Under physiological conditions, there is a steady production of mediators from both hematopoietic and non-hematopoietic cells. A second mechanism is the presence of receptors for neurotransmitters on immune cells and for immune mediators on neural structures. Complicating this interaction is the elaboration of immune mediators from nerves, and neurotransmitters from immune cells (Franco et al. 2007) (Fig. 2), and the biological responses induced by binding of these products to receptors. The distribution, location, and intracellular signaling pathways linked to these receptors are of interest. A third mechanism is enhancing the ability of immune cells to interact with nerve structures, a condition achieved during host defense by recruitment of immune cells to affected area. A fourth mechanism is the impact of the composition of the cellular infiltrate. Resident immune cells are relatively inactive, but the impact of increased numbers of activated cells dramatically alters responses of nearby hematopoietic and non-hematopoietic cells. A final

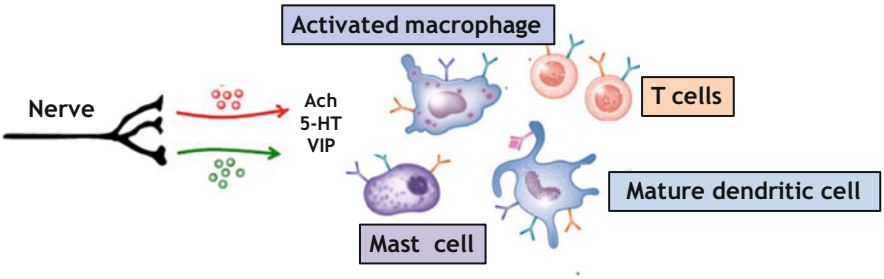

Fig. 1. The neuroimmune synapse showing that release of neurotransmitters in the proximity of immune cells binds to receptors to induce changes in the phenotype and function of these cells

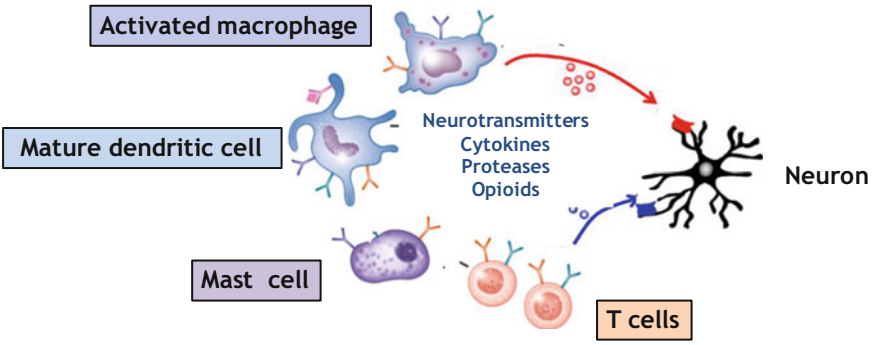

Fig. 2. Resident and recruited immune mediators bind to receptors in nerves. This may result in changes in neural sensitivity as well as activation of transcription factors (e.g., STATs) leading to altered gene expression

mechanism is that binding of mediators or neurotransmitters to receptors activates intracellular pathways that are further modulated by the proximity of other mediators and receptors resulting in activation of one of more different pathways.

4 The Impact of the Microenvironment on Neuroimmune Interactions

The microenvironment along the gut is unique, perhaps, in that there are regional differences in the presence of luminal factors such as nutrients and microbes that play an integral role in developing the GALT, maintaining mucosal homeostasis, and preserving normal GI motility patterns. Most physiological functions are optimal under a limited range of homeostatic conditions and disruption or dysregulation of homeostatic conditions is a hallmark of disease. The concept that immunological homeostasis is a goal rather than an actual condition offers a more realistic framework for neuroimmune interactions.

Immune cells are regulated by the balance of input derived from positive and negative signals. There is an appreciation that gain or loss of cell function is controlled by either suppression or inactivation of the stimulus or by activation of a compensating inhibitory signal. Loss of inhibitory signals plays a role in uncontrolled inflammation that is a hallmark of a number of autoimmune diseases. The immune cells and their products create a microenvironment that impacts the phenotype of other adjacent hematopoietic and non-hematopoietic cells, resulting in customized functions that are specific to a region of the gut or to an even more localized area. While all cells in the gut are likely modulated by neuroimmune interactions, there are several cell types that are the focus of attention because of their potential role in the initiation and chronicity of GI pathologies. There is an established role for mast cells and macrophages in neuroimmune interactions but the repertoire of cells is expanding also to include T cells and ILC. There is a growing appreciation of the impact of the microenvironment on the phenotype and function of these cells.

4.1 T Cells

A key aspect of evolution of host immunity was mounting effective responses to clear pathogens. The divergent CD4+ Th1/Th2 lineages, with their polarized cytokine profiles and counter-regulatory abilities, are the classical paradigm to explain orchestration of the host response to pathogens and establishment of memory responses. More recently, the Th17 subset was discovered as a third T effector cell lineage distinct from the Th1 and Th2 lineages (Harrington et al. 2005). First associated with autoimmune diseases including IBD (Cua and Kastelein 2006; Murphy et al. 2003; Weaver et al. 2007; Bamias et al. 2005), Th17 cells also participate in antimicrobial immunity and inflammatory pathologies (Bettelli et al. 2008). T cell differentiation into Th17 cells requires TGF-β in concert with other inflammatory mediators such as IL-1, IL-6, IL-21, or IL-23 (Volpe et al. 2008; Manel et al. 2008). Both Th1 cytokines and most members of the Th17 family, especially IL-17A and IL-17F, are involved in the pathogenesis of IBD (Fujino et al. 2003; Nielsen et al. 2003). In contrast, IL-17E (IL-25) promotes Th2 response and inhibits Th1 and Th17 cytokine responses (Fort et al. 2001; Kleinschek et al. 2007; Owyang et al. 2006). Th1, Th2, and Th17 cells all produce cytokines that impact gut function (Shea-Donohue et al. 2010a; Shea-Donohue and Urban 2004). The last major population of $CD4^+$ T cells are T regulatory cells (Tregs), which play a key role in maintaining immunological tolerance to self-antigens and in suppressing excessive immune responses (Vignali et al. 2008). The two most well-defined populations of Tregs are the forkhead box protein 3 $(Foxp3)^+$ Tregs and the interleukin-10 (IL-10)-producing type 1 Tregs (Tr1 cells). A reciprocal regulation among the Th1, Th2, and Th7 cells facilitates the exquisite balance of immunologic responses and failure of this balance can lead to chronic inflammatory pathologies. Expansion of Th1 or Th17 populations suppresses the development of Th2 responses and vice versa. Tregs are an important feature of inflammation in that they regulate effector T cells and different populations of Tregs may be involved in inflammation versus repair (Hegazy and Powrie 2015).

T cells express receptors for a number of neurotransmitters including NE, serotonin (5-HT), and ACh (Rodrigo et al. 2010). Binding of agonists to these receptors can modulate T cell phenotype and function. NE binds to α- or β-adrenergic receptors with immune cells expressing primarily β2-adrenergic receptors. Sympathetic neurotransmitters elicit migration of immune cells and at high doses activation of β-adrenergic receptors inhibits immune cell function (Straub et al. 2006). Activation of β-adrenergic receptor on naïve CD4+ T cells impacts the cytokine release of the developing Th1 or Th2 cell, and expression of these receptors is suppressed in mature Th2 cells but augmented in Th1 cells (Sanders et al. 1997). Depending on the setting, T cells can have distinct metabolic phenotypes that are important for T cell activation, function, and differentiation (O'Sullivan and Pearce 2015).

4.2 Innate Lymphoid Cells (ILC)

ILC are a sparse and discrete population of cells that communicate directly by release of cytokines that bind to receptors on hematopoietic and non-hematopoietic cells. They have lymphoid morphology but do not express surface lymphoid lineage markers and lack antigen specificity [reviewed in (Klose and Artis 2016; Sedda et al. 2014)]. They can be divided into functional subsets similar to CD4+ T cells such that the non-cytotoxic ILC1, ILC2, and ILC3 subsets elaborate cytokines similar to their respective adaptive Th1, Th2, and Th17 counterparts (Artis and Spits 2015). Like many other immune cells the functionality of the ILC is modulated by the local environment. Thus, there is a concentration of ILC2 and ILC3 at mucosal surfaces where they play a key role in innate immunity and help shape adaptive immune responses (Klose and Artis 2016). These ILC remain in the gut early in development and the presence of local tissue-resident progenitor cells allows the self-renewal of these ILC. The epithelial derived cytokines IL-33 and TSLP, and the tuft cell-derived IL-25 (Gerbe et al. 2016; von Moltke et al. 2016), are the major activators of ILC2 cells. The receptor for TSLP is a heterodimer of IL-17A and TSLPR, and is expressed by afferent neurons in the skin (Wilson et al. 2013) and GI tract (Ordovas-Montanes et al. 2015). TSLP stimulates Th2 cell production of IL-4 and IL-13 and the production of IL-5 and IL-13 by ILC2. In the gut, ILC2 contributes to the constitutive eosinophil release of IL-5 by neurotransmitter vasoactive intestinal polypeptide (VIP) (Nussbaum et al. 2013), implying that ILC are responsive to neural input. ILC are critical to the defense against enteric pathogens in experimental models and participate in restitution of the mucosal in response to DSS-induced injury (Monticelli et al. 2015), but their role in human pathologies is an area of active investigation.

4.3 Mast Cells

Mast cells are evolutionarily conserved granulocytes that are derived from CD34+ hematopoietic cells and require stem cell factor binding to c-kit receptor for differentiation. The greatest numbers of mast cell progenitors are found in the

small intestine, which may be attributed to their prominent role in the immune response to enteric nematode infection (Madden et al. 2002; Shea-Donohue et al. 2010b). The number of resident tissue mast cells is small, but large numbers are recruited (mastocytosis) in response to specific stimuli. Mast cells elaborate both preformed and newly synthesized mediators as well as chemoattractants that recruit other immune cells. The major preformed proteases in human mast cells are the β-tryptases derived from the *hTPSAB1* and *hTPSB2* genes. Their counterparts in mice are mast cell protease (mMCP)-6 and mMCP-7. Mast cells release and express receptors for neurokines such as histamine and 5-HT (Shea-Donohue et al. 2010b). The phenotype of the mast cell is influenced by the microenvironment, which ultimately modulates their activation, receptor expression, phenotype, and function. The nature and timing of release of these mediators are governed by the strength, duration, and nature of the stimuli. There are two distinct populations of mast cells, mucosal mast cells and connective tissue mast cells. The location of mast cells at mucosal surfaces is consistent with their role as immunologic "gatekeepers" (Shea-Donohue et al. 2010b). Mast cells express PRR such as TLR that are important for host defense against microbial as well as protease-activated receptors (PAR) that response to both bacterial and worm pathogens. There is evidence that long-term exposure to IL-33, a product of both epithelial and mast cells, induces mast cell hyporesponsiveness (Jung et al. 2013). Thus, under certain conditions, resident mast cells may behave like other bone marrow-derived cells such as dendritic cells and macrophages, and become less responsive to stimuli in the intestinal milieu.

4.4 Macrophages

Resident macrophages are found in nearly all tissues in the body and their heterogeneity is a reflection of the specific functions of each location. The origin of these resident mucosal macrophages is not uniform. Macrophages in the lung and peritoneal cavity are embryonically derived and self-renewing. The population of resident macrophages in the small intestine and colon are the largest population of tissue macrophages in the body (Lee et al. 1985). Postnatal gut mucosal macrophages arise from bone marrow-derived monocytes (Yona et al. 2013) and can be distinguished by the presence of surface markers $CX3CR1^+/MHCII^+/CD64^+$ in mice and $CD14^-/HLA\text{-}DR^+/CD163^+$ in humans (Gren and Grip 2016). Mucosal macrophages are positioned in the subepithelium reflecting their function in surveillance and feature a tolerant phenotype with TLR hyporesponsiveness and production of IL-10. These nonmigratory mucosal macrophages have a long half-life and are critical to the formation and maintenance of Tregs in the lamina propria (Hadis et al. 2011). The low level of inflammatory mediators in the mucosa, likely a result of the microbiota, provides a continual stimulus for the replenishment of the resident population from circulating $Ly6C^+$ blood monocytes (Bain et al. 2013). There does not appear to be a constitutive role for resident mucosal macrophages in epithelial cell function as macrophage depletion did not alter constitutive intestinal permeability or nutrient absorption (Notari et al. 2014). Resident mucosal macrophages are part of the first line of host defense. In response to

inflammatory pathogenic stimuli, there is a CC-chemokine ligand (CCL)-2- or MCP-1-mediated recruitment of $CX3CR1^{-}/Ly6C^{+}$ blood monocytes into the mucosa that do not acquire a tolerant phenotype and generate pathogen-specific cytokines (Eskandari et al. 1999), (Zigmond et al. 2012). Mucosal macrophage function is influenced heavily by the cytokine environment where development into classically activated inflammatory macrophages (M1) or alternatively activated macrophages (M2) represents the polarized extremes. Both macrophages use arginase as a substrate but express two different enzymes. The classical M1 marker is nitric oxide synthase (NOS)-2, while M2 expresses arginase (Arg)-1. In addition to Arg-1, the mannose receptor CD206 is also an established M2 marker. Th1- and Th17-dominant pathologies associated with bacterial infections and inflammatory bowel disease (IBD) promote development of M1 while Th2-dominant helminth infections and allergy lead to the M2 phenotype. The plasticity of macrophage phenotypes has spurred investigations into the phenotypes of macrophages in human disease (Zorzi et al. 2013), particularly those pathologies that feature a mixed immune environment.

In addition to resident mucosal macrophages, there is a second distinct population of macrophages located in the smooth muscle layers of the GI tract. The origin of the dense network of resident macrophage in the intestinal smooth muscle layers is less certain. They are reported to a play a constitutive role in GI motility by completing a neural circuit where they regulate peristalsis through the production of bone morphogenetic protein 2 (BMP2) that binds to bone morphogenetic protein receptor on enteric nerves (Muller Paul et al. 2014). The mechanism of the BMP2 on enteric nerves is unknown. There is evidence that this resident population expresses CX3CR1 (Muller Paul et al. 2014) and has low activity in the steady state, but in response to LPS, these resident macrophages upregulate the expression of TLR4 and produce pro-inflammatory cytokines (Hori et al. 2008). In response to inflammatory stimuli, resident muscularis macrophages produce the chemokine, monocyte chemoattractant protein (MCP)-1, leading to an increased number of macrophages in the smooth muscle. The speculated source of these recruited macrophages is the circulating $Ly6C^{+}$ blood monocytes that also replenish the resident mucosal macrophage population.

5 Vagal Cholinergic Anti-inflammatory Reflex

This reflex was first identified in the 1990s as part of a larger neural system that acts to maintain immune homeostasis (Andersson and Tracey 2012). Insights into the vagal modulation of GI motility advanced with the finding that efferent vagal activity attenuated sepsis-induced inflammation (Borovikova et al. 2000). This observation formed the underpinnings of the cholinergic anti-inflammatory reflex pathway (Tracey 2002). This hardwired rapid reflex is initiated by mediated activation of pro-inflammatory cytokine receptors on intestinal sensory vagal afferents. The efferent arm was thought originally to act via release of NE at β-adrenergic receptors on a subpopulation of memory T cells in the spleen leading to release of Ach (Rosas-Ballina et al. 2011). Binding of ACh to α7 nicotinic receptors (Wang et al. 2003) activated JAK-2/STAT3 on macrophages resulting in inhibition of the release of

pro-inflammatory cytokines such as TNFα, IL-1β, IL-18, and a DNA-binding mediator of lethal endotoxemia and sepsis called high-mobility-group box 1 (HMGB1) (Tracey 2002, 2007; Matteoli et al. 2014). There is now evidence that ACh released from vagal efferents also acts on neurons in the myenteric plexus of the smooth muscle or that the release of ACh from enteric neurons acts on α7 nicotinic receptors on adjacent macrophages (Cailotto et al. 2014).

The beneficial effects of the cholinergic anti-inflammatory reflex are mimicked by vagal simulation or administration of α7 nicotinic agonists (Borovikova et al. 2000; de Jonge et al. 2005). Despite the success of vagal stimulation in systemic diseases like sepsis that involve the gut, administration of nicotine or selective α7 nicotinic agonists to ulcerative colitis patients was ineffective against active colitis [reviewed in (de Jonge and Ulloa 2007)] and exacerbated disease severity in experimental models of colitis (Snoek et al. 2010). The lack of an effect of α7 nicotinic agonists may be explained, in part, by the finding that ACh binds to both nicotinic and muscarinic receptors on macrophages. There are a number of different G-protein-coupled muscarinic receptors (M1–M5) that activate several different second messenger systems, while nicotinic receptors are ligand-gated ion channels. IFN-γ increased the expression of muscarinic 3 receptors on bone marrow-derived macrophages and the muscarinic cholinergic agonist, bethanechol, amplified the IFN-γ-induced upregulation of the M1 marker of NOS-2 (McLean et al. 2015).

Ghrelin is in the family of hormones that includes motilin. It is elaborated by the enteroendocrine X/A cells located primarily in the upper intestine that exerts a number of orexigenic actions related to energy balance (Camilleri et al. 2009). Stimulation of the vagus leads to ghrelin release and administration of exogenous ghrelin was reported to protect against immune-mediated colitis (Di Giovangiulio et al. 2015b), endotoxin-mediated sepsis (Wu et al. 2009), traumatic brain injury (Bansal et al. 2012), and irradiation-induced damage to the GI tract (Wang et al. 2015). These data indicate that part of the beneficial effects of vagal stimulation may be mediated by ghrelin. The biological effects of ghrelin are mediated by the growth hormone secretagogue receptor type 1a (GHSR1a) located on a variety of cell types including nerves and immune cells (Camilleri and Acosta 2015). Recent data show that ghrelin has immunomodulatory effects on T cells that limits T cell proliferation in colitis (Di Giovangiulio et al. 2015b) consistent with an anti-inflammatory role.

6 Inflammation and Nerves

The close proximity of immune cells to enteric nerves, particularly mucosal afferents, is enhanced during inflammation by increased numbers of activated immune cells. Sensitization of afferents to stimulation by immune mediators modulates the magnitude or severity of the response. Immune cells release a number of neuropeptides including VIP and ACh as well as 5-HT, which is derived primarily from enteroendocrine cells. Amplification of neuroimmune interactions is the result of neuropeptide activation of immune cells as well as release of immune mediators

leading to changes in neuronal density (hyperplasia, hypertrophy) related to loss of neurons (Linden et al. 2005; Margolis et al. 2011).

There is evidence to support both pro- and anti-inflammatory role for ENS. Activation of polymodal ion channel on transient receptor potential vanilloid 1 (TRPV1) located on nerve terminals of sensory neurons leads to the release of neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) (Assas et al. 2014). Both substance P and CGRP worsen inflammation, in part, by activating immune cell mediator release (Wang et al. 2014). In contrast, ablation of sensory afferents by capsaicin also worsens inflammation as a result of the loss of protective neuropeptides. Moreover, the pro-inflammatory actions of substance P and CGRP may be modulated by VIP- and NPY-mediated anti-inflammatory effects that are linked to inhibition of pro-inflammatory cytokines. Despite the pronounced effects of neuropeptides on immune cells, the application of these interactions to the development clinical therapies has not yet been realized.

Among the most potent mediators of neurogenic inflammation in the gut are proteases. Endogenous proteases are part of the exocrine pancreatic secretion, are present in the mesenteric circulation, and are elaborated by enteric pathogens (Antalis et al. 2007). Thrombin- and trypsin-like proteases cleave PAR on primary afferent nerves resulting in sensitization of members of the transient receptor potential (TRP) family of cation channels and release of sensory neuropeptides (Cenac et al. 2007). The consensus is that TRP subfamily of V1/A1 (TRPV1/A1)-positive sensory neurons are important in neuro-inflammation (Lapointe et al. 2015).

Itch in the spectrum of pain is initiated when exogenous or endogenous pruritogens activate receptors or channels on afferent fibers of primary sensory neurons in the dorsal root ganglia. Chronic itching is linked to pain neurons and both are characterized by sensitization of afferent nerves and involve similar ion channels, neurotransmitters, and immune cell products (Liu and Ji 2013). Most of our information regarding the neural mechanisms involved in itch is garnered from research in skin and the contribution of itch receptor pathways to GI pathologies is unknown. Itch is described as sensitive or insensitive to histamine. Non-histaminergic transducers of itch include 5-HT, the (TRPV1/A1), PAR, and the Mas-related G protein-coupled family of receptors (Mrgpr) (Liu and Ji 2013) among others. These are all involved in both pain and itch (Zhao et al. 2014). TRPV1 is expressed on immune cells (Lee et al. 2012; Zhao et al. 2013) and activation of TLR4 on sensory nerves is proposed to activate TRPV1 by an intracellular signaling mechanism (Assas et al. 2014). Of interest is that both Mgrpr and PAR are activated by the PAR2 agonist SLIGRL (Liu et al. 2011). Activation of PARs is linked to visceral hypersensitivity and they are a therapeutic target in functional bowel disorders (Vergnolle 2016).

7 Plasticity of Neuroimmune Function

For many tissues, there is a resident stem cell population that differentiates into mature adult cells. Maintenance of the phenotype of differentiated non-hematopoietic cells is critical to homeostasis. Plasticity refers to the ability of a cell to change its identity and

this was thought to occur only under extreme conditions. The observation that the phenotype of the cell changes in response to injury or inflammation, however, supports the idea that dedifferentiation and trans-differentiation may be part of normal tissue regeneration (Merrell and Stanger 2016). Persistent active inflammation in the gut, such as that in IBD, leads to structural and functional remodeling in both the neural and immune systems. Immunological plasticity is part of the neural remodeling in IBD patients as the number of substance P neurons is elevated at the expense of loss of cholinergic neurons (Neunlist et al. 2003). Animal models of inflammation demonstrate post-inflammatory increases in neuronal excitability that are mediated by COX-2 (Mawe 2015).

The ability of conventional T cells to develop immunological memory is a central aspect of adaptive immunity. Immune memory is defined as the changing response to repeated antigen exposure. Differentiation of T cells progresses from naïve T cells to memory stem cells to central memory cells, that reside in secondary lymphoid structures, to effector memory T cells that are located in areas of inflammation (Mueller et al. 2013). There are higher levels of circulation memory T cells in patients with functional bowel disorders (Sundin et al. 2014) and pathogenic immunological memory cells contribute to the disease persistence in IBD (Takahara et al. 2013). Immune function also declines with age, a process termed immunosenescence, which particularly impacts the GI mucosa (Fujihashi and Kiyono 2009). In addition, there is a second process termed "inflammaging," which is associated with low-grade inflammation, enhanced intestinal permeability, decreased numbers of colonic myenteric neurons, and decreased proliferation (Man Angela et al. 2015; Sipos et al. 2011). Memory responses to previously encountered pathogens can modulate the immune response and the outcome of a subsequent infection by an unrelated pathogen by a process known as heterologous immunity. Heterologous immunity can polarize the immune response to incoming pathogens by altering the innate immune environment (Sharma and Thomas 2014).

8 Mucosal Barrier Dysfunction Modulates Neuroimmune Interactions

The mucosal barrier is an integrated system involving epithelial cells, immune cells, and the ENS. The expression and distribution of tight junction proteins (TJP) in intestinal epithelial cells play a critical role in maintaining mucosal barrier function and are a target for inflammatory mediators. The ENS also contributed to the integrity of barrier function through its ability to modulate a variety of epithelial functions including proliferation and permeability [reviewed in (Sharkey and Savidge 2014)]. As the first line of defense, epithelial cells provide critical information on the location of pathogen and inform the host of the nature and extent of the threat leading to coordinated changes in epithelial/neuroimmune interactions that are important for host defense. This process is initiated in part by activation of PRR on epithelial cells. There is a proximal to distal increase in TLR expression along the GI tract reflecting the higher concentrations of commensal and pathogenic

bacteria in the colon (Gourbeyre et al. 2015). Activation of these receptors in response to pathogenic bacteria induces the release of epithelial derived cytokines and chemokines that attract resident and recruited immune cells to promote Th1/Th17 immunity. These immune mediators also bind to receptors on epithelial cells, nerves, and other immune cells (Pastorelli et al. 2013). Bacterial products result in hallmark changes in gut function including increased small intestinal secretion to promote movement into the colon and elaboration of antimicrobial peptides from Paneth cells. This is followed by decreased secretion and motility that facilitates colonization in the colon (Shea-Donohue et al. 2010a).

Other enteric pathogens such as parasitic nematodes that preferentially colonize the upper small intestine initiate a Th2 response by precise mechanism(s) that remain largely unclear. Nematodes elicit stereotypic changes in gut function including increased intestinal permeability and hypersensitivity of enteric nerves (Madden et al. 2002; Shea-Donohue et al. 2001; Zhao et al. 2003). Many of these effects are linked to IL-13 activation of STAT6-dependent genes, which include PAR2 (Shea-Donohue et al. 2010c; Sun et al. 2016). Nematodes do elaborate a number of serine-like proteases that activate PARs on epithelial cells, enteric nerves, and immune cells. Activation of PARs is linked to both enhanced permeability and nerve sensitivity in GI pathologies such as IBS and IBD (Cenac et al. 2007; Vergnolle 2016).

Inflammation as well as enteric infections have direct effects on epithelial function and morphology that impact permeability through effects on TJP (Pastorelli et al. 2013). Increased permeability facilitates the interaction between luminal product and resident immune cells that are important in the initiation, regulation, and resolution of inflammation (Sonnenberg and Artis 2015). Changes in the composition or amount or alterations in the availability of receptors to luminal factors such as bile salts, short-chain fatty acids, proteases, and microbial products alter both epithelial and immune cell function. Recent reports showed that products that affect metabolic pathways in immune cells are a common link among a number of functions including signaling, function, fitness, and fate (Chang and Pearce 2016b). The substrate concentrations in the microenvironment modulate immune cell function and changes in immune cell metabolism impact nutrient availability (Chang and Pearce 2016b). This emphasizes again the importance of changes in microenvironment to immune cell function. There is a well-documented recognition of the contribution of "leaky gut" to autoimmune disease and GI inflammatory pathologies as well as to functional gut disorders (Pastorelli et al. 2013; Camilleri et al. 2012; Fasano and Shea-Donohue 2005). Long-term changes in epithelial permeability likely contribute to the chronicity of infection and development of drugs that can improve or restore barrier function is an emerging therapeutic option.

9 The Role of Glial Cells in Neuroimmune Interactions

The most abundant cells in the ENS are enteric glia cells (EGC). These cells are positioned to act as intermediaries between epithelial cells and enteric nerves and exhibit distinct morphologies (types 1–4) based on location within ganglia, between

ganglia, in the smooth muscle, and mucosa [reviewed in (Neunlist et al. 2014)]. EGC also express receptor for many ENS transmitters and are thought to be "innervated" by enteric nerves through neuroglial junctions. The interaction among these three structures is termed the "neuronal-glial-epithelial" unit (Neunlist et al. 2013). In response to activation, factors secreted by these cells act in a paracrine manner to exert both beneficial and deleterious effects. EGC-mediated effects on epithelial cells maintain intestinal barrier integrity following injury as mice deficient in EGC develop intestinal inflammation and disruption of barrier function (Bush et al. 1998; Pochard et al. 2016). In addition, EGC play a role in epithelial restitution (Meir et al. 2015), neuronal maturation, and survival (De Giorgio et al. 2012).

Glia cells express glial fibrillary acidic protein (GFAP) upon activation and increased numbers of GFAP-expressing cells are observed following injury and inflammation (Capoccia et al. 2015). In IBD patients the upregulation of glial cell-derived neurotrophic factor (GDNF) is linked to epithelial proliferation and preservation of mucosal integrity, while the upregulation of EGC-generated TGF-β1 is linked to inhibition of cell proliferation (Neunlist et al. 2007). GDNF is also necessary for EGC-mediated maintenance of enteric neurons (Brun et al. 2013). EGC production of S-nitrosoglutathione (GSNO) regulates epithelial permeability (Savidge et al. 2007); however, production of S100β and nitric oxide, via NOS-2, is also elevated in IBD and may drive chronic gut inflammation (Capoccia et al. 2015). Of interest is that vagal nerve stimulation activates EGC at nicotinic α7 receptors (Costantini et al. 2012) and improves intestinal barrier integrity following severe injury (Costantini et al. 2010).

10 Conclusions

The cross talk between the immune system and the nervous system in the gut has emerged as a key component in the regulation of GI homeostasis as well as in the orchestrated response to infections and inflammatory stimuli. The bidirectional communication between nerves and immune cells has been conserved throughout evolution and is implicated in allostasis (Verburg-van Kemenade et al. 2017), a process that describes the continuous adaptation to an ever-changing environment. There is a growing recognition of the importance of neuroimmune interactions in targeting the development of novel therapies for GI pathologies.

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The Role of the Gastrointestinal Microbiota in Visceral Pain

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Contents

Abstract

A growing body of preclinical and clinical evidence supports a relationship between the complexity and diversity of the microorganisms that inhabit our gut (human gastrointestinal microbiota) and health status. Under normal homeostatic conditions this microbial population helps maintain intestinal peristalsis, mucosal integrity, pH balance, immune priming and protection against invading

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pathogens. Furthermore, these microbes can influence centrally regulated emotional behaviour through mechanisms including microbially derived bioactive molecules (amino acid metabolites, short-chain fatty acids, neuropeptides and neurotransmitters), mucosal immune and enteroendocrine cell activation, as well as vagal nerve stimulation.

The microbiota-gut-brain axis comprises a dynamic matrix of tissues and organs including the brain, autonomic nervous system, glands, gut, immune cells and gastrointestinal microbiota that communicate in a complex multidirectional manner to maintain homeostasis and resist perturbation to the system. Changes to the microbial environment, as a consequence of illness, stress or injury, can lead to a broad spectrum of physiological and behavioural effects locally including a decrease in gut barrier integrity, altered gut motility, inflammatory mediator release as well as nociceptive and distension receptor sensitisation. Centrally mediated events including hypothalamic-pituitary-adrenal (HPA) axis, neuroinflammatory events and neurotransmitter systems are concomitantly altered. Thus, both central and peripheral pathways associated with pain manifestation and perception are altered as a consequence of the microbiota-gut-brain axis imbalance.

In this chapter the involvement of the gastrointestinal microbiota in visceral pain is reviewed. We focus on the anatomical and physiological nodes whereby microbiota may be mediating pain response, and address the potential for manipulating gastrointestinal microbiota as a therapeutic target for visceral pain.

Keywords
Microbiota • Brain • Gut • IBS • Pain

1 Gastrointestinal Microbiota

Within our gastrointestinal tract, we each harbour a unique microbiota signature of bacteria, archaea, yeasts, single-celled eukaryotes, as well as helminth parasites and viruses, including bacteriophage (Eckburg et al. 2005; Gaci et al. 2014; Scarpellini et al. 2015; Williamson et al. 2016). These enteric bacteria outnumber host human cells by a factor of three, with 10^{14} microorganisms estimated to populate the average human intestinal tract. With an estimated collective mass of 1–2 kg, (Frank and Pace 2008) and possessing 100 times the number of genes of the human genome (Kurokawa et al. 2007), these microbes influence virtually all aspects of human physiology and biology, through interactions with their host (Allez et al. 2007; Burokas et al. 2015; Cryan and Dinan 2012, 2015b; Dinan et al. 2015; Mayer et al. 2014a, 2015b; Moloney et al. 2014; Sampson and Mazmanian 2015; Williamson et al. 2016). This interaction between the bacteria and host is mutually beneficial with the bacteria involved in energy regulation, gut barrier function, protection from pathogens and immune system function amongst others (Bengmark 2013; Borre et al. 2014a; Burokas et al. 2015; Collins et al. 2012;

Cryan and Dinan 2012, 2015a), while the host provides the nutrients and environment in which the bacteria can thrive.

The complexity and diversity of our gastrointestinal microbiota are established early in our first few years of life and are shaped by a number of factors including mode of delivery (vaginal or caesarean section), whether we are breastfed or formula fed, timing and type of weaning, diet, medication (in particular antibiotics), exposure to viral or bacterial infections and stress (Borre et al. 2014b). It is worth noting that the critical development period, in which the seeding of our core microbiota and the development of the bacterial community in our gut happen, occurs in parallel with the growth, maturation and sprouting of neurons in the young brain (including thalamic and corticolimbic regions) (Borre et al. 2014a, b) and in the spinal cord. Similar concomitant changes occur in old age, where a decline in microbiota complexity and diversity occurs in parallel with a decrease in neuronal complexity (Biagi et al. 2013). It is plausible that this neuronal development or changes are mediated by microbiota-governed maturation and activation of spinal and central microglia (Erny et al. 2015; Rea et al. 2016). The hypothesis that gastrointestinal microbiota are involved in visceral pain has received growing interest in recent times. To date, there has been a limited number of studies investigating the interactions of the gut microbiota and its metabolites on pain and nociceptive processes. The findings from these studies suggest that a complex and diverse gastrointestinal microbiota is essential for the continuous preservation of healthy microglia, immune system and appropriate central response to physiological events throughout our lifespans (Cryan and Dinan 2015b).

2 Microbiota-Host Interaction

The environment in which our resident bacteria reside is a somewhat hostile ecosystem that is on constant immunological surveillance. The gastrointestinal tract can govern microbial density by neurally controlling gut motility, gut permeability, release of gut hormones and immune function via vagal and pelvic connections between the central nervous system (CNS), and enteric nervous system (ENS) and sympathetic prevertebral ganglia – all of which tightly regulate bacterial populations in the gut. Host-microorganism interactions occur at the luminal-mucosal interface of the intestinal tract. The intestinal epithelial cell layer contains predominantly enterocytes, secretory cells and gut-associated lymphoid tissue (GALT) (Pott and Hornef 2012). The enterocytes express innate immune receptors and can release cytokines and chemokines, while the GALT utilise lymphocytes to mount a more specific immune response if warranted. The secretory cells have multiple functions including the secretion of mucus from goblet cells, antimicrobial secretion from Paneth cells and in neuroendocrine control via the release of substances from enteroendocrine cells such as ghrelin, somatostatin, cholecystokinin, peptide YY and serotonin amongst others (Cani et al. 2013).

Epithelial pattern recognition receptors (PRRs), of which the Toll-like receptor family are the most studied, recognise molecular patterns such as peptidoglycans

(Royet et al. 2011) unique to bacteria and other microorganisms (Duerkop et al. 2009; Vaishnava et al. 2008a), and once activated can recruit inflammatory mediators, cytokine production and chemokine-mediated recruitment of acute inflammatory cells (Takeda and Akira 2004; Vaishnava et al. 2008b). This in turn regulates the number and diversity of bacteria in the gut. However, in response to invading pathogens or as a consequence of chronically high inflammatory tone, prolonged cytokine production can weaken the integrity of the intestinal barrier and mucosal layer, facilitating an increase in plasma lipopolysaccharide (LPS) levels as a consequence of increased bacteria infiltration across the gut (Souza et al. 2004). This concept of a 'leaky gut', facilitating a heightened inflammatory tone, has gained attention, and may be causal in the chronic low-grade inflammation often observed in functional gastrointestinal disorders.

Furthermore, these commensal bacteria are capable of synthesising and releasing many neurotransmitters and neuromodulators themselves, or evoke enteroendocrine cells to synthesise and release neuropeptides or hormones. For example, gamma-aminobutyric acid (GABA), noradrenaline, histamine, serotonin, dopamine and acetylcholine can all be produced by distinct gastrointestinal microbiota species (Lyte 2013, 2014); release of the gut peptides ghrelin, gastrin, orexin, galanin, cholecystokinin, leptin and neuropeptide Y can be mitigated through microbiota-enteroendocrine communication (Cani et al. 2013); SCFAs such as butyric acid, acetic acid, proprionic acid and lactic acid are bacterial metabolites derived from the fermentation of polysaccharides (Cummings and Macfarlane 1997) that can enter the blood and activate free fatty acid receptors (FFARs) or mediate epigenetic events (Kasubuchi et al. 2015; Stilling et al. 2014, 2016) and have been reported to have neuroactive properties (Russell et al. 2013). Current hypotheses suggest that these circulating cytokines, chemokines, endocrine messengers and microbial by-products can infiltrate the blood and lymphatic systems, or influence neural messages carried by the vagal and spinal afferent neurons to impact centrally mediated behavioural events, as well as impacting critical physiological systems including HPA axis activity, neuroinflammation and affective processing (Fig. 1).

3 Microbiota-Gut-Brain Axis

The microbiota-gut-brain axis comprises a dynamic matrix of tissues and organs including the brain, autonomic nervous system, glands, gut, immune cells and gastrointestinal microbiota that communicate in a complex multidirectional manner to maintain homeostasis and resist any perturbation to the system. Changes to the microbial environment, as a consequence of illness, stress or injury, can lead to a broad spectrum of physiological and behavioural effects locally including a decrease in gut barrier integrity, altered gut motility, inflammatory cytokine release and immune system activation. The neuronal architecture of the enteric nervous system, alongside the sympathetic and parasympathetic divisions of the autonomic nervous system, is intricately linked with the central nervous system. Through endocrine, immune and neuropeptide/neurotransmitter systems, the microbiota can relay information about health status of the gut. This in turn can profoundly impact the neuronal signalling in the brain and thus impact

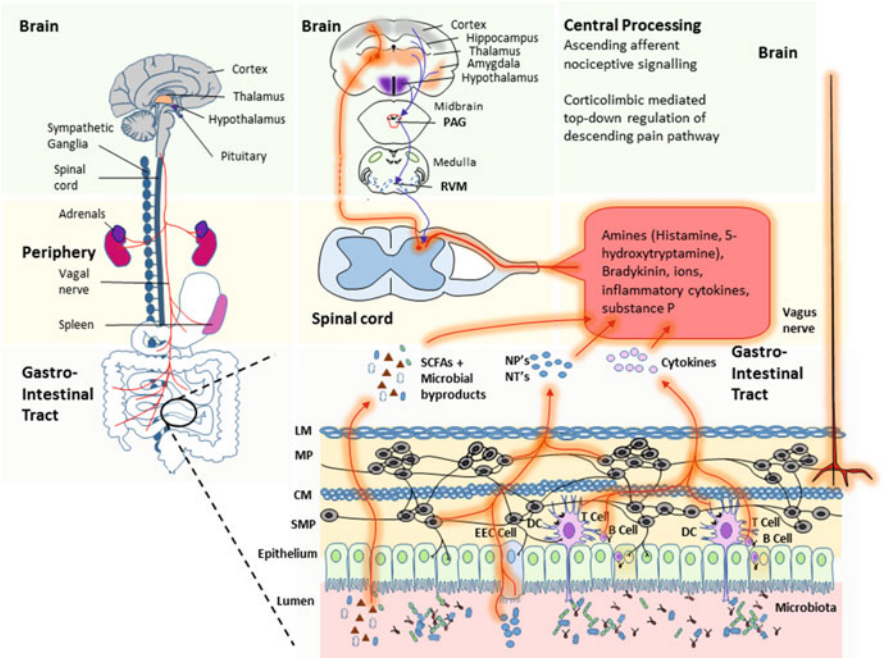

Fig. 1 Schematic for microbiota regulation of visceral pain. In the case of visceral pain, the facilitation or inhibition of pain processing is mediated at a central level, while ascending visceral afferents are mediated through the spinal cord, as a consequence of primary visceral nociceptor activation. As well as influencing central affective processing via the gut-brain axis, gastrointestinal microbiota can modulate a number of factors involved in pain through vagal nerve activation, cytokine production, neuropeptide and neurotransmitter release, SCFA release and microbial by-products. *SCFA* short-chain fatty acid, *NP* neuropeptide, *NT* neurotransmitter, *DC* dendritic cell, *EEC* enteroendocrine cell, *RVM* rostral ventromedial medulla, *PAG* periaqueductal gray, *LM* longitudinal muscle, *MP* myenteric plexus, *CM* circular muscle, *SMP* submucosal plexus

emotional systems and behavioural response (Cryan and Dinan 2015b; Mayer et al. 2014a; Rhee et al. 2009). This may be true for visceral pain, as the facilitation or inhibition of pain processing is mediated at a central level, while ascending visceral afferents are mediated through the spinal cord, as a consequence of primary visceral nociceptor activation. We will first focus on visceral pain neurotransmission and processing, and evidence for microbiota in visceral pain sensitivity, and then outline the proposed mechanisms by which gastrointestinal microbiota can regulate this response.

4 Visceral Pain

Pain is a complex multimodal experience combining a discriminative sensory component with a graded affective response. It is an innate survival facility possessed by all sentient organisms to protect against potential or existing tissue damage. While the

anatomical pathways and signalling mechanisms involved in somatic/musculoskeletal pain (skin and deep tissue) are relatively well defined, the mechanisms underlying visceral pain (internal organs) and its treatment are proving a difficult target for therapeutic intervention. Visceral pain is believed to affect up to 40% of the population at some stage in their lifetime and is commonly associated with an aching or throbbing sensation with varying degrees of discomfort, and often difficult to localise to a precise anatomical region.

Functional gastrointestinal disorders (FGIDs) including functional dyspepsia, irritable bowel syndrome (IBS) and infant colic represent one of the more common forms of visceral pain (Table 1). IBS alone affects an estimated 10–15% of the population in developed countries with an estimated economic burden to healthcare systems in the billions (Canavan et al. 2014). Other commonly reported malaises include myocardial infarction (heart attack), dysmenorrhea, appendicitis, bladder pain and pelvic pain. Notably, most patients presenting with visceral pain also experience autonomic phenomena including pallor, sweating, nausea, GI disturbances and changes in body temperature, heart rate and blood pressure. Recurrent, episodic but often unpredictable painful

Table 1 Clinical studies of visceral pain disorders and gut microbiota

Visceral pain	Microbiota involvement	Reference
Irritable bowel syndrome (IBS)	Gastrointestinal infection leads to persistent IBS symptoms	Thabane et al. (2007)
	A reduced bacterial diversity and increased Firmicutes:Bacteroidetes ratio in IBS	Jeffery et al. (2012a)
	Increased Firmicutes-to-Bacteroidetes ratio in IBS	Jalanka-Tuovinen et al. (2014) and Rajilic-Stojanovic et al. (2011)
	Correlation analysis of the microbial groups and IBS symptom scores	
	Increased *Ruminococcus* levels in IBS	Malinen et al. (2010) and Rajilic-Stojanovic et al. (2011)
	Correlation analysis of microbial load with symptoms	
	Higher Enterobacteriaceae and lower Faecalibacterium genera in IBS	Carroll et al. (2012)
	Quantitative species/OTU differences between the subgroups including IBS with and without bloating, and subtypes based on bowel characteristics	Ringel-Kulka et al. (2016)
	Higher levels of Veillonella and Lactobacillus in IBS	Lee and Tack (2010)
	Severity correlated with higher acetic acid and propionic acid levels	
	Antibiotic rifaximin reduces pain in IBS patients	Pimentel et al. (2011)
	A review of the benefit of probiotics to reduce abdominal pain	Hungin et al. (2013)
	B infantis 35624-treated patients experienced a reduction in composite and individual scores for abdominal pain and/or discomfort, bloating/distension	O'Mahony et al. (2005)

(continued)

Table 1 (continued)

Visceral pain	Microbiota involvement	Reference
Paediatric IBS	*Lactobacillus rhamnosus GG* reduces pain in paediatric IBS Prebiotics and synbiotics effective in IBS Mixture of Bifidobacterium infantis M-63®, breve M-16V® and longum BB536® improved abdominal pain in children Novel Ruminococcus-like microbe-associated with IBS; greater frequency of pain-correlated increased bacterial taxa from the genus Alistipes	Horvath et al. (2011) Curro et al. (2016) Giannetti et al. (2016) Saulnier et al. (2011)
Functional dyspepsia (FD)	Gastrointestinal infection leads to FD	Simren et al. (2013)
Functional abdominal pain	*Lactobacillus acidophilus NCFM* reduces pain in adults	Ringel-Kulka et al. (2014)
Colonic diverticulosis	Depletion of Clostridium cluster IV. Clostridium cluster IX, Fusobacterium and Lactobacillaceae were reduced in symptomatic versus asymptomatic patients	Barbara et al. (2016)
Infantile colic	Increased pathogenic bacteria, reduced lactobacilli, bifidobacteria *Lactobacillus reuteri*, PDX/GOS and *Lactobacillus rhamnosus* reduced crying	de Weerth et al. (2013) and Savino et al. (2004) Savino et al. (2007) and Partty et al. (2013)
Interstitial cystitis	Reduced levels of *E. sinensis*, *C. aerofaciens*, *F. prausnitzii*, *O. splanchnicus* and *L. longoviformis* in IC patients	Braundmeier-Fleming et al. (2016)

events can have a disabling impact on daily life and result in a reduced quality of life (Cervero 2009; Moloney et al. 2016).

The perception of gastrointestinal pain and discomfort involves complex mechanisms. These include peripheral sensitisation of sensory nerves and, at a central level, regulation of thalamic and corticolimbic signalling pathways. Of interest, there is substantial overlap in the brain areas underlying visceral pain and those that are involved in the processing of psychological stress, a key predisposing factor for visceral hypersensitivity.

5 Visceral Pain Pathways

After an event such as visceral injury, stress or infection, the nociceptive information coding for visceral pain is propagated through ascending pathways of the spinal cord to be processed at higher centres in the brain involving thalamic and corticolimbic regions to be perceived as pain (Chang 2005). In the visceral organs, nociceptors respond to mechanical stimulation such as distension or pressure, tissue

damage and chemical stimulation as a consequence of inflammation, infection or ischaemia. Nociceptors are receptors on bare nerve endings containing transient receptor potential (TRP) channels that detect tissue damage or injury. Chemicals that activate TRP channels include globulin, protein kinases, arachidonic acid, prostaglandins, histamine, nerve growth factor, substance P, calcitonin gene-related peptide, serotonin, acetylcholine, ATP and changes in pH (Cortright and Szallasi 2009; Rosenbaum and Simon 2007).

At the dorsal horn of the spinal cord, physiologically active agents including substance P, glutamate, aspartate, vasoactive intestinal peptide (VIP), cholecystokinin (CCK), somatostatin, calcitonin gene-related peptide (CGRP) and galanin are released from the nerve terminals of the visceral primary afferents to propagate the nociceptive signal to second-order neurons. Under normal physiological conditions, these neurons are under 'gated' control. However, once a certain threshold of stimulation is surpassed, these neurons are no longer suppressed and the nociceptive information coding for general location and intensity projects to supraspinal sites. The two major ascending pain pathways in mammals are the spinothalamic and the spinoparabrachial tracts, which encode the sensory-discriminatory and affective aspects of pain, respectively (Millan 1999). The descending pathways, in turn, modulate neuronal activity in ascending pathways, and can exert an inhibitory or facilitatory effect on the sensation of pain. Interestingly, the supraspinal and peripheral anatomical regions involved in facilitation and inhibition of nociception often overlap. Sensitization of receptors in these anatomical loci as a consequence of repeated or prolonged activation can often lead to chronic, repeated and often unpredictable bouts of visceral pain. Thus by targeting key bioactive chemicals or receptor systems on these sensory afferent neurons, the sensation of visceral pain could be significantly ameliorated.

6 Microbiota-Gut-Brain Axis and Visceral Pain

Alterations of intestinal microbiota have been directly linked to immunity, inflammatory, motility, and neurologic changes that are hallmark characteristic of FGIDs. A number of clinical studies have reported an altered gastrointestinal microbiota profile in patients suffering from chronic or recurrent visceral pain, including inflammatory bowel disorders (Conte et al. 2006; Frank et al. 2007; Manichanh et al. 2006) and irritable bowel syndrome (Carroll et al. 2012; Jeffery et al. 2012b; Kassinen et al. 2007; Matto et al. 2005; Noor et al. 2010; Shankar et al. 2015; Simren et al. 2013). These studies reported decreased relative abundance of the genera Bifidobacterium and Lactobacillus, and increased Firmicutes:Bacteroidetes ratios at the phylum level and are summarised elsewhere (Clarke et al. 2012). However, it is difficult to discern whether these changes are causative or deleterious to the host, or whether the altered microbiota signature is an appropriate response to tissue injury, inflammation or damage in the host. Recent success stories fuelling research into the role of human microbiota in health and disease include the treatment of peptic ulcers by suppressing *H. pylori* infection, and the use of faecal transplantation to treat *Clostridium difficile*.

As such, a number of clinical and preclinical studies have been employed to interrogate the role of the microbiota in visceral pain including prebiotic, probiotic and antibiotic intervention, faecal transplantation and the use of germ-free and specific pathogen-free animals. By and large, these studies employ visceral noxious distension as a proxy for visceral pain. Interestingly, stress (in particular early-life stress) can exacerbate this visceromotor behavioural response in adulthood (Greenwood-Van Meerveld et al. 2015; Moloney et al. 2015). Similarly, in preclinical models, active inflammation induced by chemical irritants or a pathogen causes acute hypersensitivity, with a subset of rodents developing a persistent hypersensitivity following recovery from the inflammation (Greenwood-Van Meerveld et al. 2015; Moloney et al. 2015).

7 Preclinical Evidence for a Role of Gastrointestinal Microbiota in Mediating Visceral Pain

Preclinical studies have illuminated our understanding of the role of the microbiota in behavioural responses, including response to visceral pain. Studies have shown that mice raised in a sterile environment from birth, and as such, without gastrointestinal bacteria (germ-free), exhibit an exaggerated stress response (Clarke et al. 2013; Sudo 2012; Sudo et al. 2004), and a blunted response to inflammatory pain (Amaral et al. 2008). Similarly, antibiotic-induced alteration of gastrointestinal microbiota decreased visceral pain-related response elicited by intraperitoneal acetic acid injection or intracolonic capsaicin infusion in mice (Aguilera et al. 2015), and also decreased visceral sensitivity in naïve rats (Hoban et al. 2016). Further studies have shown that exposure to antibiotics in early life can lead to subsequent sensitivity to visceral pain in adulthood (O'Mahony et al. 2014). Recently, faecal matter from IBS patients characterised by hypersensitivity to colorectal distension was transplanted to germ-free rats, and the response to colorectal distension was enhanced in these animals (Crouzet et al. 2013).

While preclinical evidence for the efficacy of prebiotics in manipulating visceral hypersensitivity is limited (Kannampalli et al. 2014; Larauche et al. 2012), there is evidence for a role of the microbiota in regulating response to visceral pain through probiotic administration. Maternal separation is a useful translational animal model to represent early-life stress in humans, with long-term changes in visceral pain sensitivity (O'Mahony et al. 2012) and gastrointestinal microbiota complexity/diversity in adulthood. Treatment with *Lactobacillus paracasei NCC2461* in mice (Eutamene et al. 2007) and the probiotic mix VSL#3 in rats (Distrutti et al. 2013) were both shown to be effective in ameliorating the maternal separation stress-induced hypersensitivity during colorectal distension. In a restraint stress-induced increase in visceral hypersensitivity to colorectal distension, *Bifidobacterium lactis* CNCM I-2494 significantly attenuated the nociceptive response (Agostini et al. 2012), while *Bifidobacterium infantis 35624* (McKernan et al. 2010), *Lactobacillus paracasei NCC2461* (Verdu et al. 2006) and *Lactobacillus reuteri* (Kamiya et al. 2006) were effective in blunting nociceptive response during colorectal distension in naïve animals. VSL#3 was also shown to prevent visceral hypersensitivity induced by inflammation via intracolonic instillation of 4% acetic acid when given prophylactically (Dai et al.

2012), while *Bifidobacterium infantis* 35624 ameliorated visceral hypersensitivity to colorectal distension in the trinitrobenzenesulphonic acid-induced (TNBS) model of colitis in rats (Johnson et al. 2011). Moreover, *Lactobacillus rhamnosus* CNCM I-3690 (Laval et al. 2015) and *Bifidobacterium animalis* ssp. *lactis* CNCM-I2494 (Martin et al. 2016) were shown to exhibit protective effects on intestinal barrier function in the TNBS model of colitis in mice by restoring barrier integrity, reducing inflammatory cytokines and increasing the levels of intestinal tight junction proteins.

8 Clinical Evidence for a Role of Gastrointestinal Microbiota in Mediating Visceral Pain

In contrast to the provocative preclinical evidence for a role for gut microbiota in visceral pain, clinical studies remain inconclusive with a large 'non-responder' population in many probiotic trials. Evidence for this range in therapeutic response is provided by studies using the somewhat controversial technique of transplantation of faecal matter from healthy donors to sufferers of FGIDs. In one study involving IBS and IBD sufferers (Borody et al. 1989), faecal matter transplant (FMT) was effective in 36% of the patients, mildly improved discomfort in 16% and non-effective in the rest 47%. Other clinical studies and case studies have utilised FMT for alleviation of chronic constipation (Andrews and Borody 1993), refractory IBS (Pinn et al. 2015) and IBD (Angelberger et al. 2013; Bennet and Brinkman 1989) with varied success. While such studies provide evidence for a role of microbiota in alleviating certain symptoms associated with gastrointestinal discomfort or visceral sensitivity, the studies provide little empirical data relating to long-lasting changes in gastrointestinal microbiota or alterations to immune, endocrine, inflammatory or neurotransmitter systems; and further double-blind randomised control trials are necessary to investigate the effectiveness of FMT in the treatment of FGIDs. However, no studies have yet investigated the effectiveness of FMT in alleviating visceral pain per se.

Prior to the recent re-emergence of the microbiota for the treatment of gastrointestinal disease, diet was key in the treatment of FGIDs, and the associated visceral discomfort and pain. The majority of studies investigating the influence of diet in FGIDs were poorly controlled and largely epidemiologically based; yet one diet that received attention and was reportedly effective in alleviating symptoms in some case studies (Halmos et al. 2014) was the low fermentable substance diet. This diet basically reduces the intake of indigestible fibre that is fermented by colonic bacteria (prebiotics). Clinical studies investigating the role of prebiotic administration for the treatment of FGIDs are somewhat limited, with little evidence for efficacy in alleviating distension or visceral pain (Olesen and Gudmand-Hoyer 2000; Silk et al. 2009; Whelan 2011).

Meta-analyses of IBS studies suggest that probiotics and prebiotics may be effective in therapeutic outcome (Mayer et al. 2014b; Moayyedi et al. 2014). However, the data used to generate these meta-analyses are compacted from various case studies where the studies were poorly designed, had low group numbers or included a number of different IBS subtypes, and they each use different bacterial strains or mixes, and with

different doses (Ford 2010). More recently, randomised double-blind, placebo-controlled studies have reported a beneficial effect of probiotics, or probiotic mixes on IBS symptomology including pain/discomfort, distension/bloating, urgency and digestive dysfunction (Guglielmetti et al. 2011; Sisson et al. 2014; Yoon et al. 2014), while other studies have reported no effect (Farup et al. 2012; Michail and Kenche 2011).

Microbiota diversity and complexity can also be altered with antibiotic administration. Anecdotal evidence originally suggested that antibiotics may be useful in treating bacterial infections that may be contributing to the discomfort and pain associated with FGID and inflammation. However, randomised double-blind placebo-controlled studies are lacking, and meta-analyses have shown only mildly therapeutic effects in treating IBS (Pimentel 2016; Sachdev and Pimentel 2012). It is clear that a longitudinal systems-based disease model (Mayer et al. 2015a, b) with complementary brain imaging is necessary to integrate central, peripheral and behavioural alterations before, during and after treatment.

Future treatment strategies for the alleviation of chronic or intermittent visceral pain in FGIDs may involve personalised microbiota alterations, i.e. the identification of patient subsets with a distinct microbiota profile, and a targeted approach to restore specific populations of beneficial bacteria.

9 Proposed Mechanisms Whereby Microbiota Alleviates Visceral Pain

Chronic visceral pain is the predominant symptom of FGIDs. It is apparent that FGIDs are multifaceted disorders involving central and peripheral mechanisms, and are often referred to as disorders of the brain-gut axis. However, it is as yet unclear how, or to what extent, microbiota confined to the gastrointestinal tract can influence visceral pain behaviour associated with FGIDs (Moloney et al. 2016).

At the source, the sensitisation of primary afferent nociceptors may lead to visceral hypersensitivity in FGIDs. A number of different receptor types are involved in the process of peripheral sensitisation including the TRPV family, proteinase-activated receptors, cholecystokinin receptors, serotonin receptors, cannabinoid receptors as well as an array of ion channels including ATP-gated ion channels, voltage-gated sodium and calcium channels and acid-sensing ion channels (Akbar et al. 2009). The gastrointestinal microbiota can activate these receptors directly or indirectly through immune responses at the mucosal surface during infection, inflammation and autoimmunity (Cassel et al. 2008; El Aidy et al. 2014; Kamada et al. 2013; Mazmanian et al. 2005; Round and Mazmanian 2009); formyl peptides (Husebye 1997) and protease (Cenac et al. 2008; Vergnolle 2009) release; polyunsaturated fatty acid (PUFA) release (Cenac et al. 2015); SCFA production (Cummings and Macfarlane 1997); neurotransmitter production (Lyte 2013, 2014); and hormone secretion (Cani et al. 2013). However, the extent to which these mechanisms either individually or collectively have a role in the aetiology of FGIDs remains unaddressed.

Gastrointestinal microbiota can also stimulate the release of the body's natural pain-suppressing biomolecules including opioids (Boue et al. 2014) from innate

neutrophils and monocytes, endocannabinoids (Muccioli et al. 2010) from colonic tissue as well as other pain modulators (Oleskin and Shenderov 2016) including monoamines.

Recently the role of spinal microglia in the mediation of visceral pain has received some attention. Repeated colorectal distension in rat neonates as a model for visceral hyperalgesia (Saab et al. 2006), chronic psychosocial stress-induced visceral hypersensitivity (Bradesi 2010) and TNBS-induced visceral hypersensitivity activated microglia in the spinal cord. Interestingly, minocycline – an antibiotic with microglia suppressant activity – was effective in reversing nociceptive responses in these three models. Microglia are critically involved in neuronal events at various stages in development and adulthood, including synaptic remodelling to improve neuronal network signalling (Schafer et al. 2012; Schafer and Stevens 2015). A recent comprehensive study (Erny et al. 2015) has highlighted a critical role for gastrointestinal microbiota in central microglia maturation, morphology and immunological function. Under normal homeostatic conditions, a diverse gastrointestinal microbiota is necessary for the maintenance of microglia in a healthy functional state. In contrast, the absence of a complex host microbiota (as evidenced by germ-free models, antibiotic suppression of microbiota or use of SPF mice) led to increased microglial populations; defects in microglia maturation, activation state and differentiation; alterations to microglia morphology (with longer processes and increased branching, terminal points and clubbing at synaptic boutons); and a compromised immune response to bacterial or viral infection (Erny et al. 2015). The observed alterations in microglial phenotype were reversed with recolonisation of gut microbiota, following 6-week cohabitation of germ-free mice with control mice. The findings from this study have redefined the ideology that the relationship between our microbiota, immune system and neurodevelopment is moot in adulthood, as a healthy and diverse gastrointestinal microbiota is essential for the continuous preservation of healthy microglia and proper brain function throughout our lifespans (Cryan and Dinan 2015a). It is plausible that such events also occur to spinal microglia. However, to date, no studies have investigated the effects of gastrointestinal microbiota modulation on spinal microglia activation in visceral pain.

10 Summary

Visceral pain is a multimodal experience combining a discriminative well-defined sensory component involving neurotransmission from the site of injury, and a less well-understood graded affective response involving corticolimbic processing with subsequent facilitation or inhibition of the incoming nociceptive information. The manifestation of pain, visceral or somatic, has been studied for decades, and many of the nociceptive pathways and chemical mediators are known; yet treatment-resistant populations still exist. In this chapter we have introduced the gastrointestinal microbiota as a potential contributor to visceral pain associated with FGIDs. Separate phyla of gastrointestinal bacteria can exacerbate or alleviate symptoms associated with FGIDs including pain, distension, bloating, constipation, diarrhoea, inflammation and immune system activation locally at the level of the gut.

However, the influence of the gastrointestinal microbiota on centrally mediated events, including the facilitation or inhibition of pain processing, cannot be overlooked. As such, further preclinical and clinical research focussing on overlapping mechanisms linking microbiota-gut-brain interactions with top-down modulation of pain processing is warranted.

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Critical Evaluation of Animal Models of Gastrointestinal Disorders

Anthony C. Johnson and Beverley Greenwood-Van Meerveld

Contents

Abstract

Preclinical research remains an important tool for discovery and validation of novel therapeutics for gastrointestinal disorders. While in vitro assays can be used to verify receptor-ligand interactions and test for structural activity of new compounds, only whole-animal studies can demonstrate drug efficacy within the gastrointestinal system. Most major gastrointestinal disorders have been modeled in animals; however the translational relevance of each model is not equal. The purpose of this chapter is to provide a critical evaluation of common animal models that are being used to develop pharmaceuticals for gastrointestinal disorders. For brevity, the models are presented

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for upper gastrointestinal disorders involving the esophagus, stomach, and small intestine and lower gastrointestinal disorders that focus on the colon. Particular emphasis is used to explain the face and construct validity of each model, and the limitations of each model, including data interpretation, are highlighted. This chapter does not evaluate models that rely on surgical or other non-pharmacological interventions for treatment.

Keywords
Colon • Construct-validity • Disease • Esophagus • Face-validity • Gastroesophageal reflux • Gastroparesis • Inflammatory bowel disease • Irritable bowel syndrome • Mouse • Peptic ulcer • Pharmacology • Postoperative ileus • Rat • Small intestine • Stomach

1 Introduction

Animal studies are the gold standard for preclinical and translational studies of gastrointestinal (GI) disorders. While there are now "organs-on-a-chip" and specialized cell lines that provide in vitro platforms that can be used for drug selectivity and specificity studies, only whole-animal physiology can approximate clinical pathology and effects of novel therapeutics. The usefulness of an animal model to correctly predict a clinical result is limited by its face and construct validity. In simple terms, face validity refers to whether an animal model actually models the particular disorder. For example, diabetic models should have elevated blood glucose, colitis models should exhibit active colonic inflammation, and peptic ulcer models should involve gastric inflammation. Face validity can either be general (i.e., colonic inflammation) or more specific (i.e., enteritis-induced colonic inflammation), which affects the translational relevance of the model. The other feature of animal models is construct validity, which refers to whether the measured effects are a valid representation of the modeled disorder. In reference to the previous examples, diabetic models with elevated blood glucose may have poor construct validity if tests are only performed with acute studies and/or if the blood glucose is not controlled with insulin therapy, as will occur in most patients. Similarly, inflammation models will have higher construct validity when using a natural pathogen or a manipulation that induces the response rather than an artificial inflammatory agent. Low construct validity does not necessarily make a poor animal model as the disease-induced changes in physiology in the model may be similar to the clinical pathology, but caution should always be taken in the interpretation of effects of novel therapeutics as a single-animal model should not be seen as representative of an entire phenotype. Additionally, each animal species has its own strengths and weaknesses that will affect the translation relevance of a study. Rodents (mice, rats, guinea pigs) have a very similar GI tract (except for the lack of a gall bladder in rats) and enteric nervous system as humans, but they lack an emetic reflex and they have a pronounced cecum that complicates whole-GI transit studies. Rodents have the additional benefits of being able to study large numbers of

animals and genetically modified strains that permit evaluation of specific disease mechanisms or present with spontaneous disease pathology with the limitations of different metabolic rates that can affect drug metabolism and reduced ability for longitudinal assessment without specialized equipment due to the small size of the animals. Rabbits, ferrets, and opossums are non-rodent species that provide larger animals for models that permit additional surgical manipulations that cannot be performed in rodents and the ability to monitor disease progression with noninvasive techniques, such as endoscopy. The use of these species can be limited by availability from accredited vendors along with increased cost and more animal welfare concerns. Dogs, cats, and pigs provide moderate to large animal models that tolerate repeated experimental testing with significantly greater per-animal expense and with significantly more regulatory oversight. Chronic surgical manipulations can also be established in these animals to either induce a disease or allow direct access to regions of the GI tract via a fistula or an endoscope. Finally, nonhuman primates are rarely used, due to ethical concerns and associated costs, for induced model of GI disease; however, spontaneous GI disease occurrences, as well as aging effects, have been studied in nonhuman primates when available. Thus, by selecting an appropriate model and species, fundamental disease processes and the effects of novel pharmaceuticals can be investigated, which can lead to the development of new clinical therapeutics.

2 Animal Models of Upper GI Disorders

2.1 Gastroparesis

Most recent literature has used mouse and rat models of gastroparesis. In mice, gastric emptying can be acutely delayed via administration of clonidine, dopamine, or m-chlorophenylbiguanide, with gastric emptying measured via a phenol red test meal (Asano et al. 2016). In healthy mice, atropine, dopamine, serotonin, or the 5-HT_3 agonist, 1-(3-chlorophenyl)biguanide, also delays gastric emptying measured with a blue dextran test meal (Kimura and Sumiyoshi 2012). Three days of dexamethasone administration also delayed gastric emptying, measured with phenol red test meal, in healthy mice via a direct effect of increasing stomach mass and indirectly through depletion of endogenous nitric oxide production (Reichardt et al. 2014). A subset of spontaneously type 1 diabetic mice, NOD LtJ, develop delayed gastric emptying as measured by [^{13}C]-octanoic acid breath test, which can be repeated multiple times over many months to study longitudinal changes in gastric emptying (Creedon et al. 2013). Nitric oxide-mediated gastric accommodation is crucial for normal gastric emptying as demonstrated in multiple knockout mouse models. Tetrahydrobiopterin (BH4)-deficient mice also display spontaneous delays in gastric emptying as pups as measured by residual stomach weight after feeding (Welsh et al. 2013, 2015). Knock-in mice with mutant-soluble guanylate cyclase that cannot respond to nitric oxide also have delayed gastric emptying measured by phenol red meal (Cosyns et al. 2013). FORKO mice deficient in follicle-stimulating hormone

receptor demonstrate delayed solid gastric emptying associated with decreases in BH4 production (Ravella et al. 2013) (Table 1).

In a rat model of Parkinson's disease induced by 6-hydroxydopamine (6-OHDA) in the substantia nigra, gastric emptying was delayed as measured by both solid emptying and via radiographic analysis of a barium meal (Zheng et al. 2011, 2014; Song et al. 2014b) and was associated with changes in beta-adrenergic receptor expression, dopamine receptor expression, and acetylcholine content within the gastric smooth muscle. In the same model, similar delays in gastric emptying were measured with the [^{13}C]-octanoic acid breath test 4 weeks after 6-OHDA treatment along with changes in vagal tone (Toti and Travagli 2014). A further study of this model demonstrated that at 6 weeks post-6-OHDA treatment, gastric emptying of a semisolid meal with nutrients was unchanged compared to healthy controls (Wang et al. 2014), but acute treatment with a combination of L-dopa/carbidopa significantly delayed emptying, which could be reversed by rikkunshito, a standardized herbal mixture, via changes in plasma ghrelin. In the uncontrolled streptozotocin (STZ) model of type 1 diabetes, at 8 weeks post-induction, approximately 50% of female rats and 25% of male rats demonstrated delayed gastric emptying measured by plasma acetaminophen levels post-liquid gavage (Showkat Ali et al. 2012). Further, treating the male diabetic rats with normal gastric emptying with estradiol for 3 weeks induced gastroparesis (Showkat Ali et al. 2012) that was associated with estrogen-mediated changes in nitric oxide synthesis. In rats with uncontrolled STZ-induced diabetes, gastric emptying was delayed 8–12 weeks after induction, as measured by a solid chow meal (Li and Chen 2014) or a semiliquid methylene blue meal (Chen et al. 2014c). At 6 weeks post-STZ, phenol red meal gastric emptying was decreased in diabetic rats and was significantly increased after chronic treatment with irbesartan, an angiotensin II receptor antagonist, which was associated with changes in nitric oxide synthesis (He et al. 2014). In STZ males, at 8 weeks uncontrolled, phenol red meal was significantly delayed, and was partially restored by chronic administration of SB203580, a p38 MAPK inhibitor (Yang et al. 2014), via changes in nitric oxide synthesis. In a model of traumatic burn, rats with a scald on ~60% of their body displayed delayed gastric emptying of both a phenol red meal (Song et al. 2013) and a solid chow meal (Li et al. 2016) due to changes in vagal nerve function. In healthy rats, acute administration of verapamil delayed gastric emptying of a solid meal (Li and Chen 2014), whereas acute water immersion-restraint stress induced a delay in gastric emptying that was reversed by mosapride ($5HT_3$ antagonist) or a standardized herbal preparation (DA-9701) (Jung et al. 2013). In rats with acetic acid-induced gastric hypersensitivity, acute administration of desvenlafaxine succinate, an SNRI, at a dose that significantly increased plasma norepinephrine concentrations, normalized gastric sensitivity, but induced a delay in solid meal emptying in both the hypersensitive and normal control rats (Dai et al. 2013). In a model of type 2 diabetes induced by high-fat diet and STZ treatment, 4 weeks post-induction, rats displayed a delayed gastric emptying of a phenol red meal that was dose-dependently normalized by chronic curcumin treatment, but not affected by rosiglitazone treatment, and associated with restoration of plasma and stomach ghrelin levels (Xu et al. 2013b).

Table 1 Animal models of gastroparesis

Assay	General method and outcomes	Limitations
Phenol red/blue dextran/ methylene blue meal	In fasted animals, a meal composed of phenol red/blue dextran/ methylene blue suspended in a methyl cellulose solution (non-nutrient) is administered via oral gavage. Stomach is isolated after a defined time period. Amount of remaining meal is assessed via spectrophotometric absorbance	Material in stomach will change emptying rate. Typical meal is in a viscous liquid without nutrients, which may not produce the same response as a solid meal or a liquid meal with nutrients. Terminal assay
Solid meal emptying	In fasted animals, a semisolid meal of a known amount of glass beads (1 mm diameter) are administered via oral gavage. Stomach is isolated after a defined time period. Total amount of remaining glass beads is assessed to measure emptying rate	Material in stomach will change emptying rate. Glass beads are delivered in a viscous carrier liquid without nutrients. Assay may approximate the effect of solid material emptying, but will not necessarily reflect emptying of a nutrient-rich meal. Terminal assay
Solid food emptying	In fasted animals, a pre-weighed amount of food pellets are provided to the animal. After a defined period of time, the stomach is removed and the contents are carefully dried to determine the amount of remaining food	Material in stomach will change emptying rate. Animal may not eat all of the presented food pellets. Drying of stomach content must use a reproducible protocol to ensure consistent results across animal cohorts. Terminal assay
[^{13}C]-octanoic acid breath test	In fasted animals, a meal of egg yolks containing [^{13}C]-octanoic acid is consumed by the animal. Expired $^{13}CO_2$ is measured over 4 h. Assays can be repeated for longitudinal studies	Animal must be habituated to consuming the test meal and the testing apparatus. Animal must be fasted before each test. The carbon isotope analyzer is expensive
Barium meal	Test meal containing barium is administered by gavage to fasted animals. After a defined period of time, X-ray image is analyzed for the amount of barium remaining in the stomach. Assay can be repeated for longitudinal studies	Radiography equipment is expensive. Animal must be fasted before each test. Assay will not necessarily reflect emptying of a nutrient rich meal
Plasma acetaminophen	Liquid suspension of acetaminophen is administered by oral gavage to fasted animals. Blood samples are taken at defined time points and assayed for acetaminophen content in the plasma following duodenal absorption. Assay can be repeated for longitudinal studies	Material in stomach will change emptying rate. Assay is limited by the amount of blood that may be withdrawn from animal

2.2 Gastroesophageal Reflux Disease (GERD)

In larger animal models (dogs, cats, pigs, rabbits) both anesthetized and ambulatory manometry, pH monitoring, and surgical manipulations such as cardiomyectomy (Hu et al. 2012; Alshehri et al. 2014) can be used to assess transient lower esophageal sphincter relaxations (TLESRs) (Plowright et al. 2013; Palheta et al. 2014) and morphological or histological evaluation of esophageal tissue following treatment with experimental therapeutics. In contrast to the larger animal models, there are limited mouse models that rely on surgical manipulations to induce GERD-like changes in esophageal histology. Some mouse models use surgery to anastomose either the duodenum (He et al. 2015) or the jejunum (Aikou et al. 2013) to the distal end of the esophagus to allow for direct reflux of digested materials onto the esophagus and the lower esophageal sphincter (LES). The model of "mixed" reflux uses a duodenal anastomose with removal of the stomach (Fang et al. 2013) to induce esophageal damage. The gastric reflux model directly removes a section of the forestomach, which compromises the ability of the LES to adequately close, thereby producing reflux of gastric contents (Chen et al. 2014b). These models were shown to have altered esophageal barrier function induced by NF-kB signaling (Fang et al. 2013), with damage mitigated by treatment with an inhibitor, and involve expression of Nrf2, demonstrated with knockout mice (Chen et al. 2014b). In contrast to the surgically induced models, smooth muscle Hgs knockout mice demonstrate a spontaneous esophageal pathology that included progressive dilation with muscle thinning, altered motility, and increased inflammatory markers (Chen et al. 2015). In rats, there are three commonly used surgical techniques that induce reflux by changing gastric accommodation and emptying. The primary model uses a single ligature of suture along the corpus-forestomach border to decrease overall stomach volume and compromise the ability of the LES to stay closed along with a second ligature at the pyloric sphincter to restrict the ability of stomach contents to empty (Singh et al. 2011; Nakano et al. 2012; Khinchi et al. 2014; Pawlik et al. 2014; Shukla et al. 2014; Zamora et al. 2014; Giri et al. 2015). The other two models modify the primary model by either removing the pyloric ligation (Kumar et al. 2014; Kwon et al. 2016) or placing a short piece of catheter tubing over the proximal duodenum instead of the pyloric ligation (Nahata et al. 2012; Masaka et al. 2013; Nakahara et al. 2014; Shimazu et al. 2014), with both modifications further restricting the ability of stomach contents to empty. In these models, the effects of novel therapeutics can be evaluated for morphologic and histologic appearance of the esophagus, effect on gastric acid secretion, and changes in inflammatory markers, with omeprazole commonly used as a positive control (Singh et al. 2011; Khinchi et al. 2014; Nakahara et al. 2014; Zamora et al. 2014). The models have also demonstrated nitric oxide-mediated worsening of esophageal damage in female rats (Masaka et al. 2013), sleep disruption (Nakahara et al. 2014), altered gastric motility associated with decreased ghrelin (Nahata et al. 2012), increased oxidative stress in the inflamed tissue with concurrent loss of Nrf2

Table 2 Animal models of GERD

Assay	General method and outcomes	Limitations
Cardiomyectomy	Surgical procedure to remove esophageal muscles immediately proximal and distal to the gastroesophageal junction, without damage to the esophageal mucosa. Induces a chronic weakening of the lower esophageal sphincter, resulting in spontaneous reflux of gastric contents into the lower esophagus	Extensive surgical procedure limited to larger animal models
Manometry	Esophageal peristalsis, lower esophageal sphincter openings (TLESRs), and pH recording are performed in anesthetized animals. The LES can be manipulated with pharmaceuticals or directly damaged and recovery of function can be monitored. Suitable for longitudinal studies	Requires a larger animal model due to size of equipment. High face validity, variable construct validity
Surgically induced reflux: *gastric, jejunal, mixed*	The section of the forestomach is removed or the duodenum or jejunum is anastomosed to the lower esophagus to allow for direct reflux of contents. Effects of compounds can be evaluated for healing esophageal damage via histology or organ bath studies	Can be used in rodent models. Reflux damage is not induced by a clinically relevant reflux pathology due to surgical manipulation of the stomach and GI tract
Stomach ligation with or without duodenal restriction	Ligation(s) of the pylorus, forestomach, and/or corpus and/or mechanical restriction of the duodenum compromises the LES and prevents normal emptying of stomach contents to allow for reflux. Effects of compounds can be evaluated for healing esophageal damage via histology or organ bath studies	Can be used in rodent models. Acute model only. Does not represent clinically relevant reflux pathology
Knockout animals	In genetically modified rodents, contractility of esophageal smooth muscle, the LES, and gastric smooth muscle can be evaluated in organ baths with/without treatment of novel therapeutics to evaluate potential mechanisms for reflux. Histology can also be evaluated in the same models	Changes in contractility and histological appearance may not reflex a clinically relevant pathology. Genetically modified models likely only present mechanisms for a subset of patients
Motility recordings	In healthy animals, contractility of esophageal smooth muscle, the LES, and gastric smooth muscle can be evaluated in organ baths with/without treatment of novel therapeutics	Ex vivo study. Changes in contractility may not induce a clinically relevant reflux pathology

expression and an increase in NF-kB expression (Kwon et al. 2016), and a decrease in esophageal damage through angiotensin-(1–7) signaling (Pawlik et al. 2014) (Table 2).

2.3 Peptic Ulcer Disease (PUD)

Due to the rapid onset of most of the ulcer-inducing models, mice and rats are often used to screen novel compounds for gastroprotective effects. In particular, there are many studies examining the effects of traditional medicines or plant-based compounds to prevent damage or accelerate healing in multiple models of induced peptic ulcers as described in Table 3 (Ali et al. 2014; Guesmi et al. 2014; Junior et al. 2014; Nordin et al. 2014; Balogun et al. 2015; Batista et al. 2015; Chang et al. 2015; Olatunji et al. 2015; Pinheiro Silva et al. 2015; Rocha Caldas et al. 2015; Wang et al. 2015a, b; Zhao et al. 2015; Chen et al. 2016). Another approach to develop new therapeutics has been to modify the structure of synthesized compounds to optimize efficacy (Agotegaray et al. 2014; Kudryavtsev et al. 2014; Rathore et al. 2014; Rossato et al. 2015; Son et al. 2015). Broadly, many other experimental compounds target established anti-inflammatory pathways, but an extensive review of potential mechanisms is beyond the scope of this chapter.

2.4 Postoperative Ileus

While larger animals have been used to develop new surgical techniques for use in clinical practice, and those animals may develop POI following the surgical manipulation, most recent literature has focused on rodent studies to evaluate mechanisms responsible for the development of POI as well as the effects of novel drugs in those models. In guinea pigs, POI was induced after brief manipulation of the cecum with upper GI transit measured after 3 or 6 h after a barium/charcoal/saline meal, which demonstrated significant decreases in total transit at both time points (Choi et al. 2013). In the same guinea pig model, acute administration of mosapride, tegaserod, and prucalopride, but not serotonin, dose-dependently increased POI-delayed upper GI transit at 3 h, whereas only prucalopride increased lower GI transit measured as total fecal pellet output (Park et al. 2013).

In anesthetized rats, POI can be induced by "running the bowel," which is accomplished by removing the proximal colon, cecum, and small intestine from the abdominal cavity, followed by gentle probing of the entire exposed GI tract with sterile cotton swabs soaked in sterile saline for approximately 5 min. The exposed intestines are then covered with sterile gauze moistened with sterile saline for an additional 10 min, following which the viscera are returned to the abdominal cavity and the incision is closed (Kalff et al. 1998; Venkova et al. 2007). Determination of delayed upper GI motility can then be assessed by administration of a test meal, followed by collection of the stomach and small intestine after a defined time period, with total transit determined by the head of the meal and/or geometric

Table 3 Animal models of PUD

Assay	General method and outcomes	Limitations
Acetic acid-induced ulcers	After an acute laparotomy to expose the stomach of the animal, 20–60% acetic acid is injected into the submucosal layer of the antrum at multiple sites. Alternately, the acid is applied to the serosal surface of the corpus. Effects of novel compounds can be monitored through endoscopic techniques in larger animals, or by measuring the size of the ulcers following treatment. Ulcers may relapse spontaneously in some animals, allowing for acute or repeated compound administration	Use of surgical manipulation to induce pathology reduces construct validity. Can be a terminal assay in smaller animals
Pylorus ligation ulceration model	After an acute laparotomy to expose the stomach, the pyloric region is ligated. Ulcer size is monitored acutely in comparison to animals pretreated with novel therapeutics	Low construct validity due to surgical induction of model balanced by use of endogenous acid to produce ulceration. Typically a terminal assay
HCl/ethanol-induced ulcers	Animals receive a gavage of 70–100% ethanol with or without 0.15–0.45 M HCl. Ulcer size is monitored acutely in comparison to animals pretreated with novel therapeutics	Moderate construct validity, but ulcers do not reoccur if allowed to heal completely. Terminal assay
Restraint stress/ cold-restraint stress-induced ulcers	Animals are placed in a tube to restrain movement for up to 24 h at room temperature, in a cold room (4 °C) for 2 h, or they are submerged to the xiphoid process in cold water for up to 20 h. Ulcer size is monitored acutely in comparison to animals pretreated with novel therapeutics	Limited construct validity not a relevant etiology for patients. No evidence for spontaneous reoccurrence after healing. Terminal assay
Acetylsalicylic acid-induced ulcers	Animals receive two gavages of 128 mg/kg acetylsalicylic acid separated by 4 h or a single 150–400 mg/kg oral dose. Ulcer size is monitored acutely in comparison to animals pretreated with novel therapeutics	Reasonable construct validity for NSAID-induced ulcers, but with a more rapid onset as dose is not in typical therapeutic range. Terminal assay
Indomethacin-induced ulcers	Animals receive a gavage of an indomethacin solution (10–100 mg/kg), a subcutaneous dose (10–30 mg/kg), multiple s.c. doses (25 mg/kg), multiple oral doses (5 mg/kg), or an intraperitoneal dose (30 mg/kg). Ulcer size is monitored acutely in comparison to	Reasonable construct validity for NSAID-induced ulcers, but with a more rapid onset as dose is not in typical therapeutic range. Terminal assay

(continued)

Table 3 (continued)

Assay	General method and outcomes	Limitations
	animals pretreated with novel therapeutics	
Piroxicam-induced ulcers	Animals receive a gavage of a piroxicam solution (100–200 mg/kg). Ulcer size is monitored acutely in comparison to animals pretreated with novel therapeutics	Reasonable construct validity for NSAID-induced ulcers, but with a more rapid onset as dose is not in typical therapeutic range. Terminal assay
Novel compound-induced ulcers	Animals are dosed with a test compound (may be any route of administration, but is typically by gavage). Any resulting ulcer size is monitored acutely	Used to screen novel therapeutics for ulcerogenic activity, so not relevant to clinical pathology. Terminal assay

center of the test meal (depending on the composition of the test meal). Stomach emptying may also be calculated if the total amount of the test meal is known and if the residual meal in the stomach can be accurately estimated. Delays in lower GI transit can be measured as total fecal pellet output per unit time, or by injecting a test substance at the start of the colon and measuring the time for the substance to exit the colon. Typically, due to the variability of material retention by the cecum, total GI transit is not practical in rodents. Using this surgical model with a test meal containing Evans blue dye, carbachol, mosapride, and jatrorrhizine increased POI-delayed gastric emptying and small intestinal transit 4 h after the POI surgery (Zhang et al. 2012). In a similar study, small intestinal transit was significantly delayed at 12 h and 1, 3, and 7 days post-surgery, which was associated with increased inflammatory cells in the tissue (Goetz et al. 2013, 2014). In an alternative procedure involving only exposing the peritoneal cavity to room-temperature air while keeping the visceral moist, there was a significant delay in upper GI transit 24 h after a 3-h air exposure that was associated with increased cytokine expression throughout the GI tract (Tan et al. 2014b). In a model of POI induced by 10 min of "running of the bowel," gastric emptying and geometric center of a phenol red meal were significantly delayed 24 h post-induction, with gastric emptying, but not geometric center, improved by pretreatment with K252a, a nerve growth factor antagonist (Berdun et al. 2015). In rat model of POI using 5 min of small intestine and cecum manipulation, delayed small intestinal transit of an Evans blue meal was dose-dependently improved by a citrus peel extract with a response similar to cisapride when measured 1 h after induction (Lyu and Lee 2013). In the "running of the bowel model," ginosenoside Rb1, an active compound from ginseng, dose-dependently reversed delayed upped GI transit of a charcoal meal along with reversing changes in serum and tissue cytokine expression induced by the POI model (Tan et al. 2014a). In a study of gastric emptying delayed by POI, POI with additional morphine treatment, or morphine alone, RM-131, a growth hormone secretagogue receptor agonist, improved or accelerated gastric emptying of a phenol red meal with more potency than exogenously administered ghrelin, and

with similar efficacy as other ghrelin mimetics at 3 h post-induction (Van der Ploeg et al. 2014). At 24 h post-induction, there was a significant delay in the geometric center of an FITC-dextran meal that was associated with increased expression of matrix metalloprotease 9 and tissue inhibitor of metalloproteinase 1 (Chang et al. 2012).

For studies of POI in mouse models, the multiple knockout strains have provided clues to the etiology of the disorder that could be relevant to new therapeutics. For example, the delay in GI transit was similar to wild-type mice in CB1 receptor knockout mice with only some additional inflammatory infiltrate in the knockout mice (Li et al. 2013). At 24 h after intestinal manipulation, small intestinal and colonic transit, measured with FITC-dextran meal, was delayed and was associated with increased cytokine expression within the smooth muscle which was dependent on mast cells, as the effects were lessened in mast cell knockout mice (Snoek et al. 2012; Vilz et al. 2012); however, a different mast cell knockout mouse line found no changes in the extent of POI (Gomez-Pinilla et al. 2014). In a similar fashion, mice with deletion of the alpha-7 nicotinic acetylcholine receptor, which is expressed on macrophages, had a similar response to intestinal manipulation as wild-type mice (Matteoli et al. 2014). Further examining the potential role of mast cells in the development of POI, GSK143, a spleen tyrosine kinase inhibitor, reversed delayed transit and decreased inflammatory markers to a similar extent as the mast cell stabilizer doxantrazole (van Bree et al. 2013). Examining the role of T-cells in the development of POI, mice lacking secondary lymph nodes or that were treated with an FTY720, a T-cell stabilizer, did not show a delay in GI transit of an FITC-dextran meal following intestinal manipulation (Koscielny et al. 2013). Furthermore, generally preventing inflammation within the GI tissues can prevent delays in GI transit as demonstrated using exogenous sodium nitrite (Cosyns et al. 2015), a $5HT_3$ antagonist (Maehara et al. 2015), a glucosylxanthone isolated from mango (Morais et al. 2015), or rikkunshito (Endo et al. 2014). However, TLR2, TLR4, or TLR2/4 knockout mice also developed POI that was similar to wild-type mice (Stoffels et al. 2014), implying that only certain immune factors meditate POI induced by intestinal manipulation.

3 Animal Models of Lower GI Diseases

3.1 Irritable Bowel Syndrome (IBS)

The study of therapies and mechanisms responsible for IBS is partially limited by the lack of animal models with high face and construct validity. Many IBS animal models can induce enhanced visceral nociceptive responses (a surrogate for visceral hypersensitivity and the chronic pain in IBS patients) with/or without changes in motility, but these phenotypes are only the minimal requirement for modeling IBS-like symptoms. In particular, for rodents, most experimental manipulations can accelerate colonic transit, which may also induce diarrhea, but there are almost no models of induced constipation, or the mixed bowel response seen in some IBS

patients. Additionally, comorbid disorders, such as anxiety and depression, are difficult to evaluate in animals, typically requiring larger experimental n-values and multiple assays to verify the behavior. Finally, most reported animal studies have used male animals, while IBS is female predominate, so the issue of sex differences within the models has largely not been investigated, although current policies from funding agencies are attempting to address this gap in the literature. Even with these limitations, many animal models have been developed that change visceral sensitivity and thereby may provide clues to the etiology of IBS which could lead to novel therapies (Table 4).

3.1.1 Early-Life Adversity Models of Colonic Hypersensitivity

Limited Nesting

The purpose of the limited nesting (LN) model is to represent early-life adversity caused by an impoverished environment (Gilles et al. 1996). By limiting the ability of the dam to create a proper nest, maternal care is altered, with less adequate nursing and more time for the pups out of the nest, without actually separating the dam from the pups. While this model has not been used extensively, a sex-specific effect on adult animals has been demonstrated with only male rats demonstrating an increase in colonic sensitivity to distension (Prusator and Greenwood-Van Meerveld 2015). The effect of LN on GI function in adolescent animals has not been evaluated.

Maternal Separation

The maternal separation (MS) model has been used extensively in the study of early-life adversity-induced changes in GI sensitivity and function (O'Mahony et al. 2011b). MS was developed to model both neglect and some abusive behavior due to the change in the maternal behavior after returning the pups to the nest (Plotsky and Meaney 1993). Since there is no standardized protocol for separation, the effect on colonic sensitivity is variable in the literature. In adult animals, some MS protocols demonstrate increased colonic sensitivity exclusively in male rats (Coutinho et al. 2002), while other protocols require an additional stressor to induce colonic hypersensitivity in MS animals (van den Wijngaard et al. 2013). A single dose of gabapentin was shown to reduce colonic sensitivity in adult male MS rats (O'Mahony et al. 2011a). Through the use of selective antagonists, neuronal nitric oxide synthase (Tjong et al. 2011), hydrogen sulfide (Li et al. 2012), and histamine type 1 receptor (Stanisor et al. 2013) have been shown to decrease MS-induced hypersensitivity. A modified MS protocol has also been used in male wild-type and $GABA_{B(1b)}$ knockout mice (Moloney et al. 2012).

Odor-Attachment Learning

Early-life adversity induced by odor-attachment learning (OAL) was developed to model an attachment to an abusive caregiver (Sullivan et al. 2000). Unlike the LN and MS models, this paradigm does not change maternal behaviors, but instead relies on paired and unpaired presentations of an odor and a shock with the pups. In

Table 4 Animal models of IBS

Assay	General method and outcomes	Limitations
Early-life adversity—limited nesting	Dams are provided with a minimal amount of nesting material and typically housed on a wire cage bottom that allows for separation of dam and litter from waste from postnatal days 2–9. Dam's quality of care is affected. Adult animals demonstrate colonic hypersensitivity to distension	Effect on colonic sensitivity seems to be predominately in male animals. Unknown effect on motility. Requires at least 70 days to reach early adulthood for behavioral studies. Face validity is relevant to an impoverished environment
Early-life adversity—maternal separation	Multiple protocols have been used in the literature. Typical protocol separates dam from the litter for 3 h per day from postnatal days 2–14. Dam's quality of care is affected. Adult animals may demonstrate colonic hypersensitivity to distension	Effect on colonic sensitivity seems to be predominately in male animals. Unknown effect on motility. Requires at least 70 days to reach early adulthood for behavioral studies. Adult animals may require an additional stressor to demonstrate hypersensitivity. Face validity is relevant to neglect
Early-life adversity—odor attachment learning	Pups are exposed to a paired odor-shock (conditioned) stimulus, an unpaired odor and shock stimulus, or the odor only from postnatal days 8–11, with learning verified on day 12. No change in the dam's care since only half of the litter is removed. Adult female rats exposed to the unpaired condition demonstrate colonic hypersensitivity	No effect on colonic hypersensitivity in male rats. Model has only been validated for Long–Evans rats. Requires at least 70 days to reach early adulthood for behavioral studies. No effect on motility. Face validity is relevant to an abusive caregiver
Early-life adversity—neonatal colonic irritation	The colon of the neonatal pups is directly exposed to chemical irritants and/or balloon distension. Extent of inflammation or damage is dependent on specific substance used (mustard oil, zymosan, acetic acid, high-pressure distension). Adult animals demonstrate colonic hypersensitivity. Some irritants also induce chronic diarrhea	Requires at least 70 days to reach early adulthood for behavioral studies. Face validity may be relevant to pediatric gastroenteritis
Restraint stress	Several variations in the literature. Typically, animal is acutely placed in a tube or cylinder to prevent locomotion and grooming for at least 1 h. Can be used in combination with different room temperatures. Induces an acute increase in colonic hypersensitivity along with changes in gastrointestinal permeability and motility	Some strains of rats and mice will habituate to repeated exposure. Effects resolve in several hours. Construct validity of physical stressors is questionable

(continued)

Table 4 (continued)

Assay	General method and outcomes	Limitations
Water avoidance stress	Some variations in the literature. Typical protocol involves placing animal on a platform surrounded by water for 1 h for 7–10 days. A single acute exposure has also been studied. Induces an increase in colonic hypersensitivity along with changes in gastrointestinal permeability and motility	Some strains of rats and mice will habituate to repeated exposure. Repeated exposure may produce a persistent phenotype, whereas acute exposure effects resolve quickly. Construct validity is low, but relevant for rodents
Brain or spinal cord manipulation	Targeted manipulation of discrete central nervous system regions with hormones, neurotransmitters, or receptor antagonists can induce changes in gastrointestinal physiology, including sensitivity, permeability, and motility	Effects on gastrointestinal function can be acute or persistent depending on region and type of manipulation. Surgical expertise and specialized instruments are necessary. Face validity will vary depending on the region targeted
Acetic acid enema	The animal receives an enema of dilute (0.6–1%) acetic acid. Gross inflammation is not evident. Colonic sensitivity is assessed by response to distension within hours of enema administration. Good for quick screens of novel therapeutics to modify sensitivity. Possible to retest animal after a suitable recovery period (at least a day, depending on half-life of tested pharmaceutical)	Transient model. Poor construct validity since acute hypersensitivity may not model chronic clinical conditions. Face validity is limited to effect on colonic sensitivity
Butyrate enema	A persistent colonic hypersensitivity can be induced in rats by repeated butyrate enema administration. Gross inflammation is not evident	Unknown construct validity. Not a rapid model as hypersensitivity develops over repeated enemas
Capsaicin, mustard oil, zymosan enema	Enema induces an acute inflammation. Can be used for rapid screening of compounds. Dose of irritant can be titrated to influence the extent of inflammation. Animals will demonstrate hypersensitivity to distension during active inflammation	Poor face validity as active inflammation does not represent an IBS-like phenotype. Limited construct validity since irritants are necessary to establish the inflammation
Post-inflammatory hypersensitivity	After recovery from the models that induce an acute inflammation (TNBS, acetic acid, etc.), a subset of animals will develop hypersensitivity to distension	Slow model due to time necessary to resolve inflammation (2–12 weeks). As few as 30% or as much as 100% of animals may develop hypersensitive response. Construct validity is mixed based on cause of initial inflammation

adulthood, this model causes a female-specific colonic hypersensitivity only in the unpaired treatment group (Chaloner and Greenwood-Van Meerveld 2013).

Neonatal Colonic Irritation

To model recovery from a neonatal gastroenteritis or possibly abuse, neonatal colonic irritation (nCI) was developed (Al-Chaer et al. 2000). In adult male rats, when the neonatal inflammation had resolved, nCI-induced colonic hypersensitivity was inhibited by antagonists of $5HT_4$ (Yan et al. 2012), CRF (Jia et al. 2013), hydrogen sulfide synthase (Qu et al. 2013), and HCN channels (Chen et al. 2014d).

3.1.2 Restraint Stress-Induced Colonic Hypersensitivity

Recent literature has shown that several targets could inhibit colonic hypersensitivity induced by acute or repeated restraint stress. A protease receptor antagonist inhibited both the hypersensitivity and stress-increased paracellular permeability (Zhao et al. 2011), and guanylate cyclase-C activation (Silos-Santiago et al. 2013) also reduced colonic hypersensitivity. Additionally, altering the colonic microbiota also prevented stress-induced colonic hypersensitivity as demonstrated by administration of an antibiotic (Xu et al. 2014) or a probiotic (Agostini et al. 2012).

3.1.3 Water Avoidance Stress (WAS)-Induced Colonic Hypersensitivity

WAS is a psychological stressor that has been used in rats and mice to explore mechanisms of stress-induced colonic hypersensitivity, altered motility, and enhanced permeability. In mice, activation of protease-activated receptor-4 (PAR-4) inhibited repeated WAS-induced colonic hypersensitivity, while a PAR-4 antagonist did not further enhance stress-induced hypersensitivity (Annahazi et al. 2012). As with restraint stress, mice pretreated with a probiotic demonstrated a decrease in WAS-induced hypersensitivity that was associated with normalization of WAS-induced changes in intestinal permeability, occludin expression, and TRL-4 expression in the colon (Nebot-Vivinus et al. 2014). In rats, repeated WAS has been shown to induce colonic hypersensitivity that is dependent on glucocorticoid receptors within dorsal root ganglion innervating the lower spinal cord (Hong et al. 2011, 2015) or with limbic brain areas such as the central nucleus of the amygdala (Myers and Greenwood-Van Meerveld 2012; Tran et al. 2013). TRPV1 antagonists (Nash et al. 2012), central CRF receptor antagonists (Tran et al. 2014), probiotics (Da Silva et al. 2014), or antibiotics (Xu et al. 2014) have also been shown to inhibit WAS-induced colonic hypersensitivity.

3.1.4 Brain or Spinal Cord Manipulation-Induced Colonic Hypersensitivity

Surgical implantation of cannula for targeting discrete brain regions or catheters to target a specific region of the spinal cord permits direct evaluation of central circuitry in the regulation of stress-induced colonic hypersensitivity. In particular, targeting the central nucleus of the amygdala (CeA) with corticosterone induces a persistent colonic hypersensitivity that is dependent on glucocorticoid receptors, mineralocorticoid receptors, and CRF (Myers and Greenwood-Van Meerveld 2007, 2010a, b, 2012a, b; Tran et al. 2015). Directly manipulating glucocorticoid receptor or CRF expression

within the CeA also changes colonic sensitivity (Johnson and Greenwood-Van Meerveld 2015; Johnson et al. 2015).

3.1.5 Acetic Acid or Butyrate Enema-Induced Colonic Hypersensitivity

Both compounds can induce a colonic hypersensitivity that is not associated with colonic inflammation. The rapidly induced hypersensitivity induced by low-concentration acetic acid (Langlois et al. 1994; Plourde et al. 1997) can be used to screen acutely administered compounds and permits retesting of the animals after a sufficient washout period. Butyrate enemas induce a persistent hypersensitivity (Bourdu et al. 2005), but require a longer time period to develop the effect. For these models, targeting serotonin receptors (Hoffman et al. 2012; Lee et al. 2012; Greenwood-Van Meerveld et al. 2014), acid-sensing ion channels (Matricon et al. 2011; Matricon et al. 2013), or mitogen-activated protein kinase (Xu et al. 2013a) has been shown to inhibit the induced colonic hypersensitivity.

3.1.6 Capsaicin-, Mustard Oil-, or Zymosan-Induced Inflammatory Colonic Hypersensitivity

While active inflammation does not model IBS, compounds that can diminish the acute pain behaviors in mice and rats may also show efficacy for chronic colonic hypersensitivity. Additionally, since these compounds are noxious, enema-induced cramping, grooming, or writhing can be measured in place of, or in addition to, the response to colonic distension. In mice, transgenic animals with a forebrain potassium channel mutation demonstrated enhanced nociceptive behaviors (Bi et al. 2011), whereas in wild-type mice a neurokinin-1 receptor antagonist (Hayashi et al. 2011), sigma-1 receptor antagonists (Gonzalez-Cano et al. 2013), a TRPA1 antagonist (Pereira et al. 2013), or a melatonin receptor antagonist (Chen et al. 2014a) decreased nociceptive behaviors or colonic hypersensitivity to distension. In rats, TRPC4 knockout animals displayed significantly decreased nociceptive behaviors (Westlund et al. 2014) and female rats showed greater mustard oil-induced colonic hypersensitivity than male rats (Ji et al. 2012). Additionally, wild-type rats had inhibited nociceptive responses following dosing with a TRPV1 antagonist (Nash et al. 2012), a $5HT_3$ antagonist (Sikandar et al. 2012), a $5HT_4$ agonist (Lee et al. 2012), or pregabalin (Sikandar and Dickenson 2011).

3.1.7 Post-inflammatory Colonic Hypersensitivity

Mouse and rat models of post-inflammatory colonic hypersensitivity attempt to mimic the chronic visceral pain that may occur following resolution of an acute GI infection through the use of an irritant that directly inflames the colon (see models of inflammatory bowel disease below). The development of post-inflammatory hypersensitivity ranges from 20 to 100% of treated animals, and the extent of the initial inflammation does not necessarily predict the final response as multiple inflammatory chemicals in multiple species and strains can produce the effect. In particular, rats that receive an enema of 4% acetic acid can develop a post-inflammatory colonic hypersensitivity within a week (due to the rapid resolution of the inflammation), whereas a TNBS enema may take up to 2 weeks to resolve the

inflammation and up to 4 weeks to develop hypersensitivity. For example, at 7–11 days post-acetic acid enema, with normal histologic appearance of the colonic tissue, rats demonstrate enhanced responses to colonic distension (Sun and Lan 2011), which was associated with increased colonic permeability, normalized with probiotics (Dai et al. 2012), and could be inhibited either with a PPARγ agonist (Paragomi et al. 2014) or berberine, a compound from a traditional Chinese herb, via a nitric oxide-dependent mechanism (Tang et al. 2013). In a similar fashion, in rats with TNBS-induced post-inflammatory colonic hypersensitivity there was an increase in NMDA receptor expression in the spinal cord (Zhou et al. 2009) and a normalization of sensitivity occurred following treatment with a TRPV1 antagonist (Nash et al. 2012), melatonin (Mickle et al. 2010), or probiotics (Johnson et al. 2011). Afferent recordings from mice with post-inflammatory colonic hypersensitivity found changes in distribution and sensitivity of the nerves (Feng et al. 2012), with prevention of hypersensitivity in GDNF family receptor a-3 knockout mice (Tanaka et al. 2011) or after treatment with a guanylate cyclase-C agonist (Castro et al. 2013).

3.2 Inflammatory Bowel Disease (IBD)

Animal models of IBD will be applicable to either ulcerative colitis or Crohn's disease if transmural inflammation is induced. A typical limitation of the exogenously induced models is that the inflammation is restricted to the colon and may be "patchy" depending on the inflammatory agent, which will affect expression of inflammatory markers if the tissue sample contains both inflamed and healthy tissue. Additionally, several mouse and rat lines have been developed that spontaneous colitis due to either gene deletion (such as IL-10 deletion) or transgene expression (such as HLA-β27). Typically, the animals will only develop colitis in adulthood and/or if they are kept in dirty cages, implying an interaction with commensal microbiota. Other genetically modified rodents may have exacerbated or blunted exogenously induced inflammation (Table 5).

3.2.1 Dextran Sodium Sulfate (DSS) Colitis

Animals that drink a DSS solution will develop an acute colitis restricted to the colonic mucosa that progressively worsens (increasing weight loss, occult or gross bleeding, diarrhea) over the period of administration. Cycles of DSS administration can also be used to develop a chronic model of colitis. Care should be taken with some mouse strains as cage mates' aggression may occur with the sickest animals. The use of genetically modified mice also allows for investigations of specific molecular mechanisms if pharmacological tools are not available. For example, mice with deletion of Runx1 in sensory nerves demonstrated decreased colonic sensitivity, but worsened inflammation, following DSS administration (Hung et al. 2014). Evaluating therapeutic effects, upregulation of glutamate transporter through administration of ceftriaxone, an antibiotic, attenuated DSS-induced colonic hypersensitivity and decreased colonic inflammation (Lin et al. 2011).

Table 5 Animal models of IBD

Assay	General method and outcomes	Limitations
Dextran sulfate sodium (DSS) colitis	Rodents consume a solution of DSS (0.5–5%) in place of their water over 3–10 days. A modified disease activity index is assessed daily that monitors stool consistency, occult bleeding, and weight loss. Colitis may resolve once DSS is discontinued, but a chronic model can be established with repeated cycles of DSS administration. Colitis is primarily restricted to the colonic mucosa, but will involve almost the entire organ	High percentage of DSS can lead to mortality and some rat strains may have an anaphylactic response. Not a spontaneous model of ulcerative colitis. Can be labor intensive with large experimental cohorts
2,4,6-Trinitrobenzene-sulfonic acid (TNBS) colitis	Rodent receives an enema of TNBS in an ethanol mixture to disrupt the colonic mucosa. TNBS concentration and ethanol concentration vary in the literature. A transmural inflammation occurs that can produce local ulcers/necrosis or a diffuse inflammation. Peak inflammatory response and duration of inflammation will vary depending on TNBS/ethanol concentration	TNBS/ethanol concentration has to be titrated to the species and strain to optimize inflammatory response. Mortality may occur from excessive necrosis. TNBS is a hazardous material. Not a spontaneous model of Crohn's disease
Infection-induced colitis	Some human pathogens (bacterial or parasitic) can directly cause colitis in animal models. Disease progression can be monitored with activity index or other physiological parameters	Pathogens within the same *Genus* may need to be substituted for the typical human pathogen. Disease progression may differ from clinical course. Route of infection may differ from clinical course

Acute administration of a TRMP8 agonist increased, while a TRMP8 antagonist decreased, nociceptive behaviors in mice with DSS colitis (Hosoya et al. 2014).

3.2.2 2,4,6-Trinitrobenzenesulfonic Acid Colitis

In mice, TNBS colitis was found to increase expression of cathepsin S, an endogenous protease-activated receptor-2 agonist, and cathepsin S knockout mice displayed less colonic hypersensitivity during active inflammation (Cattaruzza et al. 2011). In contrast, an acute enema of cathepsin G did not affect TNBS-induced colonic hypersensitivity, while a protease-activated receptor-4 (PAR4) agonist fully inhibited the hypersensitivity during active inflammation (Annahazi et al. 2012). A similar antinociceptive effect on TNBS-induced colonic hypersensitivity was demonstrated after chronic dosing of the antibiotic ceftriaxone through an upregulation of glial glutamate transporter-1 (Lin et al. 2011). Several potential mediators of active TBNS-induced inflammation and hypersensitivity have also been studied in rats. Particularly, both colonic biopsies from ulcerative colitis

patients and inflamed tissue from TNBS colitis demonstrated increases in IL-1β and prokineticin 2 expression, while a prokineticin 2 antagonist significantly inhibited colonic hyperalgesia in the rat model (Watson et al. 2012). Further, activation of guanylate cyclase-C was able to decrease colonic hypersensitivity induced by TNBS by directly decreasing colonic afferent firing frequency (Silos-Santiago et al. 2013). Finally, within the DRG or the spinal cord, TNBS-induced hypersensitivity could be significantly decreased by preventing activation of microglia (Kannampalli et al. 2014), inhibiting tumor necrosis factor-α (Song et al. 2014a), or inhibiting voltage-gated calcium channels (Qian et al. 2013).

4 Summary and Conclusions

In addition to the models described in this chapter, animals have been used to study most GI disorders, either through establishing an experimental model that can be used to test disease mechanisms and novel therapeutics or by taking advantage of naturally occurring diseases in domesticated species to study pathology. The use of animal models is a vital resource for integrating disease pathologies across multiple organ systems as well as for evaluating behavioral effects of treatments. While there are still some deficits with the construct validity of chronic diseases, most commonly used models appear to have good or excellent face validity, and as such are essential tools for developing the next generation of pharmaceutical therapies. However, the US National Institutes of Health and many international scientific journals have recently acknowledged the problem of the apparent lack of reproducibility of published preclinical studies. To address this perceived problem with reproducibility, several principles and guidelines have been established to enhance scientific rigor when reporting preclinical research (Landis et al. 2012; Clayton and Collins 2014; Collins and Tabak 2014). The lack of reproducibility in preclinical studies may have occurred not due to the animal models per se, but rather due to a bias in scientific literature that promotes publication of studies that confirm hypotheses over results that are inclusive, as well as a disincentive for reproducing previous results. As more funding agencies and journals adopt these and other guidelines to enhance scientific rigor, we can expect a positive impact on research using preclinical models that should enhance the development of effective therapeutics.

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Serotonergic Mechanisms Regulating the GI Tract: Experimental Evidence and Therapeutic Relevance

Natalie Terry and Kara Gross Margolis

Contents

Abstract

Serotonin (5-hydroxytryptamine; 5-HT) is best known as a neurotransmitter critical for central nervous system (CNS) development and function. 95% of the body's serotonin, however, is produced in the intestine where it has been

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increasingly recognized for its hormonal, autocrine, paracrine, and endocrine actions. This chapter provides the most current knowledge of the critical autocrine and paracrine roles of 5-HT in intestinal motility and inflammation as well as its function as a hormone in osteocyte homeostasis. Therapeutic applications in each of these areas are also discussed.

Keywords
Bone • Enteric nervous system • Intestinal inflammation • Intestine • Motility • Serotonin

1 Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is best known as a neurotransmitter critical for central nervous system (CNS) development and function (Kepser and Homberg 2015; Brummelte et al. 2016). 95% of the body's serotonin, however, is produced in the intestine where it has been increasingly recognized for its hormonal, autocrine, paracrine, and endocrine actions (Fig. 1) (Gershon 2013). The critical importance of 5-HT as a modulator of hormonal communication is evidenced by its presence in this role in primitive organisms, without a nervous system (i.e., sponges), that evolved over 500,000 years ago (Mukherjee et al. 2015). Despite this historical presence in animals, 5-HT wasn't identified until the 1940s (Erspamer 1940) and its vast roles in gastrointestinal (GI) function have begun to be elucidated relatively recently. Intestinal 5-HT has been found to modulate enteric nervous system (ENS) development and neurogenesis, motility, secretion, inflammation, sensation, and epithelial development (Gershon 2013; Margolis et al. 2014, 2016; Hoffman et al. 2012; Mawe and Hoffman 2013). Current knowledge of 5-HT in these areas, as well as its hormonal role in bone formation, is reviewed. A summary of relevant potential therapeutic applications is also discussed.

2 Background

Serotonin derives its name from its origin, the serum, and its ability to increase tone or vasoconstriction, hence the name "sero-tonin" (Page 1976). Its discovery was first noted by Dr. Vittorio Erspamer who discovered "enteramine" in gastrointestinal extracts from enterochromaffin (EC) cells in a rabbit (Erspamer 1940). Within a decade, the group of Rapport, Green, and Page were studying the serum vasoconstrictor, named "serotonin," for its potential role in hypertension, ultimately publishing its isolation in 1948 and its structure as 5-HT (Rapport et al. 1947, 1948; Rapport 1949). Drs. Espamer and Rapport's findings merged in 1952 when Erspamer purified and identified "enteramine" as Rapport's previously identified 5-HT (Erspamer and Asero 1952). Intestinal 5-HT would likely have garnered more immediate attention had 5-HT in the central nervous system (CNS) not been found so shortly after its

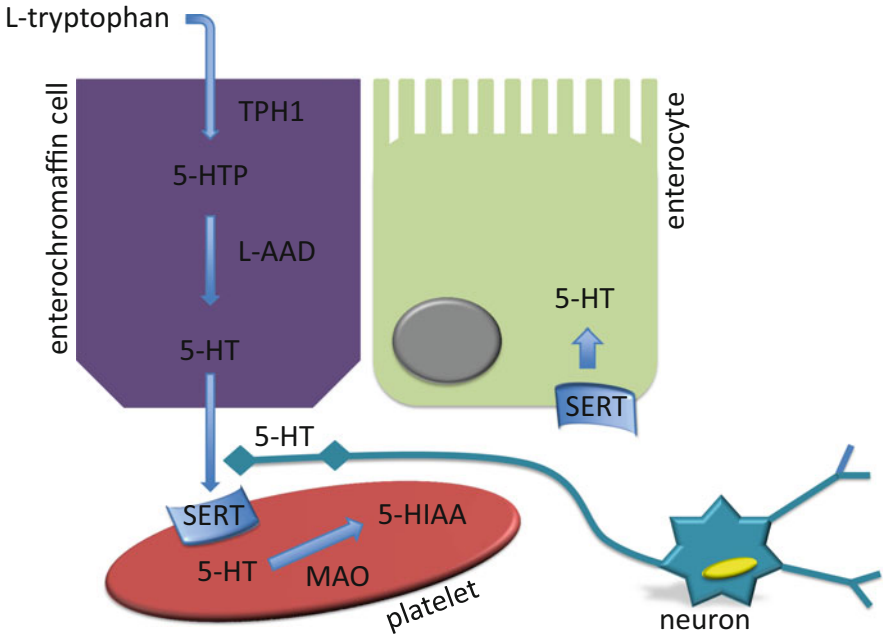

Fig. 1 5-HT biosynthesis. L-tryptophan is taken up by enterochromaffin (EC) cells (purple) where it is converted by tryptophan hydroxylase 1 (TPH1) to 5-hydroxytryptophan (5-HTP). The enzyme L-amino acid decarboxylase (L-AAD) then produces 5-hydroxytryptophan (5-HT) which is released into the extracellular space and can either act locally in the intestine, via its receptors in the intestinal mucosa or intercalated dendrites of the submucosal and myenteric plexuses, or be taken up by platelets (*red*) via the serotonin transporter (SERT). Locally acting 5-HT will be taken up by the enterocytes, via SERT, where it is broken down by monoamine oxidase (MAO) and metabolized to 5-hydroxyindoleacetic acid (5-HIAA). The 5-HT taken up by platelets is either released at a distal site, where it can execute hormonal actions, or metabolized, as in the enterocytes, by MAO within the platelet, and later excreted by the kidneys

discovery in the intestine (Twarog 1954). Given the key functions that CNS-derived 5-HT plays in the modulation of brain development, sleep, mood, appetite, and temperature regulation, it is understood why it has attained so much relative fame (Brummelte et al. 2016). The prestige regarded to these CNS-related functions, however, has decreased the attention paid to intestinal 5-HT, likely leading to its delay in understanding of function.

3 5-Hydroxytryptophan Homeostasis and Signaling

5-HT synthesis begins with its precursor amino acid L-tryptophan that is converted by the rate-limiting enzyme tryptophan hydroxylase (TPH), to 5-hydroxytryptophan (5-HTP). 5-HTP is then converted by aromatic L-amino acid decarboxylase to 5-HT

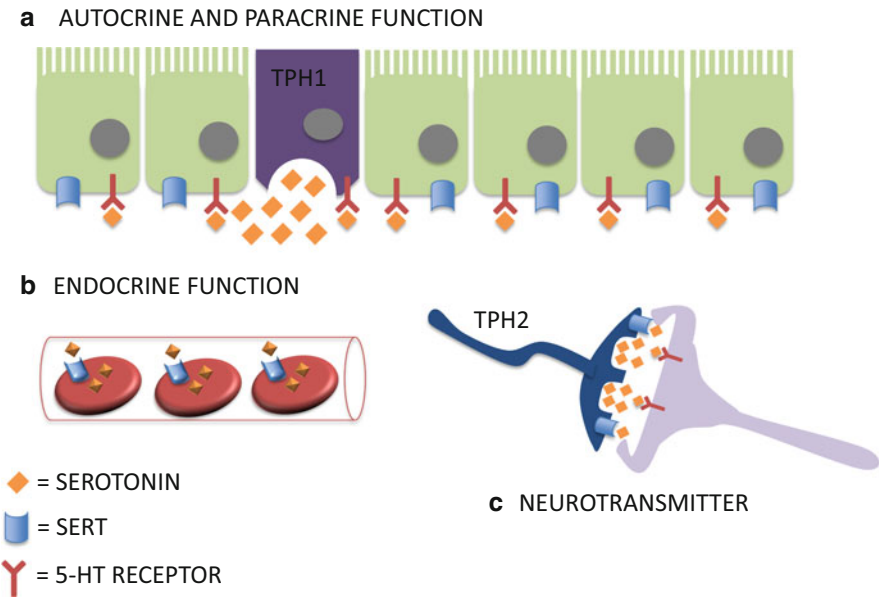

Fig. 2 5-HT signals in an autocrine, paracrine, and endocrine fashion. (**a**) In the intestinal epithelium, mucosal 5-HT (*orange diamonds*) is produced by TPH1 in the enterochromaffin cells (*dark purple*) where, once secreted, it will signal in an autocrine or paracrine fashion, to itself or to neighboring enterocytes (*green*), respectively, via 5-HT receptors (pictured in *red*). Once 5-HT has perpetrated its actions it needs to be inactivated or receptor desensitization can occur. In order to undergo inactivation, mucosal 5-HT must be taken up by the serotonin reuptake transporter (SERT; *blue spheres*), located on intestinal epithelial cells, where, once intracellular, it can be broken down by monoamine oxidase. (**b**) 5-HT secreted into the intestine can also be taken up via SERT (*blue sphere*) in platelets to be transported in the bloodstream to distal sites for endocrine function. (**c**) In the enteric nervous system, 5-HT is produced by TPH2 in 2–3% of enteric neurons. 5-HT is released into the synapse and activates postsynaptic 5-HT receptors. It is then taken up by SERT in the presynaptic neuron for deactivation

(Gershon 2013; Gershon and Tack 2007) (Fig. 2). There are two different isoforms of TPH that are separate gene products, tryptophan hydroxylase 1 (TPH1) and TPH2. In the intestine, TPH1-dependent 5-HT biosynthesis occurs in the enterochromaffin cells of the mucosal epithelium and in mast cells of mice and rats, and accounts for 90% of intestinal 5-HT production (Gershon and Tack 2007; Walther and Bader 2003). TPH2 is located in the neurons of the enteric nervous system (ENS) and the central nervous system (CNS) and accounts for the remaining 10% of intestinal 5-HT production (Zhang et al. 2004; Walther et al. 2003; Sakowski et al. 2006). Loss of TPH1, as seen in TPH1 global loss-of-function (TPH1 knockout; KO) mice, thus leads to an almost complete loss of intestinal 5-HT production but does not affect the brain in any discernible fashion whereas TPH2 global loss of function (TPH2 knockout; KO) leads to a loss of both brain and enteric neuronal 5-HT biosynthesis

(Walther and Bader 2003). The differences of TPH1 and TPH2 in 5-HT production in location and function serve to play major and very different roles in intestinal development and function (Yadav et al. 2008). For example, although the minority of intestinal 5-HT is synthesized by TPH2, its absence has profound effects on ENS development and many of the GI functions discussed below that are distinct from TPH1 (Margolis et al. 2014; Li et al. 2011a; Heredia et al. 2013).

The actions of 5-HT are mediated by 15 different receptors that have been identified in mammals, separated into seven different subclasses. Six of the seven subclasses are G-protein-coupled receptors while the 5-HT_3 receptors are ligand-gated ion channels (Hannon and Hoyer 2008). In the intestine, five receptor classes (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, and 5-HT_7) are expressed (Hoyer et al. 2002). The most well-studied intestinal receptors are 5-HT_3 and 5-HT_4. 5-HT_4 receptors are localized to presynaptic enteric neurons while 5-HT_3 receptors are localized to sensory and myenteric neurons (Gershon 2004). Once activated, the receptors serve to affect a variety of intestinal functions (see below for details).

Once 5-HT is synthesized by TPH1 or TPH2, and stimulates one or more of its receptor subtypes, mechanisms must exist for deactivation. If 5-HT is not inactivated in a timely fashion, persistent extracellular 5-HT can have toxic effects and mediate receptor desensitization (Chen et al. 1998; Gershon and Ross 1962; Wade et al. 1994). The enzyme required for 5-HT inactivation, monoamine oxidase (MAO), is intracellular. Further, because 5-HT is a highly charged molecule, an active transporter is thus required for its intracellular transport and subsequent inactivation. The plasmalemmal serotonin transporter (SERT) is the primary transporter by which 5-HT is transported intracellularly (Wade et al. 1996). Intestinal SERT is expressed in neurons of the ENS and in enterocytes of the intestinal mucosa (Gershon 2013; Gershon and Tack 2007). Selective serotonin receptor inhibitors (SSRIs) reduce the activity of SERT, thus increasing available 5-HT. In animal models, the study of SERT global loss-of-function (SERT KO) mice, which possess increased levels of extracellular 5-HT, and the study of mice possessing a constitutively hyperactive SERT (SERT Ala56), which decreases the extracellular 5-HT pool, have led to valuable insights into neurogenesis and motility as reviewed below (Gershon 2013; Gershon and Tack 2007; Margolis et al. 2016).

Platelets, which continuously circulate throughout the intestine, also express SERT (Beikmann et al. 2013). Platelet uptake of 5-HT thus contributes to the termination of its enteric activity (Lesch et al. 1993; Morrissey et al. 1977; Hughes and Brodie 1959; Matondo et al. 2009). Further, because platelets take up peripheral 5-HT, and the majority of peripheral 5-HT comes from the intestine, platelet-derived 5-HT provides a mechanism for long-range signaling by transporting 5-HT to distant targets, such as liver (Lesurtel et al. 2006) and bone (Kode et al. 2014). Importantly, because platelets take up peripheral 5-HT but do not synthesize it, blood 5-HT levels are an indicator of intestinal 5-HT production (Foley et al. 2011). On the other hand, neuronal SERT is important in regulating serotonergic neurotransmission both in the central and enteric nervous system.

4 5-HT and Neurogenesis

In addition to its function as a neurotransmitter, neuronal 5-HT is also a growth factor for neurons of the ENS, both prenatally and in adult life (Li et al. 2011a; Liu et al. 2009). 5-HT is present in the earliest born enteric neurons where it has been demonstrated to promote neuronal development and is, therefore, in a position to shape the development of the ENS. In vitro, 5-HT has also been shown to promote development of dopaminergic neurons in cultures of isolated enteric neural crest-derived precursor cells (ENCDCs) (Li et al. 2011a). The major source of 5-HT necessary for enteric neuronal development is neuronal. In TPH1KO mice, which lack the 5-HT produced by enterochromaffin cells, there are no evident abnormalities in neural subpopulations (Li et al. 2011a). In TPH2KO mice, however, the absence of neuronal 5-HT causes gross ENS hypoplasia results with severe deficits of later developing 5-HT-dependent enteric neuronal subsets including those expressing gamma-aminobutyric acid (GABA), nitric oxide (NO), calcitonin gene-related peptide (CGRP), and tyrosine hydroxylase (TH; indicative of dopaminergic neurons) (Li et al. 2011a). ENS hypoplasia is also present in SERT Ala56 mice, a mouse model with an alanine substituted for a glycine on the SERT locus, in which SERT hyperactivity causes enhanced 5-HT clearance and thus reduced serotonin available for signaling (Margolis et al. 2016). Consequently, the enteric neuroanatomy of SERT Ala56 mice is similar to that of TPH2 KO; total enteric neurons, as well as GABA-, CGRP-, and TH-expressing neuronal subsets, are significantly less abundant in SERT Ala56 mice (Margolis et al. 2016). In contrast, ENS hyperplasia results when an overabundance of enteric 5-HT is present during development, as demonstrated in SERT KO mice and mice exposed to the SSRI, fluoxetine, during embryogenesis (Margolis et al. 2016). These models support the idea that neuronal 5-HT activity is necessary for neurogenesis.

5-HT promotes enteric neurogenesis and differentiation during development and postnatally at least partly through its actions on the 5-HT_{2B} and/or 5-HT_4 receptors (Liu et al. 2009; Fiorica-Howells et al. 2000). Stimulation of 5-HT_{2B} receptors enhances the differentiation of enteric neurons from both dissociated cultures of mixed fetal gut cells and in cultures of isolated ENCDCs (Fiorica-Howells et al. 2000). 5-HT_4 agonism is neurogenic and neuroprotective; 5-HT4 agonists increase enteric neurogenesis in vivo and in vitro, and postnatal enteric neurogenesis is deficient in 5-HT_4 KO mice (Liu et al. 2009). 5-HT_4 KO and wild-type (WT) mice have the same number of enteric neurons at birth but the neuronal accumulation that occurs during the first 4 months of life in wild-type (WT) mice does not occur in those mice without 5-HT_4 receptors (Liu et al. 2009). 5-HT_4 signaling directly impacts postnatal gut-derived enteric neural stem/progenitor cell (ENSCs) proliferation as well. Neuronal proliferation and neuronal differentiation increase significantly in postnatal ENSCs and colon explants cultured with 5-HT_4 receptor agonist (RS67506)-loaded liposomal nanoparticles (Hotta et al. 2016). Results are mimicked in vivo as co-transplantation of ENSCs with 5-HT_4 receptor agonist-loaded nanoparticles leads to significantly increased neuronal density and proliferation (Hotta et al. 2016).

5 Therapeutic Implications for Neurogenesis

Enteric neuropathies and degenerative diseases are not often diagnosed until after birth. It is thus exciting that 5-HT_4 agonists may have therapeutic benefits in the postnatal period. Older 5-HT_4 agonists, such as cisapride and tegaserod, have been restricted in use secondary to adverse cardiovascular side effects based on their off-target effects on hERG potassium channels (Tack et al. 2012). Newer, more highly selective 5-HT_4 agonists, however, have not been associated with these side effects (Shin et al. 2014). Prucalopride, a newer, more targeted 5-HT_4 agonist, has been found to increase neurogenesis in vivo though it has not yet been trialed in neuropathic conditions (Margolis et al. 2016; Gershon 2016).

6 Modulation of Intestinal Motility

6.1 The Peristaltic Reflex

The peristaltic reflex is a fundamental manifestation of propulsive motility, consisting of oral contraction and aboral relaxation, which occurs in response to elevations of intraluminal pressure. The purpose of the peristaltic reflex is to increase movement of GI contents down the intestinal tract in a sequential manner. The reflex was first described by Bayliss and Starling, at the end of the nineteenth century, when they found in a dog's intestine that elevated intraluminal pressure would evoke a reproducible oral contraction and anal relaxation in vivo (Bayliss and Starling 1899, 1900, 1901). Because this reflex persisted, even when deprived of all of the intestine's extrinsic innervation, they attributed the activity to a local intrinsic intestinal mechanism that they termed the "local nervous mechanism" (Bayliss and Starling 1899, 1900, 1901). Trendelenburg confirmed that the peristaltic reflex was indeed due to a "local nervous mechanism" in 1917, when he demonstrated that the reflex could be elicited in vitro (absent of the brain, spinal cord, and sensory ganglia) (Trendelenburg 2006). He coined this behavior the "peristaltic reflex" and "the local nervous mechanism" that Bayliss and Starling referred to is now called the ENS.

It was the work of Edith Bülbring and colleagues that first highlighted the link between 5-HT, the peristaltic reflex, and the stimulation of intestinal propulsion. Bülbring showed, in isolated preparations of guinea pig ileum, that either applied or endogenously synthesized intestinal 5-HT instigates the peristaltic reflex and, further, that pressure or stimulation of the peristaltic reflex causes 5-HT to be secreted from the intestine (Bulbring and Crema 1958, 1959a, b; Bulbring and Lin 1958; Bulbring et al. 1958). While these data have been relatively conclusive in implicating 5-HT in colonic motility, there is a continuing controversy regarding whether mucosal or neuronal 5-HT is necessary for the initiation or propagation of propulsive contractions. Clinical data supports the idea that it is specifically EC cell derived, and not enteric neuronal, 5-HT that stimulates peristaltic reflexes. Carcinoid tumors, which are derived from EC cells and secrete copious amounts of 5-HT, are associated with severe diarrhea and enhanced peristaltic activity (Ahlman

1985). Moreover, serotonin receptor 3 (5-HT_3) receptor antagonists oppose this carcinoid-associated diarrhea, supporting the idea that mucosal 5-HT secretion promotes motility.

6.2 The Roles of Neuronal and Enteroendocrine Cell-Derived 5-HT in Motility

The relative importance of mucosal versus neuronal 5-HT in motility has been examined more thoroughly in recent years with mice in which mucosal and/or neuronal 5-HT synthesis has been selectively blocked either genetically or pharmacologically (Margolis et al. 2014; Li et al. 2011a). The genetic models studied, TPH1 KO, TPH2 KO, and the double knockout (TPH1/2dKO), have made it easier to investigate whether EC cell-derived, neuronal, or both depots of intestinal 5-HT participate in the modulation of peristalsis and GI motility (Li et al. 2011a). Neuronal 5-HT is critical to GI motility; small intestine, colonic, and total GI transit (GIT) are significantly slower in TPH2 KO and TPH1/2dKO mice compared to their WT littermates (Li et al. 2011a). Whether these abnormalities are due solely to the associated developmental defects in neurogenesis is not yet known. These observations are consistent, however, with prior suggestions that 5-HT mediates propagating contractile complexes, as well as slow and fast excitatory synaptic transmission (Wade et al. 1994; Erde et al. 1985; Neal et al. 2009; Takaki et al. 1985). A contrasting feature of the TPH2 KO mice, however, is an increase in gastric emptying despite the otherwise diffuse hypomotility. This observation is consistent with prior reports, using pharmacologic blockade, that neuronal 5-HT excites gastric inhibitory neurons, which promote accommodation and delay gastric emptying (Booth et al. 1986; Bulbring and Gershon 1968; Coleman et al. 2003; Mawe et al. 1989; Takahashi and Owyang 1997). Although the quantity of enteric 5-HT neurons is small (2–3%), motility could easily be influenced by the extensive projections that these neurons have with critical effectors of gut motility, including other 5-HT neurons, nitric oxide synthase-containing neurons that are the main inhibitory neurons of the myenteric plexus, and/or the interstitial cells of Cajal (ICC). It thus makes sense that the motility defects in these mice are the consequence of disrupted neurogenesis and an ongoing loss of serotonergic signaling.

Although initial studies did not support evidence of an essential role for enteric mucosal 5-HT in GI motility (Margolis et al. 2014; Bian et al. 2011), more recent data has provided evidence for a subtle, yet distinct, role for mucosal 5-HT in GI peristalsis; contracting migrating motor complexes (CMMCs), the motor complexes that trigger peristaltic waves, that are elicited in isolated TPH1KO colons of male and female mice are poorly disseminated and tend to move in the retrograde direction (Heredia et al. 2013; Smith et al. 2014; Balasuriya et al. 2016).

6.3 The Role of SERT in Motility

The roles that 5-HT play in GI motility have been further elucidated by transgenic mouse models in which SERT activity is altered or absent, and pharmacologic studies involving the selective serotonin reuptake inhibitor (SSRI), fluoxetine. The SERT Ala56 mutation, that causes SERT to become hyperactive, leads to less available 5-HT for neurotransmission (Margolis et al. 2016). The SERT Ala56 mouse exhibits not only a neuronal hypoplasia but a decrease in in vivo and in vitro intestinal motility as well (Margolis et al. 2016). These observations further suggest, as noted in the section above on neurogenesis, that it is the ENS abnormality (in this case hypoganglionosis) that impairs the generation and conduction of peristaltic reflexes and also affects in vivo GI motility, as there are similar effects to these parameters in the ENS-hypoplastic TPH2 KO mouse (Li et al. 2011a).

In contrast to the SERT Ala56 mice, genetic ablation of SERT (SERT KO mice) or antagonism to SERT (after developmental fluoxetine exposure) decreases 5-HT inactivation and thus increases its signaling impact. Accordingly, SERT KO and SSRI-exposed mice possess a hyperinnervated ENS; the resulting motility patterns are both abnormal yet more complex (Margolis et al. 2016). Mice exposed to fluoxetine during development demonstrate significantly slower total GI, small intestinal, and colonic motility compared to nonexposed controls (Margolis et al. 2016). Although SERT KO mice also have slower colonic transit, total GI transit is similar to control mice. One critical difference between these models is that, in fluoxetine-treated animals, SERT is not inhibited at the time that motility is tested (6–8 weeks of age) because fluoxetine administration ceases after the mice are weaned from breastfeeding at 3 weeks of age. In contrast, in SERT KO mice, the deletion of SERT is not time limited, which may lead to desensitization of 5-HT receptors (Margolis et al. 2016). Alternation of diarrhea and constipation in SERTKO mice, for example, has been attributed to bouts of receptor desensitization in these animals and thus may account for the lack of change in average total intestinal transit time (Chen et al. 2001).

The abnormal motility parameters normalized in both SERT KO and fluoxetine-exposed animals after chemical sympathectomy with 6-hydroxydopamine (Margolis et al. 2016). This suggests that sympathetic slowing of GI motility can exert a substantial effect on in vivo measurements of murine GI motility and that both the deletion of SERT and its inhibition during development increase central sympathetic input to the intestine (Adamec et al. 2006).

There are several potential areas of clinical relevance that are related to the SERT-based abnormalities defined in these studies. The SERT Ala56 mutation is the most common SERT-based mutation overexpressed in children with autism spectrum disorders (ASD) (Veenstra-VanderWeele et al. 2012). In addition to ENS hypoplasia and the GI abnormalities described above, SERT Ala56 animals display increased blood 5-HT (hyperserotonemia), increased clearance of 5-HT, supersensitivity at central 5-HT_{1A} and 5-HT_{2A} receptors, as well as communication deficits and repetitive behaviors that are reminiscent of ASD (Sutcliffe et al. 2005; Veenstra-Vanderweele et al. 2009; Veenstra-VanderWeele et al. 2012; Kerr et al.

2013). Other hyperefficient SERT-coding variants have also been found in subjects with ASD (Sutcliffe et al. 2005; Prasad et al. 2005, 2009). Interestingly, GI motility problems, and especially constipation, are over fourfold more common in children with ASD, implying that the findings in the SERT Ala56 mouse may implicate developmental perturbations of 5-HT signaling to both the behavioral and medical features of ASD (McElhanon et al. 2014). Further, hyperserotonemia, secondary to high-platelet 5-HT, is present in about 30% of individuals with ASD (Mulder et al. 2004; Cook et al. 1993; Geller et al. 1988; Ritvo et al. 1970), and is also consistent with a GI abnormality.

Another clinical parallel has been demonstrated in humans exposed to SSRIs in utero; a retrospective clinical study showed that children exposed to tricyclic antidepressants and SSRIs in utero were tenfold more likely to require laxatives for constipation (Nijenhuis et al. 2012a, b). As demonstrated in the preclinical studies, it is likely that antenatal exposure to these medications causes defects in ENS development (i.e., hyperplasia) that lead to long-lasting changes in GI motility.

6.4 The Roles of 5-HT Receptors in Motility

Once 5-HT is released, it then binds to specific receptors to initiate gut motility; the 5-HT_3 and 5-HT_4 receptors have been the most widely studied in regard to gut motility (Mawe and Hoffman 2013). 5-HT_3 antagonists and 5-HT_4 agonists both evoke peristaltic reflexes (Hoffman et al. 2012; Bertrand et al. 2000; Galligan et al. 2000; Grider et al. 1998). Both are present on neurons within the myenteric and submucosal plexuses of the ENS, intrinsic and extrinsic sensory neurons, and EC cells. The stimulation of 5-HT_3 receptors results in the activation of intrinsic and extrinsic afferent nerves and also the stimulation of a small number of excitatory postsynaptic potentials (EPSPs) (Paintal 1973; Hillsley and Grundy 1998; Hillsley et al. 1998; Ireland and Tyers 1987). 5-HT_4 receptor agonists augment the peristaltic reflex pathways by acting presynaptically on nerve terminals to enhance the release of acetylcholine (Tonini et al. 1989; Galligan et al. 2003; Pan and Galligan 1994; Liu et al. 2005; Fang et al. 2008). By acting in this manner, 5-HT_4 receptor agonists are thought to enhance naturally occurring reflex activity rather than to generate neurotransmission (Hoffman et al. 2011). Further, colonic epithelial cells also express the 5-HT_4 receptor where they have been demonstrated to activate 5-HT release, mucus discharge from goblet cells, and chloride secretion by enterocytes (Hoffman et al. 2012). These actions, as a whole, can alleviate constipation and visceral pain, a component of irritable bowel syndrome (IBS). Intraperitoneal or intraluminally administered tegaserod, which stimulates 5-HT_4, attenuated nociceptive responses in a rat visceral hypersensitivity model, though the mechanism by which this occurs has not been fully elucidated (Hoffman et al. 2012; Greenwood-Van Meerveld et al. 2006).

Less is known about 5-HT_{2B} receptor modulation of motility. The 5-HT_{2B} receptor is also expressed by interstitial cells of Cajal (ICC), and is important for the integrity of the ICC network. 5-HT promotes the survival of ICC in culture via actions on 5-HT_{2B}

receptors, and the density of ICC is decreased in 5-HT_{2B}-receptor-deficient mice (Tharayil et al. 2010). Because ICCs serve as a GI "pacemaker" that leads to contraction of the smooth muscle, alterations of the ICC network might impair slow-wave propagation and, as a consequence, slow intestinal motility (Du et al. 2010).

7 Therapeutic Implications of 5-HT Modulation for Intestinal Motility

Drugs targeting the 5-HT3 and 5-HT4 receptors have been demonstrated to be effective in the treatment of functional diarrhea, functional constipation, or irritable bowel syndrome (IBS). IBS is a disorder in which diarrhea (IBS-D), constipation (IBS-C), or alternating types of both (IBS-M) are accompanied by abdominal discomfort or pain. 5-HT_3 receptor antagonists have been shown to be effective in treating both the diarrhea and abdominal discomfort symptoms of IBS-D (Andresen et al. 2008; Rahimi et al. 2008). One such example is alosetron, which has been used to treat diarrhea, presumably by blocking 5-HT_3 receptors on intrinsic neurons that stimulate motility. Although the precise pain-relieving mechanisms of 5-HT_3 antagonists have not been determined, it is possible that they inhibit pain by blocking the effects of 5-HT on extrinsic sensory neurons that signal pain and discomfort (Barbara et al. 2007; Mangel and Northcutt 1999; Johanson 2004). Efforts have focused on partial agonists because stimulation of 5-HT_3 receptors on vagal and spinal afferent fibers can lead to nausea and abdominal discomfort, respectively. On the other hand, since stimulation of extrinsic sensory neurons can exacerbate nausea and emesis, specific 5-HT_3 receptor antagonists are being used to inhibit chemotherapy- and radiation-induced nausea and emesis, in part due to their activation of the vagal afferents, in addition to the area postrema in the CNS (Bertrand et al. 2000; Tyers and Freeman 1992; Evans et al. 2007; McCallum et al. 1988). One partial 5-HT_3 antagonist, pumosetrag, has also been shown to reduce reflux events in individuals with gastroesophageal reflux disease (GERD) (Evangelista 2007; Choung et al. 2008; Costall et al. 1986).

5-HT_4 receptor agonists alleviate constipation and abdominal pain that are associated with IBS-C and also accelerate the rate of gastric emptying (Evans et al. 2007; McCallum et al. 1988). In humans, oral administration of the 5-HT_4 receptor agonist tegaserod reduces rectal sensitivity to distension in individuals with IBS (Sabate et al. 2008). While the older generation of 5-HT_4 agonists, including tegaserod, were effective against chronic constipation and constipation-predominant IBS, they were removed from the consumer market because of their adverse effects. These side effects were thought to result from nonspecific actions on other targets, including the cardiac hERG potassium channels, dopamine receptors, and 5-HT_1 and 5-HT_2 receptors (Tack et al. 2012; De Maeyer et al. 2008). More specific 5-HT_4 antagonists, such as prucalopride, naronapride (ATI-7505), mosapride, and velusetrag (TD-5108), have been developed to fill this need and some have been demonstrated, in clinical studies, to effectively treat constipation without associated adverse cardiac events (Tack et al. 2012; Manabe et al. 2010). Prucalopride is currently available for therapeutic use in Europe and Canada (Gershon 2016).

Since 5-HT signaling can be disrupted in the CNS and ENS, therapeutic agents with either functional specificity to the intestine or regional specificity (i.e., do not cross the blood–brain barrier) are necessary for the treatment of 5-HT-driven gastrointestinal disorders.(Margolis et al. 2014; Crane et al. 2015) Results of a double-blind, placebo-controlled study of one of these compounds, LX-1031, indicate that this approach shows promise for treating the symptoms of non-constipating IBS (Brown et al. 2011).

8 5-HT and Inflammation

As a critical entrance to the body from the external world, the intestine is a "bodyguard" for the mammalian immune system. It must contend with the continuous assault of a vast number of potentially pathogenic organisms while simultaneously maintaining balance of a commensal microbiome (Kedees et al. 2013) (Goyal et al. 2015; Sanchez de Medina et al. 2014). Immune defenses against microbial invasion are thus well developed and neuroimmune interactions, between enteric neurons and immune cells, are important in regulating and integrating these defenses (Margolis and Gershon 2009). When this process goes awry, intestinal inflammatory disease can develop. The neuroimmune interactions that underlie intestinal inflammation involve the action of neuromodulators and cytokines that carry signals, often bidirectionally, between enteric neurons and immune cells (Buhner and Schemann 2012; Verheijden et al. 2015). One of the most fundamental paracrine/neurocrine messengers that participate in this cross talk is 5-HT. 5-HT modulates immune cell trafficking, chemotaxis, activation, and proliferation (Arreola et al. 2015; Baganz and Blakely 2013; Askenase et al. 1980, 1991; Gershon et al. 1975). 5-HT influences these actions of immune cells, in part, by stimulation of the 5-HT receptor subtypes they express, including 5-$HT_{2A, 2B, 2C}$, 5-HT_3, 5-HT_4, and 5-HT_7 (Shajib and Khan 2015). Macrophages and T cells also produce 5-HT themselves (Baganz and Blakely 2013) while rat and mouse mast cells synthesize, store, secrete, and take up 5-HT (Manning et al. 2015). The vast interactions that take place between the immune system and 5-HT are highlighted in greater detail outside of this chapter (Baganz and Blakely 2013; Worthington 2015). This evidence, however, has led to a research focus of 5-HT in intestinal inflammatory diseases. 5-HT has been implicated in the modulation of several intestinal inflammatory diseases, including Crohn's disease, ulcerative colitis, diverticulitis, and celiac disease (Foley et al. 2011; Coleman et al. 2006; Costedio et al. 2008; Magro et al. 2002; El-Salhy et al. 1997).

Data from both humans and animal models support a proinflammatory role for mucosal 5-HT in intestinal inflammatory disease. EC cells, the major producers of enteric 5-HT, are increased in intestinal biopsy specimens taken from individuals with Crohn's disease and ulcerative colitis (El-Salhy et al. 1997; Belai et al. 1997; Coates et al. 2004a). Further, the EC cells isolated from individuals with Crohn's disease secrete significantly more of the proinflammatory cytokine interleukin (IL)-1β and also exhibit more lipopolysaccharide-induced 5-HT secretion, relative to individuals without Crohn's disease (Kidd et al. 2009). The effects of adenosine receptors that drive 5-HT secretion from EC cells are amplified in IBD, providing a

mechanism for its continuous increased release (Chin et al. 2012). Enteric 5-HT levels and duodenal EC cells are also increased in patients with celiac disease, and a significant correlation is observed between peak postprandial 5-HT levels and postprandial dyspepsia scores, suggesting a role for 5-HT in promoting associated pain-related symptoms (Coleman et al. 2006). Postinfectious irritable bowel syndrome (PI-IBS), which has been suggested to represent a low-grade inflammatory state, has also been associated with an increase in the peak of postprandial 5-HT release and EC cell hyperplasia (Spiller 2007; Gershon 2005). All of the conditions described above, as well as diverticulitis, are also associated with decreased epithelial SERT expression levels (Coates et al. 2004b). A decrease in SERT, the major transporter responsible for the inactivation of 5-HT, would further increase enteric 5-HT availability. Taken together, this evidence suggests that increased levels of available 5-HT contribute to the pathogenesis of intestinal inflammation and/or to the severity of GI symptoms.

Animal models of intestinal inflammation have both confirmed the data from human studies and expanded our knowledge of the potential mechanisms underlying the role of mucosal 5-HT in enteric inflammation and/or infection. The inflammatory bowel disease models studied have included two forms of chemical colitis: trinitrobenzene sulfonic acid (TNBS)-induced colitis, that shares pathologic features of Crohn's disease, and dextran sodium sulfate (DSS)-induced colitis, a colonic inflammation that more closely resembles ulcerative colitis. Infections that have been studied include *Citrobacter rodentium*, *Trichinella spiralis*, and enteropathogenic *E. coli* (Buhner and Schemann 2012; O'Hara et al. 2006, 2007; Wheatcroft et al. 2005; Bertrand et al. 2010). The common feature underlying these disease models is that all result increased EC cell numbers and decreased levels of epithelial SERT. Not surprisingly, where measured, 5-HT levels are also increased.

The role of increased mucosal 5-HT thus appears to be proinflammatory in its contribution to the pathogenesis of intestinal inflammation. It thus makes sense that inflammation is ameliorated when mucosal 5-HT production is eliminated. When mucosal 5-HT is selectively depleted, in TPH1 KO mice and in animals that receive a nonabsorbable TPH antagonist (LP-920540), the effects are anti-inflammatory; the severity of dinitrobenzene sulfonic acid- (DNBS; similar to TNBS) or DSS-induced colitis is significantly diminished (Shajib and Khan 2015; Ghia et al. 2009) (Margolis et al. 2014). Downregulation of SERT, however, may be both a consequence and cause of inflammation. SERT expression and function are downregulated in an inflammatory microenvironment; SERT is decreased in the Caco-2 human epithelial cells exposed to the proinflammatory cytokines interferon gamma (IFN-γ) and tumor necrosis factor (TNF), or by conditioned medium from activated lymphocytes (Bayliss and Starling 1900). Importantly though, studies evaluating mice that lack SERT (SERTKO) support the idea that decreased levels of SERT, with a consequent increase in levels of 5-HT, are the cause of intestinal inflammation rather than its consequence; these mice develop an increased severity of TNBS-induced or IL-10 KO-associated colitis (Bischoff et al. 2009). While the precise mechanisms underlying the proinflammatory effects of mucosal 5-HT have yet to be fully elucidated, it is evident that after inflammation has been initiated, the participation of 5-HT gathers force in a positive

feedback loop. Inflammation leads to the downregulation of SERT and an increase in EC cell quantity, which presumably further increases the amount of 5-HT available for signaling (Linden et al. 2003, 2005; Spiller et al. 2000; O'Hara et al. 2004).

5-HT may modulate inflammatory signaling via the 5-HT_4 receptor in both pro- and anti-inflammatory ways. In animal models of colitis, 5-HT_4 receptor activation on the colonic epithelium reduces the development of, and accelerates recovery from, colitis (Spohn et al. 2010). Beyond the epithelial layer, colitis promotes enteric neurogenesis in the adult colon through a serotonin-dependent activation of 5-HT_4 that drives glial cells to transdifferentiate into neurons (Belkind-Gerson et al. 2015). Though the effect of this 5-HT_4-induced neurogenesis is not yet known, ENS hyperplasia is repeatedly found in inflamed segments of bowel in patients with Crohn's disease and has been shown in animal models to increase predisposition to both TNBS- and DSS-induced colitis (Margolis et al. 2011). The neurogenic actions of 5-HT_4 may actually be protective; 5-HT_4 receptors promote neuroprotection specifically against the oxidative stress that is released during inflammation (Liu et al. 2009; Belkind-Gerson et al. 2015; Goto et al. 2016). Other 5-HT receptors may be involved in the inflammatory cascade, such as dendritic cell 5-HT_7 receptors (Shajib and Khan 2015; Li et al. 2011b; Kim et al. 2013). Unfortunately, the studies evaluating the effects of 5-HT on dendritic cells are conflicting; one group has demonstrated proinflammatory effects of 5-HT_7 receptor activation while the other group showed anti-inflammatory effects (Idzko et al. 2004).

9 Therapeutic Implications for 5-HT Signaling in Inflammatory Bowel Disease

Small-molecule drugs that modulate 5-HT signaling pathways have been developed for the treatment of IBS, but despite their potential, the utility of related neuroprotective agents in intestinal inflammatory diseases has not yet been trialed (Brown et al. 2011; Hornby 2015).

10 Serotonin and Bone Mass Accrual

The association between 5-HT and osteoporosis was made over a decade ago in patients on SSRIs for depression and continues to be extensively studied with a variety of clinical studies trying to reduce confounding variables (Bruyere and Reginster 2015; Diem et al. 2007; Haney et al. 2007). Recent meta-analyses continue to support the association of patients on SSRIs having decreased bone mineral density and increased fracture risk. Even in a pediatric population, treatment with SSRIs is linked to low bone mass (Eom et al. 2012; Feuer et al. 2015; Rabenda et al. 2013).

The effect of SSRIs on blocking bone mineral accrual has been recapitulated in mice treated with SSRIs (Warden et al. 2005). Similar to SSRI treatment, deletion of

the 5-HT transporter, SERT, also leads to reduced bone mass, indicating that it is SERT inefficacy, rather than off-target drug effects of SSRIs, that accounts for this decrease in bone density (Warden et al. 2005). The mechanism of serotonin's effect on bone has been actively studied, with effects from brain and gut 5-HT. Interestingly, brain 5-HT is downstream of leptin that is secreted from adipocytes. Receptors in the brain stem respond to leptin to produce 5-HT which in turn decreases sympathetic tone via 5-HT2c (Ducy 2011). In the Tph2 KO mice, depletion of central 5-HT and increased sympathetic tone leads to decreased bone formation and increased bone resorption (Yadav et al. 2009). The SSRI-mediated increase of central 5-HT should thus improve bone mass instead of precipitating osteoporosis, suggesting a role for peripheral 5-HT.

The role of intestinal serotonin as a hormone affecting bone homeostasis has been suggested by studies of the LDL receptor-related protein 5, LRP5, that works with its co-receptors, members of the frizzled family, to activate the Wnt–β-catenin signaling pathway, which is crucial for bone formation (Baron and Rawadi 2007). Loss-of-function mutations in LRP5 are implicated in osteoporosis pseudoglioma (OPPG), an autosomal recessive disorder of blindness, and low bone mass while high-bone-mass syndrome is related to gain-of-function mutations in LRP5 (Boyden et al. 2002; Gong et al. 2011). A mouse model of global LRP5 loss recapitulates the eye and bone findings of OPPG, and links the bone phenotype to decreased osteoblast proliferation and function (Kato et al. 2002). Whether or not the effect of LRP5 is cell or non-cell autonomous is still controversial. One group has reported no change in bone mass in intestinal specific Lrp5 knockouts with a decrease in bone mass with an osteocyte-specific Lrp5 knockout (Cui et al. 2011). Conversely, separate studies with a conditional Lrp5 knockout have demonstrated low bone mass in the intestinal specific Lrp5 knockout (Yadav et al. 2008).

In order to more directly test the role of intestinal 5-HT in bone growth, both groups tested bone mass in TPH1 KO mice and again received similar conflicting results. Three 5-HT receptors are expressed in bone with 5-HT_{1b} the most prevalent (Yadav et al. 2008; Westbroek et al. 2001). In one study, osteoblast-specific HT_{1b} mutant mice, in combination with TPH1 intestinal mutants and Lrp5 mutants, among others, support a model in which LRP5 expression in the intestinal 5-HT-expressing EC cells inhibits TPH1 (Yadav et al. 2008). This decreased serotonin production causes a decreased activation of 5-HT_{1b} in the bone to inhibit osteoblast proliferation (Yadav et al. 2008). A second study, however, did not detect significant changes in 5-HT content or bone mass after *Lrp5* deletion or when a high bone mass-causing allele was expressed in intestinal epithelial stem cells using a villin promoter to drive Cre expression (Cui et al. 2011). Different outcomes of the two studies have been hypothesized to be due to the use of different mouse models, the structure of transgenes, and other differing experimental conditions (Bonewald 2011).

11 Therapeutic Modalities for Osteoporosis

Therapeutics that modulate 5-HT may hold promise in the treatment of osteoporosis. Nonselective 5-HT_{1b} modulators have potential off-target effects (Ducy 2011). The nonabsorbable TPH inhibitor, LP533401, however, was found to reduce serum and intestinal 5-HT levels and have a rescuing bone effect in ovariectomized mice with osteoporosis (Yadav et al. 2008, 2010; Shi et al. 2008). No clinical trials with either therapeutic option have been trialed yet.

12 Conclusions

The expansive roles that 5-HT play in ENS neurogenesis and GI function have been increasingly recognized. The recognition of 5-HT as being more than just a modulator of motility and secretion is likely to provide critical insights into many disorders whose etiologies are not fully elucidated, including those involving abnormal ENS development and intestinal inflammatory diseases. A major obstacle to understanding the physiologic roles of enteric 5-HT includes the multiplicity of responses to applied 5-HT. Because of the widespread actions of 5-HT, drugs that target specific functions must be focused to specific receptors. Even receptor targets are complex, however, because of the large number of enteric 5-HT receptors that can serve different, and even contrasting, roles. Another important goal is to understand more precisely the distributions of 5-HT receptors and to determine which receptors in specific locations are physiologically relevant. Many of these receptors are located in the CNS as well as the gut. Study of these receptors may lead to an increased understanding of brain–gut interactions and treatments for brain–gut axis disorders such as IBS. Further, specific targeting of serotonin signaling exclusively in the gut, as was accomplished with the development of a nonabsorbable tryptophan hydroxylase inhibitor, may help us to target disease more effectively while limiting off-target drug effects. Areas of importance that could not be covered in the space limitations of this chapter, but are ongoing areas of investigation, include the role of 5-HT signaling in enteric epithelial homeostasis, microbiota composition, immunity, and liver regeneration.

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Cannabinoid Receptors in Regulating the GI Tract: Experimental Evidence and Therapeutic Relevance

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Contents

Abstract

Cannabinoid receptors are fundamentally involved in all aspects of intestinal physiology, such as motility, secretion, and epithelial barrier function. They are part of a broader entity, the so-called endocannabinoid system which also includes their endocannabinoid ligands and the ligands' synthesizing/degrading enzymes. The system has a strong impact on the pathophysiology of the gastrointestinal tract and is believed to maintain homeostasis in the gut by controlling hypercontractility and by promoting regeneration after injury. For instance, genetic knockout of cannabinoid receptor 1 leads to inflammation and cancer of

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the intestines. Derivatives of Δ^9-tetrahydrocannabinol, such as nabilone and dronabinol, activate cannabinoid receptors and have been introduced into the clinic to treat chemotherapy-induced emesis and loss of appetite; however, they may cause many psychotropic side effects. New drugs that interfere with endocannabinoid degradation to raise endocannabinoid levels circumvent this obstacle and could be used in the future to treat emesis, intestinal inflammation, and functional disorders associated with visceral hyperalgesia.

Keywords

Cannabinoid receptors • Colon cancer • GPR55 • IBD • IBS • Intestinal inflammation • PPARα • TRPV1

Abbreviations

2-AG	2-Arachidonoyl glycerol
Δ^9-THC	Δ^9-Tetrahydrocannabinol
AA	Arachidonic acid
ACEA	Arachidonyl-2′-chloroethylamide
ACF	Aberrant crypt foci
AEA	Arachidonoyl ethanolamine
CB	Cannabinoid
CBD	Cannabidiol
CD	Crohn's disease
CNS	Central nervous system
CRC	Colorectal cancer
DAGL	Diglyceride-specific lipase
DG	Diglyceride
ENS	Enteric nervous system
FAAH	Fatty acid amide hydrolase
GI	Gastrointestinal
GPR55	G protein-coupled receptor 55
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
MGL	Monoglyceride lipase
NANC	Non-adrenergic non-cholinergic
NAPE	N-Arachidonoyl phosphatidylethanolamine
NAPE-PLD	NAPE-selective phospholipase D
OEA	Oleoylethanolamide
PEA	Palmitoylethanolamide
PPAR	Peroxisome proliferative activated receptor

TLESR	Transient lower esophageal sphincter relaxation
TNFα	Tumor necrosis factor alpha
TRPV1	Transient receptor potential cation channel subfamily V member 1
UC	Ulcerative colitis

1 Introduction

Marijuana has been used for centuries as a traditional medicine for a variety of disorders, in particular, those affecting the gastrointestinal (GI) tract. Today it is established that the therapeutic effects of cannabinoids in the GI tract include stimulation of appetite, normalization of gut motility, regulation of gastric secretion, and ion transport (Izzo and Sharkey 2010). The cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of the *Cannabis sativa* plant (Gaoni and Mechoulam 1964). Δ^9-THC binds and activates cannabinoid (CB) receptors. A few years after the identification of CB receptors, their endogenous ligands were characterized, i.e., arachidonoyl ethanolamine (AEA, or anandamide) and 2-arachidonoyl glycerol (2-AG), both of them derivatives of arachidonic acid (AA), and were termed endocannabinoids (Devane et al. 1992; Mechoulam et al. 1995; Sugiura et al. 1995). After more than 2 decades of research, studies have demonstrated that cannabinoids (synthetic, plant derived, and endogenous) affect major physiologic functions of the gut. Cannabinoid 1 receptors (CB_1) most likely represent physiological "brakes" within the GI tract suggesting that intestinal motility is under the control of endocannabinoids (Galligan 2009). However, more functions of CB receptors in the GI tract, such as the control of the epithelial barrier and the interaction with the gut microbiome, are emerging (Cani et al. 2016).

This chapter provides a brief overview of the role of CB receptors in the physiology and pathophysiology of the GI tract and the possible application of cannabinoids for GI disorders.

2 Cannabinoid Receptors: Part of the Endocannabinoid System

CB receptors, endocannabinoids, and the enzymes involved in endocannabinoid synthesis and degradation together constitute the "endocannabinoid system." The best known endocannabinoids, AEA and 2-AG, are synthesized from membrane-bound lipid precursors. Although various synthesis pathways have been described, the major pathway of AEA synthesis is the cleavage of its phospholipid precursor N-arachidonoyl-phosphatidylethanolamine (NAPE) by a Ca^{2+}-activated NAPE-selective phospholipase D (NAPE-PLD) (Okamoto et al. 2004). After internalization, AEA is rapidly degraded by enzymatic hydrolysis. The major enzyme in this process is fatty acid amide hydrolase (FAAH) which generates AA and ethanolamine (Deutsch and

Chin 1993). 2-AG is predominantly generated from diglycerides (DGs) by membrane-associated Ca^{2+}-sensitive DG-specific lipases (DAGLα and DAGLβ) (Bisogno et al. 2003). After binding to CB receptors and their activation, 2-AG is rapidly hydrolyzed, mainly by monoglyceride lipase (MGL; also monoacylglycerol lipase, MAGL), to AA and glycerol. MGL is the major monoglyceride hydrolase in the rat brain (Dinh et al. 2002). In contrast to other signaling lipids, endocannabinoids are produced "on demand" in response to depolarization and Ca^{2+} influx. Because of their high hydrophobicity, they act in close proximity to their site of synthesis in a paracrine and autocrine fashion. Generally, activation of CB receptors is involved in a variety of (patho-)physiological processes, such as regulation of gut function, feeding behavior, activity, emotions, pain processing, and intestinal inflammation (Cota et al. 2003; Izzo and Sharkey 2010; Silvestri et al. 2011). To date, two CB receptors have been characterized. The CB_1 receptor is mainly expressed in the central nervous system (CNS) to regulate neurotransmitter release (Herkenham 1991). However, expression has also been reported in peripheral tissues such as liver, gastrointestinal tract, and adipose tissue (Cota et al. 2003; Osei-Hyiaman et al. 2005; Duncan et al. 2005). In contrast, the CB_2 receptor is mainly expressed on immune cells and is often referred to as the peripheral CB receptor (Munro et al. 1993). There are also other receptors, such as the peroxisome proliferator-activated receptor alpha (PPARα), the G protein-coupled receptor 55 (GPR55), and the transient receptor potential cation channel subfamily V member 1 (TRPV1), all of which are responsive to endocannabinoids and/or exogenous cannabinoids and are therefore additional players in the action of the endocannabinoid system.

3 Cannabinoid Receptors in the Regulation of the GI Physiology

3.1 GI Motility

Motility of the gut is directly controlled by the enteric nervous system (ENS), a ganglionic network of neurons that expresses all components of the endocannabinoid system. Natural and synthetic cannabinoids affect motility mainly by activating CB_1 receptors which are highly expressed in the myenteric and submucosal plexus neurons of the intestine (Coutts and Izzo 2004). Activation of CB_1 receptors results in the inhibition of acetylcholine release from enteric cholinergic neurons that consequently causes a decrease in electrically evoked smooth muscle contractility and hence in peristalsis (Pertwee 2001). Although inhibition of acetylcholine release has been demonstrated to be the major effect of CB_1 receptor activity in the ENS, other mechanisms have also been proposed. Izzo et al. (1998) and others (Storr et al. 2004; Mulè et al. 2007) demonstrated that CB_1 receptors were involved in the inhibition of non-adrenergic non-cholinergic (NANC) excitatory transmission, and also in inhibitory transmission. In addition, CB_1 receptors seem to modulate endogenous purinergic effects via P2X receptors (Begg et al. 2002; Baldassano et al. 2009). Only a few studies so far have investigated the underlying mechanisms of CB_1 receptor

activation in the ENS at the cellular level. In primary cultures of myenteric plexus neurons, Boesmans et al. (2009) showed that blockade of CB_1 receptors increased while inhibition of FAAH decreased the spontaneous neural network activity, confirming the aforementioned findings. The authors also observed that CB_1 receptors were involved in the regulation of vesicle release at enteric nerve terminals (Boesmans et al. 2009).

Not only synthetic CB receptor agonists but also naturally occurring cannabinoids were shown to reduce upper GI transit and colonic propulsion (rev. in Izzo and Sharkey (2010)). Early studies demonstrated that Δ^9-THC decreases intestinal transit and inhibits electrically evoked contractions in guinea pig explants (Roth 1978; Izzo et al. 1998). A herbal non-psychotropic cannabinoid, cannabidiol (CBD), was able to normalize intestinal hypermotility under inflammatory conditions (Capasso et al. 2008a, b). The effects of CB receptor agonists on GI motility are similar to the effects observed for agonists of μ-opioid receptors or α2-adrenoceptors in the intestine suggesting synergism or even interaction of these systems (Izzo and Sharkey 2010). In line with the inhibiting effects of CB_1 agonists on motility, rimonabant (SR141716), an antagonist/inverse agonist of CB_1 receptors, increased electrically evoked contractions and peristalsis in intestinal explants in vitro (Pertwee et al. 1996; Izzo et al. 1998), and colonic propulsion in vivo (Pinto et al. 2002).

While effects of acute CB_1 receptor activation on gut motility have been studied in some detail, less is known about chronic stimulation of CB_1 receptor activity, which may likely occur in *Cannabis* users or after long-term therapy with CB_1 receptor agonists. In a recent study, Abalo et al. (2009) could show that a single administration of the CB receptor agonist WIN 55,212-2 attenuated intestinal motility, whereas repeated administration caused tolerance to the agonist in the whole GI tract, but not in the stomach. Interestingly, mice treated for 3 days with rimonabant also developed tolerance (Carai et al. 2004).

Under pathophysiological conditions, the endocannabinoid system probably displays its full significance when it is activated and many of their components are upregulated (Alhouayek and Muccioli 2012; Izzo and Sharkey 2010). Under these conditions, CB_1 receptor agonists have been demonstrated to be more potent in reducing intestinal transit than under physiologic conditions (Izzo et al. 2001). Although CB_2 receptors are also expressed in the ENS, they are suggested to play a minor role in the regulation of gut motility under physiological conditions but might become important in inflammatory states (Duncan et al. 2008a). Indeed, the CB_2 receptor agonist JWH-133, but not a CB_1 receptor agonist, delayed the transit in the inflamed gut of rats in a dose-dependent way, an effect that was blocked by a CB_2 receptor antagonist (Mathison et al. 2004). Recent reports suggest that GPR55, a receptor responsive to certain cannabinoids but phylogenetically different to the classical CB receptors, is involved in the regulation of gut motility because O-1602, a GPR55 agonist, slowed down whole-gut transit in mice in vivo (Li et al. 2013).

The acute inhibition of enzymes responsible for endocannabinoid synthesis or degradation can modify intestinal motility. Thus, in a mouse model of genetically induced constipation, pharmacological inhibition of DAGLα led to a normalization of motility (Bashashati et al. 2015). In addition, the inhibition of either of the two

major endocannabinoid-degrading enzymes, FAAH or MGL, attenuated intestinal motility through an increase in AEA or 2-AG levels, and, in consequence, through activating CB_1 receptors (Duncan et al. 2008b; Bashashati et al. 2012; Taschler et al. 2015). Interestingly, neither FAAH-deficient nor MGL-deficient mice showed changes in motility under physiological conditions (Bashashati et al. 2012; Taschler et al. 2015). However, pharmacological inhibition or genetic deletion of FAAH normalized endotoxin-induced hypermotility (Bashashati et al. 2012). A recent study by Taschler et al. (2015) convincingly demonstrated that genetic knockout of MGL in mice caused desensitization of intestinal CB_1 receptors. The intestines of these mice were insensitive to CB receptor agonist treatment in vitro and in vivo (Taschler et al. 2015).

Next to AEA, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) belong to the group of acylethanolamides. They share similar biosynthesis and degradation pathways with AEA but are not ligands of CB receptors; thus, they may be classified as endocannabinoid-like compounds. OEA and PEA, however, influence signaling of AEA via an entourage effect (Borrelli and Izzo 2009). OEA is able to reduce gastric emptying and GI transit, and further inhibits accelerated upper GI transit at a dose that is ineffective to modulate transit under physiological conditions (Cluny et al. 2009). Similarly, PEA is able to reduce GI motility under basal conditions via CB_1-independent mechanisms (Capasso et al. 2001, 2005, 2014). Additionally, PEA reduces inflammation-accelerated GI transit, most probably by modulation/activation of TRPV1 (Capasso et al. 2014). TRPV1 is expressed throughout the gut with its highest expression located in extrinsic nerve fibers of the ENS (Ward et al. 2003). Also, PPARα might be involved in these effects, since both OEA and PEA can activate PPARα, although to a different extent (Izzo and Sharkey 2010).

Gut motility is most likely also regulated by cannabinoids that act via the gut–brain axis. For instance, the CB receptor agonist WIN 55,212-2 attenuated whole-gut transit in mice when injected intracerebroventricularly (Izzo et al. 2000). Additional evidence was provided by a recent study showing that deletion of CB_1 receptors specifically in the vagal nerves of mice caused an acceleration of GI motility (Vianna et al. 2012).

Like in rodents, CB_1 receptors are also functionally present in human small and large intestines (Croci et al. 1998; Manara et al. 2002; Guagnini et al. 2006a). Thus, WIN55,212-2 and the CB_1 receptor agonist arachidonyl-2′-chloroethylamide (ACEA) inhibits electrically evoked contractions in a healthy human colon and this effect is completely blocked by rimonabant (Guagnini et al. 2006b). 2-AG and AEA were shown to inhibit acetylcholine-induced contractions in explants of human colonic longitudinal and circular muscle; however, this effect was found to be independent of CB_1 and CB_2 receptors (Smid et al. 2007) suggesting that a non-CB effect may be via GPR55 receptors. Clinical studies in human subjects revealed that dronabinol, a synthetic derivative of Δ^9-THC, slowed gastric emptying and caused relaxation of the colon (Esfandyari et al. 2006, 2007). These data suggest that the human gut is highly amenable to treatment with cannabinoids in order to regulate GI motility.

3.2 Gastric and Intestinal Secretion

There is evidence that cannabinoids play an important role in the regulation of gastric and intestinal secretion in rodents and humans. Already more than 30 years ago, first studies suggested that Δ^9-THC was able to reduce the gastric juice volume and ulcer formation in rats (Sofia et al. 1978). This observation was supported by further experiments showing that CB_1 receptor activation decreases the production of gastric acid secretion (Adami et al. 2002). In mice, activation of CB_1 receptors reduced intestinal hypersecretion induced by cholera toxin (Izzo et al. 2003). These antisecretory effects might have been brought about by inhibition of neurotransmitter release from intrinsic neurons and extrinsic primary afferents (Tyler et al. 2000; MacNaughton et al. 2004). In accordance with these findings, pharmacological inhibition and genetic deletion of FAAH or MGL provided beneficial effects against diclofenac-induced gastric irritation (Naidu et al. 2009) and proved highly gastroprotective (Warzecha et al. 2011; Kinsey et al. 2011). In contrast, CB receptor antagonists stimulated gastric acid secretion in vitro (Coruzzi et al. 2006; Borrelli 2007). Moreover, enhanced secretion was also observed in humans treated with the CB_1 receptor antagonist rimonabant (Fernandez and Allison 2004).

Collectively, activation of CB_1 (or CB_2) receptors or inhibition of endocannabinoid-synthesizing or -degrading enzymes could be beneficial for patients with dysregulated gut functions. Modulation of the acylethanolamides OEA and PEA, or administration of the non-psychotropic cannabinoids like CBD, represents an additional valuable approach for the treatment of GI motility disorders.

4 Cannabinoid Receptors: Role in GI Diseases

4.1 Emesis and Nausea

It is known that CB receptors and endocannabinoids play an essential role in the regulation of emesis (Izzo and Sharkey 2010). A synthetic Δ^9-THC derivative, nabilone, is currently used as an antiemetic agent against chemotherapy-induced vomiting; however, due to its side effects, it is not a first-choice therapeutic and is only applied in combination with other antiemetics. The beneficial effect of the drug is based on the suppression of the vomiting reflex through CB receptor activation which is, under physiological conditions, achieved by endocannabinoids, in particular, by AEA, which seems to maintain an "endocannabinoid tone" (Sharkey et al. 2007). Raising the levels of AEA and 2-AG through inhibition of endocannabinoid-degrading enzymes has been suggested as a strategy to reduce emesis. The findings that the FAAH inhibitor URB597 was able to reduce experimentally induced emesis via CB_1 and CB_2 receptors (Van Sickle et al. 2005), and the MGL inhibitor JZL184 via CB_1 pathways, strongly support this therapeutic concept (Sticht et al. 2012).

Mechanisms and brain areas responsible for nausea are less elucidated (Rock et al. 2014). Nevertheless, also in models of nausea, blockade of FAAH or MGL produces beneficial effects (Cross-Mellor et al. 2007; Parker et al. 2015). A study in

rodents demonstrated that exogenous 2-AG infused into the visceral insular cortex dose-dependently suppressed signs of nausea (conditioned gaping) (Sticht et al. 2015), suggesting a role of endocannabinoids in the control of nausea. Pharmacological interference of endocannabinoid degradation through FAAH and MGL inhibitors, therefore, could represent a worthwhile form of therapy against emesis and nausea.

4.2 Functional Bowel Disorders

4.2.1 Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) and functional dyspepsia are the most frequent forms of functional bowel disorders. A meta-analysis revealed an 11.2% global prevalence for IBS, but the number varies depending on the country (1.1–45%; Lovell and Ford 2012). Differences in region and criteria definitions also cause strong variation in the prevalence of functional dyspepsia (5–40%; Mahadeva and Ford 2016). Although IBS has been termed a functional disorder, pathophysiologic changes, such as infiltration of immunocytes, low-grade inflammation, bacterial overgrowth, and epithelial barrier dysfunction, have been observed in the mucosa of IBS patients (Ohman and Simrén 2010). According to Rome III criteria, IBS is a multifactorial disease that includes symptoms of abdominal pain, bloating, and altered bowel habits and occurs in the following forms: IBS-C (constipation predominant), IBS-D (diarrhea predominant), mixed, and un-subtyped (Longstreth et al. 2006). Visceral hypersensitivity and a distorted processing of visceral sensory input to the brain are likely causes for the abdominal pain experienced by IBS patients.

The main physiologic tasks of the GI tract, i.e., motility, secretion, and maintenance of the intestinal barrier, are all under the regulation CB receptors and their endocannabinoid ligands to promote homeostasis in the gut. A change in CB receptor signaling could be therefore involved in the development of IBS. CB receptors are distributed all along the gut–brain axis, particularly in the ENS (Trautmann and Sharkey 2015) and in vagal afferent fibers (Vianna et al. 2012), capable of modulating the flow of information between the gut and the brain. Due to the localization of CB receptors, endocannabinoids can act at the gut–brain interface and influence, next to IBS, the development of other disorders, such as nausea and vomiting, GI inflammation, and obesity (Sharkey and Wiley 2016).

Regulation of Motility and Secretion in IBS

Disturbances in gut motility patterns are typical features of IBS. Patients with IBS-D display high-amplitude propagated contractions and accelerated colonic transit (Chey et al. 2001). CB_1 receptors are located prejunctionally in ENS neurons and control acetylcholine release in order to dampen exaggerated muscle contractility (Boesmans et al. 2009). Based on preclinical data that showed a decrease in hypermotility through activation of CB_1 receptors (Izzo et al. 1999; Capasso et al. 2014; Izzo and Sharkey 2010), the CB receptor agonist dronabinol was investigated in a trial of healthy volunteers. In this study, dronabinol postprandially produced relaxation of colonic tone

and motility (Esfandyari et al. 2007). The study subjects, however, reported increased sensory ratings to distension during the course of the treatment, which may have been caused by heightened awareness, a phenomenon that has already been observed in Δ^9-THC treatments (Buggy et al. 2003). Dronabinol was then further tested in IBS patients and revealed inhibitory effects on fasting colonic motility, but not on tone, especially in patients with IBS-D and alternating (Wong et al. 2011). Dronabinol at the same dose showed no effect on gastric, small bowel, and colonic transit in a later published study (Wong et al. 2012). Next to a disturbed motility, an unbalanced fluid household of the gut is another feature of IBS. Although preclinical data point at an essential role of CB_1 receptors in the regulation of gastric and intestinal secretion, their involvement in secretion during IBS has not yet been addressed in detail.

Visceral Hypersensitivity

Abdominal pain is one of the main symptoms of IBS, owing to the sensitization of visceral sensory afferents or the derangement of sensory processing along the gut–brain axis. In particular, CB_1 receptors, located in the CNS and/or at peripheral sites, may be involved in the development of hypersensitivity. Cannabinoids have been traditionally used for alleviating abdominal pain. Indeed, animal models demonstrated that pain responses during colorectal distension were inhibited by WIN55,212-2 in a CB_1-mediated fashion (Sanson et al. 2006; Brusberg et al. 2009). CB_1 receptor agonists applied into the lumen of the rat duodenum were also shown to attenuate 5-HT-induced hyperalgesia (Feng et al. 2014). However, in a double-blind, randomized, crossover human trial with IBS patients that had undergone colonic distension to elucidate whether dronabinol may decrease pain perception, no differences were observed to placebo (Klooker et al. 2011). Another trial failed to demonstrate an effect of dronabinol on sensation scores for pain and gas in response to high distension pressures (Wong et al. 2011). Maybe simultaneous activation of other analgesic receptors like opioid receptors that interact with CB receptors is necessary to effectively decrease visceral hypersensitivity. In this context it is of interest that CB receptors colocalize with opioid receptors in enteric neurons (Poonyachoti et al. 2002) or form heteromers, as previously shown for CB_1 and delta opioid receptors (Rozenfeld et al. 2012). A kappa opioid receptor agonist, asimadoline, has been shown to reduce sensation to colonic distension at subnoxious pressures in IBS patients (Camilleri 2008). In animal models, the drug salvinorin A (from *Salvia divinorum*) was able to reduce secretion and motility through involvement of both kappa opioid and CB receptors (Fichna et al. 2009; Capasso et al. 2008a, b).

Another possibility is that low endocannabinoid levels may contribute to visceral hypersensitivity. Assessment of endocannabinoids in IBS-D patients revealed that levels of PEA, however not of AEA, were decreased in comparison to healthy individuals (Fichna et al. 2013). Similar results were reported in a mouse model of inflammation-induced hypermotility (Izzo et al. 2012). PEA has also been shown to significantly alleviate experimental colitis via PPARα activation (Esposito et al. 2013). The FAAH inhibitor PF 3845 and the dual-FAAH/MGL inhibitor JZL195 both alleviated inflammatory- and mechanically evoked visceral pain in rodent models (Sakin et al. 2015). These compounds also raised the levels of AEA and PEA which could have

contributed to the beneficial effects of the drugs (Sakin et al. 2015). Thus, a decrease in abdominal pain could be achieved by an increase in endocannabinoid levels, for instance, through inhibition of endocannabinoid-degrading enzymes, such as FAAH and MGL.

It is also known that chronic stress adds to IBS symptoms. Recent studies in rodents have suggested that reciprocal changes of components of the endocannabinoid system (i.e., levels of 2-AG and COX-2/FAAH, and of CB_1 and TRPV1), located in sensory fibers that innervate the distal colon, occur during chronic stress (Zheng et al. 2015). These changes may be due to epigenetic regulations, as shown for the promoter regions of the *Cnr1* (encodes CB_1) and TRPV1 genes (Hong et al. 2015).

Despite promising preclinical data, human trials so far suggest that dronabinol given alone may be little useful in reducing visceral hypersensitivity in IBS patients; however, FAAH inhibitors or combinations of cannabinoid and opioid compounds are well worth exploring as new treatment options.

Cannabinoid Receptors and Microbiota in IBS

The development of IBS has been linked to an altered composition of gut microbiota (dysbiosis) (Kassinen et al. 2007). There is increasing evidence that ECs and CB receptors may be involved in the control of the epithelial barrier and thus in the entry of microbial products into the bloodstream (Cani et al. 2016). Rousseaux et al. (2007) could show that CB_2 receptors were induced by *Lactobacillus acidophilus* NCFM in colonic epithelial cell lines and that they could play a role in the regulation of colonic hypersensitivity. Induction of CB_2 receptors in colon epithelium was, however, not observed in humans that were given *Lactobacillus acidophilus* NCFM over a few weeks (Ringel-Kulka et al. 2014). In light of a recent study in a rodent model of visceral pain, in which antibiotic treatment caused a reduction of pain responses and a slight increase in CB_2 receptors (but downregulation of colonic CB_1 and mu opioid receptors; Aguilera et al. 2015), the role of microbiota-induced CB_2 receptor expression and the impact on abdominal pain in IBS patients still remain to be elucidated.

Cannabinoid Receptors and Genetic Variations in IBS

Polymorphisms of the *CNR1* gene have been associated with IBS symptoms. A significant association of the *CNR1* polymorphism rs806378 with colonic transit, sensation rating of gas, but not with pain, was observed by Camilleri et al. (2013), particularly in patients with IBS-D. With regard to AAT triplet repeats in the *CNR1* gene, a gene-by-phenotype interaction was observed for colonic transit and gas, strongest in IBS-D patients (Camilleri et al. 2013). Two other studies reported that allele frequencies of AAT triplet repeats in the *CNR1* gene were associated with symptoms and development of IBS (Park et al. 2011; Jiang et al. 2014).

4.2.2 Gastroesophageal Reflux, Functional Dyspepsia, and Noncardiac Chest Pain

In dogs and humans, Δ^9-THC inhibits meal-induced transient lower esophageal sphincter relaxations (TLESRs), the main mechanism underlying gastroesophageal reflux disease (Beaumont et al. 2009). In addition, Lehmann et al. (2002) could

demonstrate that CB_1 receptor activation by Δ^9-THC in TLESR was not mediated via gastric vagal afferents and suggested the involvement of central CB_1 receptors.

CB receptors may also play a role in functional dyspepsia, which is likely caused by a disturbed gastric accommodation (Tack et al. 1998). Meal-induced accommodation (but not gastric balloon distension) was sensitive to treatment with the rimonabant, suggesting that CB_1 receptor activity regulates accommodation (Ameloot et al. 2010). A recent study in patients with functional dyspepsia showed that brain areas involved in visceral nociception and homeostatic and hedonic regulation of food intake had a higher CB_1 receptor availability to CB_1 receptor radioligands than control subjects (Ly et al. 2015).

With regard to other functional disorders, dronabinol was evaluated in a double-blind, placebo-controlled prospective study of functional chest pain and revealed significantly increased pain thresholds and reduced pain intensity in comparison to placebo (Malik et al. 2016). To summarize, current data suggest that central CB receptors could play an important role in the development of functional and non-functional disorders of the upper GI tract and could be therefore targeted with cannabinoid compounds.

4.3 Inflammatory Bowel Disease

Interest in the therapeutic potential of the endocannabinoid system in inflammatory bowel disease (IBD) was sparked when patients anecdotally reported symptom relief after taking cannabis. A questionnaire on cannabis use amongst 291 IBD patients revealed that 33% of ulcerative colitis (UC) and 50% of Crohn's disease (CD) patients had used cannabis to ameliorate symptoms like abdominal pain and diarrhea (Lal et al. 2011). A retrospective observational study reported that 21 of 30 CD patients had significantly improved symptoms after treatment with cannabis, i.e., patients that used cannabis revealed a strongly decreased Harvey–Bradshaw index and a reduced necessity to undergo surgery (Naftali et al. 2011). Correspondingly, yet another survey study among 292 IBD patients found that cannabis users experienced a great relief of symptoms (Ravikoff Allegretti et al. 2013). The first prospective placebo-controlled study then showed a clinical response (i.e., a decrease in Crohn's disease activity index score by >100) to Δ^9-THC administration in ten of 11 CD patients (Naftali et al. 2013). Finally, Storr et al. (2014) reported that cannabis consumption ameliorated abdominal pain and cramping, diarrhea, and joint pain in IBD patients, and among them, 17.6% specifically used cannabis for symptom relief. Interestingly, however, this study also found that prolonged cannabis use was a strong predictor in CD patients for requiring surgery (Storr et al. 2014).

The therapeutic relevance of cannabinoids and their receptors is further supported by a plethora of preclinical studies on rodent experimental colitis models. As reviewed by Izzo and Sharkey (2010) and Alhouayek and Muccioli (2012), it has been shown that most of the major components of endocannabinoid system are differentially expressed in the inflamed colon when compared to controls. Both CB receptors, as well as their ligand AEA, were found to be upregulated in experimental colitis (Massa et al.

2004; D'Argenio et al. 2006; Kimball et al. 2006; Storr et al. 2009). Expression of the AEA-degrading enzyme FAAH, on the other hand, was decreased (Storr et al. 2008). Together, these changes in expression levels suggest an enhancement of CB signaling during colitis. Since the endocannabinoid system is thought to be involved in GI homeostasis, an increase in CB signaling might be a means to restore balance (Schicho and Storr 2011). This hypothesis is corroborated by findings that treatment with CB_1 or CB_2 receptor agonists is protective in various preclinical models of colitis (Massa et al. 2004; Kimball et al. 2006; Storr et al. 2009; Singh et al. 2012). Accordingly, genetic deletion or pharmacological antagonism of either of the CB receptors left mice more susceptible to intestinal inflammation (Massa et al. 2004; Storr et al. 2009; Engel et al. 2010). As for human data, CB_2 receptor expression was found increased in sections of the colon from patients with IBD (Wright et al. 2005; Marquez et al. 2009) suggesting a role of CB_2 receptors in inflammatory conditions in humans.

The exact mechanisms through which the endocannabinoid system affects IBD have not been elucidated yet, but evidence gathered from preclinical experiments that show improvement of disease via CB receptor signaling appears to be very promising. Further research on potential therapeutic effects of cannabinoids and related substances is thus highly warranted.

4.4 Colon Cancer

Cannabinoids have been reported to exert anticarcinogenic effects on various cancer hallmarks, such as proliferative signaling, resisting cell death, angiogenesis, and metastasis. As reviewed by Velasco et al. (2012), activation of CB receptors can induce cell death in a multitude of cancer cell lines. In colorectal cancer (CRC) cells, activation of CB_1/CB_2 receptors inhibits RAS-MAPK and PI3K-AKT survival signaling (Greenhough et al. 2007), induces tumor necrosis factor alpha (TNFα)-mediated de novo synthesis of the proapoptotic sphingolipid ceramide (Cianchi et al. 2008), induces the downregulation of the inhibitor of apoptosis protein survivin (Wang et al. 2008), and promotes endoplasmic reticulum stress, which leads to autophagy-mediated cell death (Pellerito et al. 2014). Further evidence for an involvement of the endocannabinoid system in the progression of CRC emerged after it was found that inhibition of the 2-AG-degrading enzyme MGL, either pharmacologically or through siRNA interference, attenuated the invasion of CRC cells (Ye et al. 2011). Additionally, angiogenesis could be inhibited by a cannabinoid-like compound, LYR-8, in a xenograft model using the chick chorioallantoic membrane.

The anticarcinogenic effects by cannabinoids observed in vitro were corroborated by animal models of colon cancer. When *Cnr1* (the gene coding for CB_1) was knocked out in mice carrying a germline mutation in the adenomatous polyposis coli gene ($Apc^{Min/+}$ mice), intestinal polyp burden increased drastically. Activation of the CB_1 receptor with methanandamide, on the other hand, significantly reduced the number of polyps in $Cnr1^{+/+}$ $Apc^{Min/+}$ mice (Wang et al. 2008). Accordingly, in a model of chemically induced colon cancer, treatment with the nonselective CB_1/CB_2 receptor agonist HU210 decreased the

development of aberrant crypt foci (ACF) (Izzo et al. 2008). Interestingly, in the same model, treatment with the CB_1 receptor antagonist SR141716 (rimonabant) reduced ACF formation (Santoro et al. 2009).

Studies in biopsies from CRC patients revealed that the expression level of the CB_1 receptor was lower in tumor tissue than in the adjacent nonneoplastic mucosa (Cianchi et al. 2008; Wang et al. 2008). The reason for this downregulation was found to be hypermethylation of CpG islands in the promoter region of *CNR1* (Wang et al. 2008). A large study examining the correlation between CB_1 receptor expression and CRC patient survival outcome, however, reported no differences in overall post-surgery survival between patients with high or low CB_1 receptor expression (Jung et al. 2013). In patients with high CB_1 receptor expression, distant metastases were observed to a lesser degree, but no differences were detected for tumor size or lymph node metastasis (Jung et al. 2013). In stage II microsatellite stable CRC patients, high CB_1 receptor expression even correlated with poorer disease-specific survival (Gustafsson et al. 2011). A correlation between survival and a single-nucleotide polymorphism of *CNR1* has also been demonstrated. Patients with a hetero- or homozygous genotype for the 1359 G/A nucleotide change had a shorter overall survival than patients with G/G genotype although it is not yet elucidated how this polymorphism affects CB_1 signaling (Bedoya et al. 2009). CB_2 receptor expression in CRC is less investigated. One study reported that CB_2 mRNA expression was present in 28.6% of the investigated tumor specimens and correlated with reduced overall and disease-free survival (Martínez-Martínez et al. 2015).

In summary, despite the preclinical advances in the understanding of CB signaling in cancer cells, the role of CB receptors in human colon cancer needs further investigation.

5 Concluding Remarks

Cannabinoid receptors and the endocannabinoid system are crucially involved in the regulation of gastrointestinal function under physiological and pathophysiological conditions. Research efforts during the last 2–3 decades unmasked a great potential to use this knowledge for future treatment in gastrointestinal disease. Due to the complexity of the endocannabinoid system beneficial effects may be achieved by targeting the endocannabinoid system at various sites. Receptor agonist and antagonists for the different cannabinoid receptors as well as blockers or enhancers of synthesis and degradation of the endocannabinoids may be employed. Especially in the field of functional bowel disorders, inflammatory bowel disease, and gastrointestinal cancer there is overwhelming evidence suggesting these states as promising targets where the cannabinoid knowledge may be further exploited and finally harvested by conducting the now-needed clinical trials.

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Insights into the Role of Opioid Receptors in the GI Tract: Experimental Evidence and Therapeutic Relevance

James J. Galligan and Catia Sternini

Contents

Abstract

Opioid drugs are prescribed extensively for pain treatment but when used chronically they induce constipation that can progress to opioid-induced bowel dysfunction. Opioid drugs interact with three classes of opioid receptors: mu opioid receptors (MORs), delta opioid receptors (DOR), and kappa opioid receptors (KORs), but opioid drugs mostly target the MORs. Upon stimulation, opioid receptors couple to inhibitory Gi/Go proteins that activate or inhibit downstream effector proteins. MOR and DOR couple to inhibition of adenylate cyclase and voltage-gated Ca^{2+} channels and to activation of K^+ channels resulting in reduced neuronal activity and neurotransmitter release. KORs

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couple to inhibition of Ca^{2+} channels and neurotransmitter release. In the gastrointestinal tract, opioid receptors are localized to enteric neurons, interstitial cells of Cajal, and immune cells. In humans, MOR, DOR, and KOR link to inhibition of acetylcholine release from enteric interneurons and motor neurons and purine/nitric oxide release from inhibitory motor neurons causing inhibition of propulsive motility patterns. MOR and DOR activation also results in inhibition of submucosal secretomotor neurons reducing active Cl^- secretion and passive water movement into the colonic lumen. Together, these effects on motility and secretion account for the constipation caused by opioid receptor agonists. Tolerance develops to the analgesic effects of opioid receptor agonists but not to the constipating actions. This may be due to differences in trafficking and downstream signaling in enteric nerves in the colon compared to the small intestine and in neuronal pain pathways. Further studies of differential opioid receptor desensitization and tolerance in subsets of enteric neurons may identify new drug or other treatment strategies of opioid-induced bowel dysfunction.

Keywords
Constipation • Drug tolerance • Enteric nervous system • Opiates

1 Introduction

Opioid receptor agonists are very effective in treating pain and they have powerful effects on gastrointestinal functions. Opioid receptor agonists produce their effects by interacting with opioid receptors, the MOR (the predominant receptor), DOR, and KOR, that belong to the family of G protein-coupled receptors (GPCRs) (Alex et al. 2002; Williams et al. 2013). Some opioid receptor agonists can also be used to treat gut motor and secretory disorders, especially diarrhea. However, chronic administration of opioid agonists causes constipation and in severe cases these drugs can cause the narcotic bowel syndrome (Grunkemeier et al. 2007). With the increased use of prescription opioid receptor agonists for pain treatment, there has been a parallel increase in the number of peripherally restricted opioid receptor antagonists that are used to reverse the gastrointestinal effects of opioid receptor agonists. At the same time peripherally restricted opioid receptor antagonists preserve the central nervous system-mediated analgesic effects of the agonists. Finally, there are peripherally restricted opioid receptor agonist drugs that are used to treat diarrhea but have no abuse potential. This chapter reviews the physiology and pharmacology of opioid receptors in the gut and the mechanisms by which opioid receptor agonists and antagonists alter gut function.

2 Localization of Opioid Receptors in the Gastrointestinal Tract

MOR, DOR, and KOR are expressed by myenteric and submucosal plexus neurons in the enteric nervous system (ENS) (Bagnol et al. 1997; Poonyachoti et al. 2002; Ho et al. 2003; Sternini et al. 2004; Gray et al. 2006; Poole et al. 2011; Lay et al. 2016), with some species differences. In the rat, MOR neurons are more abundant in the submucosal than myenteric plexus, whereas KORs are more numerous in the myenteric plexus, with both MOR and KOR myenteric neurons being more abundant in the stomach and proximal colon compared to other regions of the GI tract (Bagnol et al. 1997). MOR and KOR are not co-expressed in the same rat enteric neurons (Gray et al. 2006), whereas MOR and DOR extensively colocalize in rat and mouse enteric neurons (Gray et al. 2006; Poole et al. 2011). All three opioid receptors are expressed by interstitial cells of Cajal (ICCs) where MOR colocalizes with DOR or KOR in the rat GI tract (Bagnol et al. 1997; Gray et al. 2006). In the mouse GI tract, MOR and DOR but not KOR are expressed by ICC (Kim et al. 2016). In the guinea pig, MORs are localized to interneurons controlling the peristaltic reflex and to submucosal secretomotor neurons (Ho et al. 2003; Lay et al. 2016). In the guinea pig stomach, MORs are located in nitrergic inhibitory motor neurons and cholinergic secretomotor neurons (Lay et al. 2016). In the guinea pig ileal myenteric plexus, MORs are localized to cholinergic, excitatory motor neurons and vasoactive intestinal peptide (VIP) expressing inhibitory motor neurons, and excitatory, cholinergic/VIPergic interneurons (Ho et al. 2003; Lay et al. 2016). The density of MOR nitrergic neurons is much higher than that of MOR cholinergic neurons in the myenteric plexus of both the proximal (Anselmi and Sternini unpublished observations), and distal colon (Anselmi and Sternini unpublished observations; Lay et al. 2016). In the submucosal plexus, MORs are confined to VIP non-cholinergic secretomotor neurons of the ileum and distal colon (Lay et al. 2016). MORs are not expressed by intrinsic primary afferent neurons (IPANs) at least in the guinea pig intestine (Ho et al. 2003; Lay et al. 2016). Finally, MOR nerve fibers are dense in the muscle layers often in close association with ICCs (Ho et al. 2003). Both DOR and KOR have been reported in guinea pig enteric neurons with DOR being localized to myenteric and submucosal neurons and to varicose nerve fibers surrounding nerve cell bodies and the mucosal glands. KOR is confined to the myenteric plexus, where it is localized to neurons and nerve fibers supplying the muscle layers (Sternini et al. 2004). In the mouse gut, DOR-expressing neurons are most abundant in the small intestine and include secretomotor and vasomotor neurons of the submucosal plexus and excitatory and inhibitory myenteric motor neurons in the small intestine, but DOR are expressed mostly by inhibitory motor neurons in the colon myenteric plexus (Poole et al. 2011). Finally, in the human gut, MOR is localized to neuronal cell bodies and nerve fibers in both submucosal and myenteric ganglia of the small and large intestine (Sternini et al. 2004). The overall distribution of opioid receptors is consistent with their role in modulating gastrointestinal motility and secretion (see below).

Opioid receptors, particularly MOR, are expressed by immune cells, supporting a role of the opioid system in regulating intestinal inflammation and intestinal

ischemia (Stefano et al. 1996; Madden et al. 1998; Philippe et al. 2003, 2006; Sternini et al. 2004; Saccani et al. 2012; Anselmi et al. 2015). Opioid receptor activation on immune cells can have indirect actions on enteric nervous system function by suppressing synthesis or release of inflammatory mediators (Hughes et al. 2016) (Table 1).

3 Actions of Opioid Drugs on Myenteric Neurons and Gut Motility

Opioid receptors are G protein-coupled receptors that activate multiple effector molecules. MOR and DOR expressed by myenteric nerve cell bodies couple to the G_i G protein to cause inhibition of adenylate cyclase, reduced cyclic 3′,5′ adenosine monophosphate (cAMP) and reduced levels of protein kinase A (PKA) activation (Liu and Anand 2001; Christie 2008). MOR and DOR also couple to the G_o subtype of G protein which links MOR and DOR to inhibition of Ca^{2+} channels and activation of K^+ channels via a membrane-delimited mechanism (Morita and North 1982; Mihara and North 1986; North et al. 1987; Surprenant et al. 1990; Tatsumi et al. 1990; Shen and Surprenant 1991). Inhibition of Ca^{2+} channels decreases neurotransmitter release from enteric nerves while activation of K^+ channels causes membrane potential hyperpolarization and inhibition of action potential firing. KOR links via G_o to inhibition of nerve terminal Ca^{2+} channels causing decreased neurotransmitter release (Cherubini et al. 1985; Cherubini and North 1985).

Myenteric neurons control gut motility by releasing acetylcholine and substance P to cause muscle contraction (Brookes 2001) and ATP/βNAD, nitric oxide, and VIP to cause muscle relaxation (Jin et al. 1996; Brookes 2001; Hwang et al. 2011). Motor neurons are controlled by interneurons which coordinate the timing of contraction and relaxation required for propulsive motility patterns such as peristalsis. Interneurons use acetylcholine as the primary excitatory neurotransmitter but ATP and 5-HT also contribute to myenteric fast excitatory synaptic transmission (Galligan et al. 2000).

Studies in human colon have shown that morphine acts at MOR and DOR to inhibit inhibitory neuromuscular transmission causing an increase in muscle tone and a decrease in propulsive motility (Bauer et al. 1991). This is an important mechanism for the constipating effects of opioid receptor agonists. In addition, suppression of inhibitory neuromuscular transmission is likely responsible for the abdominal cramps caused by opioid receptor agonists. DOR also mediated inhibition of excitatory cholinergic and non-cholinergic neuromuscular transmission in the human distal colon (Chamouard et al. 1994). Studies done by the same group also showed that KORs mediate inhibition of excitatory cholinergic and non-cholinergic and inhibitory neuromuscular transmission in the human colon (Chamouard et al. 1993).

Studies done in the mouse myenteric plexus have revealed additional mechanisms of opiate action on enteric neurons (Smith et al. 2012). Whole-cell patch-clamp studies using mouse myenteric neurons maintained in primary culture showed that morphine could reduce action potential firing by coupling to inhibition of voltage-gated Na^+ channels. This effect would suppress interneuronal and neuromuscular transmission

Table 1 Summary of opioid receptor localization and function in the gut

Opioid receptor	Cell type expression	Molecular targets	Functional consequence
MOR	Myenteric inhibitory motor neurons (guinea pig stomach and small intestine)	K^+ channel activation	Inhibit action potential firing and neurotransmitter release
		Ca^{2+} channel inhibition	Decreased propulsive motility
	Myenteric excitatory motor neurons, inhibitory motor neurons, and interneurons (guinea pig small intestine)	K^+ channel activation	Inhibit action potential firing and neurotransmitter release
		Ca^{2+} channel inhibition	Decreased propulsive motility
	Myenteric interneurons (guinea pig)	K^+ channel activation	Inhibit action potential firing and neurotransmitter release
		Ca^{2+} channel inhibition	Decreased propulsive motility
	Submucosal secretomotor neurons (guinea pig ileum and distal colon)	K^+ channel activation	Decreased water and electrolyte secretion
		Ca^{2+} channel inhibition	
	Myenteric neurons (mouse); primary culture	Na^+ channel inhibition	Inhibit action potential firing
			Decreased propulsive motility
	Interstitial cells of Cajal (ICC) (rat, mouse)	K^+ ATP channel activation (mouse)	Inhibit pacemaker potentials
	Myenteric and submucosal neurons (human small and large intestine)	Not determined	Decreased water and electrolyte secretion
	Inhibitory motor neurons (human)	Not determined	Decreased muscle relaxation
	Rat myenteric and submucosal neurons	Not determined	Not determined
DOR	Submucosal secretomotor neurons (guinea pig ileum)	K^+ channel activation	Decreased water and electrolyte secretion
		Ca^{2+} channel inhibition	
	Submucosal secretomotor and vasomotor neurons (mouse small intestine)	Not determined	Not determined
	Excitatory and inhibitory motor neurons (mouse small intestine)	Not determined	Not determined
	Inhibitory motor neurons (mouse colon)	Not determined	Not determined

(continued)

Table 1 (continued)

Opioid receptor	Cell type expression	Molecular targets	Functional consequence
	Interstitial cells of Cajal (ICC) (rat, mouse)	K^+ ATP channel activation (mouse)	Inhibit pacemaker potentials
	Excitatory motor neurons (human colon)	Not determined	Decreased neurogenic contraction
	Inhibitory motor neurons (human)	Not determined	Decreased propulsive motility
KOR	Myenteric motor neurons (guinea pig)	Ca^{2+} channel inhibition	Inhibit neurotransmitter release
			Decreased neurogenic contraction and relaxation
	Excitatory and inhibitory motor neurons (human colon)	Not determined	Decreased propulsive motility
	Interstitial cells of Cajal (ICC) (rat)	Not determined	Functional data unavailable

(Smith et al. 2012). Myenteric neurons in the guinea pig and mouse small intestine express nicotinic receptors composed of α3 and β4 subunits and these receptors mediate most fast excitatory postsynaptic potentials in the myenteric plexus (Zhou et al. 2002; Gade et al. 2016). Studies of mouse small intestinal myenteric neurons maintained in primary culture showed that nicotine-induced inward currents were larger in neurons exposed to morphine for 16–24 h compared to neurons exposed to morphine for 1 h. An α3β4 nicotinic receptor agonist increased fecal pellet output in mice treated chronically but not acutely with morphine. These data suggest that tolerance to the inhibitory effects of morphine on gut motility may be due in part to upregulation of α3β4 nicotinic receptors on small intestinal myenteric neurons (Gade et al. 2016).

Recent studies have shown that activation of MOR and DOR but not KOR inhibits pacemaker potentials in mouse intestinal ICC maintained in primary culture. This effect was blocked by glibenclamide, a K^+ ATP channel inhibitor, and by guanylate cyclase and protein kinase G (PKG) inhibitors. These data indicate that MOR and DOR agonists activate K^+ ATP channels via a cGMP/PKG-dependent pathway to inhibit ICC function at least in the mouse intestine (Kim et al. 2016). Disruption of pacemaker potentials would disrupt propulsive motility patterns and this would contribute to the constipating effects of opioid receptor agonists.

4 Actions of Opioid Drugs on Submucosal Neurons and Intestinal Secretion

Opioid receptor agonists inhibit colonic water and electrolyte secretion which contributes to opioid-induced constipation. Water and electrolyte (Cl^-) secretion by enterocytes is stimulated by submucosal secretomotor neurons that release Ach and VIP from nerve endings in close apposition to the enterocytes (Brookes 2001).

Enterocytes express muscarinic cholinergic receptors and VPAC1 and VPAC2 receptors for VIP (Banks et al. 2005). Intestinal water secretion occurs in response to activation of enterocyte Cl^- channels including the cystic fibrosis transport regulator (CFTR) and ClC2 channels (Fei et al. 2010; Kopic et al. 2010). Opioid agonists acting at MOR and DOR on secretomotor neurons suppress Ach and VIP release resulting in a decrease in Cl^- secretion and osmotic water movement (North et al. 1987; Fei et al. 2010; Kopic et al. 2010).

5 Opioid Receptor Trafficking, Signaling Cascades, and Tolerance Development

MOR agonists activate GPCR-dependent pathways that regulate ion channels and adenylyl cyclase, or G protein-independent pathways that include scaffolding molecules and kinases (ERK and JNK). Activation of multiple signaling pathways may reflect agonist selectivity for GPCRs or agonist-selective MOR signaling (Williams et al. 2013). Opioid receptor agonists initiate a cascade of events including phosphorylation of the opioid receptor by G protein receptor kinases (GRKs) that promote receptor interaction with β-arrestins (β-arrestins 1 and 2). Activation of β-arrestin-2 in the ENS uncouples MOR from G proteins, causing internalization of the MOR through clathrin-coated pits and subsequent intracellular trafficking to the endosome (Sternini 2001; Claing et al. 2002; Williams et al. 2013). The receptor is dephosphorylated in the endosome causing β-arrestin-2 to fall off the receptor which is then recycled back to the plasma membrane (Sternini 2001; Claing et al. 2002; Williams et al. 2013). Phosphorylation, endocytosis, intracellular sorting, and recycling are important regulatory processes that mediate desensitization, downregulation, and resensitization, events that modulate cellular responsiveness. However, there are differences in the trafficking and recycling pattern depending on the neuron, agonist, and duration of stimulation. In enteric neurons, in vitro and in vivo, MORs undergo rapid concentration-dependent and ligand-selective internalization that persists for as short as 2 h (Lay et al. 2016) or can last 4–6 h (Minnis et al. 2003). Internalized MORs can recycle back to the cell surface, a process that does not require new receptor synthesis. When recycled back to the membrane, MOR can internalize again with little or no loss of total receptor numbers (Minnis et al. 2003). MOR internalization is induced by endogenous opioids, such as enkephalins and endomorphins, by the MOR agonist DAMGO (D-Ala2,N-Me-Phe4,Gly-ol5 enkephalin) that does not cross the blood-brain barrier, by most opiates, including etorphine and fentanyl (Sternini et al. 1996; Minnis et al. 2003) and by loperamide, a peripherally acting MOR agonist (Lay et al. 2016). By contrast, morphine does not induce receptor internalization under the same conditions, even at concentrations that exceed those inducing maximal inhibition of neurogenic stimulation-evoked smooth muscle contraction or inhibition of cAMP formation. The resistance of morphine-activated MORs to internalization has led to the proposal that internalization might protect against tolerance, since morphine has higher propensity to induce tolerance than opiates which induce efficient MOR internalization (e.g., etorphine or fentanyl) (Martini and Whistler 2007). However, this idea has been challenged by the

observation that morphine acquires the ability to induce pronounced MOR internalization in enteric neurons chronically treated with morphine in vivo (guinea pig) and in vitro (rat) (Patierno et al. 2011; Duraffourd et al. 2014). These differences in MOR internalization in response to morphine might be due to differences in the intracellular levels of proteins involved with receptor trafficking. Indeed, the internalization of morphine-activated MORs in neurons chronically exposed to morphine is accompanied by increased dynamin expression and translocation from the intracellular pool to the membrane. This response is prevented by treatment with a dynamin inhibitor or neuronal transfection with a mutant dynamin, in the absence of a change in β-arrestin levels (Duraffourd et al. 2014). By contrast, in enteric neurons chronically exposed to fentanyl, morphine-activated MORs do not internalize and dynamin localization and levels are unchanged (Anselmi et al. 2013). DAMGO, a selective MOR agonist with high internalizing efficiency, retains its ability to induce MOR internalization in neurons chronically stimulated with either morphine or fentanyl. This result indicates that chronic activation of MOR does not impair receptor trafficking in enteric neurons (Patierno et al. 2011; Anselmi et al. 2013). Different ligands might also affect the recycling pathways as suggested by the report that internalized MORs remain in the cytoplasm for at least 2 h following stimulation with loperamide while DAMGO- and morphiceptin- (MOR agonist) activated receptors recycle back to the membrane within 2 h (Lay et al. 2016). Others have shown that agonist-stimulated MOR can remain internalized up to 6 h before recycling back to the membrane (Minnis et al. 2003), Interestingly, MOR is more abundant in the cytoplasm of unstimulated enteric neurons in the colon compared to the small intestine (Anselmi and Sternini unpublished observations). This finding, together with the different proportion of MOR excitatory and inhibitory enteric neurons in the small and large intestine, with higher ratio of excitatory MOR neurons in the ileum and higher percentage of MOR inhibitory neurons in the colon, could provide some explanation why the ileum but not the colon develops tolerance following chronic treatment with morphine (Ross et al. 2008) (see below).

DORs also undergo agonist-stimulated internalization in enteric neurons. Internalization occurs in the soma and neurites and is blocked selectively by a DOR antagonist and is dynamin dependent (Poole et al. 2011). However, unlike MOR, DORs do not recycle to the cell surface and are degraded in lysosomes. Replenishment of DORs at the cell membrane occurs 6–16 h later and requires synthesis of new receptors. The sustained receptor downregulation might play a role in long-lasting tolerance to DOR agonists (Poole et al. 2011). Furthermore, since MOR and DOR colocalize in enteric neurons, we can speculate that heterodimerization might play a role in regulating the neuronal response to chronic use of opioid drugs, since there is evidence that opioid receptor dimerization or heterodimerization modulates receptor function (Jordan and Devi 1999; Gomes et al. 2004).

As described above, receptor phosphorylation results in acute receptor desensitization that develops within 1 min of receptor activation. Phosphorylated receptors then undergo internalization and intracellular trafficking that induce late desensitization, which is followed by recycling so the receptors can be reactivated or degradation into lysosomes, thus resulting in downregulation (Martini and Whistler 2007; Williams et al. 2013). This canonical pathway for receptor desensitization

does not follow for all opioid agonists as exemplified by morphine, which produces profound tolerance yet results in little or no internalization. Several theories have been proposed to explain these differences. This includes different signaling pathways of MOR phosphorylation by low- and high-efficacy opioid agonists. Morphine-induced analgesic tolerance can be reversed by protein kinase C (PKC) inhibitors suggesting that PKC and not GRK phosphorylation mediates morphine-induced tolerance, while the high-efficacy opioid agonist DAMGO-induced tolerance is mediated via GRK (Bailey et al. 2006). MOR agonists can be distinguished by their internalization profiles and downstream effectors, which reflects functional selectivity at GPCR or ligand-directed signaling. For instance, morphine, unlike DAMGO or fentanyl, does not induce phosphorylation of the downstream signaling mitogen-activated protein kinase/extracellular signal-regulated kinases 1 and 2 (MAPK/ERK) in normal enteric neurons. By contrast, both internalizing (e.g., morphine and derivatives) and non-internalizing opioids (e.g., DAMGO, fentanyl) activate MAPK/ERK in enteric neurons chronically treated with morphine (Duraffourd et al. 2014), further emphasizing ligand and cell type differences in MOR signaling. Together, these findings support the concept that different receptor and signaling mechanisms contribute to the regulation of opioid drug actions in the gut.

6 Tolerance Mechanisms and Opioid-Induced Constipation

Tolerance develops quickly to the analgesic but not constipating effects of opioid receptor agonists. In addition, tolerance develops to the anti-transit effects of morphine in the small intestine but not in the colon (Ross et al. 2008; Galligan and Akbarali 2014). The mechanism responsible for this effect was addressed in experiments measuring contractions caused by repeated morphine applications to in vitro circular muscle/myenteric plexus rings from wild-type mouse small intestine and colon (Kang et al. 2012). Morphine-induced contraction of small intestinal circular muscle, but not colon circular muscle, declined in amplitude with repeated morphine applications suggesting that morphine tolerance developed in the small intestine but not colon. However, when the same experiment was repeated in tissues from β-arrestin-2 knockout mice, morphine tolerance developed in small intestinal *and* colon circular muscle rings (Kang et al. 2012). Similar data were obtained in guinea pig ileum and colon preparations in vitro where tolerance to the inhibitory effects of morphine on electrically evoked circular muscle contractions developed in ileal but not colon tissues (Kang et al. 2012). It has also been found that intestinal levels of β-arrestin do not change following chronic (4–7 days) morphine treatment in vivo in guinea pigs or in vitro in rat ileum (Patierno et al. 2011; Kang et al. 2012; Duraffourd et al. 2014) suggesting the involvement of additional mechanisms that do not depend on β-arrestin-2 levels. Chronic morphine treatment triggers changes in proteins involved in MOR trafficking such as dynamin upregulation and translocation, and downstream signaling including ERK phosphorylation-dependent activation of the transcription factor, CREB. Furthermore, blockade of this signaling pathway prevents the development of gastrointestinal motility impairment induced by chronic morphine treatment (Duraffourd et al. 2014). Thus chronic morphine treatment alters

MOR downstream signaling in enteric neurons leading to opioid-induced constipation. There are ~31 splice variants of the OPRM-1 gene that encodes for the MOR (Pan 2005). Alternative splicing occurs in humans and rodents suggesting that multiple mechanisms can contribute to tolerance to different opioid receptor agonists. Differences in the carboxy-terminal (a target for GRK phosphorylation) in different receptor isoforms may activate different intracellular signaling pathways that can explain why cross-tolerance to analgesia does not occur among different opioid agonists (Pasternak 2001). The presence of differences in MOR mediating the central inhibition of GI transit was suggested by earlier pharmacological studies with MOR antagonist naloxonazine (Heyman et al. 1988) where anti-transit effects of intrathecally administered morphine were blocked by the antagonist but not when morphine was delivered at supra-spinal levels via intracerebroventricular injections. This was in contrast to the inhibition by naloxonazine of the analgesic effects. More recently, Mori et al. (Mori et al. 2013) suggested the differential activation of MOR at central and peripheral sites by morphine, oxycodone, and fentanyl. Thus, identifying the specific isoforms in the gastrointestinal tract will be important to establish new receptor targets for treating opioid-induced bowel dysfunction.

A significant development in opioid receptor pharmacology has been the identification of biased agonism where drug-induced stimulation of G protein signaling differs from its efficacy for β-arrestin-2 signaling (Violin and Lefkowitz 2007) or its bias towards the Gi/o proteins (Manglik et al. 2016). As stated above, persistent opiate receptor signaling in the colon via β-arrestin-2 contributes to opioid-induced constipation. Likewise, prolonged MOR signaling and MAPK/ERK activation induced by long-term opioid treatment in the ileum together with the induction of CREB phosphorylation might also contribute to the development of opioid-induced constipation (Duraffourd et al. 2014). Opioid agonists with reduced efficiency in recruiting β-arrestin-2 have strong analgesic properties with reduced side effects such as respiratory depression and constipation (Raehal et al. 2011). TRV130 is a G protein-biased ligand that causes less β-arrestin-2 recruitment than morphine and with higher potency towards analgesic effects and reduced constipation (DeWire et al. 2013). Similarly, a new drug, PZM21, has been discovered utilizing structure-based computational screening methodology, which has strong bias activity for Gi/o signaling and is an effective analgesic with reduced constipation and abuse liability (Manglik et al. 2016). The potential role of biased ligands in long-term use is under investigation.

7 Drugs Acting at Opioid Receptors in the Gastrointestinal Tract

Loperamide (Imodium) Loperamide is an MOR agonist used to treat diarrhea with limited abuse liability. Loperamide is used to treat occasional episodes of diarrhea (traveler's diarrhea) but it is also used to treat some IBS patients with diarrhea as their predominant symptom. Loperamide is a substrate for P-glycoprotein which is a widely expressed transporter protein (Vandenbossche et al. 2010). Loperamide

has limited oral bioavailability due to the activity of P-glycoprotein expressed by mucosal epithelial cells and it has limited blood-brain barrier permeability due to the action of P-glycoprotein expressed by astrocytes and endothelial cells in the cerebral circulation (Davis et al. 2014). Loperamide acts at MOR in the ENS causing decreased propulsive motility and intestinal secretion (Awouters et al. 1983; Ho et al. 2003; Lay et al. 2016).
While loperamide has been used safely for many years, there has been a recent increase in the number of loperamide overdoses and fatalities (Bishop-Freeman et al. 2016). The increase in loperamide overdoses parallels the rise in the use and abuse of prescription opiate pain medications and the related resurgence in heroin addiction (Daniulaityte et al. 2013). At supra-therapeutic blood levels, loperamide can block cardiac HERG K^+ channels leading to potentially fatal arrhythmias (Eggleston et al. 2017; Kang et al. 2016; Vaughn et al. 2016).

Naloxegol (Movantik) Naloxone is a potent and very selective antagonist of opioid receptors, especially MOR. Naloxone is used by first responders to reverse the potentially fatal effects of an opiate overdose. Naloxone readily crosses the blood-brain barrier to block central sites of action of opioid drugs responsible for the lethal effects of an overdose (cardiovascular and respiratory centers). Naloxone also blocks peripheral sites of opiates including the enteric nervous system. Naloxegol is a pegylated modification of naloxone. Naloxegol is a substrate for the blood-brain barrier P-glycoprotein transporter and together with its large molecular weight (652 g/mol) limits naloxegol penetration across the blood-brain barrier (Bui et al. 2016; Leppert and Woron 2016). Naloxegol is approved for treatment of opioid-induced constipation especially in non-cancer pain patients (Chey et al. 2014; Leppert and Woron 2016).

Methylnaltrexone (Relistor) Methylnaltrexone is a naltrexone analog with a quarternary amine group that is positively charged and this limits its blood-brain barrier permeability (Bader et al. 2013; Webster et al. 2015). Therefore, methylnaltrexone can block peripheral MOR without affecting centrally mediated analgesia. Methylnaltrexone is effective in treating opioid-induced constipation in cancer and non-cancer chronic pain patients.

Eluxadoline (Viberzi) GPCRs can form heterodimeric complexes that increase signaling options and pharmacological responses. For example, MOR and DOR form heteromeric complexes throughout the nervous system (Fujita et al. 2015). MOR or DOR ligands can bind individually to the heteromeric receptor complex to activate the dimeric receptor but binding of a DOR antagonist will increase the activity of agonists at the MOR-binding site (Gomes et al. 2004). Eluxadoline has been approved recently for the treatment of diarrhea-predominant IBS (Lacy 2016). Eluxadoline (known as mu/delta in the earlier literature) is a mixed MOR agonist/DOR antagonist (Wade et al. 2012). Preclinical studies showed that eluxadoline had limited systemic bioavailability after oral administration and its actions were restricted to the gut wall. Eluxadoline inhibited propulsive motility in vivo and

intestinal secretion in vitro in mice, but it did not inhibit the visceromotor response to colorectal balloon distention in rats in vivo. These results are consistent with a local gastrointestinal action of eluxadoline. Eluxadoline reduces diarrhea in IBS-diarrhea patients and constipation is rare (Lembo et al. 2016). The beneficial effects of eluxadoline on gut motility may be related to biased signaling due to the mixed MOR agonist/DOR antagonist properties of the drug. Although evidence documenting MOR/DOR dimers in the gut is not available both receptors are expressed in the ENS. Agonist activation of enteric neuronal MOR initiates β-arrestin signaling and ERK phosphorylation which are likely to cause constipation. However, simultaneous ligand binding to an MOR/DOR heterodimer is coupled to G protein signaling pathways not linked to constipation (Wade et al. 2012).

8 Summary and Conclusions

Morphine and other MOR agonists cause constipation by disrupting neurotransmission in the ENS. This causes a reduction in propulsive motility and colonic secretion. Morphine and other agonists act at MOR, DOR, and KOR to inhibit Ca^{2+} and Na^{+} channels and to activate K^{+} channels on enteric neurons. Receptor desensitization is a key component regulating opiate receptor signaling in the nervous system and β-arrestin and dynamin binding to activated opiate receptors causing receptor internalization, intracellular trafficking, and desensitization. There are differences in β-arrestin signaling that are important for tolerance development to the analgesic effects of opioid receptor agonists. However, β-arrestin signaling does not link to opioid receptor desensitization and tolerance in the colon. This is likely one cellular/molecular mechanism responsible for opioid-induced bowel dysfunction. In addition, other proteins involved in receptor trafficking such as dynamin and GRKs are likely to play an important role in the mechanisms initiating compensatory downstream events that contribute to opioid agonist-induced side effects (Finn and Whistler 2001; Martini and Whistler 2007; Williams et al. 2013). Development of opioid receptor agonists with biased agonism and identification of receptor isoforms as well as a better understanding of downstream signaling pathways in enteric neurons of different regions of the gastrointestinal tract require further study.

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Ghrelin and Motilin Control Systems in GI Physiology and Therapeutics

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Abstract

Ghrelin and motilin are released from gastrointestinal endocrine cells during hunger, to act through G protein-coupled receptors that have closely related amino acid sequences. The actions of ghrelin are more complex than motilin because ghrelin also exists outside the GI tract, it is processed to des-acyl ghrelin which has activity, ghrelin can exist in truncated forms and retain activity, the ghrelin receptor can have constitutive activity and is subject to biased agonism and finally additional ghrelin-like and des-acyl ghrelin receptors are proposed. Both ghrelin and motilin can stimulate gastric emptying, acting via different pathways, perhaps influenced by biased agonism at the receptors, but research is revealing additional pathways of activity. For example, it is becoming apparent that reduction of nausea may be a key therapeutic target for ghrelin receptor agonists and perhaps for compounds that modulate the constitutive activity of the ghrelin receptor. Reduction of nausea may be the mechanism through which gastroparesis symptoms are reduced. Intriguingly, a potential ability of motilin to influence nausea is also becoming apparent. Ghrelin interacts with digestive function through its effects on appetite, and ghrelin antagonists may have a place in treating Prader-Willi syndrome. Unlike motilin, ghrelin receptor agonists also have the potential to treat constipation by acting at the lumbosacral defecation centres. In conclusion, agonists of both ghrelin and motilin receptors hold potential as treatments for specific subsets of digestive system disorders.

Keywords

Appetite • Colon • Constipation • Des-acyl ghrelin • Gastrointestinal tract • Gastroparesis • Ghrelin • Human • Motilin • Nausea • Obestatin • Prader-Willi syndrome • Receptor • Stomach

1 Introduction

The gastrointestinal (GI) tract is the major source of the structurally related hormones ghrelin and motilin (Itoh 1997; Kojima et al. 1999; Wierup et al. 2007), and also des-acyl ghrelin, which like obestatin is an additional product of the preproghrelin gene. Motilin is largely restricted to the upper regions of the GI tract, its receptor does not exhibit constitutive activity and it appears to be the only

functional product of the motilin gene. Ghrelin, by contrast, is also found in smaller amounts in non-GI regions, its receptor has significant constitutive activity and additional bioactive products are derived from its precursor protein. These simple observations suggest different levels of control exerted by the motilin and ghrelin systems. In the following sections the expression and release of these peptides are summarised prior to a discussion on the pharmacology of their receptors and the potential pathophysiological roles of this "ghrelin-motilin system."

2 Expression Patterns of Ghrelin and Motilin

The major active products of the ghrelin gene are ghrelin, a 28-amino acid peptide with an eight-carbon side chain on the serine at position three (Kojima et al. 1999); des-acyl ghrelin (or unacylated ghrelin) in which this acyl group has been removed or not added (Toshinai et al. 2006; Delhanty et al. 2014); and obestatin, a 23-amino acid peptide formed from the C-terminal of proghrelin (Soares and Leite-Moreira 2008). A number of ghrelin gene splice variants have also been described (Seim et al. 2016). In rats and mice, for example, an additional 27-amino acid splice variant of ghrelin (Gln14 deleted) exists, which in cows and pigs is the only form of ghrelin (Sato et al. 2012). Some variants, including "mini-ghrelin," a C-terminus truncated form of ghrelin, have functional activity similar to that of ghrelin itself and are expressed in both humans and mice, illustrating conservation of alternative splicing during evolution (Seim et al. 2016).

Although widely present in small amounts outside the GI tract (Ghelardoni et al. 2006; Chen et al. 2009) the largest source of ghrelin in humans (approximately 80–90% of total) is the endocrine cells in the oxyntic glands of the gastric fundus and corpus, with smaller amounts present in endocrine cells within the gastric antrum, duodenum, jejunum, ileum, and colon (Date et al. 2000). Des-acyl ghrelin is similarly distributed within the GI tract. Indeed, in rat stomach, the amount of des-acyl ghrelin is higher than that of ghrelin (Hosoda et al. 2000; Soares and Leite-Moreira 2008). Obestatin has been detected in certain rat stomach endocrine cells and within the myenteric plexus (Soares and Leite-Moreira 2008) but in the human gastric fundus the amount is very low, relative to ghrelin (Mondal et al. 2008).

In humans, motilin is found within endocrine cells of the duodenum and jejunum, with smaller amounts in the antrum (Polak et al. 1975; De Clercq et al. 1995); very little motilin mRNA has been reported elsewhere (McKee et al. 1997). Notably and in contrast to all other mammals studied, laboratory rodents lack a functional form of motilin and indeed a motilin pseudogene has been detected, indicative of a loss of function for this hormone during the evolution of these animals (He et al. 2010; Sanger et al. 2011). Interestingly, this does not appear to be true for all rodents. For example, the North American kangaroo rat (*Dipodomys*) and mouse (*Microdipodops*) possess a functional form of motilin but a non-functional motilin receptor pseudogene, suggesting the potential for motilin to operate via non-motilin receptors

in at least some rodents that are not typically studied within the laboratory (He et al. 2012).

3 Release of Ghrelin and Motilin

Circulating concentrations of ghrelin and des-acyl ghrelin increase gradually during fasting, along with an increase in ghrelin receptor sensitivity (Scott et al. 2007), peaking just before a meal and falling back after eating at a rate that depends not on gastric distension but on nutrient content (Callahan et al. 2004; Cummings et al. 2004). By far the largest contribution is made by des-acyl ghrelin, consistent with a shorter half-life of ghrelin and its metabolism to des-acyl ghrelin and short peptide fragments, in addition to a higher degree of binding of ghrelin to lipoproteins in the blood (Soares and Leite-Moreira 2008; Chen et al. 2009; Satou et al. 2011). This metabolism of ghrelin can be achieved within blood and tissues by several different enzymes including acyl protein thioesterase and in humans, butyrylcholinesterase (Satou et al. 2011). The amount of obestatin within blood circulation is again small compared with ghrelin (Mondal et al. 2008).

For ghrelin, factors that increase its release from endocrine cells include stimulation by glucagon, the sympathetic nervous system acting at ß-adrenoceptors, or by the vagus acting via muscarinic receptors; factors which inhibit release include the sympathetic nervous system acting at α-adrenoceptors, insulin, leptin and the presence of glucose, protein and to a smaller degree lipids (Hosoda and Kangawa 2008; Yin et al. 2009) (Fig. 1). Interestingly, a recent study using human stomach has shown that both ghrelin and the anorexigenic peptide nesfatin 1 co-express within the same gastric endocrine cells, with the ratio of their expression changing in favour of nesfatin 1 in obese patients, suggesting an adaptive change of expression related to changes in body weight (Stengel et al. 2013). Finally, it is of value to note that in dogs, ghrelin may be released by motilin (Zietlow et al. 2010), suggesting that ghrelin may contribute to an ability of motilin receptor agonists to affect sensations such as satiety and/or nausea (Sanger and Furness 2016).

In humans, the release of motilin from endocrine cells also occurs when the stomach and duodenum become empty of nutrients. The release is cyclical and synchronised with the gastric migrating motor complex (Bormans et al. 1987; Sanger et al. 2013b; Deloose et al. 2015) (see below), controlled in part, by prior mechanical stimulation induced by a cyclical release of 5-HT (Nakajima et al. 2010) (Fig. 2). The release of motilin may also be increased by vagal nerve activity acting via muscarinic receptors, by duodenal acidification (Mitznegg et al. 1976) and by the presence of fat within the stomach (Diamant 1990).

Interestingly, both motilin and ghrelin may be secreted together from the human duodenum and jejunum (Wierup et al. 2007). In the porcine gastrointestinal tract the zinc transporter, ZnT8, has been shown by nested RT-PCR and immunostaining to be co-localized with cells expressing ghrelin and motilin, but not neurotensin, 5-HT or glucagon-like peptide 1 (Schweiger et al. 2016). ZnT8 is a major autoimmune target in human type 1 diabetes.

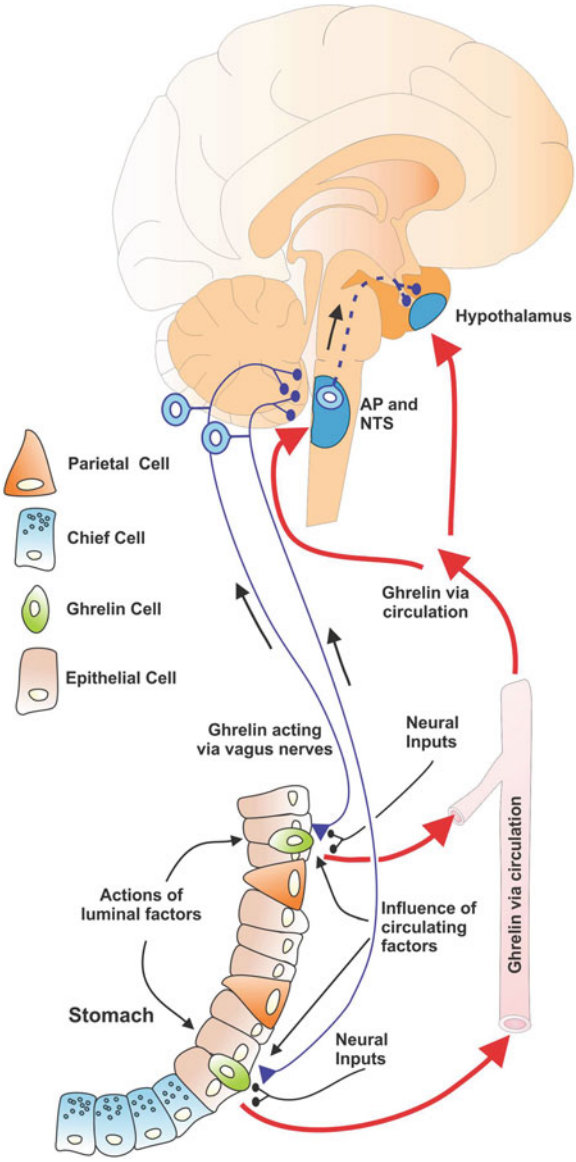

Fig. 1 Pathways through which endogenous ghrelin modulates upper gastrointestinal function. The principal way in which ghrelin stimulates appetite is through the activation of endings of vagal afferent neurons in the stomach. The vagal axons provide signals to the nucleus tractus solitarius (NTS) in the lower brainstem. Nerve pathways from the NTS innervate the feeding centres in the hypothalamus. Appetite can also be facilitated by circulating ghrelin acting on hypothalamic neurons. Ghrelin diminishes nausea, which may be mediated at least in part by circulating ghrelin acting on the area postrema (AP), a brain region that is poorly protected by the blood–brain barrier

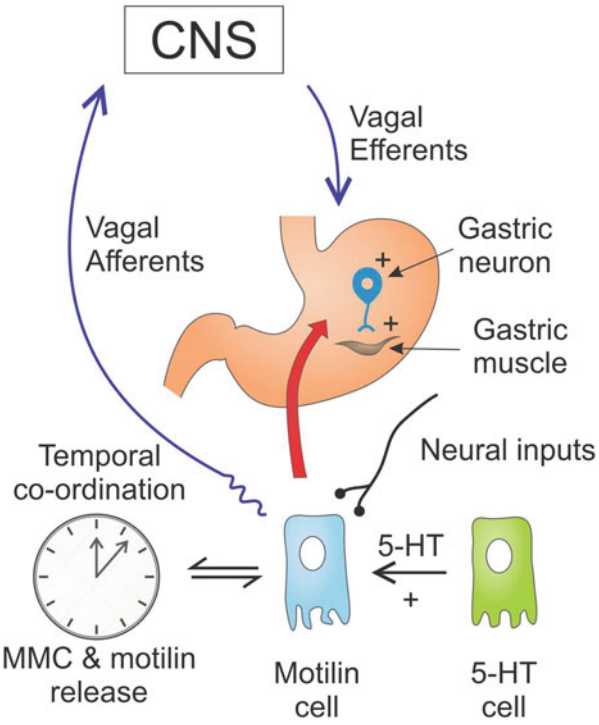

Fig. 2 Mechanisms of action of motilin. Motilin is released from enteroendocrine cells (EEC) in the proximal intestine in time with the phase III of the migrating myoelectric complex. Its release is enhanced by 5-HT released from a different population of EEC. Motilin acts on the stomach to enhance emptying, through actions on gastric enteric neurons. At higher concentrations it can directly excite gastric muscle. Motilin can also activate vagal afferents. These may in turn increase activity in vagal efferent pathways

4 Ghrelin and Motilin Receptors

Ghrelin and motilin receptors belong to a family of G protein-coupled receptors (GPCRs) that include GPR39, neurotensin and neuromedin-U receptors (McKee et al. 1997; Holst et al. 2004). The human ghrelin and motilin receptors have seven transmembrane (TM) regions and share 52% overall amino acid identity and 86% in the TM regions (Folwaczny et al. 2001; Sanger et al. 2011; Sanger et al. 2013b). Nevertheless, differences between the N-terminus regions of ghrelin and motilin ensure that for the human receptor at least, each peptide does not have appreciable ability to activate the receptor of the other [e.g. compared with motilin, >300 times the concentration of ghrelin was without activity at the recombinant human motilin receptor (Dass et al. 2003a; Nunoi et al. 2012)]. The ghrelin and motilin receptors also exist as 5-TM variants. Further, additional recognition sites for ghrelin and ghrelin-related peptides have been proposed. At present, these have not been molecularly identified or cloned, but here they are referred to as receptors because they recognize specific, naturally occurring ligands and must have structure.

4.1 Ghrelin Receptor

The first ghrelin receptor agonists were developed as growth hormone secretagogue receptor (GHSR) agonists, before the discovery of ghrelin and the cloning of its receptor. Small-molecule ghrelin receptor agonists from different chemical templates now exist with varying degrees of oral bioavailability and potency (Callaghan and Furness 2014). For some of these molecules it is important to recognise their non-selectivity of activity. These include ulimorelin (also active at α1-adrenoceptors), 4-[(aminocarbonyl)amino]-*N*-[4-(2-aminoethyl)phenyl]benzenesulfonamides (β3-adrenoceptors), spiro-azetidine-piperidine analogues (muscarinic receptors) and perhaps the synthetic pentapeptide relamorelin, which may act at sites other than the ghrelin receptor and whose structure has not been disclosed (Camilleri and Acosta 2015).

Ghrelin recognises a seven-transmembrane GPCR originally called growth hormone secretagogue receptor 1a (GHSR1a) which was cloned before ghrelin was found to be its natural ligand (Howard et al. 1996; Kojima et al. 1999). This receptor is now formally known as the ghrelin receptor (Davenport et al. 2005). The ghrelin receptor interacts with the Gαq/11, and Gαi/o, and Gα12/13 G proteins. Ghrelin has also been reported to signal through a G protein-independent mechanism involving β-arrestin (Evron et al. 2014). The receptor has high constitutive activity (that is, the receptor can produce a biological response without being bound by ghrelin), an ability to form heterodimers with other GPCRs, and is susceptible to functional or biased agonism (that is, different receptor ligands can activate the same receptor in ways which favour transmission via one or another intracellular signalling pathway, evoking different responses), arguably consistent with the diversity of actions of ghrelin. A truncated 5-TM receptor, referred to as GHSR1b, is also known. Truncated GPCRs exist as a result of either faulty or normal post-translational modifications of the receptor, being retained within the endoplasmic reticulum and not transported to the cell surface (Wise 2012). However, the 5-TM ghrelin receptor is inserted in the surface membrane where it has been shown to form heterodimers (Takahashi et al. 2006). Co-expression of the ghrelin receptor (GHS-R1a) and GHS-R1b, both in HEK-293T cells and in striatal and hippocampal neurons in culture, indicates that GHSR1b at low expression potentiates ghrelin receptor function by facilitating trafficking of this receptor to the plasma membrane and at high expression inhibits signal transduction by exerting a negative allosteric effect on the ghrelin receptor. Dimerisation of the ghrelin receptor with GHSR1b in HEK-293 cells results in coupling to Gαi/o; however the same dimerisation in striatal and hippocampal neurons couples to Gαs/olf (Navarro et al. 2016).

A large number of structural studies have been performed to investigate the ligand-binding sites of the ghrelin receptor. These include the use of truncated peptides and structure-activity relationships (to determine the regions of ghrelin and other ligands required for pharmacological activity), and alanine scanning experiments (in which putative key amino acid residues are replaced with the relatively small and inert amino acid alanine) to determine the binding site of ghrelin, the important residues that interact with peptide and non-peptide ghrelin receptor ligands, and the residues that

are important for the efficacies of these ligands. In summary, the part of ghrelin essential to agonism at the receptor is the N-terminal (Bednarek et al. 2000; Matsumoto et al. 2001). As the molecule is relatively large, ghrelin interacts with the outer portion of the transmembrane regions of the receptor. In particular the extracellular regions of TM3, 6 and 7 residues (Gln05 and Glu09 in TM 3; Phe16, Trp13 and Arg20 in TM6; and Asn02 in TM7) are reported to be important for binding. Interestingly, Glu09 in TM 3 (Glu124) is equivalent to Glu09 in TM 3 (Glu119) of the structurally similar motilin receptor, which is important for erythromycin binding (Xu et al. 2005). The binding of ghrelin is thought to activate the receptor through a "toggle switch" mechanism (Schwartz et al. 2006), in which a conformational change in the receptor is induced by ligand binding, leading to the activation of the G protein. Ghrelin can cause activation of the G protein even in a naturally occurring mutant that lacks constitutive activity (Pantel et al. 2006).

A great deal of work on the ghrelin receptor residues involved in agonism, inverse agonism and antagonism has been performed using experimental modification of the wFw (D-Trp, L-Phe, D-Trp) peptide motif (with the D-isomer residues in lowercase). This amino acid sequence was identified as the important interacting motif of the inverse agonist [D-Arg1, D-Phe5, D-Trp7, 9, Leu11] substance P [the amino acid sequence of substance P is RPKPQQFFGLM, so the undecapeptide inverse agonist sequence is rPKPfQwFwLL; (Holst et al. 2003)] through the use of structure-activity analysis of progressively truncated peptides. It was initially discovered that the C-terminal heptapeptide (fQwFwLL) was the key region for inverse agonist activity at the receptor, but more detailed analysis indicated that the pentapeptide wFwLL was sufficient for the inverse agonist properties (Holst et al. 2006). The wFw motif interacts with the aromatic central cluster of residues in the core of the ghrelin receptor between TM6 and TM7, a region previously demonstrated to be important for constitutive activity of the ghrelin receptor. This peptide (wFwLL) has a biphasic concentration response curve, acting as a partial agonist at low nanomolar concentrations, and a partial inverse agonist at micromolar concentrations. The addition of a positively charged Lys residue to the N-terminal end of the peptide removes this agonist activity making KwFwLL a full inverse agonist. Conversely, extending the N-terminal with an apolar Ala residue (AwFwLL) changes the peptide into an agonist (Holst et al. 2007). Mutagenesis experiments have demonstrated that changing the residues in the binding site of the receptor for these compounds leads to a shift in the efficacy of these compounds, with AwFwLL becoming an inverse agonist if Ser08 in TM3 is changed to Ala, and KwFwLL acting as an agonist if Phe04 in TM 3 is changed to Ser. These experiments display some of the ligand-specific effects on the ghrelin receptor, and demonstrate the binding pocket interactions that are responsible for these effects, facilitating rational design of putative ghrelin receptor agonists and inverse agonists.

It should be noted that ghrelin receptor agonists are invariably identified by measuring functions of isolated cells, possessing the recombinant or perhaps the naturally expressing receptor. However, the existence of constitutive activity for the receptor and more especially the potential for functional or biased agonism at the receptor means that more emphasis needs to be placed on developing assays for ghrelin receptor agonists which use the native receptor (preferably human) and its

linked second messenger systems, preferably expressed in a therapeutically relevant cell type, to identify compounds with different profiles of activity (Sanger 2014). These may include ligands interacting with the receptor as (partial) agonists or antagonists in an orthosteric or allosteric fashion, ligands decreasing the constitutive activity by acting as inverse agonists or ligands acting via a combination of these modes. A potential example of the need to correlate ligand-receptor studies with functional studies using native tissues is provided by a series of experiments in which single, maximally effective intravenous injections of ulimorelin were found to cause long-lasting stimulation of colorectal propulsion in rats, acting within the lumbosacral spinal cord, whereas responses to other receptor agonists fade more quickly and in a manner not explained by pharmacokinetic reasons (Shimizu et al. 2006; Ferens et al. 2010; Pustovit et al. 2014). The reason for such marked differences in duration of activity now needs to be determined.

The use of mutagenesis studies which stabilise receptors in conformations that preferentially signal through particular pathways has provided opportunities to demonstrate ghrelin receptor agonists which may induce biased signalling. For example, mutating the conserved proline reside 148 in the intracellular loop 2 region of the receptor prevents signalling through β-arrestin whilst maintaining the ability of the receptor to increase intracellular [Ca^{2+}], whereas mutating residue L149 prevents increases in [Ca^{2+}] but maintains β-arrestin-mediated internalisation (Evron et al. 2014). Ligands have been identified which show functional bias at the ghrelin receptor. These ligands are based upon the [D-Arg1, D-Phe5, D-Trp7, 9, Leu11] substance P peptide series. The wFw motif linked to isonipecotic acid at the N-terminal of the molecule (referred to as wFw-Isn-NH_2) displayed good potency (40 nM) and efficacy (80%) at inositol phosphate accumulation and was able to induce ERK phosphorylation. However, this molecule was completely unable to activate the serum response element or RhoA, presumably through an inability to activate the Gα12/13 G protein (Sivertsen et al. 2011).

4.1.1 Ghrelin Receptor Summary

- Conservation: Generally well conserved across species (Sato et al. 2012).
- Natural ligand: nM affinity for ghrelin and the 27-amino acid splice variant; poorly responsive to des-acyl ghrelin (1000-fold lower potency than acylated ghrelin) (Fujimiya et al. 2008; Sato et al. 2012; Callaghan and Furness 2014).
- Constitutive activity: High level (up to 50% total activity), inhibited by inverse agonism (Holst et al. 2004).
- Biased agonism: Demonstrated, although not clearly in functions affecting the gastrointestinal system; involves recruitment of Gαq, Gαi/o, Gα12/13 and arrestin (Sivertsen et al. 2011; Sivertsen et al. 2013).
- Dimerisation: Several identified and some have been demonstrated to exist in native tissues (Callaghan and Furness 2014). For example, in mice, a dimer of ghrelin and dopamine D_2 receptors in hypothalamic neurons affects feeding behaviour (Kern et al. 2012).

4.1.2 Five-Transmembrane Ghrelin Receptor (GHSR1b) Summary

- Highly conserved.
- Non-responsive to ghrelin, or other agonists when transfected into host cells (Leung et al. 2007).
- Constitutive activity: May have constitutive activity in the endoplasmic reticulum (Chow et al. 2012).
- Biased agonism: Not known.
- Dimerisation: Can dimerise with ghrelin receptor and other seven-transmembrane GPCRs to alter their cell surface expression and/or sensitivity to ligands (Chow et al. 2012).

4.2 Proposed Ghrelin Receptor-Like Receptor

- Ghrelin and des-acyl ghrelin exert activity at nM concentrations in tissues lacking the ghrelin receptor, suggesting the existence of a third receptor (Callaghan and Furness 2014; Delhanty et al. 2012).

4.3 Proposed Des-Acyl Ghrelin Receptor

- The existence of a receptor has been proposed but to date it has not been cloned. This putative receptor is currently known as the unacylated ghrelin or UAG receptor (Delhanty et al. 2012; Callaghan and Furness 2014), at which acylated ghrelin is a poor agonist (Delhanty et al. 2012).
- Natural ligand: In tissues not expressing ghrelin receptors des-acyl ghrelin can exert activity at 10–100 nM concentrations (Toshinai et al. 2006; Callaghan and Furness 2014).

4.4 Proposed Obestatin Receptor

- The receptor has not yet been identified. Suggested pairings of obestatin with different receptors (Seim et al. 2011) include GPR39 (Zhang et al. 2005) and the glucagon-like peptide 1 receptor (Granata et al. 2008), but these have not been sustained by other experiments. Activation of the corticotropin-releasing factor CRF_1 receptor remains a possibility (Ataka et al. 2008; Fujimiya et al. 2008).

4.5 Motilin Receptor

This hormone recognises a seven-transmembrane GPCR previously known as GPR38 but now formally known as the motilin receptor. A truncated 5-TM motilin receptor has also been described (Feighner et al. 1999).

The motilin receptor is made of 412 amino acids. Cysteine deletion and alanine replacement strategies have indicated the key roles of several residues in the N-terminus and the first, second and third extracellular loops for receptor activation (Matsuura et al. 2006). Deletion of the cysteine residues in the amino terminal (Cys 25 and 30), the first extracellular loop (Cys 111) and the second extracellular loop (Cys 235) leads to diminished affinity and efficacy of motilin and erythromycin. This indicates the critical role for these residues producing disulphide bonds for both peptide and non-peptide binding and activation. Similarly, the intracellular residues Tyr66, Arg163 and Val229 within the first, second and third intracellular domains are critical for responses to both motilin and erythromycin. In contrast, many of the residues critical for motilin binding and activation (in the amino terminus Gly36; in the first extracellular loop Pro103 and Leu109; in the second extracellular loop Val179, Leu245 and Arg246; and in the third extracellular loop Phe332) did not significantly affect the binding and activation of the receptor by erythromycin. Rather the residues responsible for erythromycin binding and activation are located in the transmembrane (TM) helical bundles, in TM2 (Asp94, Leu95, Arg97 and Trp99), 4 (Ser169) and 6 (Tyr321 and Glu325), where mutation of these residues selectively ablated responses to erythromycin but not motilin (Utsunomiya et al. 2013).

Among the rodents, evolutionary pressures have resulted in the loss of a functional motilin system, at least for those species which have been evaluated (He et al. 2010). This change, associated with dramatic changes in upper GI functions, including the loss of an emetic reflex, created motilin and motilin receptor pseudogenes (Sanger et al. 2011). Thus, in most experiments using laboratory rodents motilin fails to induce a response, although notable and as-yet unexplained exceptions exist in the literature (e.g. Fang et al. 2004). Interestingly and as previously discussed, these evolutionary changes did not always occur in a uniform manner, with the kangaroo mouse and rat being identified as examples of rodents possessing motilin receptor pseudogenes but an apparently functional motilin gene (He et al. 2012). It is not clear if the functions of ghrelin can, or indeed should, substitute for the loss of motilin. Nevertheless it is interesting to note that ghrelin stimulates gastric movements by acting on vagal and enteric nerve systems in rodents, whereas ghrelin has no clear ability to increase enteric nervous system function in humans (Broad et al. 2014a), a difference that may again reflect the different physiology of rodents.

4.5.1 Motilin Receptor Summary

- Natural ligand: nM affinity for motilin.
- Species variation: In laboratory mice, rats and guinea pigs the receptors are non-functional pseudogenes (Sanger et al. 2011). There is also a significant structural variation in dog compared with human, changing ligand affinities (Ohshiro et al. 2008; Leming et al. 2011).
- Constitutive activity: None (Holst et al. 2004).
- Biased agonism: Not proved, but cell signalling via G protein and arrestin recruitment has been demonstrated and different receptor-binding sites are recognised for different ligands (Mitselos et al. 2007; Sanger 2014).
- Dimerisation: Not known.

4.5.2 Five-Transmembrane Motilin Receptor

- When transfected into host cells this receptor was not responsive to motilin (Feighner et al. 1999).

5 Appetite and Upper GI Functions of Ghrelin and Motilin

5.1 Physiological Sites of Action of Ghrelin Affecting Appetite and Upper GI Function

A primary physiological role of ghrelin is to increase food intake (Wren et al. 2000; Asakawa et al. 2001; Cummings et al. 2001; van der Lely et al. 2004) and the release of total ghrelin (ghrelin and des-acyl ghrelin) correlates with hunger scores in humans (Cummings et al. 2004). There are two principal sites at which exogenous ghrelin or ghrelin receptor agonists stimulate appetite (Hosoda et al. 2006), the hypothalamus, specifically the arcuate nucleus (Schaeffer et al. 2013), and vagal nerve endings in the stomach (Fig. 1). There are also ghrelin receptors in the area postrema through which ghrelin might increase food intake, perhaps by reducing nausea (see below). A major physiological role of the vagus in eating behaviours is indicated by lesion experiments. In human volunteers who had previously undergone total vagal transection for gastric or esophageal dysfunction, ghrelin (1 or 5 pmol/kg/min on 3 separate days) caused no change in energy intake from a buffet meal, although growth hormone release was increased in a dose-dependent manner (le Roux et al. 2005). Infusion raised plasma ghrelin by 4- and 15-fold, levels that increase food intake in individuals without vagotomy. Transection of the vagus also prevents the orexigenic effect of ghrelin in rodents (Asakawa et al. 2001; Date et al. 2002). In humans with tumours of the dorsal medulla oblongata, where vagal afferents enter the CNS, there is reduced feeding and weight loss (Masdeu and Ross 1988), and lesions of this region in rats also reduce food intake and body weight (Hyde and Miselis 1983). Nerve pathways from the medulla oblongata project to the feeding centres in the hypothalamus (Morton et al. 2014) (Fig. 1). It is feasible that higher doses of ghrelin could act on the hypothalamus (Schaeffer et al. 2013) or area postrema (Fry and Ferguson 2010) to potentiate hunger signals. Moreover, constitutive activity of hypothalamic ghrelin receptors may act as, or contribute to, a set point for appetite (Holst and Schwartz 2004).

In contrast to the release of motilin, ghrelin and des-acyl ghrelin release are not associated with human migrating myoelectric complex (MMC) activity (Deloose et al. 2015) [although in dogs, phase I of the MMC is associated with peak plasma concentrations of ghrelin (Zietlow et al. 2010; Ogawa et al. 2012), which are insufficient to induce contractions (Ohno et al. 2006), but by inhibiting the release of motilin may reduce phase III activity (Ogawa et al. 2012)] and it is thought unlikely that ghrelin is normally released in sufficient concentrations to affect gastric motility; physiological doses of ghrelin or low doses of ghrelin receptor antagonists that modulate food intake do not appreciably affect gastric motility (Camilleri et al. 2009). Nevertheless, exogenously administered ghrelin can initiate gastric phase III MMC activity in humans (Tack et al. 2006) and increase gastric emptying of meals

when given for up to 4 weeks (Sanger 2008; Avau et al. 2013). In rats, ghrelin can evoke phase III-like activity after endogenous release during phase I (Ariga et al. 2007, 2008) or after intravenous administration (Edholm et al. 2004) even after feeding (Fujino et al. 2003; Edholm et al. 2004). In rats and mice, gastric motility is increased by ghrelin stimulating enteric cholinergic activity and activating vagal motor neurons within the brainstem (following stimulation of the vagal afferent nerves and/or signalling via the area postrema) (Bassil et al. 2006; Sanger 2008). However, ghrelin does not facilitate enteric cholinergic activity in rabbit (Dass et al. 2003b) and human stomach (Broad et al. 2014a), which suggests that in humans, ghrelin increases gastric emptying via the central nervous system.

Finally, it needs to be noted that des-acyl ghrelin is reported to have variable or no effects on food intake in rats and mice (Asakawa et al. 2005; Chen et al. 2005), but increases neuronal activity in arcuate, paraventricular and solitary tract nuclei in rats (Stevanovic et al. 2014). Des-acyl ghrelin has been shown to inhibit gastric emptying in mice (Asakawa et al. 2005) and inhibit gastric motility in rats during fasting but not after eating, an activity dependent on activation of corticotropin-releasing hormone receptor 2 in the brain (Chen et al. 2005).

Thus the primary influence of ghrelin on the upper gut is to increase appetite, which may be related to an inhibition of nausea as discussed below. Effects on gastric motility are likely to be indirect, at least in humans, and in contrast to motilin (see below) a clear role of ghrelin in controlling the MMC has not been demonstrated. Increased gastric emptying in human might be caused by supraphysiological doses of ghrelin or by ghrelin receptor agonists acting non-physiologically.

5.2 Physiological Site of Action and Functions of Motilin in the Upper Digestive Tract

Motilin is released in humans during fasting in association with phase III of the MMC, a pattern of movements in the stomach and small intestine that progress down the digestive tract and is terminated by ingestion of food in human, but not all species (Sanger et al. 2013b). Phase I of the MMC represents a relatively long period of quiescence, which is followed by phase II, a shorter period of irregular contractile activity, and then phase III, a burst of high-amplitude contractions propagating distally and followed by recovery (phase IV). In humans, this cycle repeats every 90–120 min. Notably, the association between motilin release and phase III activity appears to be true for those MMCs which begin in the stomach and not those which begin in the small intestine (Bormans et al. 1987; Deloose et al. 2015). Interestingly, people older than 65 years may have higher blood concentrations of motilin with less marked fluctuations in motilin concentrations during the MMC (Bonora et al. 1986). Patients with obesity may also have lower plasma concentrations of motilin during the MMC and less frequent gastric phase III activity compared with individuals of normal body weight (Pieramico et al. 1992). Injection of motilin can initiate the MMC in fasted humans (Boivin et al. 1997).

The phase III propulsive movements help clear any undigested material and prevent bacterial overgrowth in the upper gut (Deloose et al. 2012). More recently, it has been shown that both the phase III MMCs which begin in the stomach and the gastric phase III-like activity induced by erythromycin can each be correlated with feelings of hunger (Tack et al. 2014). Hunger may be initiated by motilin-releasing ghrelin to enhance appetite (Zietlow et al. 2010) and/or by motilin directly activating the vagus nerve (Javid et al. 2013), transmitting information about the empty stomach to the brain. The mechanisms leading to the cyclic release of motilin are not clear. Nevertheless, a study in dogs suggests that a gradual release of 5-HT from enterochromaffin cells initiates the phase II contractile activity which in turn stimulates motilin release and together the initiation of phase III activity (Nakajima et al. 2010). The phase III contractions are caused by motilin strongly facilitating gastric antrum myenteric cholinergic activity for a short duration (Dass et al. 2003a; Broad et al. 2012), perhaps with additional stimulation of vagus nerve activity (Javid et al. 2013).

5.3 Links Between Reduced Nausea, Gastric Discomfort, Increased Appetite

A number of studies suggest that the mechanisms of nausea, appetite and gastric discomfort might be linked in some way, perhaps involving the actions of ghrelin and motilin. Understanding these links is important for patients, for example those with gastroparesis, a disorder commonly associated with nausea, gastric discomfort and delayed gastric emptying (Morley 1990; Ellacott et al. 2010; Sanger et al. 2013a).

5.3.1 Ghrelin and Nausea

Studies indicate that when nausea is present in humans or nausea-like behaviours are observed in rodents (e.g. inhibition of gastric emptying or reduction in feeding evoked by a stimulus which would cause emesis in humans) treatments which increase appetite may reduce nausea (or nausea-like behaviours). Thus, the effective appetite stimulant, ghrelin, and synthetic stimulants of the ghrelin receptor, can concomitantly reduce nausea (see below) and increase the pleasurable effects of eating (Mason et al. 2014), actions that are possibly linked physiologically and behaviourally.

In volunteers exposed to a sensation of motion sickness (Farmer et al. 2015), subjects who developed nausea were compared with those who did not become nauseous. Subjects with nausea had tachygastria and elevated blood pressure. Subjects with lower nausea scores had significantly higher plasma ghrelin concentrations.

In ferrets, intracerebroventricular ghrelin significantly reduced the number of retches and vomits induced by the cancer chemotherapy agent, cisplatin, although peripherally administered ghrelin was ineffective (Rudd et al. 2006). In mice, dyspepsia indicated by reduced food intake and slowed gastric emptying after cisplatin was reversed fully for food intake and partly for gastric emptying by ghrelin given i.p. (Liu et al. 2006). In cancer patients with cachexia the ghrelin receptor agonist,

anamorelin, increased appetite and reduced nausea symptoms (Temel et al. 2010). Ghrelin itself (3 μg/kg), administered by intravenous infusion, twice daily for a week, also reduced nausea and increased appetite in patients undergoing cancer chemotherapy (Hiura et al. 2012). In a study in patients with diabetic gastroparesis, the ghrelin receptor agonist, TZP102, given orally over a period of 28 days, reduced nausea, without any significant change in gastric emptying (Ejskjaer et al. 2013). The related compound, TZP101 (ulimorelin), given by intravenous infusion on 4 successive days, reduced vomiting and increased appetite in patients with diabetic gastroparesis, but also did not improve gastric emptying (Ejskjaer et al. 2010). A further investigation found that ulimorelin (intravenous) substantially reduced the frequency and severity of nausea and vomiting in patients with gastroparesis associated with severe nausea and vomiting (Wo et al. 2011). The effect of ulimorelin on gastric emptying was not measured in this study. Animal studies indicate that ulimorelin is centrally penetrant (Pustovit et al. 2014). Another ghrelin agonist, a peptide analogue of ghrelin, relamorelin, administered by subcutaneous injection, suppressed vomiting events by 60% and reduced the gastric emptying time ($t_{1/2}$) by 12–20% in diabetic gastroparesis (Lembo et al. 2016). In patients with baseline vomiting, nausea was significantly reduced.

Some studies suggest that suppression of nausea may be exerted in the central nervous system, and that centrally penetrant compounds would be the best to use as anti-nauseants, whereas others suggest a peripheral site of action. The stimulation of neurons in the area postrema by ghrelin (Fry and Ferguson 2010) suggests a central site to modify nausea and to link control of appetite and nausea. The area postrema is the key trigger zone for humoral initiation of emesis, and vagal projections converge with inputs from area postrema in the *nucleus tractus solitarius* to influence nausea and vomiting (Carpenter 1990). Further investigations of the sites through which ghrelin receptor agonists inhibit nausea and vomiting, of compounds that are the most potent anti-nauseants and anti-emetics and of the relations between effects on nausea, appetite and gastric emptying need to be conducted.

In terms of molecular mechanisms, the following are worthy of consideration:

1. The constitutive activity of the ghrelin receptor may suppress nausea during eating. This possibility arises because after eating when ghrelin release is low, the constitutive activity could, hypothetically, promote consumption of additional food at the end of a meal or as postprandial snacks. In this way, the ghrelin receptor would be linked with the experience of pleasurable or hedonic eating (Holst and Schwartz 2004).
2. The ghrelin receptor could function as a dimer with the dopamine D_2 receptor. Thus, in mice (animals which cannot vomit but instead stop feeding in response to treatments which induce emesis in humans, including D_2 receptor agonists), the ability of a D_2 receptor agonist to inhibit feeding was prevented by ghrelin receptor antagonism suggesting dimerisation of the D_2 and ghrelin receptors in the hypothalamus (Callaghan and Furness 2014; Kern et al. 2014) and pointing to additional ways in which ghrelin could influence nausea.

3. Interactions between the functions of ghrelin and the bitter taste receptor: This possibility is included because substances with bitter taste increase ghrelin release from endocrine cells in mouse stomach (Janssen et al. 2011) and in humans sensitivity to bitter taste predicts nausea susceptibility (Benson et al. 2012). Consistent with bitter tastants stimulating ghrelin release, they are common components of pre-meal appetisers.

5.3.2 Motilin and Nausea

It is well established that erythromycin and other "motilides" can cause nausea and vomiting when these drugs are used at doses required for antibiotic treatment (e.g. Desautels et al. 1995; Boivin et al. 2003). However, lower doses are often used to stimulate gastric motility and treat patients with delayed gastric emptying, and at these lower doses, some evidence is emerging of a potential anti-nauseogenic activity of motilin receptor agonists. This includes a reported ability of erythromycin to control symptoms, which include nausea, in patients with delayed gastric emptying and also to reduce vomiting in patients with cyclic vomiting syndrome (Vanderhoof et al. 1993; Sanger et al. 2013a; Franco and Koulaeva 2014). In *Suncus murinus*, erythromycin has been shown to inhibit vomiting caused by abnormal motion and nicotine (Javid et al. 2013).

How could motilin influence nausea? This is a difficult question to answer as the mechanisms of nausea itself are poorly understood (Andrews and Sanger 2014). Possible mechanisms include the ability of motilin to activate vagal nerve firing, transmitting information to the brain (Javid et al. 2013), directly promote appetite (see earlier section: Physiological Site of Action and Functions of Motilin in the Upper Digestive Tract), release ghrelin to enhance appetite (Zietlow et al. 2010), or perhaps an ability to influence the function of the interstitial cells of Cajal (ICC) within the stomach wall. ICCs provide electrical pacemaker activity, regulating rhythmic propulsive contractions. They may be dysregulated or damaged during gastroparesis and chronic unexplained nausea and vomiting (Forster et al. 2005; Faussone-Pellegrini et al. 2012; Grover et al. 2012; Angeli et al. 2015). In rabbits, ICCs express motilin receptors (Xu et al. 2012) and an ability to disrupt coordination of ICCs within human gastric antrum muscle has been proposed as the mechanism by which motilin can induce alternating small and large cholinergically mediated contractions evoked in response to a standard burst of electrical field stimulation, following fade of the initial strong facilitation of the amplitude of these contractions (Broad et al. 2012). Further research is clearly required. However, it is interesting to note that in fasted patients with scleroderma and gastric dysrhythmia (measured using surface electrogastrography) associated with nausea, abdominal bloating and pain, there was a positive correlation between blood plasma concentrations of motilin and periods when gastric movements appeared to be well coordinated (McNearney et al. 2009).

6 Therapeutic Targeting of GI Functions by Ghrelin Receptor Ligands

The great majority of investigations, in animals and humans, have involved ghrelin receptor agonists, whereas there has been little progress in using ghrelin receptor antagonists to further understand GI pathophysiology or target disorders. Similarly, inverse agonists at the ghrelin receptor, which shows a high level of constitutive activity (Cameron et al. 2014), have not been fully evaluated for activity within the GI tract.

6.1 Changed Ghrelin Levels During Upper GI Disease

Increased plasma concentrations of ghrelin have been associated with chemotherapy-associated dyspepsia, whereas reduced plasma concentrations have been associated with gastroesophageal reflux disease and gastritis with or without *Helicobacter pylori* infection; variable changes have been reported in association with functional dyspepsia (Sanger and Furness 2016). However, since these disorders are sometimes difficult to define and have been associated with different possible causes, it is difficult to interpret such data in terms of mechanisms of action. More precise experiments are needed to link these changes to defined mechanisms.

6.2 Appetite and Upper GI Functions

Ghrelin receptor agonists are currently being evaluated in clinical trials for the treatment of conditions such as anorexia nervosa, cancer cachexia, sleep-wake regulation, chronic heart failure, gastroparesis, chronic constipation and postoperative ileus (Callaghan and Furness 2014). Trials have, for example, demonstrated great promise in their ability to increase appetite and reduce cachexia in cancer patients (Camilleri and Acosta 2015). The development of ghrelin receptor agonists simply as stimulants of gastric emptying is currently proving difficult. Trials with ulimorelin, for example, in patients with postoperative ileus have proved unsuccessful (Shaw et al. 2013). Further, in other conditions associated with delayed gastric emptying, such as gastroparesis, the poor correlation between symptoms and rate of gastric emptying (Kashyap and Farrugia 2010) begins to question the use of gastric prokinetic drugs, leading to exploration of alternative therapeutic approaches. The focus is, therefore, shifting to the appetite-promoting and nausea-reducing potential of ghrelin (e.g. relamorelin reduced nausea and vomiting in patients with this disorder; see earlier section), opening up new possibilities for the treatment of patients with a range of conditions (see Sect. 5.3.1).

In terms of animal health it is important to note that Aratana Therapeutics, a company specialising in veterinary medications for companion animals, is marketing an oral formulation of capromorelin under the trade name, Entyce, for appetite stimulation in dogs. Entyce has been given FDA Center for Veterinary Medicine (CVM) approval (May 2016).

6.3 Prader-Willi Syndrome (PWS)

PWS, the most common form of human syndromic obesity, has been associated with disordered ghrelin and/or ghrelin receptor function. It is also characterised by short stature, hypogonadism, dysmorphic features and cognitive impairment. PWS is associated with loss of function of a region of chromosome 15 (15q11.2-q13). Normally the maternal copy of this region of chromosome 15 is imprinted. PWS can occur in one of three ways: paternal deletion of a segment of chromosome 15, maternal uniparental disomy or an imprinting defect of paternal chromosome 15 (Cassidy et al. 2011). PWS is a multiphase syndrome with hypotonia and decreased appetite in neonates followed by onset of severe hyperphagia that occurs with a median age of 8 years. Non-PWS obesity is almost always associated with low ghrelin levels, but plasma ghrelin levels are three- to fivefold higher in PWS subjects compared to lean and BMI-matched controls (Haqq et al. 2003). Two studies published in 2002 reported that fasting and satiated levels of plasma ghrelin are significantly higher in individuals with PWS (Cummings et al. 2002; DelParigi et al. 2002) but can be suppressed by meal consumption (Haqq et al. 2003; Kuppens et al. 2016). Circulating ghrelin levels are elevated in young children with PWS before the onset of hyperphagia, especially during the early phase of poor appetite and feeding (Kweh et al. 2015). The overeating behaviour results from an abnormal response to food intake with limited changes in feelings of hunger and fullness after normal food intake, and an absence of changes in patterns of brain activation, in response to food. Individuals with PWS also experience temperature dysregulation, high pain threshold, an inability to vomit, scoliosis, sensory impairment, a risk of seizures, thick saliva and dental problems. Constipation and prolonged gastrointestinal transit time are highly prevalent in PWS (Kuhlmann et al. 2014) despite higher levels of ghrelin, which if anything would be expected to accelerate transit (see previous section: Physiological Sites of Action of Ghrelin Affecting Appetite and Upper GI Function). CCK, which tends to slow gastric emptying, is also elevated (Griggs et al. 2015). Despite ghrelin normally stimulating growth hormone (GH) secretion, GH deficiency is also frequent in PWS and GH replacement, considered gold standard therapy, provides improvement in growth, body composition and physical attributes (Cassidy et al. 2011). Desensitisation of the ghrelin receptor in the pituitary has been suggested as an explanation for the reduction of GH (Holland et al. 2003).

The mechanism underlying the increase in ghrelin in PWS is unlikely to reflect mutation of the genes encoding ghrelin or its receptor, as these are not contained within the locus responsible for PWS. However, several affected genes in that region encode factors that could indirectly affect ghrelin expression (Cummings et al. 2002). These factors include C/D box small nucleolar RNAs (snoRNAs), including SNORD116 (which is encoded within the complex), and the paternally expressed SNRPN (small nuclear ribonuclear protein N) locus that are involved in the alternative splicing of mRNA (Holland et al. 2003). The Snord116 deletion mouse model manifested a subset of PWS symptoms including hyperphagia and hyperghrelinemia; however the hyperphagia in this model is balanced by increased energy expenditure, so that the mice are not obese (Lin et al. 2014). Octreotide, a

somatostatin receptor agonist that suppresses ghrelin levels by fivefold in healthy adults, was investigated for therapeutic potential in PWS. A pilot study found that the high fasting-plasma ghrelin concentrations of children with PWS are markedly suppressed by short-term octreotide administration (Haqq et al. 2003). A later prospective randomized crossover trial found that octreotide treatment caused a prolonged decrease in ghrelin concentrations in adolescents with PWS but did not improve body mass or appetite (De Waele et al. 2008). Moreover, ghrelin receptor antagonists may not be an effective therapy for PWS as studies in the Snord116 deletion mouse model showed that three different inhibitors of this receptor, namely [D-Lys 3]-GHRP6, the inverse agonist SPA and YIL-781, suppressed acute food intake in fasted wild-type mice but showed reduced efficacy in Snord116del mice (Lin et al. 2014). These findings again suggest that elevated endogenous ghrelin results in ghrelin receptor desensitisation and lower levels of active receptor (Aakerlund et al. 1990). As far as we can determine, quantitative measurement of the expression of the ghrelin receptor, whose reduction could be associated with desensitisation, has not been examined, neither in the Snord116 deletion mouse model nor in tissue of PWS subjects.

7 Therapeutic Targeting of Upper GI Function by Motilin Receptor Agonists

Motilin receptor agonists have long been targeted as a potential means of stimulating the rate of gastric emptying. For this purpose, motilin receptor agonists were available before the motilin receptor was identified and cloned, and before techniques became available to determine the extent of selectivity or non-selectivity of these ligands forthe receptor. This began with the discovery that the antibiotic drug erythromycin stimulates gastric motility in a manner consistent with activation of the motilin receptor (Peeters et al. 1989), confirmed once the motilin receptor had been cloned (Feighner et al. 1999). Considerable efforts were then made to identify non-antibiotic variants (known as "motilides" because of their biological activity and the complex macrolide structure of erythromycin) and peptides as motilin receptor agonists (Westaway and Sanger 2009). For the motilides this was not a simple task, due to their complex structures, making it difficult to eliminate off-target activities and optimise the oral pharmacokinetic properties of the molecules. Erythromycin, for example, interacts with cytochrome P450 3A4, inhibits calcium influx through P2X purinoceptor channels at similar concentrations to those which activate the motilin receptor, and has further actions at higher concentrations, most likely inhibition of L-type calcium channels (Sanger et al. 2013b). Erythromycin and other macrolides and motilin receptor agonists such as azithromycin (Broad and Sanger 2013) are also antibiotic drugs, so their use risks further exacerbating the development of antibiotic resistance by prescribing for non-infective disorders.

Non-macrolide motilin receptor agonists have been described, including a small-molecule/peptide hybrid [BMS-591348; (Li et al. 2004)], a small-molecule selective motilin receptor agonist [GSK962040; camicinal; (Sanger et al. 2009)] and a

non-macrolide, non-peptide motilin receptor agonist, RQ-00201894, the structure of which is not yet in the public domain (Broad et al. 2016).

7.1 Motilin Receptor Desensitisation and Tachyphylaxis

The actions of motilin are often thought to readily desensitise, perhaps consistent with its role in mediating phase III of the gastric MMC, a short-lived burst of intense contractile activity migrating down the GI tract and needing to limit its duration of activity. Attempts to understand this process of desensitisation, so that longer lasting therapeutic drugs could be designed, were accelerated after the failure of the motilin receptor agonist ABT229 as a treatment of gastroparesis and gastroesophageal reflux disease (Westaway and Sanger 2009). Critically, these early studies were conducted using cells or preparations in which the motilin receptor was expressed in smooth muscle cells or in host cells expressing the recombinant receptor. In muscle, a down-regulation of the motilin receptor (or a loss of responsiveness to agonist application) was demonstrated in rabbit colonic myocytes following chronic oral erythromycin treatment (Bologna et al. 1993) or intravenous treatment of erythromycin (Depoortere et al. 1991). In CHO cells expressing recombinant motilin receptors, pretreatment with motilin concentration-dependently decreased the E_{max} of a subsequent motilin concentration response curve. This effect was mimicked by ABT-229, but not by erythromycin (Thielemans et al. 2005). ABT-229 also caused the greatest decline in acute muscle strip contractility following a 1 h pre-incubation, its ability to induce tachyphylaxis correlating with the its ability to induce receptor internalisation but not with the EC_{50} (Lamian et al. 2006). There have also been reports of alternative signalling leading to different mechanisms of receptor internalisation. Phosphorylation of the receptor, in preparation for internalisation (although not lysosome trafficking), is thought to be predominantly mediated through PKA. ABT-229 was found to activate phosphorylation through PKC (Mitselos et al. 2008), a mechanism known to cause different levels of tachyphylaxis in mu opioid receptors and others (Bailey et al. 2006). Re-sensitisation of the receptor occurred with significantly different half-lives following stimulation with ABT-229 (26 h), motilin (3 h) or erythromycin (1 h). Together, these data indicate scope for development of efficacious motilin receptor agonists that do not have the same propensity of ABT-229 to undergo tachyphylaxis.

The translational importance of these data, obtained using muscle cells or recombinant receptors, was questioned by Westaway and Sanger (2009) who noted that the apparent desensitisation profiles of different motilin receptor agonists varied according to the assay. The motilin receptor agonist mitemcinal, in particular, demonstrated a greater degree of tachyphylaxis than ABT-229 in a rabbit duodenal muscle preparation, but a relatively smaller degree of desensitisation in a CHO cell-based assay, and an ability to stimulate human gastric emptying and relieve symptoms of gastroparesis when repeatedly dosed. Consequently it was argued that better, more clinically relevant data might be found by studying how motilin receptor agonists act on enteric neurons to facilitate cholinergically mediated contractions of the muscle. Such a model would find more in common with the ability of low doses of erythromycin to stimulate gastric

movements in human volunteers, operating via a mechanism inhibited by atropine, as opposed to the excitatory effects of higher doses of erythromycin on gastric movements which were not inhibited by atropine (Coulie et al. 1998). Consistent with these observations, a number of studies have shown that repeated low doses of erythromycin increase gastric emptying and relieve symptoms of gastroparesis, whereas higher doses more readily induce nausea and stomach cramping and increase meal-induced satiety (Sanger et al. 2013b).

In rabbit and human isolated gastric antrum, motilin receptor agonists facilitate cholinergically mediated contractions evoked by electrical stimulation of the intrinsic neurons, but major differences were noted between the durations of these responses when motilin or non-peptide ligands were applied. In the rabbit antrum both erythromycin and camicinal caused long-lasting facilitation of this response, whereas motilin had a short half-life (Dass et al. 2003a; Jarvie et al. 2007). Motilin also displayed a comparatively short duration of activity in the human antrum, whereas camicinal had a longer duration of action; interestingly, in the human assay erythromycin, azithromycin (Broad et al. 2012; Broad and Sanger 2013) and also the motilin receptor agonist RQ-00201894 (Broad et al. 2016) induced activity of longer duration than motilin but shorter than that to camicinal. The reason for these differences in these neuromuscular models is not known. Binding of agonists stabilises receptors in a configuration that is different from the agonist-unbound state. One hypothesis is that certain non-peptide motilin receptor agonists stabilise the receptor in the active orientation which is more stable than that held by motilin, possibly by binding to different sites on the receptor (see above). Another possibility is that functional or biased agonism is possible (see Sect. 4.1), introducing different ligand-dependent degrees of receptor activation and desensitisation (Sanger 2014). Further research is required.

7.2 Use of Motilin Receptor Agonists as Gastric Prokinetic Drugs

Motilin has only weak ability to modulate neuromuscular function in the human distal oesophagus and gastric fundus (Broad et al. 2014b). In healthy volunteers, erythromycin has usually been found to have no effect on movements of the oesophagus, although in some studies oesophageal transit time was shortened (Kao et al. 1995; Chang et al. 2003). Single doses of the motilin agonist atilmotin decreased distal oesophageal contraction amplitudes whilst increasing proximal gastric and lower esophageal sphincter pressures (Korimilli and Parkman 2010). Camicinal increased gastric emptying but did not increase human esophageal motility in healthy volunteers (Hobson et al. 2015). Nevertheless, erythromycin has been shown to reduce reflux in some (e.g. Hara et al. 2006) but not all studies (e.g. Champion et al. 1994). Perhaps, by accelerating gastric emptying, acid clearance may be improved by compounds such as camicinal, reducing the number of acid reflux events (Hobson et al. 2015). If confirmed, there may be a role for selective motilin receptor agonists in the treatment of GERD refractory to treatment with proton pump inhibitors, especially in patients with slow gastric emptying. The motilin receptor agonist ABT-229 had no effects on

oesophageal movements or gastric emptying in patients with GERD (Netzer et al. 2002), but doubt has been cast on the doses used for these studies (Camilleri 2002).

Because erythromycin increases gastric emptying, even after repeated dosing, and especially when given in low doses to avoid causing nausea, this drug (and also azithromycin) is used to facilitate emergency surgery or gastric intubation during critical care, reduce aspiration and facilitate nutrition during enteral feeding, and help regulate blood glucose levels in patients with diabetes. The optimal doses of erythromycin and azithromycin for use as gastric prokinetic agents have not been determined; they are assumed to be lower than antibacterial doses, minimising adverse effects and optimising tolerance to repeat dosing (Sanger et al. 2013b). Notably in nondiabetic individuals, when gastric emptying and hunger are reduced during hyperglycaemia, erythromycin has reduced ability to increase gastric emptying (Jones et al. 1999; Petrakis et al. 2002). These observations, obtained using 3 mg/kg or 200 mg intravenously administered erythromycin, now need further study with lower doses of erythromycin known to increase gastric movements in an atropine-sensitive manner [40 mg intravenously (Coulie et al. 1998)].

In terms of new and selective motilin receptor agonists, camicinal has been reported to significantly accelerate gastric emptying in patients with diabetic gastroparesis (Hellström et al. 2016). At a single oral dose of 125 mg, half emptying of a solid meal was reduced from 147 to 52 min. This study was not powered to investigate clinical outcomes. In a previous report, from the same group, camicinal, at doses that did not accelerate gastric emptying, improved appetite and symptoms related to fullness (Barton et al. 2014).

8 Lower Gastrointestinal Function

8.1 Ghrelin Receptor Agonists

Studies using centrally penetrant ghrelin receptor agonists in animal models and in humans clearly show that the agonists stimulate colorectal motility and cause defecation (Fig. 3).

8.1.1 Animal Experiments

It was first discovered in 2005 and published in 2006 that centrally penetrant agonists of ghrelin receptors activate propulsive reflexes in the colorectum of rat and cause defecation (Shimizu et al. 2006). Ghrelin does not share this action, when given peripherally (intravenously or orally), because it does not penetrate into the spinal cord in sufficient quantity, but it does stimulate the defecatory pathways when it is injected directly into the spinal cord in the region of the spinal defecation centres, L6-S3 in the rat (Hirayama et al. 2010). Subsequently, ghrelin receptor agonists have been shown to be effective defecation stimulants in mice (Charoenthongtrakul et al. 2009) and in humans (Ejskjaer et al. 2010; Ellis et al. 2015; Acosta et al. 2016).

In the original study, the small molecule, non-peptide agonist, CP-464709, administered to conscious rats by subcutaneous injection caused defecation, and in

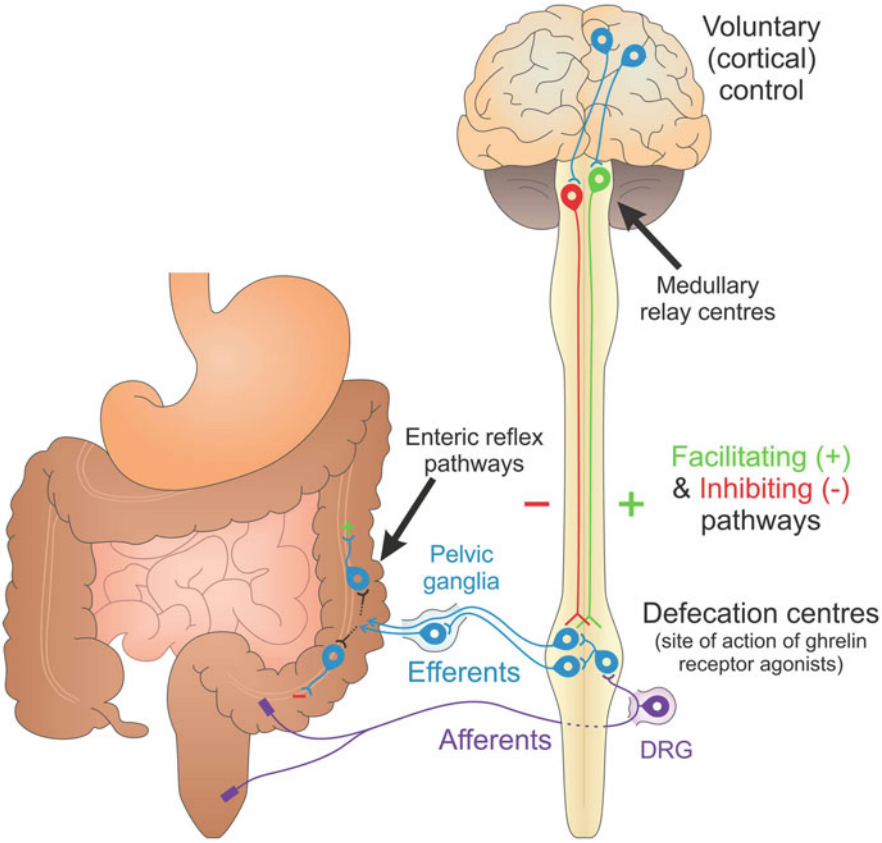

Fig. 3 The nerve circuit for control of defecation and faecal continence. Ghrelin receptor agonists act at the lumbosacral defecation centre in the defecation control pathway. Voluntary control (faecal continence and faecal release) is directed from the cerebral cortex through pathways that inhibit (−) and activate (+) the defecation centre. Pathways from these spinal centres activate enteric nervous system nerve circuits to cause colonic propulsion and bowel emptying. Sensory pathways signal colorectal fullness to the defecation centre. Colonic propulsion is coordinated with internal and external sphincter relaxation

anaesthetised rats, intravenous injection caused propulsive contractions of the colorectum (Shimizu et al. 2006). Hexamethonium, an antagonist of excitatory synaptic transmission in autonomic ganglia, prevented the response to CP464709. Ghrelin or CP464709 given intrathecally at L6-S1 evoked propulsive contractions, but contractions were not elicited by application at ponto-medullary levels or to the thoracic spinal cord (Ferens et al. 2010; Naitou et al. 2015). Propulsive contractions in response to ghrelin agonists were prevented when pelvic nerve outflows were severed, but not when the spinal cord was cut rostral to the defecation centre at L6-S3 (Shimizu et al. 2006; Naitou et al. 2015). In rodents, ghrelin receptor expression was detected in autonomic preganglionic neurons in the lumbosacral spinal cord and some

of these neurons were found to project to the pelvic ganglia, which are in the spinal cord to colorectum pathway (Ferens et al. 2010; Furness et al. 2012). The selective ghrelin receptor antagonist, YIL-781, prevented the initiation of propulsive contractions of the colorectum by the potent ghrelin receptor agonist, ulimorelin, given directly to the lumbosacral spinal cord by intrathecal injection (Pustovit et al. 2014). Ulimorelin given intravenously to conscious rats increased faecal output.

A central site of the colokinetic actions of ghrelin receptor agonist is supported by observations in rats, mice and humans that ghrelin exhibits no direct ability to contract colon muscle in vitro and does not facilitate muscle contractions caused by electrical field stimulation of the intrinsic nerves of the colon (Dass et al. 2003b; Bassil et al. 2005). Moreover, ghrelin applied peripherally in vivo has no effect on the contractile or propulsive activity of the colon (Ohno et al. 2006; Trudel et al. 2002; Hirayama et al. 2010).

Several small-molecule ghrelin receptor agonists are orally effective in causing defecation. EX-1314 (Elixir), given orally to fed mice, increased faecal output (Charoenthongtrakul et al. 2009). Oral gavage with GSK-894281 (GlaxoSmithKline) caused a dose-related production of faecal pellets in rats; at a dose of 10 mg/kg faecal output was four times greater than after the carrier (Shafton et al. 2009). Faecal production was greatest during the initial 30 min and declined over the next 90 min. GSK894281 was effective on eight successive days at an oral dose of 10 mg/kg. Another small-molecule ghrelin agonist, capromorelin, causes defecation when administered orally to rats (Pustovit et al. 2015) or humans (Ellis et al. 2015). The conclusion from these studies is that centrally penetrant ghrelin receptor agonists stimulate receptors in the lumbosacral spinal cord to cause defecation (Fig. 3).

Currently there is no evidence that the effects of ghrelin agonists to activate defecation reflect a physiological role of ghrelin, because there is no evidence that ghrelin is contained in neurons that impinge on the defecation centre or is even present in the spinal cord (Furness et al. 2011).

8.1.2 Actions at Higher Centres That Influence Colorectal Function

Faecal output is increased by about 2.5 times when the ghrelin agonist, EX-1314, or ghrelin itself is given into the cerebral ventricles of rodents (Charoenthongtrakul 2009), and colonic transit is accelerated when ghrelin is administered intracerebroventricularly (Tebbe et al. 2005a, b). Microinjection of ghrelin into the paraventricular nucleus also elicited colonic propulsion (Tebbe et al. 2005a, b). This was prevented by applying a neuropeptide Y_1 receptor antagonist to the paraventricular nucleus or by an antagonist of the corticotropin-releasing hormone receptors 1 and 2.

8.1.3 Animal Models of Colorectal Dysfunction

A number of animal models of constipation have been used to test the effectiveness of centrally penetrant ghrelin receptor agonists. These include the constipation of opiate usage (Charoenthongtrakul et al. 2009), ileus of the colon in combination with morphine treatment (Fraser et al. 2009), constipation of spinal cord injury

(Ferens et al. 2011), constipation in the 6-hydroxydopamine model of Parkinson disease (Karasawa et al. 2014) and low-fibre diet-induced constipation (Pustovit et al. 2015). In all cases ghrelin agonists triggered defecation or relieved the constipation. The agonist EX-1314 accelerated small intestine transit in mice in which transit had been slowed by morphine treatment, but effects on the large intestine were not reported (Charoenthongtrakul et al. 2009). In rats with generalised ileus plus morphine treatment, ulimorelin decreased colonic transit time and increased faecal output (Fraser et al. 2009).

A majority of people living with spinal cord injury have dysregulated colonic function, including constipation (Widerström-Noga et al. 1999; Snoek et al. 2004), because the pathways that are used to voluntarily empty the bowel are severed (Fig. 3). The agonist capromorelin was as effective in stimulating colorectal propulsion in rats in which the descending control pathways in the spinal cord were disrupted, as it was in intact rats (Ferens et al. 2011). The centrally penetrant ghrelin receptor agonist, HM-01, restored faecal pellet output in rats in which 6-hydroxydopamine was injected unilaterally into the medial forebrain bundle to produce a model of Parkinson disease (Karasawa et al. 2014). Furthermore, capromorelin stimulated colorectal activity and restored defecation in rats constipated by being fed a low-fibre diet (Pustovit et al. 2015). These findings suggest that ghrelin receptor agonists could be effective in the treatment of constipation caused by a range of conditions.

Different ghrelin receptor agonists have different durations of action on colorectal function. For example, the effects of capromorelin are brief (Carpino et al. 2002), whereas ulimorelin has long-lasting effects, and does not desensitise to the extent of other compounds (Pustovit et al. 2014). The biological basis of these differences, which are suggestive of allosteric variations, is discussed in Sect. 4.1.

8.1.4 Observations in Humans

Although only a few studies have been conducted in humans to date, the studies all indicate that ghrelin agonists could prove effective in constipation or delayed colonic transit. The number of bowel movements per day was significantly increased by injection of 80 mg/kg ulimorelin ($P = 0.0032$), although this was in a trial for the treatment for gastroparesis (Ejskjaer et al. 2010). Relamorelin, trialled in a single-centre, randomized, double-blind, placebo-controlled trial, relieved constipation and accelerated colonic transit during 14 days of treatment (Acosta et al. 2016). In an unblinded dose-ranging and pharmacokinetic trial, capromorelin triggered defecation in patients with constipation caused by spinal cord injury (Ellis et al. 2015). Both the animal proof-of-principle studies and the limited number of clinical trials indicate that further investigation of the therapeutic value of ghrelin receptor agonists in constipation is warranted.

8.2 Motilin Receptor Agonists and Lower Bowel Functions

The muscle and myenteric plexus of human colon express motilin receptors (Feighner et al. 1999; Ter Beek et al. 2008; Broad et al. 2012) but motilin receptor agonists do not influence movements of human isolated colon (Broad et al. 2012) or intact colon in patients with chronic constipation (Venkatasubramani et al. 2008). Nevertheless, intravenously administered motilin has been reported to increase human rectum volume and compliance without affecting rectal sensations (Kamerling et al. 2003). In other species, motilin receptor agonists increase cholinergic motor function and stimulate defecation in rabbit colon (Dass et al. 2003a; Sanger et al. 2009) and preliminary experiments suggest that the motilin receptor antagonist TZP-201 may reduce anticancer chemotherapy-induced diarrhoea in dogs (Westaway and Sanger 2009).

9 Conclusions

Ghrelin is released from gastric enteroendocrine cells to promote appetite, primarily through the vagus nerve, to reduce nausea and to increase the rate of gastric emptying. A functional link between the ability of ghrelin to improve appetite and to control nausea has been proposed. Confirmation and exploitation of this link could change how ghrelin receptor agonists are developed for upper gastrointestinal disorders. Thus reduction of nausea is a key therapeutic target for ghrelin receptor agonists and perhaps for compounds that modulate the constitutive activity of the ghrelin receptor. Ghrelin also increases human gastric motility by mechanisms which, in contrast to rodents, do not depend on stimulation of enteric motor neuron activity. However, the type of receptor activation (e.g. short-lasting or long-lasting) required to evoke a sustained increase in gastric emptying in humans is difficult to determine without understanding the pathways involved or possessing an animal model which reflects the mechanisms by which ghrelin increases gastric emptying in humans. Reduction of ghrelin's stimulation of appetite may find a place in the treatment of Prader-Willi syndrome.

Motilin receptor agonists increase gastric emptying by facilitating enteric cholinergic activity in a short-lasting or long-lasting manner, indicative of the potential for biased agonism. Compounds with long-lasting activity might be useful in treating patients who require increased gastric emptying, for example, during enteral feeding. In other conditions in which the relationships between gastric emptying and symptoms are unclear, such as diabetic gastroparesis, low doses of motilin receptor agonists reduce appetite and symptoms of bloating. Possibly, at these low doses motilin receptor agonists act via the vagus nerve and/or improve gastric rhythmicity without appreciably modifying the rate of gastric emptying. A potential role for motilin in influencing appetite and nausea requires further research.

Unlike motilin, ghrelin receptor agonists also have the potential to treat constipation. The lumbosacral defecation centres, which are not exposed to endogenous ghrelin, are activated by ghrelin receptor agonists that penetrate into the spinal cord or are applied directly to this area. Different ghrelin receptor agonists induce

tachyphylaxis or evoke sustained activity, suggesting the existence of ligand-specific biased agonism.

In conclusion, agonists of both ghrelin and motilin receptors hold potential as treatments for specific subsets of digestive system disorders. Selective agonists for both receptors were developed before their possible therapeutic roles were fully understood and, indeed, this work is still in progress. As an example, questions remain concerning the extent to which optimal activity is dependent on biased receptor activation and in addition the therapeutic potential of modulators of the constitutive activity of the ghrelin receptor requires further research. When drugs from each receptor class have been registered for clinical use, potential synergies of therapeutic actions could be explored by using motilin and ghrelin receptor agonists together.

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Centrally Targeted Pharmacotherapy for Chronic Abdominal Pain: Understanding and Management

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Abstract

Chronic abdominal pain has a widespread impact on the individual and the society. Identifying and explaining mechanisms of importance for the pain experience within a biopsychosocial context are central in order to select treatment that has a chance for symptom reduction. With current knowledge of brain–gut interactions, chronic abdominal pain, which mostly appears in functional gastrointestinal disorders, to a large extent involves pain mechanisms residing within the brain. As such, the use of centrally targeted pharmacotherapy as an effective treatment option is obvious in a selected number of patients. The antidepressants are most common, but also other classes of medications can be used, either alone or in combination. The latter option refers to when there is insufficient effect of one drug alone or side effects limiting dosage, and when combined in lower doses, certain drugs give rise to augmentation effects. This chapter outlines basic mechanisms of importance for the understanding of chronic abdominal pain and the pharmacologic treatment options.

Keywords

Abdominal pain • Antidepressants • Brain–gut axis • Functional gastrointestinal disorders • Treatment

The impact of chronic abdominal pain upon society goes beyond the patient, and includes family and friends and increased use of health care services and costs. Chronic abdominal pain may occur with frequent recurrence of these symptoms or as a more constant abdominal pain, and is best recognized as a functional gastrointestinal disorder (FGID or disorder of gut–brain interaction), though it may also occur with other gastrointestinal disorders attributed to specific organ systems. This can include as examples painful chronic pancreatitis, or inflammatory bowel disease (IBD-IBS), where the pain is not well correlated with the identified clinical features of the underlying disease. When there is a more or less continuous abdominal pain without any structural correlate, it is now defined as a centrally mediated abdominal pain syndrome (CAPS) (Keefer et al. 2016). Associated with this disorder is evidence that health care utilization is high (Drossman et al. 1993; Koloski et al. 2002) and quality of life as well as work productivity are low (Drossman et al. 1993). Thus, there is a need for efficient and rational treatment in order to avoid a vicious cycle of chronic symptoms leading to health care seeking with unneeded diagnostic studies, ineffective treatments, and increased health care use and costs.

The definition of abdominal pain according to the International Association for the Study of Pain (IASP) refers to "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." Everyone experiences this from time to time, most often in a setting that is understandable or at least of a duration that is short enough for not causing the individual to fear a serious underlying condition. This chapter deals with the situation where the duration of abdominal pain is long enough to justify the label of chronicity. The time factor varies in different scientific settings, but at least 3 months are required as a minimum and for the FGIDs 6 months, when defining patients included in intervention studies and basic research about these conditions.

Centrally targeted pharmacotherapy for chronic abdominal pain must not be considered as a singular option for pain relief; rather it should be looked upon as one of a series of interventions that can be tailored by use of clinical, biological, and psychosocial factors combined in a multidimensional approach. One must also remember that the definition of a clinical outcome related to pain response in a clinical drug trial often only involves partial pain relief even in those characterized as responders. The recommendations of the US Food and Drug Administration and the European Medicines Agency for IBS trials are that for a responder definition, abdominal pain needs to be reduced by 30% compared to baseline; therefore a substantial component of the symptom treated will still be present. So for clinical care this response must be reconciled with patient satisfaction in order to achieve a successful treatment outcome, and this may involve components other than pharmacotherapy.

Disseminating knowledge about pharmacologic treatment of chronic abdominal pain must also consider the risk of exacerbating the pain problem by the wrongful use of opioid analgesics. There is no scientific data that support the use of opioids as a successful treatment modality in chronic pain (Dowell et al. 2016); and in treating chronic abdominal pain, the use of opioids leads to unwanted side effects such as opioid-induced constipation but also the paradoxical condition of opioid-induced central hyperalgesia, also known as the narcotic bowel syndrome (Keefer et al. 2016; Kurlander and Drossman 2014).

Within this chapter, we review background data for providing tools needed for safe and efficient treatment of chronic abdominal pain with centrally targeted pharmacological agents.

1 The Biopsychosocial Model and Neurogastroenterology

The biopsychosocial model has emerged during the last 4 decades as a means to address clinical problems dominated by disorders of gut–brain interaction (previously called FGIDs), in particular chronic abdominal pain, which is not explained by obvious evidence of biologic alterations in tissue structure (Engel 1977; Drossman 1998). In essence, this model integrates the biological data with the social, psychological, and behavioral dimension to produce a composite understanding relating the perception of illness, symptom severity, and clinical outcome. By describing the patient in such an integrated manner, the traditional dilemma of attempting to

dichotomize the disorder into psychiatric or medical is not only unnecessary but also insufficient. Of central importance when using this model in a clinical situation is also the need for a good patient–doctor relationship, which forms a basis for the development of a mutual understanding. By use of a patient-centered communication skill, both the doctor and patient are provided a better chance for understanding expectations and needs and identifying important factors involved in a biopsychosocial context. The value of this model has been further strengthened by the advent of modern brain imaging (Tillisch and Labus 2011), which supports the proposed nature of bidirectional ("brain–gut") communication and introduces new dimensions of assessment of brain–gut dysfunction and potentially the effects of interventions. To understand the rationale for centrally acting pharmacotherapy, a basic knowledge of the brain–gut axis is needed along with the evidence that chronic pain is associated with changes in neural function and anatomy, changes that even may be reversed by a careful use of selected drugs or behavioral treatments.

2 The Brain–Gut Axis

2.1 Anatomy of Gut Sensory Pathways

It is relevant, and in fact popular, to describe the brain and gut as being "hard-wired." It is the embryonic development of direct connections that originate when the neural crest differentiates into the brain and spinal cord, and the latter sends out ganglia into the evolving endoderm to form the myenteric plexi of the gastrointestinal (GI) tract, the enteric nervous system that motivates the description. This differentiation creates a network of neurohumoral connectivity that is unique for the brain and the gut and also gives the opportunity to affect chronic abdominal pain by medications aiming at neurotransmitters with neural effects, regardless if they are predominantly peripheral or central.

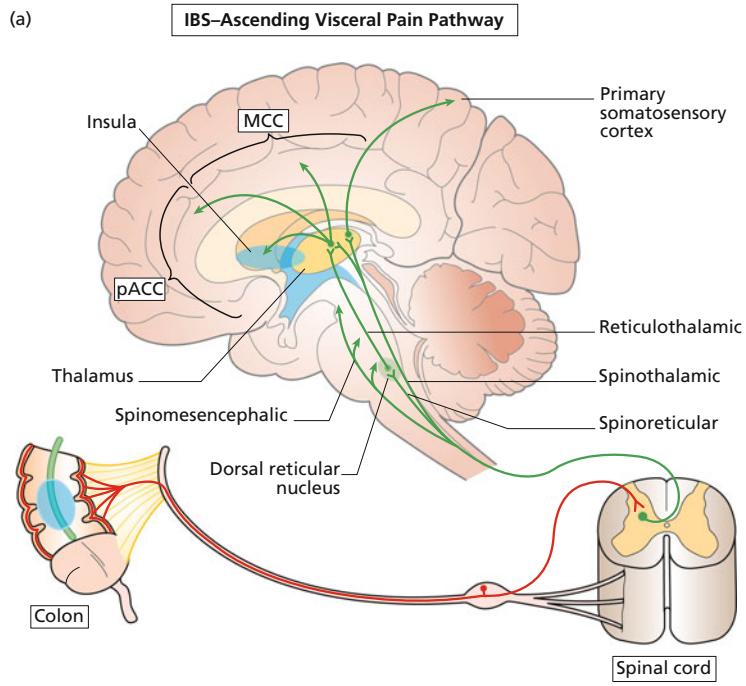
The afferent nerve signals conveying pain reach the brain by transmission through a three-neuron chain: the primary spinal afferent neuron with its cell body in the dorsal root ganglion that terminates in the dorsal column laminae of the spinal cord, the second-order afferent neuron that projects from the dorsal horn of the spinal cord to the thalamus and the midbrain, and the third-order neuron that can diverge into three main transmission routes, the spinothalamic tract, the spinoreticular tract, and the reticulothalamic tract. These last three main routes of transmission involve different aspects of sensory function where the spinothalamic tract communicates with the primary somatosensory cortex for basal discrimination and localization of visceral and somatic stimuli, and the spinoreticular tract communicates with the reticular formation of the brainstem and is part of the emotional component of pain. Finally, the reticulothalamic tract has complex projections from the reticular formation to the medial thalamus on the left, and further to the cingulate cortex and insula. The cingulate cortex is divided into components that include the perigenual anterior cingulate cortex (pACC) involved in affect and the midcingulate cortex (MCC) involved in behavioral response modification. This multicomponent integration of nociceptive information, dispersed to the

somatotypic intensity area (lateral sensory cortex) or the insula for visceral input and to the emotional or motivational affective area of the medial cortex, explains variability in the experience and reporting of pain (Mayer and Tillisch 2011; Keszthelyi et al. 2012). Whether or not there is a "primary pain region" in the brain that is activated regardless of bodily location of origin is a matter of debate. A meta-analysis of brain-imaging studies indicates that there might be a central role for the ACC and insular regions regardless of pain modality studied (Jensen et al. 2016), but in general, the concept of networks of brain activation where the magnitude of response determines symptom experience has expanding data supporting it (Mayer et al. 2015). This type of salience network activation differs comparing patients with IBS and healthy controls by to a larger degree involving emotional arousal centers in IBS patients (Tillisch et al. 2011).

Finally, another modulatory system for pain is important to understand. This is the descending fibers from the brain stem, such as those originating in the periaqueductal gray that can affect the sensitivity of the dorsal horn neurons, i.e., the first- and second-order sensory neuron conduction site. This modulation has the potential to alter sensitivity of the dorsal horn neuron and act as a central control mechanism of pain perception. Serotonin and norepinephrine are two key neurotransmitters involved in this process and are as such available for being affected by pharmacotherapy. See Fig. 1 for a schematic representation of the gut sensory pathways.

2.2 Determinants of Pain Sensation

There is not a direct connection between the objective intensity of a gut sensory stimulus and the sensation experienced by the patient. On the contrary, there are data indicating that those affected by the most debilitating type of abdominal pain, CAPS, are not hypersensitive (increased sensation of stimuli) to peripheral stimuli when tested by use of a rectal barostat (Nozu and Kudaira 2009). This means that a lowered peripheral threshold for visceral pain cannot be the only reason for the experience of long-standing abdominal pain, which has also been shown in studies of patients with functional dyspepsia (Stanghellini et al. 2016), even if a proportion of patients with FGIDs qualify for the label visceral hypersensitivity or as having allodynia (reporting a stimuli as painful that previously was not) (Bouin et al. 2002; Tornblom et al. 2014; van der Veek et al. 2008; Tack et al. 2001). Determinants for more severe or constant pain seem to involve components of central sensitization and also a more complex network of interacting mechanisms like psychological distress and maladaptive coping strategies with a resulting need for health care utilization and the experience of poor health-related quality of life (Drossman et al. 2000). Psychological distress has the potential to lower the threshold for afferent signals to reach conscious awareness and also for being a risk factor for an acute peripheral insult leading to mucosal afferent neuron sensitization that produces pain, to develop into a chronic pain situation. A clinical example of this is when an acute gastroenteritis precedes the development of IBS (postinfection IBS, PI-IBS) (Spiller and Garsed 2009) where the risk for chronicity is increased if a significant life event coexists causing emotional distress (Neal et al. 2002). From this,


(a)
IBS–Ascending Visceral Pain Pathway
Insula
MCC
Primary somatosensory cortex
pACC
Reticulothalamic
Thalamus
Spinothalamic
Spinomesencephalic
Spinoreticular
Dorsal reticular nucleus
Colon
Spinal cord

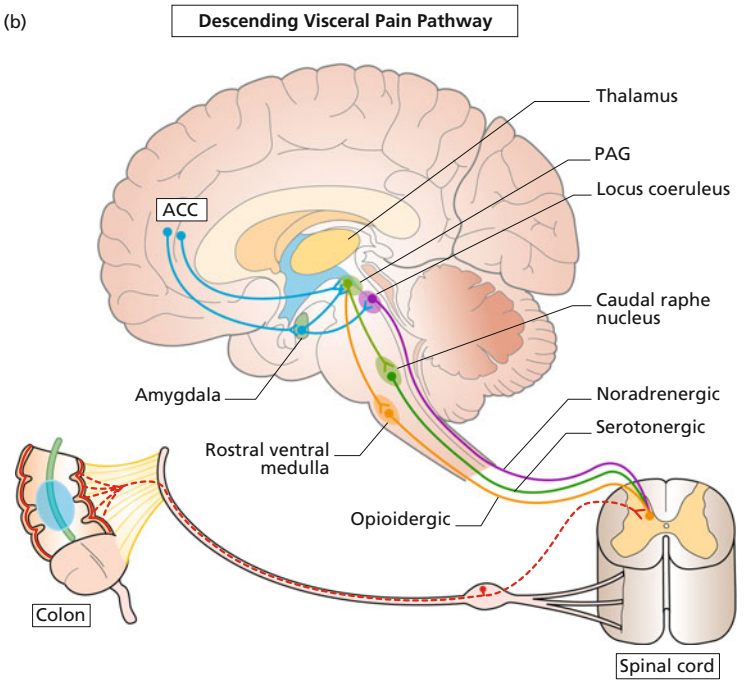

(b)
Descending Visceral Pain Pathway
Thalamus
PAG
Locus coeruleus
ACC
Caudal raphe nucleus
Amygdala
Noradrenergic
Serotonergic
Rostral ventral medulla
Opioidergic
Colon
Spinal cord

Fig. 1 (**a**–**b**) Ascending (**a**) and descending (**b**) neural pathways involved in visceral perception. The afferent nerve signals conveying pain reach the brain by transmission through a three-neuron chain. The descending fibers can affect the sensitivity of the dorsal horn neurons, i.e., the first- and second-order sensory neuron conduction site. This modulation has the potential to alter sensitivity of the dorsal horn neuron and act as a central control mechanism of pain perception. Serotonin and norepinephrine are two key neurotransmitters involved in this process

peripheral sensitization can influence the onset of pain and the CNS appears involved in the predisposition and perpetuation of pain where activation of the salience network involves more emotional arousal (Tillisch and Labus 2011) when a more severe chronic pain condition develops. This can be translated into a model where the chronic pain situation more likely is predominantly centrally mediated and depending upon not only disease-specific factors, but also psychosocial factors. A defective pain inhibition involving not only the descending pathways to the spinal dorsal horns but also the anterior cingulate cortex (ACC) region (Ringel et al. 2008) has the potential to interact and create a negative spiral of pain amplification. As an example the ACC, which as pointed out originates the descending pain inhibitory pathway, shows greater activation on fMRI in IBS patients relative to healthy controls (Mertz et al. 2000). By understanding these type of mechanisms where the peripheral sensory input becomes less important for the chronic pain, it is also logic that drugs with predominant peripheral analgesic effects, such as linaclotide in IBS with constipation or the antispasmodics, have less of a chance to result in a meaningful pain relief on their own.

2.3 Neurodegenerative Pathology in Chronic Abdominal Pain

Both in somatic and visceral pain of chronic character, signs of an altered brain structure have been reported. A reduced volume of regional gray-matter density in areas of importance for pain modulation in IBS (Blankstein et al. 2010), chronic pancreatitis (Frokjaer et al. 2012), and generalized pain disorders (Valet et al. 2009), also after controlling for anxiety and depression as covariates in the analysis (Seminowicz et al. 2010), indicates the importance of neural structural changes accompanying chronic pain. Also methods to examine white matter tracts have shown signs of alterations in multiple brain areas in IBS (Ellingson et al. 2013). The functional importance of structural variations in the CNS by data that links functional brain abnormalities to those anatomical variations visualized in the context of pain is largely still missing even if a few observations exist. In patients who underwent hip replacement surgery, a follow-up study showed that the structural gray-matter alterations in pain-transmitting networks were partly reversed 4 months post-surgery when pain was gone (Rodriguez-Raecke et al. 2009). Another important observation is that these variations in CNS structure do not only strictly relate to chronic pain situations, rather also to differences in pain thresholds in healthy

individuals that were reported to be linked to variations in gray-matter density without causing an illness (Elsenbruch et al. 2014). Also the observed differences in white matter have been associated with symptom severity and psychological variables (Chen et al. 2011).

As an indirect way of understanding the pathophysiologic link between neurodegeneration and symptoms, clinical data from research in depression is a good example. Signs of neuron proliferation can be measured by use of serum levels of brain-derived neurotrophic factor (BDNF), and these levels have been found to correlate with status of clinical response. BDNF levels are low in depressed patients and its levels are increased with antidepressive treatment where the outcome is positively associated with BDNF levels; that is increasing levels predict clinical improvement (Brunoni et al. 2008). This indicated neurogenesis as part of a healing process and that neurogenesis actually can occur has been shown in animal models where an injected thymidine analog co-labels with markers of mature neurons (Gould 2007). The regional reductions in gray-matter density associated with depression, severe psychological stress such as post-traumatic stress disorder, and chronic pain syndromes (Fuchs et al. 2001) would therefore suggest that neuronal regeneration might be of benefit for initiating a clinical response (Malberg and Duman 2003; Perera et al. 2007). The lag phase lasting for some weeks before improvement occurs after starting pharmacologic treatment of depression fits well with the rise of BDNF levels in blood and the observed time that a "newborn" neuron needs for maturation.

Turning to rat experiments, the effects on neurogenesis by antidepressants can further be visualized. In a traumatic brain injury model where a reduction in cognitive function was caused by brain cell loss, treatment with the tricyclic antidepressant (TCA), imipramine, when compared to a group of rats not given the TCA, was associated with signs of a greater posttraumatic neurogenesis and in parallel with that also associated with greater improvement in cognitive function over time (Han et al. 2011).

3 The Therapeutic Context in Treatment of Chronic Pain

3.1 A Basic Model of Pain Development

When treating a patient with chronic pain, a basic model of pain development is practical in order to cover different aspects of possible interventions. The model presented in the following section not only provides an understanding of the benefit of centrally acting pharmacologic treatments in reducing chronic painful conditions via neurogenesis, but it also legitimizes the physiological reality of these relationships rather than being considered "psychiatric."

Some factors like genetics, early family modeling, or environmental exposures including infection and traumatic events such as abuse (Drossman 2011) or the influence of the family on enabling pain behavior and health care seeking (Drossman 2014) can be considered as predisposing. This means that they pose a risk for a vicious cycle of pain in a situation where a number of precipitating factors that might

trigger transient pain, like an acute infection, major psychological stressors, medical illness, drug abuse, and ongoing or unresolved abuse(Drossman 2011, 2014), can be more intensely expressed. Finally, a chronic illness state is more easily maintained if perpetuating factors like psychological comorbidities such as anxiety and depression are present, with resultant cognitive responses including helplessness, vulnerability and low self-esteem, maladaptive coping, and poor social support.

From a conceptual point of view, and similar to a traumatic brain injury model, these stressful factors and exposures to medical illness and abuse can lead to neuronal vulnerability and in areas such as the hippocampus are associated with suppression of neurogenesis, which in turn can lead to the development of depression with an uncoupling of affect from context and impaired segregation of irrelevant stimuli from relevant ones (Perera et al. 2008). If intermittent abdominal pain is involved as a major stimulus during an uncoupling process, it may well be driving a central affective component resulting in the development of a chronic pain experience that no longer is linked to the initial trigger; rather pain persists and acquires an independent existence. It is likely that in addition to antidepressants other enriching processes such as physical exercise, psychological treatments, and also an effective patient–physician relationship can help to reverse this uncoupling process, leading to clinical improvement.

3.2 Patient–Physician Interaction

Without a good patient–physician relationship, there will be major problems in treating a chronic pain condition. An effective communication skill forms the basis for understanding why a patient that in many cases has had a symptom for a long period of time now is addressing health care for help (Drossman 2013). It also aids in the understanding of factors that might have an impact on symptom development and persistence like in the model depicted in the previous section. In order to obtain as many facets as possible of the full biopsychosocial context, this requires a patient-centered approach that allows for a mutual trust to develop by involving the patient in the clinical management with continual reassurance of benefits to ensue a partnership in the care that is presented as an ongoing one. It has been shown that such an approach where simple efforts to educate on diagnosis, interact on management, and provide reassurance and continuity of care (Drossman and Thompson 1992) will diminish the need for excessive health care consumption (Owens et al. 1995).

Often, stressors are not initially verbalized by the patient but should always be considered as they may be reported later as a sense of trust is established with the provider like the perpetuating factors that might contribute significantly to why a condition persists. With chronic pain, changes and adaptations occur in the patient that can lead to feelings of helplessness, vulnerability, low self-esteem, and psychological concomitants such as anxiety and depression. This keeps the patient in a vicious cycle where the symptom is seen as a threat to oneself and the ensuing emotional distress and sense of inability not to control the symptoms lower symptom threshold even more leading to greater pain, and so it continues. Further methods

to help develop an effective patient–provider relationship can be reviewed elsewhere (Drossman 2013).

4 The Role of Centrally Targeted Pharmacotherapy in Chronic Abdominal Pain

It must be remembered that the evidence for effectiveness of centrally targeted therapy in chronic abdominal pain to a large extent is extrapolated from research in other types of chronic pain where central mechanisms have been shown to be of importance. On the other hand, many of the reasons for the empirical use of antidepressants and other psychotropic classes of drugs emanate from assumptions given by their use in clinical practice that involves many other factors of importance for a positive clinical outcome as outlined above. By a careful selection, these drugs can be motivated not only for their central actions, but also in many instances for their effects on other pathophysiologic aberrations that may be of importance in a patient where the clinical problem involves abdominal pain, like a disturbed bowel habit, primary and secondary psychological problems, as well as "true" psychiatric comorbidity. When the psychiatric indication is obvious, the choice of drug should mainly be based on that indication. Otherwise, a more complex decision making based on multiple factors as outlined in the sections to come can be used.

Like in all treatments, but particularly of importance when starting centrally acting treatments, a good and reliable explanation for the rationale is important as well as a credible description of side effects. It is also wise to bring up the possibility of experiencing an early adverse effect, perhaps already after one tablet, where there is a good chance that it more probably is caused by a nocebo effect (the opposite situation from a placebo effect) rather than a true pharmacologic side effect. In case of the former, this type of reactions often subsides within the first 2 weeks of treatment and is associated with psychological distress and a greater tendency in groups of patients with abdominal pain to report multiple somatic symptoms (Thiwan et al. 2009). Dose escalation can be decided upon with the patient on an individual basis, but it is recommended to have an early follow-up for screening of both positive and negative effects of treatment, before recommendations are made.

5 Tricyclic Antidepressants

5.1 Evidence for Efficacy in Chronic Abdominal Pain

This is the most studied antidepressant class of drugs for treatment of abdominal pain without a psychiatric indication. Their mode of action involves mechanisms beyond serotonin and norepinephrine, like blockage of voltage-gated ion channels, opioid receptor activation, and also a possibility of neuroimmunologic anti-inflammatory effects (Dharmshaktu et al. 2012). The major receptor effects are summarized in Fig. 2.

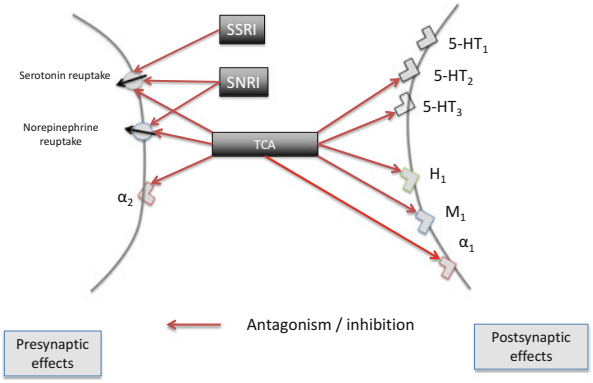


Fig. 2 Pre- and postsynaptic effects of tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin and norepinephrine reuptake inhibitors (SNRIs). The SSRIs only inhibit the presynaptic serotonin reuptake mechanism and the lack of effect on norepinephrine levels limits its use in treatment of abdominal pain unless anxiety is a prominent feature for pain experience. The additive effects of inhibiting reuptake of both serotonin and norepinephrine make the SNRIs more suited in pain treatment, having important effects on descending modulatory pain pathways. The TCAs have multiple effects, and among them, a presynaptic α_2 antagonism increases serotonin and norepinephrine release and together with inhibitory effects on serotonin and norepinephrine reuptake these mechanisms both increase levels of serotonin and norepinephrine in the synaptic cleft. Pharmacologic side effects from TCAs are mainly related to α_1 antagonism (hypotension), histamin$_1$ (H_1) antagonism (sedation), and muscarinic (M_1) receptor antagonism (anticholinergic side effects like dry mouth, sedation, blurred vision). Blockage of sodium-gated ion channels (not shown) is thought to cause the sometimes-lethal cardiac arrhythmias and seizures in situations of overdose

Most studies of TCAs in abdominal pain are small and include different subsets of patients with abdominal pain as one of many GI symptoms. In one of the early, randomized double-blind placebo-controlled studies, trimipramine was superior to placebo in a group of 61 IBS patients with respect to symptom reduction where abdominal pain was not specifically presented (Myren et al. 1982). In a later study by Greenbaum et al., desipramine in a crossover comparison was superior to both atropine and placebo in reducing abdominal pain in 28 IBS patients (Greenbaum et al. 1987). The largest study until now on the effects of a TCA with regard to abdominal pain in IBS patients was performed in 216 women with IBS and showed numerical but not statistically significant benefit from 12-week treatment in the intention-to-treat analysis for a composite symptom score and abdominal pain (Drossman et al. 2003). In the per protocol analysis of patients who stayed on treatment until the end of the study, there was significant benefit against placebo. Furthermore in a subanalysis when benefit was assessed specifically for those patients with detectable desipramine plasma levels, there was an even greater and highly significant composite score response to desipramine with a number needed to treat (NNT) of 4.3 for satisfaction with the treatment. This last study indicated one of the problems with psychotropic medications, the relatively high prevalence of perceived side effects causing a substantial risk for termination of treatment before any positive effects have had a chance to occur. In this particular study,

25% of participants were lost primarily because of side effects. Interestingly, the more favorable response in patients without concomitant depression suggests that the beneficial effect observed was not due to improvements in psychiatric comorbidity. This suggests that effort must be made to address patient concerns related to taking antidepressants through the patient–provider interaction.

After this study, some more have followed in gastroenterologic diagnoses that include chronic abdominal pain. In functional dyspepsia (FD) a small study including 38 patients showed benefit for amitriptyline over placebo in improving total symptom score and nausea, as well as upper abdominal pain (Braak et al. 2011), and a more recent multicenter study in FD also indicates a positive effect of treatment with amitriptyline (Talley et al. 2015). An interesting aspect of this last study is that patients with ulcer-like dyspepsia have the most favorable response with a more than threefold higher likelihood of adequate relief compared with placebo or escitalopram treatment. This effect was not seen in dysmotility like dyspepsia and overall this recent and well-designed study supports the use of TCAs for painful FGIDs, which also meta-analyses do with stable outcomes over time (Ford et al. 2009, 2014). It also shows that SSRIs per se are not effective in treatment for chronic pain with presumed central origin if psychological distress is not a major factor contributing to the pain experience as discussed below.

Apart from studies in FGIDs, patients with fibromyalgia also benefit from TCA treatment (Arnold et al. 2000) as well as peripheral neuropathy and other somatic pain states (Bryson and Wilde 1996).

5.2 Practical Approach for Use in Chronic Abdominal Pain

Many, if not most, doctors are familiar with the use of TCAs for the treatment of chronic pain. Low- to modest-dose regimens in the range of 25–50 or 75 mg/day regardless of substance have been used in studies apart from the Drossman study where a wider dose regimen was allowed, escalating desipramine up to 150 mg/day if tolerated (Drossman et al. 2003). The evidence for relevant analgesic effects from doses lower than 25 mg is lacking. The major drawback of TCAs is the side effects related to anticholinergic and anti-histaminic actions such as drowsiness, xerostomia, and palpitations, which is most pronounced when a tertiary amine (amitriptyline, imipramine) is used compared to the secondary amines (desipramine, nortriptyline). Some of these side effects can in clinical situations also be therapeutically useful, like for improvement of bad sleep quality or for decreasing diarrhea. In situations where patients perceive non-anticholinergic side effects early during treatment, the often recommended ultralow dosage of 10 mg can be used in order to help patients overcome the first weeks of treatment after which these usually subside and a more adequate dosage can be reached. This strategy can be motivated by the finding reported by Thiwan et al. which showed that most symptoms that were not of anticholinergic character, but perceived as side effects, were present also before start of TCA treatment (Thiwan et al. 2009). Patients can be allowed to adjust within the first 4–6 weeks of treatment within the dose range of 25–75 mg at night, where most often the occurrence of anticholinergic side effects sets natural limits.

6 Selective Serotonin Reuptake Inhibitors

6.1 Evidence for Efficacy in Chronic Abdominal Pain

There are relatively few data supporting a role for selective serotonin reuptake inhibitor (SSRI) drugs specifically for treatment of abdominal pain. The SSRIs do not have much noradrenergic effect and are thus less beneficial for pain. However, the importance of treating concurrent anxiety and psychological distress as factors determining pain experience may still lead to improvement particularly for global scores in patients with pain.

From a GI physiologic point of view, SSRIs in the form of citalopram have been shown to increase colonic contractility and reduce colonic tone during fasting conditions and reduce the colonic tone increase after meal ingestion (Chial et al. 2003a). In IBS the same drug did decrease scores for abdominal pain as well as bloating independent of anxiety, depression, and colonic sensorimotor function in a small crossover study involving 23 patients at a tertiary referral center (Tack et al. 2006). Also improvements in overall well-being regardless of coexisting depression with paroxetine treatment in IBS patients (Tabas et al. 2004) and decreased abdominal discomfort in IBS-C patients treated with fluoxetine (Vahedi et al. 2005) lend support that subgroups of patients benefit from mechanisms other than the antidepressive and anxiolytic ones. Also fluoxetine reduced abdominal pain scores in nondepressed IBS patients with rectal hypersensitivity (Kuiken et al. 2003) in a small placebo-controlled study. Of importance to take note of from an evidence-based approach is that the study with most robust design, placebo-controlled multicenter including almost 300 patients, did not show escitalopram to be superior to placebo in terms of improved symptoms of functional dyspepsia, including the epigastric pain component (Talley et al. 2015). This speaks against the use of SSRIs in clinical situations with abdominal pain without psychological comorbidity.

6.2 Clinical Application for Chronic Abdominal Pain

This group of psychotropic agents is to be looked at as best suited when anxiety, depression, and phobic features are present in parallel with and likely contributing to the abdominal pain. Treating these symptoms comes with a good chance of a reduction in pain as a secondary effect. Unlike the TCAs, the serotonergic related side effect profile includes symptoms like diarrhea, sexual dysfunction, tremor, insomnia, and nightmares. Thus patients with constipation as a comorbid problem might benefit from the tendency of SSRIs to shorten gut transit time. From a practical point of view, in the patient where anxiety is judged to be of central importance for pain experience, treatment with an SSRI is recommended to start with. In those where the anxiolytic and antidepressive effects do not result in pain control, low-dose TCA treatment can be added for augmentation with a better central analgesic effect. The doses of SSRI used for treatment of patients with chronic pain are in the same range as in treatment of anxiety and depression.

7 Serotonin-Norepinephrine Reuptake Inhibitors

7.1 Evidence for Efficacy in Chronic Abdominal Pain

Considering the central roles of serotonin and norepinephrine in the descending modulatory nerve pathways, SNRIs seem like an ideal class of drugs to modulate pain sensation by blocking presynaptic transporters for these transmitters, thereby increasing the postsynaptic stimulatory effect (see Fig. 2 for comparison of synaptic effects of SSRIs and TCAs). In the clinical setting, duloxetine and milnacipran have formal approvals for treatment of chronic pain conditions and the latter is exclusively marketed as a pain medication in the United States. Venlafaxine is indicated for depression though it is used off label for pain. The agents differ between each other by differences in serotonin reuptake inhibition, venlafaxine being the most, duloxetine intermediate, and milnacipran the least potent (Stahl et al. 2005). The simultaneous effect of increasing both serotonin and norepinephrine is better pain-relieving properties compared with increases in one or the other neurotransmitter alone in rat models (Iyengar et al. 2004). There is no indication though that there are significant effects on acute pain, rather only chronic pain (Iyengar et al. 2004; Jones et al. 2005).

In a recent Cochrane review, duloxetine was found to have adequate evidence for the treatment of peripheral diabetic neuropathic pain, but also moderate-quality evidence for its use in fibromyalgia (Lunn et al. 2014), a common extraintestinal manifestation in the generalized pain situation seen in severe FGIDs. There are also some data to support their role as having dual treatment effects in patients suffering from both depression and at least moderate pain of unknown etiology (Brecht et al. 2007). When it comes to GI function, venlafaxine has been shown to increase compliance, relax tone, and reduce the postprandial colonic contraction as well as have a tendency for increasing the sensory thresholds in response to balloon distensions (Chial et al. 2003b). There is also a slight effect on postprandial gastric volume change from venlafaxine (Chial et al. 2003a).

7.2 Clinical Application for Chronic Abdominal Pain

In pain-predominant FGIDs, SNRIs have the advantage compared to TCAs of less side effects. Even so, there might be an issue when it comes to the adherence to treatment in the studies involving FGID patients because of side effects. The most common is nausea for which the risk can be reduced by starting treatment in the low-dose range, (75 mg/day for venlafaxine, 30 mg/day for duloxetine), and supporting patients to overcome the first week of treatment where nausea is most common; then the medication can be increased to effective levels. Other side effects include palpitations, sweating, sleeping disorders, dizziness, and visual impairment (van Kerkhoven et al. 2008; Brennan et al. 2009). There is also a possibility of anticholinergic side effects mediated through a modulation of sympathetic tone by norepinephrine that some patients can experience. Comparing the different SNRIs, venlafaxine could be more prone to a side effect problem when treating pain, including a risk for increased diastolic blood pressure

which is recommended to be monitored at least in the higher dose range of treatment (Thase 1998). In the lower dose range for venlafaxine, the serotonergic effects dominate and dosage needs to be escalated to 150–225 mg/day in order to reach norepinephrine effects sufficient for pain modulation.

8 Tetracyclic Antidepressants

8.1 Evidence for Efficacy in Chronic Abdominal Pain

Mirtazapine has multiple receptor activities resulting in an increased noradrenergic and serotonergic activity. The latter is dominated by a 5-HT_{1A} agonism, and antagonism of 5-HT_2 and 5-HT_3 receptors (Stimmel et al. 1997), but there is also antagonism at α_2-adrenergic receptors, histamine H_1-receptors, and the muscarinic receptor (see Fig. 3). However, human data of effects on GI function is limited, but dose-dependent effects resulting in an accelerated gastric emptying (Yin et al. 2014) and a decreased acute response to colorectal distension (Yin et al. 2010) in animal experiments have been published. From aspects like these, pain-predominant FGIDs would be a putative indication, but data from clinical situations involving abdominal pain is limited apart from case reports in IBS (Thomas 2000; Spiegel and Kolb 2011). A study in FD patients with weight loss without coexisting anxiety or depression also showed that mirtazapine 15 mg in the evening for 8 weeks was superior to placebo in improving

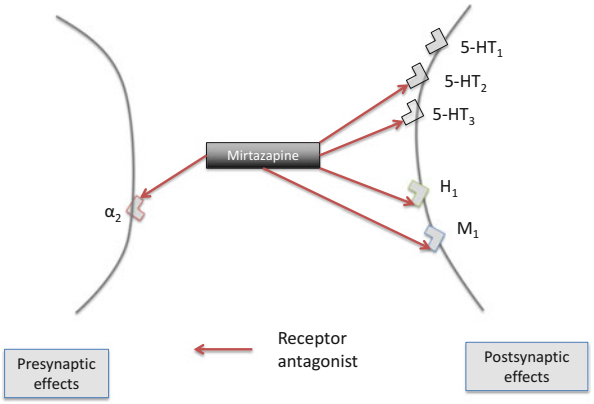


Fig. 3 Mirtazapine has multiple antagonistic effects on neurotransmission. The presynaptic α_2 antagonism results in disinhibition of both serotonin and norepinephrine release which are key factors for its analgesic effects. The antagonistic effects on 5-HT_2 and 5-HT_3 receptors result in an increased serotonin effect on the 5-HT_1 receptor, a mechanism of theoretical advantage in situations dominated by nausea. Side effects (constipation and sedation) are mainly related to the antagonistic effects on histamin$_1$ (H_1) and muscarinic (M_1) receptors. From a clinical point of view, some of these side effects can be used as positive therapeutic effects in patients with disturbed sleep and diarrhea

the overall symptom score, which represents the part where abdominal pain is rated, early satiation, nutrient tolerance, and weight recovery (Huynh Giao et al. 2013).

8.2 Clinical Application for Chronic Abdominal Pain

Since the GI side-effect profile is favorable, the use of mirtazapine in clinical situations involving abdominal pain and in particular nausea and vomiting can be considered. The sedative effect from the antihistaminic action also reduces the latency for sleep, at least in patients treated for depression (Winokur et al. 2003), which can be of help when abdominal pain disturbs the sleep pattern. The regular dose range is from 15 to 45 mg/day given in the evening in order to decrease the risk for daytime sedation.

9 Other Centrally Targeted Pharmacologic Compounds

9.1 Bupropion

This antidepressant has no serotonergic effects but instead inhibits presynaptic reuptake of noradrenalin and dopamine. The use of bupropion in chronic abdominal pain has no formal evidence but can be considered from extrapolation of SNRI effects on descending inhibitory nerve fibers which is involved in the antihyperalgesic effect of bupropion as shown in animal models (Hoshino et al. 2015). There is one small, randomized placebo-controlled crossover study in humans with neuropathic pain of different etiology showing a significant pain reduction during bupropion treatment compared with placebo (Semenchuk et al. 2001), but none of the participants suffered primarily from abdominal pain. A theoretical advantage could be to use bupropion if there is a problem with fatigue and sleepiness involved in the total clinical picture, since the treatment effects of bupropion from this respect are better compared with the SSRIs (Papakostas et al. 2006). Like for the SNRIs, nausea is a common side effect which makes bupropion less suitable if this symptom already is at hand as part of a chronic abdominal pain problem. Dosage is the same as for the psychiatric indications, i.e., 150–300 mg/day.

9.2 Azapirones

These are anxiolytic agents with relatively few side effects and without a potential for physical dependence to develop. There is limited formal evidence of direct effects on abdominal pain, but their 5-HT_{1A} agonism has the potential to result in important effects on gastric physiology mechanisms seen in patients with FD in particular. In a 4-week study of buspirone in FD patients, dyspeptic symptoms decreased more than during placebo treatment, mainly involving uncomfortable sensations not directly expressed as pain such as early satiety, fullness, bloating, and nausea. Interestingly this was associated with greater increases in postprandial gastric volumes, a finding that indicates that effects on gastric accommodation are the physiologic background

for this positive effect to occur (Tack et al. 2012). The same result on symptoms has been seen after a nutrient satiety drink test as well (Chial et al. 2003a). Tandospirone is an azapirone that is not available in large parts of the world apart from China and Japan. When tested in patients with IBS, positive effects on abdominal pain ratings were seen as expressed by the fraction of patients reporting reductions in pain scores of at least 50% in the tandospirone treatment arm, but the interpretation of this result is complicated by a simultaneous use of pinaverium, an antispasmodic that might have had significant effects on pain as well (Lan et al. 2014). This is further emphasized by a randomized controlled study that was not able to show any effects superior to placebo in patients with FD (Tack et al. 2009). If buspirone is used in the setting of an FGID with abdominal pain, the dosage should be the same as in treatment of anxiety, i.e., 30 mg divided two or three times daily, but can be increased to 60 mg. From a theoretical point of view and with the existing studies in mind, it is probably best suited in patients where the symptoms also involve early satiety, fullness, and nausea.

9.3 Anticonvulsants

Pregabalin or gabapentin has been shown to reduce visceral hypersensitivity (Houghton et al. 2007; Diop et al. 2002; Eutamene et al. 2000). This has resulted in a clinical use that to a large extent has been aiming for the treatment of neuropathic pain but also for pain associated with fibromyalgia. It acts by binding to the $\alpha_2\delta$ site, a subunit of the voltage-gated calcium channels, and modulates the release of excitatory neurotransmitters involved in nociception such as norepinephrine, substance P, glutamate, and calcitonin gene-related peptide. Results from brain-imaging studies in fibromyalgia suggest that the analgesic effects of pregabalin also have a central component involving reductions in brain insula glutamate levels (Harris et al. 2013). Studies in FGIDs are largely lacking, but its clinical use in certain situations, like when a general anxiety disorder or fibromyalgia/abdominal wall pain coexists, is reasonable in a dosage between 150 and 600 mg/day where treatment effects should be expected within a month in order to continue therapy.

9.4 Atypical Antipsychotics

Quetiapine is an atypical antipsychotic where there is some experience from treatment in situations involving chronic pain. It has a mechanism of action that includes a weak D_2 receptor antagonism and 5-HT_{2A} receptor antagonism, partial 5-HT_{1A} receptor antagonism, H_1 receptor antagonism, and moderate to high affinity for α_1 and α_2 adrenoceptors (Ichikawa et al. 2002). These complex actions at the receptor level can result in clinical effects like anxiety reduction and establishment of a normal sleep pattern (Calandre and Rico-Villademoros 2012). Its main metabolite also has effects as a noradrenaline transporter inhibitor, which is of theoretical advantage for analgesic effects (Jensen et al. 2008). Most data for pain reduction is

to be found in the treatment of fibromyalgia where a controlled study reported effects superior to placebo on the pain domains (McIntyre et al. 2014), but with effects inferior to duloxetine in yet another study (Calandre et al. 2014). Quetiapine has also been shown to improve pain when used to augment the use of a TCA and SNRI (Grover et al. 2009) as discussed below. A major drawback is poor tolerability with a high proportion of patients experiencing sedation, dizziness, and weight gain leading to discontinuation of treatment when used in the higher dose ranges of 200–400 mg/day or greater.

10 Augmentation Therapy

The concept of augmentation refers to the clinical situation where individual therapeutic effects from the most common drugs like TCAs, SSRIs, and SNRIs are either insufficient or complicated by side effects that restrict dosage to a suboptimal level. Instead of totally abandoning the drug that results in this situation, it is also possible to choose adding other drugs in order to combine their effects and also be able to use a lower dosage to minimize the risk for side effects. This concept was first reported and studied in the treatment of depression (Trivedi et al. 2006). By careful selection and dosage, a reduction in anxiety that mediates a tendency to perceive side effects can also be the goal, as exemplified by low doses of an atypical antipsychotic (Grover et al. 2009). In the ideal situation the mechanisms of action should be complementary when combining drugs in order to optimize the chance for positive additive effects. Even if it is seldom seen by use within normal dose ranges, the possibility of provoking a serotonergic syndrome (hyperreflexia, spontaneous clonus, muscle rigidity, and fever) warrants caution when combining centrally acting medications. The doctor prescribing combinations of medications should be familiar with their effects and interaction potential, both from the positive and negative aspects. In reality, also non-pharmacologic treatment options are to be considered as augmentation.

A simplified model of augmenting effects that can be useful in the treatment of chronic abdominal pain is summarized in Fig. 4.

11 Relapse Prevention

In patients responding to the initial (2–3 months) treatment of chronic abdominal pain with centrally acting drugs, the following question arises: For how long should the treatment go on before tapering the dose and finally stopping it? If returning to the initial description of a neuroplastic effect from antidepressants, the duration of use should probably be extended in the same manner as is recommended for the treatment of major depressive disorders, i.e., approximately another 4–9 months at least. A short initial treatment period, less than 4–6 months, is followed by an increased relapse risk in the treatment of depression (Baldessarini et al. 2015), and in general, the risk of relapse is higher after stopping treatment compared to if treatment continues (Sim et al. 2016). Having yet another non-pharmacologic treatment option (hypnotherapy,

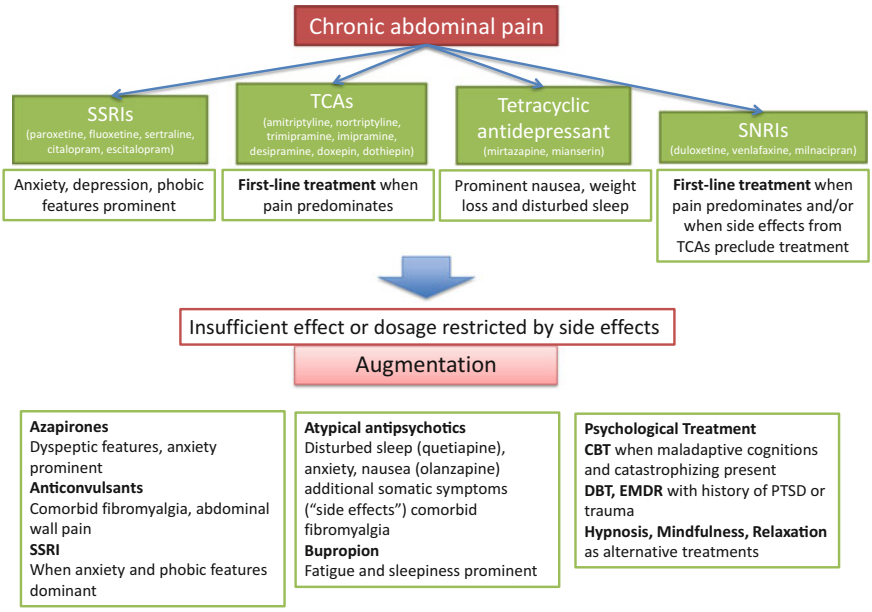


Fig. 4 Summary of the clinical characteristics in a situation of chronic abdominal pain that can be used when selecting pharmacotherapy. Those drugs in the upper part of the figure can be considered as first-line options. In the lower part of the figure, the pharmacologic options most often used to augment treatment effects are depicted as well as some non-pharmacologic treatment alternatives

cognitive behavioral therapy, etc.) available for the patient probably decreases the relapse risk after stopping medications. In those patients who have risk factors for recurrence such as ongoing psychosocial stressors, the choice between stopping and continuing pharmacologic treatment favors the latter.

12 Summary

Chronic abdominal pain is a considerable treatment challenge. By understanding the complexity of mechanisms that is of importance in the progression and maintenance of long-standing pain in general and GI pain in particular, a rational and careful use of centrally acting drugs can be of considerable help in both reducing pain and improving social and mental functions. The basic evaluation includes factors that need to be addressed before pharmacologic therapy can be decided upon, such as understanding the biopsychosocial context of pain and establishing a patient–doctor relationship dominated by trust. Most doctors that have experience in treatment with antidepressants can safely use them also in situations dominated by chronic abdominal pain as outlined in this chapter. In a selected number of patients who respond poorly or perceive side effects, using other drug classes than the

antidepressants and also augmenting effects by combining drugs in lower doses are also possibilities.

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